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The methods of vegetative propagation of useful agroforestry species in the Peruvian Amazon

M.Sc. Thesis

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Declaration

I hereby declare that the M.Sc. Thesis „The methods of vegetative propagation of useful agroforestry species in the Peruvian Amazon" has been written by myself indepently, only with the expert guidance of my thesis supervisor Bohdan Lojka, Ph.D., without any external unauthorized help.

I further declare that all data, figures, tables and information I have used in this thesis come from initiate sources stated in the references.

Jiří Lipenský

Prague, April 20, 2010 ……

signature

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ABSTRACT

Reforestation in Peruvian Amazon depends on succesful propagation of the trees. Vegetative propagation may serve as a good alternative to propagation by seeds. This study is focused to determine effects of Indole-3-butyric acid (IBA) to rooting parametres of semihardwood leafy stem cuttings of *Calycophyllum spruceanum* (Bentham) Hooker f. ex Schumann (Rubiaceae) and *Simarouba amara* Aubl. (Simaroubaceae). The concetrations 1000, 2000, 4000, and 5000 ppm of IBA, and one control group without IBA were used. For *C. spruceanum*, the number and percentage of rooting, calluss formation, number of roots, total root length, length of the longest root, and vigor was significantly higher, while mortality and leaf abscission was significantly lower in group treated with 2000 ppm of IBA, than in control group with untreated cuttings ($P < 0.05$). However no significant differences were found in these parametres between the group treated with 2000 ppm and groups treated with 4000, and 5000 ppm, except for the leaf abscission parametre. No significant differences were recorded in number of callusses per cutting between the five groups tested. *S. amara* presented (98.5 %) mortality of both, treated and untreated cuttings, and did not present any callussing, or rooting success. Future study with more juvenile material and/or different type of cutting is recommended.

Keywords: Leafy stem cutting, Indole-3-butyric acid (IBA), Rhizogenesis, Subirigated polyethylene polypropagator.

ABSTRAKT

Opětovné zalesňování v peruánské Amazonii závisí na úspěšném množení stromů. Vegetativní rozmnožování může sloužit jako dobrá alternativa k rozmnožování pomocí semen. Tato práce je zaměřena na zjištění účinků kyseliny indolyl-3-máselné (IBA) na parametry zakořenění polodřevitých olistěných řízků druhů *Calycophyllum spruceanum* (Bentham) Hooker f. ex Schumann (Rubiaceae) and *Simarouba amara* Aubl. (Simaroubaceae). Byly použity koncentrace 1000, 2000, 4000 a 5000 ppm a kontrolní skupina bez ošetření IBA. Pro *C. spruceanum* byl počet a procentuelní zastoupení zakořeněných řízků, řízků s přítomností kalusu, počet kořenů na řízek, celková délka kořene, délka nejdelšího kořene a vitalita významně vyšší, zatímco mortalita a opad listů byl významně nižší u skupiny ošetřené 2000 ppm IBA, než u skupiny bez IBA ošetření. ($P < 0.05$). Nicméně nebyly nalezeny žádné významné rozdíly v těchto parametrech mezi skupinou ošetřenou 2000 ppm IBA a skupinami ošetřené 4000 a 5000 ppm IBA, vyjma parametru opadu listu. V počtu kalusů na řízek nebyly zaznamenány žádné významné rozdíly mezi těmito pěti testovanými skupinami. *S. amara* ukázala vysokou mortalitu (98.5 %) u ošetřených i neošetřených řízků a nebyla prokázána přítomnost kalusu ani kořene. Pro budoucí výzkum je doporučuno použití juvenilnějšího materiálu, popřípadě jiný typ řízku.

Klíčová slova: Olistěný polodřevitý řízek, Kyselina indolyl-3-máselná (IBA), Rhizogeneze, Polyetylénový polypropagátor se spodním zavlažováním.

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LIST OF ABREVIATIONS

asl	above sea level
CIDRA	Centro de Investigacion y Desarollo Rural Amazónico
CULS	Czech University of Life Sciences
IIAP	Instituto de Investigaciones de la Amazonía Peruana
IBA	Indole-3-butyric acid
ITS	Institute of Tropics and Subtropics
KW ANOVA	Kruskal-Wallis Analysis of Variance
NAA	α-naphalene acetic acid
RH	Relative Humidity
UNU	Universidad Nacional de Ucayali

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1 INTRODUCTION

The small-scale farming communities in the Peruvian Amazon depend upon more than 250 tree species for construction material, energy fuel, fibres, fence posts, resins, fruits, medicines, and other functions such as soil conservation and shade (Sotelo-Montes and Weber, 1997). These trees are logged directly from primary and secondary forest. Ucayali is a region in where settlers have traditionally practiced slash-and-burn agriculture, where the farmers cut and burn the forest to release accumulated nutrients in the woody biomass to produce annual crops.

The slash-and-burn agriculture methods are no more suitable and sustainable on such scale there because of high deforestation and soil degradation (Fig.1). In general, 60% of the deforestation of tropical rain forests is carried out by farmers for agriculture settlement. In addition, national and global environmental benefits of forests are reduced (Denevan and Padoch, 1988). The soils are acid without sufficient nutrients for sustainable, repeated harvests of annual crops. The fields are left to fallow or are converted to pasture. Slash-and-burn agriculture also leads to poor natural regeneration of many valuable tree species. Farmers and loggers cut the best timber trees, without leaving high-quality trees to produce seed for natural regeneration (Weber *et al.*, 1997).

