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Grafting Success of *Pinus caribaea* under Varying Shade Intensities at National Tree Seed Center, Namanve, Uganda

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ABSTRACT

The effect of shade intensity and grafting methods on grafting success and growth rate of *Pinus caribaea* was assessed in Uganda by subjecting grafts to different shade intensities. Scion growth and number of new leaves produced were counted and recorded for a period of 16 weeks. One-way ANOVA was used to statistically analyze the results. Shade intensity had a significant effect on height growth (P=0.000) and leaf production (P=0.027) but not on scion diameter growth (P=0.401). Different grafting methods showed a significant effect on scion height growth (P=0.000). Higher mean graft survival was observed with the top wedge method (49%). 95% shade intensity had the highest mean survival with a 38% graft survival. The mean scion height growth rate per week was 0.30 and 0.21 cm for top wedge and splice method, respectively. The mean scion diameter growth rate was higher with the splice method (0.032 cm) than with the top wedge method (0.017 cm). Shade intensity had a significant effect on mean height and diameter growth of scions for 0, 50 and 95% shade intensities, respectively. Although *P. caribaea* grafts' survival and scion growth rate are highly influenced by both grafting method and shade intensity, a more extended study is still needed to elucidate the survival and growth rate of grafts in the field.

Keywords: asexual propagation, grafting method, growth rate, plantation species, tree

INTRODUCTION

Development of planted forests in tropical countries is needed to satisfy the ever-growing global demand for wood products as well as to improve the local environment, livelihoods of poor people and combat desertification. In response to this, plantation of tree species such as *Pinus caribaea* has been recommended (Evans 1992). *P. caribaea* is commonly known as Bahamas pitch pine (English), Caribbean pine (English) and pin des Caraïbes (French). Even if *P. caribaea* has been recommended for plantation, its establishment in Uganda has been constrained by difficulties associated with low seed supply and a high cost of imported seeds. This is so because an increasing local demand for its seed outmatches the decreasing supply from seed-exporting countries such as Australia and Brazil (NFA and SPGS 2005).

To meet the increasing tree seed demand in Uganda, there is now a need to establish a national seed orchard of major important species such as this one. Since seed orchard establishment in Uganda has been limited by lack of a formal tree improvement programme for various species (SPGS 2007), development of appropriate vegetative propagation techniques of timber species like *P. caribaea* is overdue.

Although the causes of *P. caribaea* grafting failures in Uganda are not yet clear and undocumented, available studies by different scientists have explored the effects of pathological incidences (Gómez *et al*. 2004; Reddall *et al*. 2004), graft incompatibility (Moore 1984; Brennan *et al*. 1998; Errea *et al*. 2001; Peil 2003; Suguino *et al*. 2003) and application of different levels of plant hormones on grafting (Fernández *et al*. 2004; Akaneme and Ene-Obong 2008). A report by Felker *et al*. (2000) aimed to study the influence of environmental factors (full-sunlight, half-shade and 'tent' treatment, i.e. 30×40 cm black plastic shade) on grafting success of *Prosopis ruscifolia* onto *Prosopis alba* indicates that grafts exposed to low light intensity can give greater success than those under high light intensities. Furthermore, a study investigating the influence of shade on the growth of seedlings of three Amazon species (*Jacaranda copaia*, *Hymenaea courbanil* and *Ochroma lagopus*) showed that seedling growth varied with different shade levels (Campos 2002).

Even when the top wedge method has been reported as a better method than the tube grafting method for tomatoes (Kacjan *et al.* 2004), top wedge grafting has been reported to be superior to whip and inverted T budding methods for grafting *Spondias tuberosa* (Araújo 2002). Compared to tongue, top wedge, chip budding and side veneer grafting methods, the side (cleft) method reportedly resulted in a higher graft success of *Vitellaria paradoxa* grafts (Sanou *et al*. 2004).