In order to reduce problems associated with unsuitable agriculture practices, there have been increasing efforts for the adoption of agroforestry, which can be a suitable natural resource management system. The agroforestry methods can also increase farm productivity and profits. Therefore, they reduce subsistence land needs and subsequent deforestation. When crop yield and value decrease, farmers often migrate to open up more land, repeating the cycle of deforestation, forest fragmentation, soil degradation *etc*. Forestry or agroforestry, based on good knowledge, represents the only sustainable use of the land. Currently, farmers do not have access to high-quality tree transplants for their agroforestry systems. The seeds are not often available and some tree species have low germination rate. An alternative to sexual propagation can be propagation asexual (vegetative).

The vegetative propagation can produce cheap, fast, mass genetic copies of selected individuals for planting with many desirable traits for simultaneous selection and improvement in clonal agroforestry or plantation systems. Further it can speed up domestication to allow urgently needed tree planting to be carried out with mixtures of

superior selections and allows new trees to be raised at any time, so that seed problems need not affect the choice of suitable planting stock (Longman, 1993).

Reforestation efforts in the Peruvian Amazon have had little success to date because they have usually concentrated on slow-maturing timber tree species. *Calycophyllum spruceanum* (Capirona) and *Simarouba amara* (Marupa) are two underutilized multipurpose tree species that are suitable for domestication and implementation in agroforestry systems. Both species are relatively fast growing and can be harvested in 3-20 years, depending on the product desired. These tree species are also valued for firewood, charcoal production, and construction poles (Weber *et al*., 2002). Although charcoal production in the forest of Pucallpa has created another incentive to convert the rainforest, this activity is slowing the rate of deforestation in the area (Labarta *et al.*, 2007).

This study aims to determine the effect of different concentrations of Indole-3-butyric acid (IBA) on the rooting ability of leafy stem semihardwood cuttings of *Calycophyllum spruceanum* (Capirona) and *Simarouba amara* (Marupa).

Fig. 1. (a),(b),(c),(d): Different levels of deforestation caused by slash-and-burn agriculture around the city Pucallpa in Peruvian Amazon.

2 LITERATURE REVIEW

2.1 Vegetative propagation of tropical trees

The vegetative (asexual) propagation of plants is the process, when an exact copy of the genome (clone) of a mother plant (ortet) is made and continued in new individuals. It is ensured by meristematic, undifferentiated cells that can differentiate to the various organs necessary to form a whole new plant (Wiesman and Jaenicke, 2002). Clone is a group of plants derived from a single ortet by asexual reproduction. All members (ramets) of a clone have an identical genotype to a single source plant and tend to be uniform. Propagule is a plant derived from vegetative propagation including tissue culture and rooted cutting, capable of developing into an adult (Burley *et al.*, 2004).

Clonal forestry is an operational deployment of a few (5-50) proven superior clones for reforestation (Burley *et al.*, 2004). Clonal approaches to forestry have been practised for hundreds / thousands of years in Europe with willows (*Salix* spp.) and poplars (*Populus* spp.), and with Sugi (*Cryptomeria japonica*) in Japan and China (Ohba, 1993), some very old fruit and ornamental plants (Hartmann *et al.*, 1996).

According to (Leakey *et al*. 1982b, 1987) the clonal multiplication has the potential to exploit the considerable amount of genotypic variation present within tree populations. Vegetative propagation further can increase yields and quality, and shorten rotations alleviate of biological problems (*e.g.*, seed storage and poor viability) hindering reforestation with hardwood species. Other potential advatages according to (Libby, 1985) are the ability to rapidly capture a greater proportion of the additive and non-additive genetic variation and the elimination of inbred individuals from plantations. Further belongs there production of valuable, expensive genotypes and of those rare individuals which have two or more favourable characteristics by hybridization or biotechnology. It is possible to select and utilize greater genetic diversity than is normally found in a single progeny, and to use clones that are well adapted to a particular site. It can further offer the greater simplicity and flexibility of managing sets of stockplants and the increasing superiority of clones passing through multiple-trait selection programmes. The possibility of use of mature tissues can also be an advantage.

There are several disadvanteges of a clonal forestry that shouldn´t be overlooked: The single genotype demands the same things at the same time, and thus utilises the site

worse than a mixture of genotypes. The genetic diversity may decrease. In a mix, another genotype may take over the ecological space left by a failed genotype. Another disadvatege is that some desease can spread faster in a uniform stand (Lindgren, 2002).

2.1.1 Methods of vegetative propagation

The classical method is propagation by stem cuttings, which encourages roots to form on a piece of stem or twigs detached from the donor plant. In some species, stem sections, root sections, and sprouts can also serve as cuttings (Libby, 2004). More than 80% of tropical forest trees can be rooted as leafy stem cuttings (Fig. 2) which are also called softwood, greenwood, semi-hardwood, ripewood, or summercuttings (some of these terms are not suitable for the tropical trees). Leafy stem cuttings are smaller and can be taken from softer shoots. Leafless stem cuttings, sometimes called hardwood or wintercuttings; stakes or poles are larger and of firmer wood. They do not dry up fast, and can survive in moist soil until roots are formed. With these 1-2 m long poles is possible to establish “live fences” to support climbing crop plants, *e.g.*, in *Cassia siamea, Gliricidia sepium* and species of *Bombacopsis* and *Spondias* (Longman, 1993).

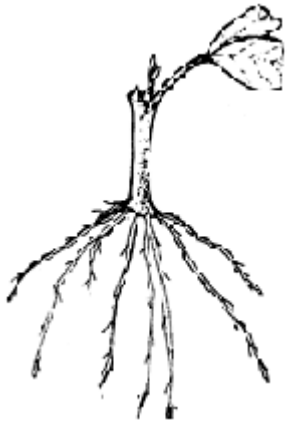

Fig. 2. Leafy stem cutting (Longman, 1993).

Another tree propagation technique is taking root suckers, or separating shoots (sometimes with root section) that have been produced on roots (Fig. 3). It is possible with *Acacia dealbata, A. melanoxylon, Chlorophora* spp., *Cinnamomum camphora, Cordia alliodora, Melia azedarach, Millingtonia, Ocotea usambarensis* and *Populus canescens* (Longman, 1993).

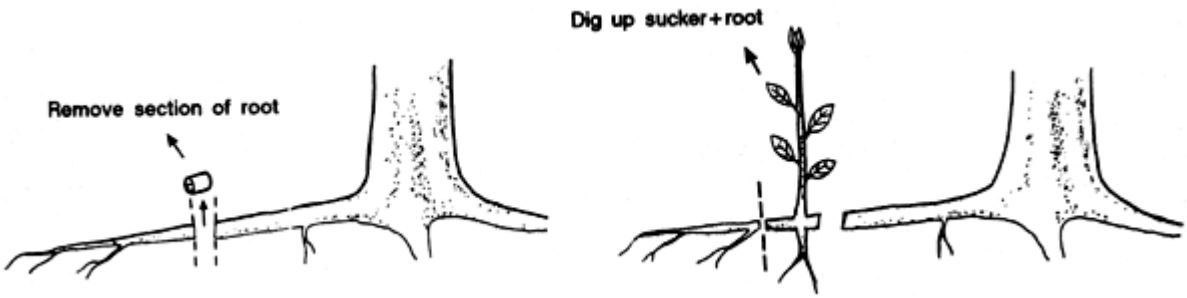

Fig. 3. Taking root suckers (Longman, 1993).

Marcotting is propagation on the intact plant by airlayering (area where bark has been removed is enclosed in propagation medium), and it is used as a tool for capture mature genotypes (Burley *et al.*, 2004).

Grafting is quite a special propagation method. Grafted plants (grafts), are compound organisms consisting of a rootstock (which may be a clonal propagule or a seedling) and a scion (a twig or bud from the desired donor plant), (Fig. 4). The rootstock becomes the root system and sometimes the basal part of the bole, while the scion becomes the upper part of the tree. Techniques of grafting are based on principle of matching the cambium of the scion to the cambium of the rootstock to promote early fusion and healing. Grafting is commonly used to produce highly uniform selected genotype clones of fruit or nut trees (Libby, 2004). Grafting has been successful with *e.g., Cedrela, Cordia, Mangifera, Pinus, Tectona, Terminalia, Treculia, Triplochiton*, and is likely to be possible with most tropical trees. It is used especially for seed orchards (Longman, 1993).

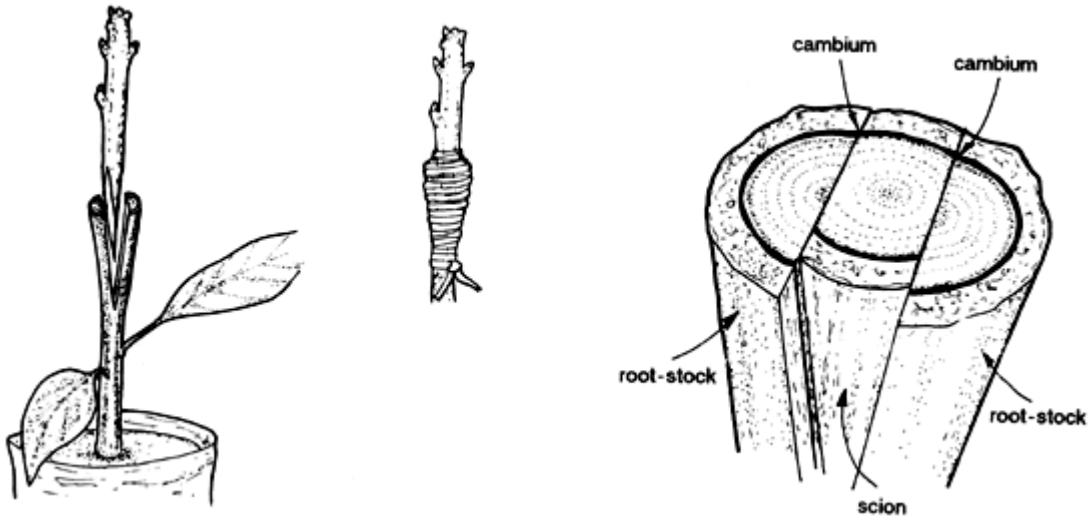

Fig. 4. Grafting (Longman, 1993).