Since shade intensity and grafting methods have been cited as factors that affect grafting success of some species, this study investigated the effect of these two factors on grafting success of *P. caribaea* in Uganda. At present the demand of planting material (seeds) of *P. caribaea* is overwhelmingly increasing, so the need for establishment of national seed orchards of this species through grafting is increasingly important.

MATERIALS AND METHODS

Study area

A grafting experiment was set up at the National Tree Seed Center (NTSC), Namanve located 12 Km along the Kampala-Jinja highway at an altitude of 1150 meters above sea level (m.a.s.l). The area has a mean annual temperature of 22-28°C and of 1750 mm annual rainfall (Mugisha 2005). The site was chosen because of the availability of grafting equipment and material such as the grafting shade and rootstocks, respectively.

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Original Research Paper

Table 1 The complete randomized block (CRB) experimental design

Treatment / Replicate	0	50	95
Splice method	S, 0	S, 50	S, 95
Top wedge	T,0	T, 50	T, 95

S and T refer to splice and top wedge grafting methods respectively.
Number of plants (n) = RT, n =20 × 6 =120 plants where R and T are number of replicates and treatments, respectively.

Experimental design

The experiment was set up in a complete randomized block design (CRBD) with two factors: method of grafting (splice and top wedge grafting methods) and shade intensity. Shade intensity was controlled by the use of 0, 50 and 95% shade intensity grafting nets. The experiment had 6 treatments replicated 20 times making a total a number of 120 grafts (**Table 1**).

Experimental set up

One hundred and twenty (120) unlignified scions at the dormant growth stage were collected from a tagged branch in the canopy of identified superior mature *P. caribaea* trees. These trees were selected based on good physical and sanitary characteristics such as tree form and absence of parasites. The scions were collected from a 5-year old F1 *P. caribaea* stand in Kifu Central Forest Reserve, Mukono district between 8:00 and 9:00 am on each collection day. The scions were 5-17 cm long with a diameter of 0.5-1.0 cm at the base.

After collection, the scions were immediately placed in a cool box at 20°C during transportation from Kifu to the Namanve experimental site. One hundred and twenty (120) one-year-old *P. caribaea* seedlings sown from seed of Brazilian origin were used as rootstocks. The rootstocks were obtained from the NTSC. The rootstocks ranged between 10 and 15 cm in height and 0.5 and 1.0 cm in diameter at the root collar and were selected based on their straightness and absence of parasites. These characteristics were judged visually.

Grafting onto rootstocks was done on the same day immediately after scion collection so as to minimize physiological stress (Jaenicke and Beniest 2002). Sixty grafts were grafted using each grafting method. The grafting procedures for top wedge and splice method were the same as described by Jaenicke and Beniest (2002).

For top wedge grafting (**Fig. 1**), the stem of the scion and the rootstock were cut at right angles at a point where it is about 0.5-cm thick and 25 cm above the soil line. Thereafter scions of the same size were cut in a wedge shape. After the tapered end was fitted into a cleft (2.5 cm down through the rootstock), the graft was then held firm with a polyethylene strip. This was to ensure that the scion remained in place during tying in. With splice grafting (**Fig. 2**) rootstocks were topped at a point where it is about 0.5 cm thick and 25 cm above the soil line. A slanting cut of 2 cm was made into the rootstock. After a similar cut was made on the scion, both the rootstock and scion were inserted with the tongues interlocking and matching the cambium layers. In the same way, the grafts were held firm with a polyethylene strip while ensuring that the scion did not slip during tying in.