Tissue culture is a generic term that includes *in vitro* growth and proliferation of relatively unorganized cells (cell culture), of callus (callus culture), of particular tissues, and of organized organs such as shoots or roots (organ culture). Organ culture (Fig. 5) is the form of tissue culture most commonly used to propagate forest trees (Libby, 2004). Main steps of this method are: (i) setting up the tissue culture laboratory, (ii) preparing the media, (iii) taking the micro-cuttings, (iv) setting the micro-cuttings, (v) shoot multiplication, (vi) sub-culturing, (vii) rooting, (viii) transfer of plantlets to soil, (ix) veaning, (x) re-potting and (xi) hardening (Longman, 1993).

Fig. 5. *In vitro* organ culture (Longman, 1993).

2.1.2 The rooting process of the cuttings

The rooting of stem cuttings is a complex process in which the genotype of the parent plant is exactly duplicated. It is possible because of two unique plant characteristics: *Totipotency* is the property of vegetative plant cells to carry all of the genetic information necessary to regenerate the original plant. *Dedifferentiation* is the ability of mature (differentiated) cells to return to a meristematic condition and produce a new growing point (Hartmann and Kester, 1983). The rooting of cuttings starts with a healing and the formation of new cells (Fig. 6). It continues by the induction of root formation, and the linking up or bridging of these roots with the existing vascular tissue of the cutting stem. These newely formed roots further elongate and finally develope to a new functional plant. Several exogenous and endogenous factors influence the success of this process (Wiesman and Jaenicke, 2002). These factors are described in following text.

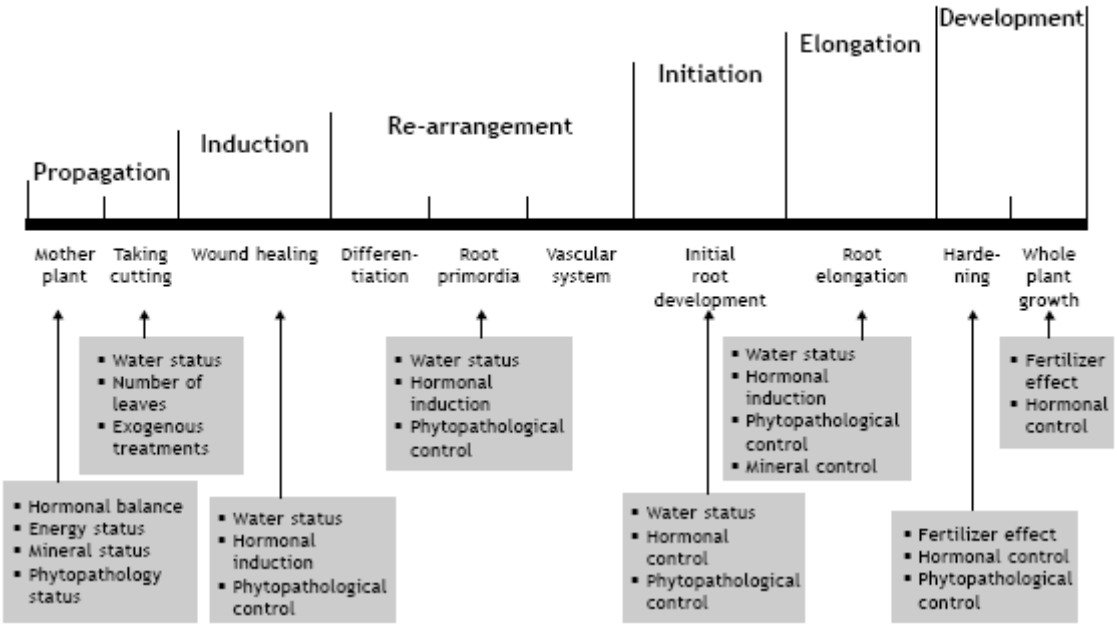

Fig. 6. Different stages in the rooting process, and the factors influencing them (Wiesman and Jaenicke, 2002).

2.1.3 Donor plant factors – cyclophysis and rejuvenation

Cyclophysis refers to the maturation state of the terminal meristem and the effects of that on propagation and subsequent development. In many tree species, the maturation state of the cutting is of great importance. Juvenile cuttings typically root easily and grow well, but mature cuttings root with difficulty, grow weakly, and often maintain branch form for several or even many years (Libby, 2004).

Sexual maturation can be understood as a developmental genetic process, proceeding more or less continuously from embryonic through various juvenile, adolescent, and mature phases, to a late-mature phase (Libby, 2004). Juvenile phase can be short (1-2 years) or long (greater than 30 years). Sexual maturation includes morphological changes (growth rates, plagiotropism, foliar morphology, reproductive competence) as well as physiological and biochemical changes. While maturation is likely under genetic control, the effect of environment on gene expression is also significant (Webber, 2004).

The change from juvenile vegetative growth stage to sexually mature growth stage is called Phase change. It can be accelerated by grafting seedlings onto mature rootstocks (Lewandowski and Zurawicz, 2000). The ability of terminal shoot to produce roots rapidly declines with tree age and it is very difficult to root cuttings from the crown of a mature tree, with some exceptions. In *Eucalyptus deglupta*, the maturation of trees propagated

vegetatively usually occurs rapidly and appears to be due to the production of a rooting inhibitor in mature apical or epicormic leaves (Potts, 2004).