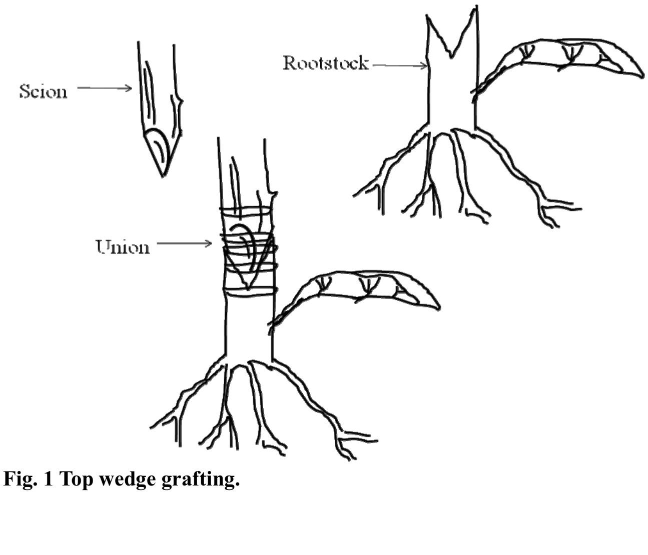

Fig. 1 Top wedge grafting.

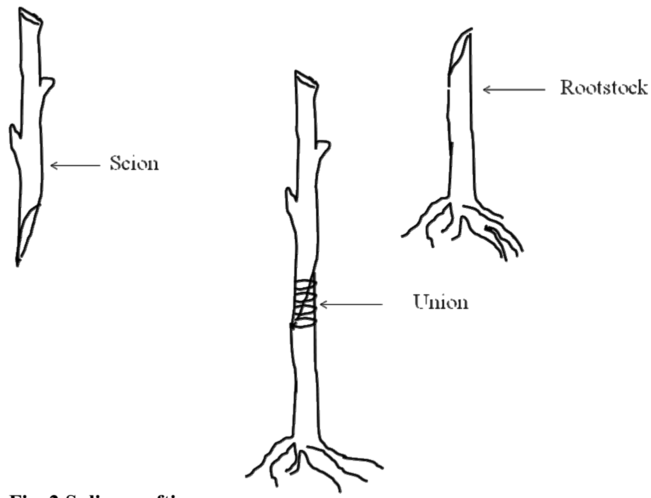

Fig. 2 Splice grafting.

Fig. 3 Grafts placed on a wire mesh over bricks.

After grafting with the different methods, the grafts were covered with transparent polythene bags in which water had been sprinkled. The polythene bags were tied tightly around the stem. Air was blown into the polythene bags so as to prevent them from clinging to the scion, thus avoiding possible infections. Twenty grafts of each method were put into an open area (0% shade), a black net of 50% and 95% shade intensity, respectively and watered regularly. Temperature was recorded in each shade type 7 days after the start of the experiment and continued for 16 weeks. Grafts were then placed on a wire mesh raised from the ground using bricks (**Fig. 3**) to minimize damping off and pest attack. Side shoots (water suckers) that developed below the union point and weeds that were growing in the pots were regularly removed from all grafts. Polythene bags were removed gradually as new leaves began to grow. Use of disinfected equipment and maintenance of dryness inside the grafting shade were also implemented to avoid pathogen attack (Hartman 1997).

Data collection

Changes in height and diameter of scions were recorded at 14, 21, 28, 35 and 42 days for another 16 weeks after grafting. A minimum period of 21 days was needed to ensure a sufficient difference in growth of all grafts in terms of scion length, scion diameter and production of new leaves (Moore 1984; Errea *et al.* 2001). The length of the scions was measured using a ruler and new leaves were counted for each graft type. The diameter of scions was measured using a vernier caliper and recorded.

Fig. 4 A successful Pine graft just after removal of the polythene covers.

Data on height, diameter and number of new leaves produced on the scions were collected only from living grafts. The effect of shade intensity and grafting method on growth rate of scions was determined by randomly selecting two grafts for each method and shade intensity. Since the polythene covers had to be removed after the new leaves had begun to grow (Jaenicke and Beniest 2002), in this study the polythene covers were removed at 7 weeks, a period when the new leaves had begun to grow (**Fig. 4**).