Rejuvenation is the opposite process to maturation or ageing. The rejuvenation of *Eucalyptus* shoots from the crown of mature trees is possible through rapid 'cascade' grafting (including micrografting) on juvenile rootstocks or micropropagation (five to six transfers are usually required); (Potts, 2004). Some forest-tree species naturally sprout at or near the root collar when the top is killed, from the stump when cut, or from roots (Fig. 7). When applied on purpose, this regeneration technique is called coppicing (or pollarding if the stem is cut 2-3m above ground). It results in maintaining many of the same (usually well-adapted) genotypes in the subsequent forest. Root-sprouting results in spreading the clone over short distances, and it can be stimulated by ripping the soil to cut up the root systems, or by otherwise damaging the roots. Sprouts can also serve as good sources of cuttings or tissues for subsequent cloning to other sites. They are particularly effective because traits of the mature tree can be evaluated, and because the sprouts are usually at a juvenile or early adolescent maturation state (Libby, 2004).

The stockplant, established by felling a selected mature tree, and repeatedly harvesting the coppice shoots it produces, is considered as the best way to return to the juvenile state (Leakey, 2004). In *Milicia excelsa*, the rooting ability of cuttings from coppice shoots was negatively correlated with the age of the stump (Ofori *et al*., 1997), and in *Vochysia guatemalensis* increasing stump diameter had a negative effect on rooting too, with larger stumps produced more shoots (Dick *et al*., 1998).

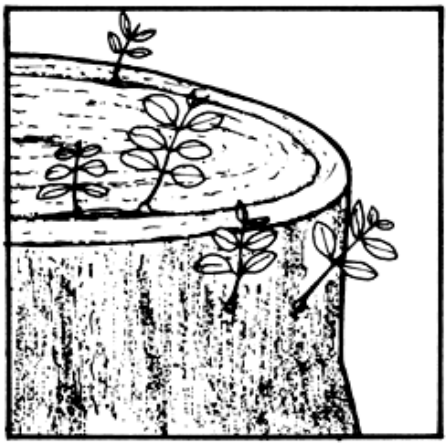

Fig. 7. Stump sprouts (Longman, 1993).

Chronological age of the tree refers to information concerning the time passed since germination. The oldest part of a in terms of chronological age can be found near the base, while the chronologically youngest parts are the terminal meristems. Ontogenetical age means if the tree is juvenile or adult, and the different stages of development from germination to senescence. The shoot sprouting near the base is now ontogenetically youngest and the most juvenile, while the terminal meristems are ontogenetically oldest. Physiological age describes when a plant is improving or deteriorating, and informs in regards to vigour and susceptibility patterns or weakening of the plant (Fortanier and Jonkers, 1976). The poor rooting ability of mature shoots can be attributed to physiological ageing rather than to ontogenetic ageing. Experiments within the crown of mature trees are difficult to do, because requires the formation of physiologically young shoots within an ontogenetically mature crown (Leakey, 2004).

2.1.4 Donor plant factors – topophysis and plagiotropism

Topophysis has elements of cyclophysis, but adds an effect of the additional differentiation that occurs after lateral meristems are produced and grow into a branch hierarchy, plus the different physiological conditions of branches in different parts of the tree. It is effect of the position in the parent's crown from which the plant material is collected. Different parts of a large tree are typically at different maturation states. The more juvenile (usually early adolescent) states are found low in the tree or in the roots, and with cumulative distance along the stem are the most mature occurring in the terminal meristem(s). It is possible to influence the maturation state of propagules by choosing the location on the donor plant (Libby, 2004).

From the top of the plant to the bottom, there is a within-shoot gradient in age that affects the leaf size, leaf water potential, leaf carbon balance, leaf senescence, internode length, internode diameter, stem lignification, nutrient and stem carbohydrate content, respiration, *etc*. This mean that no two cuttings are physiologically identical with the same rooting capacity. In *Triplochiton scleroxylon* stockplants, there were found strong gradients within a stem node position in rooting ability and cutting mortality, and strong relationship between cutting length and rooting ability (Fig. 8). This may be due to the need for storage capacity for current assimilates until the new roots form a sink for these carbohydrates (Leakey and Mohammed, 1985). Tchoundjeu and Leakey (1996) further showed a negative relationship between leaf area and cutting length, suggesting that short cuttings can't

provide the storage capacity for assimilates coming from a large leaf. Relative concentrations of carbohydrates and nutrients in cutting tissues as stored reserves for successfull rooting vary between node positions and over time in *Khaya ivorensis* (Tchoundjeu and Leakey, 2000).