Data analysis

Data obtained for both treatments were entered into Ms Excel from which percentages were calculated and histograms drawn. One-way ANOVA was used to test for the effect of shade intensity and grafting method on increase in height and diameter plus new leaf production of scions. All statistical tests were performed at the 5% level of significance using CRBD MINITAB v. 12. Shade intensity and grafting method were considered to be the only sources of variation in this study.

RESULTS

Shade intensity had a significant influence on the survival of scions and their growth rates. 95% shade intensity resulted in a higher survival percentage with a 38% scion survival followed by 50% shade intensity with a 8.6% scion survival (**Fig. 5**). The height and diameter of scions was higher under 95% shade intensity (**Table 2**). There was a significant difference in mean height growth (**Table 3**) and mean new leaf production (**Table 4**) but not in mean diameter growth of the grafts (**Table 5**). The mean temperature recorded was 28, 23 and 19°C for 0, 50 and 95% shade intensity, respectively.

The highest scion mean survival percentage was achieved with the top wedge grafting method with a 49% scion survival percentage compared to the splice method in which a 24% scion survival percentage was observed (**Fig. 6**).

The height and diameter of scions was higher in the top wedge grafting method than in the splice method (**Table 6**).

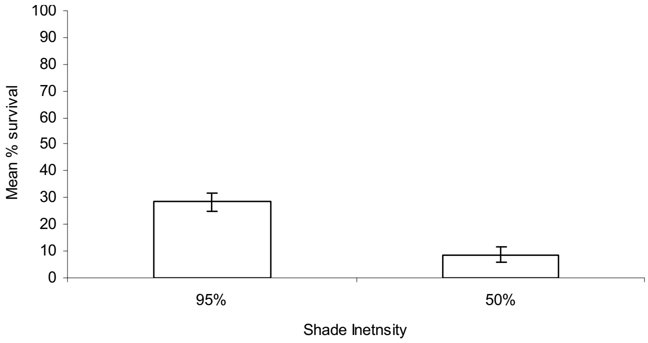

Fig. 5 Effect of shade intensity on scion survival percentage.

Table 2 Effect of shade intensity on mean scion growth rate.

Shade intensity (%)	Mean change in height (cm)	Mean change in diameter (cm)
50	0.307	0.027
95	0.380	0.050

There was a significant difference in scion mean height growth (**Table 7**) but not in mean diameter growth and mean new leaf production.

DISCUSSION

Scion survival percentage (**Fig. 5**) and growth rate (**Table 2**) were significantly influenced by different shade intensities. This can be attributed to the influence of shade on callus formation and the general wound healing process at the graft union point and also its role in controlling evapo-transpiration and desiccation (Hartman 1997). Very poor scion survival percentage and growth rates were recorded for scions under 0% shade intensity. As reported by Felker *et al.* (2000) and Caponetti *et al.* (1971) shade intensity influences effective plant growth by influencing the rates of evapo-transpiration, desiccation, callus formation and healing process of the union point.

According to Felker *et al.* (2000), *Prosopis mlifolia* and *Prosopis alba* grafts exposed to a low light intensity (tent treatment) gave a better success of 90% as compared to 70% and 40% successes for those under higher (half- and full-sun) light intensities, respectively. On the other hand, Caponetti *et al.* (1971) showed that the callus of grafted black cherry (*Prunus serotina*) became larger in darkness than they did in light. This implies that shade intensity can affect plant growth by influencing the rates of evapo-transpiration, desiccation, callus formation and healing process of the union point (ICRAF 2000).

The significant differences in mean scion height growth (**Table 3**) and mean new leaf production (**Table 4**) could be attributed to the ability of plant shoots to elongate and produce more leaves, which can be used to obtain sufficient solar energy for various plant processes such as photosynthesis. A report by Ribeiro and Gomes (2003) indicates that limited solar radiation (90% shade intensity) decreased photosynthetic and transpiration rates. This in turn increased

Table 3 Effect of shade intensity on mean height growth of scions.

Source	DF	SS	MS	F	P	Significance
Shade	2	575.7	287.8	20.33	0.000	**
Error	443	6273.1	14.2			
Total	445	6848.8		Individual 95% CIs for mean based on pooled StDev		
Level	**N**	**Mean**	**StDev**	-----+---------+---------+--------+-		
1	48	9.323	1.736	(-------*------)		
2	162	13.184	4.068	(---*-)		
3	236	11.840	3.841	(--*--)		
Pooled StDev = 3.763				-----+---------+---------+--------+------		
				9.0 10.5 12.0 13.5		

** = Significant at $P \leq 0.01$, * = Significant at $P \leq 0.05$, ns = not significant, -----+----- = regions of significance or no significance

Table 4 Effect of shade intensity on mean new leaf production of scions.

Source	DF	SS	MS	F	P	Significance
Shade	2	1522	761	3.65	0.027	*
Error	443	92284	208			
Total	445	93806		Individual 95% CIs for mean based on pooled StDev		
Level	**N**	**Mean**	**StDev**	--+---------+---------+--------+---		
1	48	0.00	0.00	(------------*---------)		
2	162	6.06	16.83	(-----*-----)		
3	236	5.88	14.09	(----*----)		
Pooled StDev = 14.43				--+---------+---------+---------+---		
				-3.5 0.0 3.5 7.0		

** = Significant at $P \leq 0.01$, * = Significant at $P \leq 0.05$, ns = not significant, -----+----- = regions of significance or no significance

Table 5 Effect of shade intensity on mean diameter growth of scions.

Source	DF	SS	MS	F	P	Significance
Shade	2	17.54	8.77	0.92	0.401	ns
Error	443	4245.62	9.58			
Total	445	4263.16		Individual 95% CIs for mean based on pooled StDev		
Level	**N**	**Mean**	**StDev**	-----+---------+---------+--------+----		
1	48	0.640	0.093	(----------------*----------------)		
2	162	1.082	5.133	(----------*--------)		
3	236	0.676	0.109	(-------*------)		
Pooled StDev = 3.096				-----+---------+---------+--------+----		
				0.00 0.50 1.00 1.50		

Note: Level 1 (0% shade intensity), 2 (50% shade intensity), 3 (95% shade intensity)
** = Significant at $P \leq 0.01$, * = Significant at $P \leq 0.05$, ns = not significant, -----+----- = regions of significance or no significance

plant height growth and leaf production to augment photosynthetic and transpiration surface area, respectively. The generally low survival percentage and growth rate of the grafts could be attributed to low rainfall and high temperatures, which might have been experienced after grafting. According to Hartman (1997) and Evans and Turnbull (2004), callus formation in grafts is optimum under conditions of high moisture and low temperature, which are useful in preventing the scions from drying out.

As reported by Celık *et al.* (2006), cambial connectivity is related to callus formation and is mostly affected by temperature and humidity around the graft union. Grafts exposed to high temperatures transpire a lot of water into the atmosphere resulting in desiccation. This occurs because the newly formed cells of important callus tissues have relatively thin and tender cell walls with no provision of resisting desiccation. If such cells are exposed to dry air for a very long time, they are more likely to die. The implication of this is that the graft union needs to be kept sufficiently moist if the survival percentage and growth rate are to be enhanced (Hartman 1997; Jaenicke and Beniest 2002).

Scions mean survival percentage (**Fig. 6**) and growth rate (**Table 6**) can also be significantly influenced by different grafting methods. Although the effect of grafting method on scion survival percentage and growth rate are unclear, it could be related to the ease with which graft contact is developed (Sanou *et al.* 2004) and the time taken to develop callus tissues at the union point (Araújo 2002) for survival and growth rate, respectively.