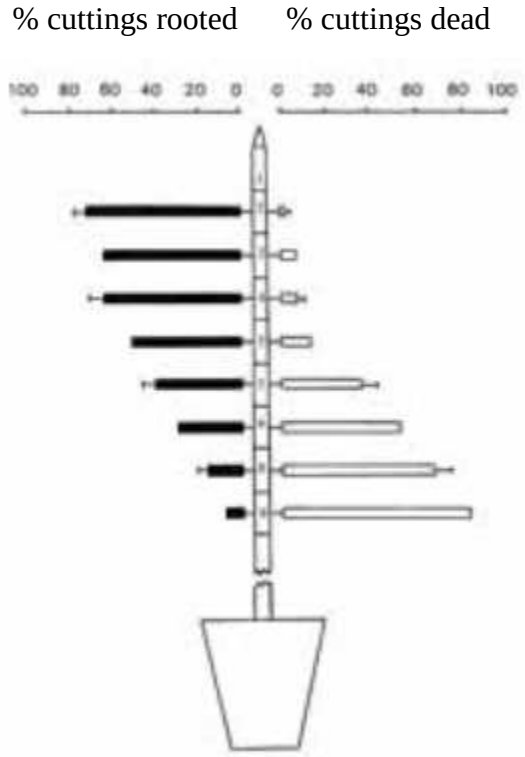

Fig. 8. Mean effects, after 10 weeks, of node positions on rooting and death of single-node leafy mainstem cuttings from undecapitated stockplants of three *T. Scleroxylon* clones (Leakey and Mohammed, 1985).

There are differences in rooting ability between the lateral shoots (even in the simplest type of stockplant). In *T. Scleroxylon*, there was found a relationship between percentage rooting and the number of shoots per plant. Two-shoot stockplants had the highest rooting ability. The percentage of cuttings rooted declined as the height of the stockplant increased. The rooting ability can be enhanced by the use of fertilizers, or by re-orienting the stockplant (angled or horizontal). The upper shoot had the highest rooting ability and it may be enhanced by removal of the lower shoots. The intershoot competition can be the factor in the determination of rooting ability. Basal shoots had a higher rooting ability than upper shoots in situations of low but equal competition. The rooting ability of basal and upper shoots was similar under similar light conditions (Fig. 9); (Leakey, 1983).

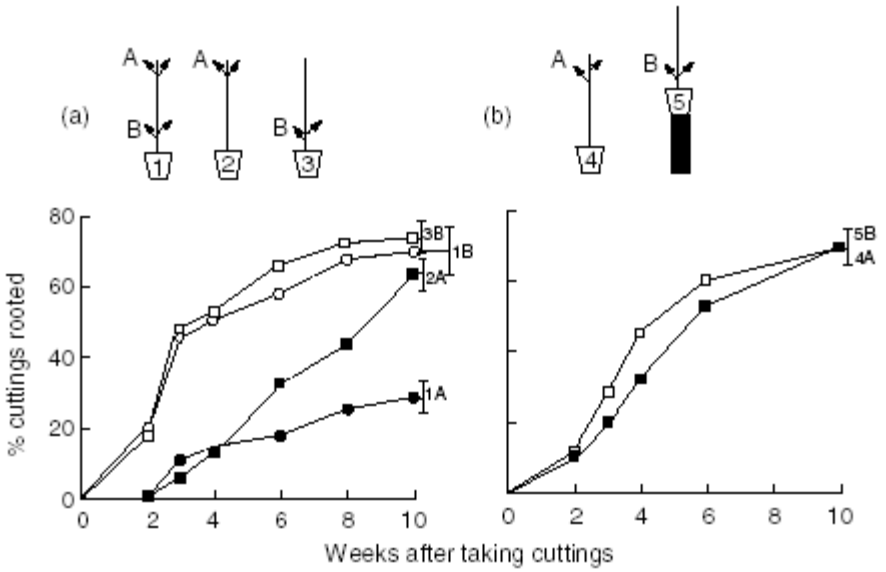

Fig. 9. Effects of the number and position of shoots in stockplants of *T. scleroxylon*, relative to a light source (Leakey, 1990).

Non-erect propagules obtained from older ortets have usually a tendency to plagiotropism. Plagiotropism indicates that the main stem of the growing propagule develops at an angle and rather like a branch (often with bilateral symmetry), as contrasted to vertical, radially symmetric orthotropic growth typical of seedlings and other juvenile propagules (Libby, 2004). Species vary in the strength of plagiotropism (Zobel and Talbert, 1991).

2.1.5 Donor plant factors – periphysis and genetic factors

Periphysis refers to the effects of the operational environment of the donor plant on the physiological condition of the parts sampled from the donor (Libby, 2004). In *T. Scleroxylon,* there was found complex of interactions between nutrients and the quantity and quality of light, which affected photosynthesis and the carbohydrate status of cuttings. High irradiance with low nutrients resulted in high starch content. Cuttings with high starch concentrations, associated with low rates of photosynthesis appeared to inhibit rooting. Active photosynthesis was associated with good rooting. Both low irradiance and low red:far-red ratios independently enhanced rooting ability (Leakey and Storeton-West, 1992). In *Eucalyptus grandis,* the cuttings from shaded stockplants had longer internodes, greater specific leaf area, greater codominance between shoots, lower rates of pre-severance net photosynthesis, lower chlorophyll concentration, but higher rates of net photosynthesis per unit of chlorophyll and other differences (Hoad and Leakey, 1994).

These gas exchange characteristics subsequently enhanced the cuttings post-severance physiological status and promoted high rooting ability (Hoad and Leakey, 1996).