The height of scions was significantly affected by the grafting method (**Table 7**). This could be due to the kinds of wound created on the rootstock during grafting. Since the depth of the wound on a rootstock would vary with the grafting method used (to ensure cambial union), methods which create deep wounds on the rootstock during grafting can have a negative effect on graft healing, cambial connectivity and shoot growth development (Celık *et al.* 2006). Subsequently, the quick and strong union formation provided by a given grafting method determines uptake of water and nutrients hence scion survival and high growth rate.

Although in the present study the top wedge grafting method gave higher scion mean survival percentage and growth rate, it contrasts with the findings of Kapoor (1981) in which the splice method was reported to be a better method for grafting *P. caribaea* in India.

Table 6 Effect of grafting method on scion growth rate.

Method	Mean change in height (cm)	Mean change in diameter (cm)
Top wedge	0.307	0.027
Splice	0.380	0.050

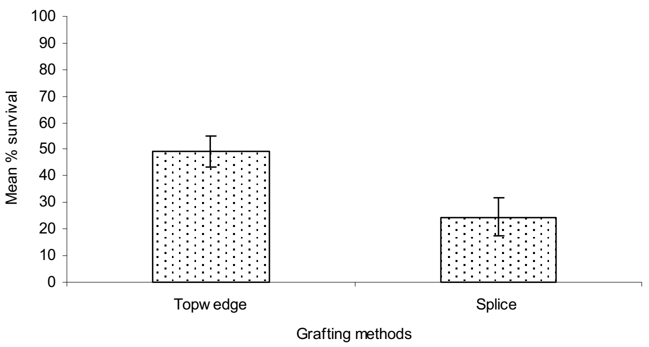

Fig. 6 Effect of grafting method on scion survival percentage.

Table 7 Mean height growth of scions for different methods after every 7 days.

Source	DF	SS	MS	F	P	Significance
Method	1	554.9	554.9	39.14	0.000	**
Error	444	6293.9	14.2			
Total	445	6848.8		Individual 95% CIs for mean based on pooled StDev		
Level	**N**	**Mean**	**StDev**	-+---------+---------+---------+-----		
Cleft	288	12.883	4.001	(----*----)		
Splice	158	10.551	3.290	(-----*----)		
Pooled StDev = 3.765				-+---------+---------+---------+-----		
				10.0 11.0 12.0 13.0		

** = Significant at $P \leq 0.01$, * = Significant at $P \leq 0.05$, ns = not significant, -----+----- = regions of significance or no significance

CONCLUSION

The survival percentage and growth rate of *Pinus caribaea* grafts can be significantly influenced by shade intensity. In this study, the highest survival percentage and growth rate of *P. caribaea* grafts was under 95% shade intensity. Higher survival percentage and growth rate of *P. caribaea* grafts was realized with the top wedge grafting method more than with the splice method of grafting. Thus, to enhance grafting success in *P. caribaea*, shade intensity > 90% and the top wedge grafting method should be promoted.

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REFERENCES

Akaneme FI, Ene-Obong EE (2008) Tissue culture in *Pinus caribaea* Mor. var. *Hondurensis* Barr. and Golf. II: Effects of two auxins and two cytokinins on callus growth habits and subsequent organogenesis. *African Journal of Biotechnology* **7**, 757-765

Araújo de Castro Neto FP (2002) The effect of physiological factors of mother plants and timing of grafting with different methods of Umbu (*Spondias tuberosa*). *Revista Brasileira de Fruticultura* **24**, 752-755

Brennan EB, Mudge KW, Sherrer A (1998) Open-pollinated, site-specific F1 hybrid seed orchards of *Leucaena leucocephala*: Rootstock effect. *Agroforestry Systems* **41**, 221-223

Caponetti JD, Hall GC, Farmer RE Jr. (1971) *In vitro* growth of black cherry callus: Effects of medium, environment and clone. *Botanical Gazette* **132**, 313-318