2.1.6 Stockplant management

The good donor plant or stockplant management gives the opportunity for enhancing the rooting ability of cuttings by promoting the appropriate morphological and physiological conditions of the shoots (Leakey, 2004). The shoots from within about one metre of the ground are usually juvenilie, vigorous and easy-rooting (like shoots from seedlings). The stockplant can be established by felling a selected tree, or by planting or potting clonal cutting. Coppice stumps need frequent harvesting and pruning to prevent tall shoots and those with branch structure, instead of vertical main stems developing (Fig. 10); (Longman, 1993). Methods like hedging, or shading by nitrogen-fixing species like *Leucaena leucocephala* can be very beneficial (Leakey, 2004). Leguminous shrub *Flemingia rhodocarpa*, is used in stockplant areas for Robusta coffee. Other species like *Acacia, Delonix, Cassia, Erythrina, Gliricidia, Parkia, Prosopis* and *Sesbania* could also be tried. The stockplant qualities and thereby rooting ability can be also enchanced by good watering, mulching, use of fertilizers, or inoculation stockplants with the appropriate mycorrhizal fungi (Longman, 1993). In *T. scleroxylon*, the injection of auxins into the stockplants enhanced rooting ability of cuttings (Leakey, 1992).

Fig. 10. Pruning stump sprouts (Longman, 1993).

2.1.7 The propagation environment for leafy stem cuttings

Leafy cuttings would dry out very quickly and need to be kept very high air humidity. Other conditions recommended are moderately low light intensity; equable temperatures, suitable rooting medium, and protection from wind, heavy raindrops, pests and diseases (Longman, 1993). The propagation environment should encourage

meristematic activity (mitosis and cell differentiation) in the stem, and physiological activity (photosynthesis and transpiration) in the leaf to minimise the physiological stresses experienced by the tissues, from transpiration and respiration. The transport of assimilates and nutrients from the leaf to the base of the stem, and of water from the base of the stem to the leaf, are also important (Leakey, 2004).

The most common types of propagation systems are (i) fogging systems, (ii) intermittent mist controlled by a range of different sensors and (iii) air-tight, water-tight, high humidity, non-mist propagators (Fig. 11). These systems vary in cost, sophistication and effectivity (Leakey, 2004).

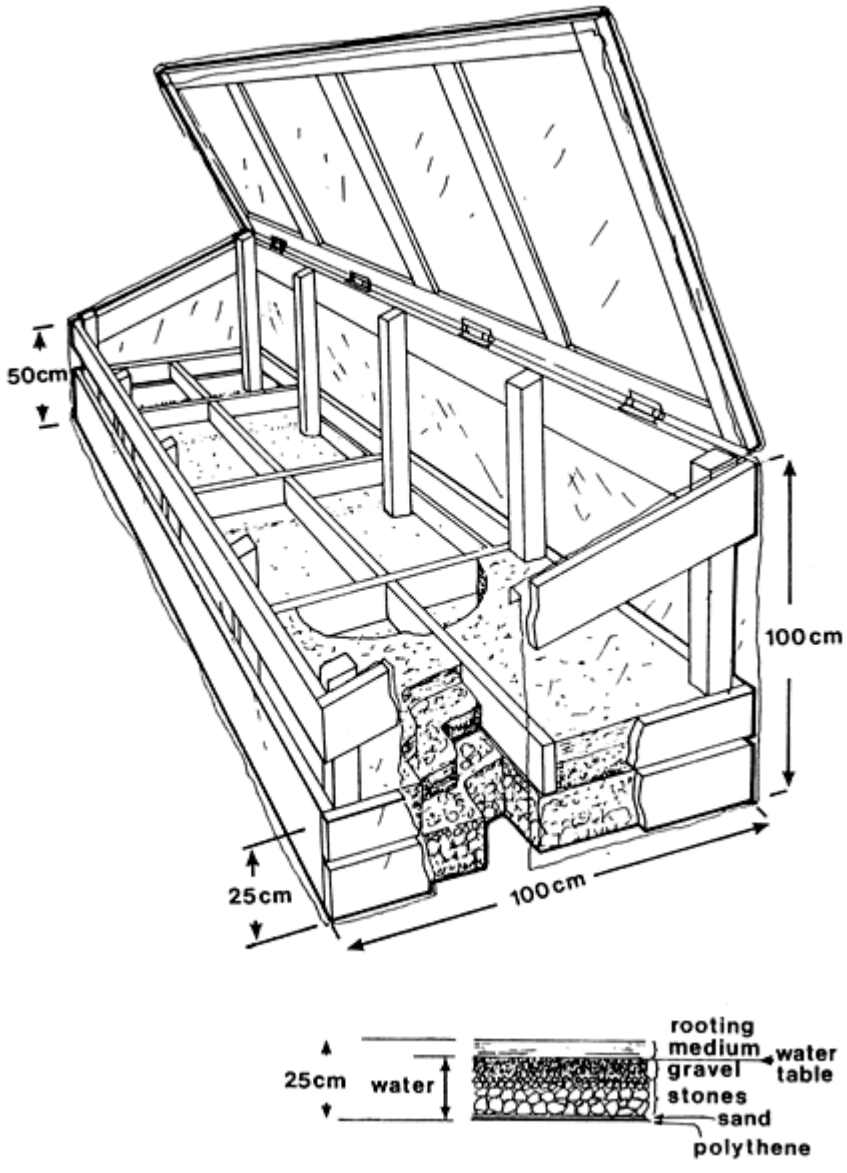

Fig. 11. Subirigated non mist polypropagator (Leakey *et al.*, 1990).