Campos MAAU (2002) Influence of shade on growth of seedlings of three Amazon species. *Pesquisa Agropecuaria Brasileira* **37**, 281-288

Celık H, Zengınbal H, Ozcan M (2006) Effect of budding performed by hand and with manual grafting unit on kiwifruit propagation in the field. *Horticultural Science (Prague)* **33**, 57-60

Errea P, Garay L, Marín JA (2001) Early detection of graft incompatibility in apricot (*Prunus armeniaca*) using *in vitro* techniques. *Physiologia Plantarum* **112**, 135-141

Evans J (1992) *Plantation Forestry in the Tropics* (2nd Edn), Oxford University Press, 403 pp

Evans J, Turnbull J (2004) *Plantation Forestry in the Tropics* (3rd Edn), Oxford University Press, pp 188-195

Felker P, Ewens M, Ochan H (2000) Environment influence on grafting success of *Prosopis ruscifolia* onto *Prosopis alba*. *Journal of Arid Environments* **4 (46)**, 433-449

Fernández GN, Carvajal M, Olmos E (2004) Graft union formation in tomato plants: Peroxidase and Catalase involvement. *Annals of Botany* **1 (93)**, 53-60

Gómez O, Piñón M, Martínez Y, Quiñónes M, Fonseca D, Laterrot H (2004) Breeding for resistance to begomovirus in tropic-adapted tomato genotypes. *Plant Breeding* **123**, 275-279

Hartman HT (1997) *Plant Propagation: Principles and Practices* (6th Edn), Prentice-Hall Inc, Eaglewood Cliffs, 770 pp

Jaenicke HE, Beniest J (2002) *Vegetative Tree Propagation in Agroforestry. Training Guidelines and References*, World Agroforestry Centre (ICRAF), Nairobi, Kenya, 95 pp

Kacjan MN, Osvald J (2004) The influence of grafting on yield of two tomato cultivars (*Lycopersicon esculentum*). *Actaagriculturae Slovenica* **83**, 243-249

Kapoor ML (1981) A note on subsequent results of field grafting trails with Caribbean pine. *Indian Forester* **107**, 60-62

McKeand SE, Raley EM (2000) Interstock effects on strobilus initiation in top grafted loblolly pine. *Forest Genetics* **7**, 179-182

Moore R (1984) The role of direct cellular contact in the formation of compatible autografts in *Sedum telephoid. Annals of Botany* **54**, 127-133

Mugisha OR (2005) *Uganda Districts Information Handbook*, Fountain Publishers, Kampala, Uganda, 182 pp

National Forestry Authority, Saw log Production Grant Scheme (2005) *Tree Species for Commercial Timber Production in Uganda*, Number 5, 18 pp

Peil RM (2003) Grafting of vegetable crops. *Ciência Rural* **33 (6)**, 1169-1177

Reddall A, Ali A, Able JA, Stonor J, Tesoriero L, Wright PR, Rezaian MA, Wilson LJ (2004) Cotton bunchy top: an aphid and graft transmitted cotton disease. *Australian Plant Pathology* **33**, 197-202

Ribeiro AM de A, Gomes JC (2003) Physiological characteristics and growth of coffee plants grown under shade of pigeon pea and unshaded. *Pesquisa Agropecuaria Brasileira* **38**, 1131-1137

Saw log Production Grant Scheme (2007) *SPGS Plantation Guidelines*, February, 15 pp

Suguino E, Appezzato-da-Glória X, Araújo B, Simão PSR (2003) Vegetative propagation of Camu-Camu by intergeneric grafts in Myrtaceae family. *Pesquisa Agropecuária Brasileira* **38**, 1477-1482

Sanou H, Kambou S, Teklehaimanot Z, Dembele M, Yossi H, Sina LDS, Bouvet JM (2004) Vegetative propagation of *Vitellaria paradoxa* by grafting. *Agroforestry Systems* **60**, 93-99