Subirigated polyethylene sheet non-mist polypropagator provides a very uniform and humid environment if the box is airtight and water-tight. The advantage of these low cost and low-tech propagators is, that can be used in situations, where electricity and piped water are not available (Leakey *et al*., 1990). The cuttings can be well supplied with water at the cutting base while the leaves are in a cool, shady environment with low vapour pressure deficit (VPD) to minimise water stress and overheating (Leakey, 2004). When the lid of the propagator is opened and in the middle part of the day the cuttings have to be sprayed, because 90 % RH (relative humidity percent) will soon dry up (Fig. 12), even when a hand-sprayer is used (Longman, 1993).

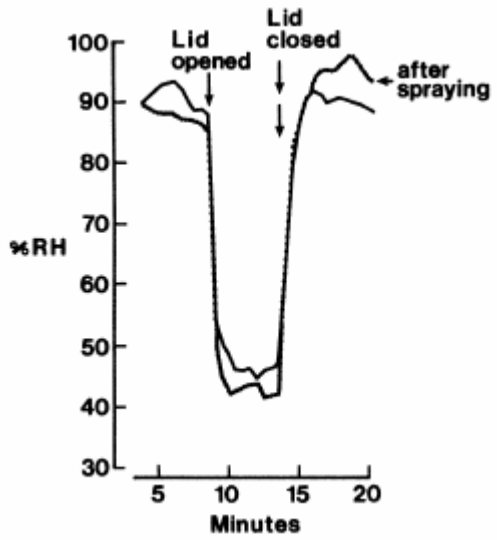

Fig. 12. Rapid humidity decrease in non mist polypropagator, when the lid is open (Leakey, 1990).

For shading, there can be used materials such as matting, woven bamboo, palm-leaf, plastic shadecloth or metal sheets with some translucent panels. It should avoid large sun-flecks and areas of dark shade especially in the mornig and in the afternoon (Longman, 1993). While shading is beneficial, leafy cuttings need enough light to photosynthesise. Interestingly, the highest values of photosynthesis in severed cuttings have been found at relatively low levels of irradiance (Leakey, 2004).

The rooting medium is usually sand, gravel, grid, sawdust or mixtures. Sand, grit and gravel should be washed before use, and other components should not be full of troublesome weeds, animal pests or disease-causing moulds (Longman, 1993). The composition of the rooting medium is often critical for rooting and can vary between

species, and cultivars/clones. In addition to holding the cutting firm, it has to provide moisture retention/drainage and allow respiration from the tissues and root penetration. The the optimal air:water ratio (gas:filled pore space ratio) of the medium tends to be species specific (Leakey, 2004). In *Milicia excelsa*, the moisture content of the medium was positively related to the numbers of roots formed and negatively related to mortality and leaf abscission (Ofori *et al*., 1997).

2.1.8 Leaf area of the cuttings

The rooting of softwood cuttings is usually dependent on the presence of a leaf and physiological processes inside. Death of the leaf due to rotting, necrosis, bleaching or to leaf abscission is usually most common reason for these cuttings failing to root (Leakey, 2004). Trimming the leaves by sharp scissors is important for leafy cuttings of species with leaves longer than about 8-10 cm, before the shoots are detached from the stockplant. Large leaves may lead to more loss of water by the unrooted cutting. On the other hand, small leaves may not produce enough sugars and other substances needed for the cutting to survive (Fig. 13). The best size of the leaf area varies from one tree species to another (Fig. 14). It is also influenced by the type of cutting, auxin application used, propagation environment, *etc*. Experiments with *Triplochiton* have shown that rooting is better when the leaf-blade is neither too big nor too small (Longman, 1993). Rooting appears to be promoted by the production of specific sugars during the time in propagator. There is a relationship, which develops after severance, in cuttings with differing leaf areas, between rooting ability and the content of reflux-extracted soluble carbohydrates (Leakey and Storeton West, 1992).

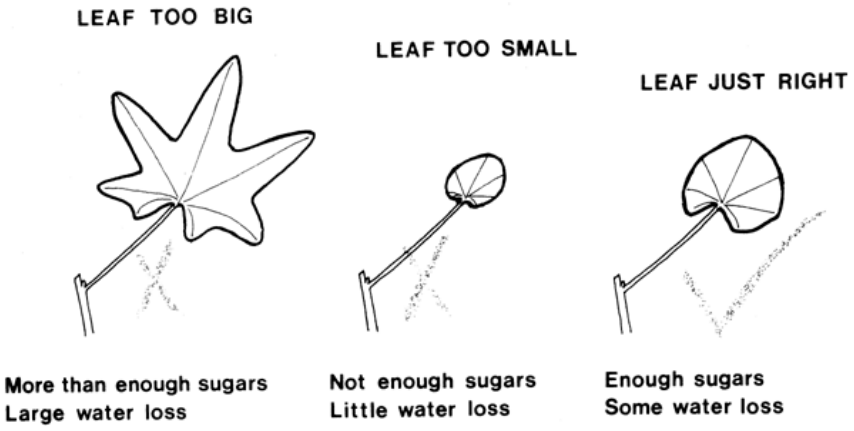

Fig. 13. Comparation of three different leaf areas of *T. scleroxylon* (Longman, 1993).

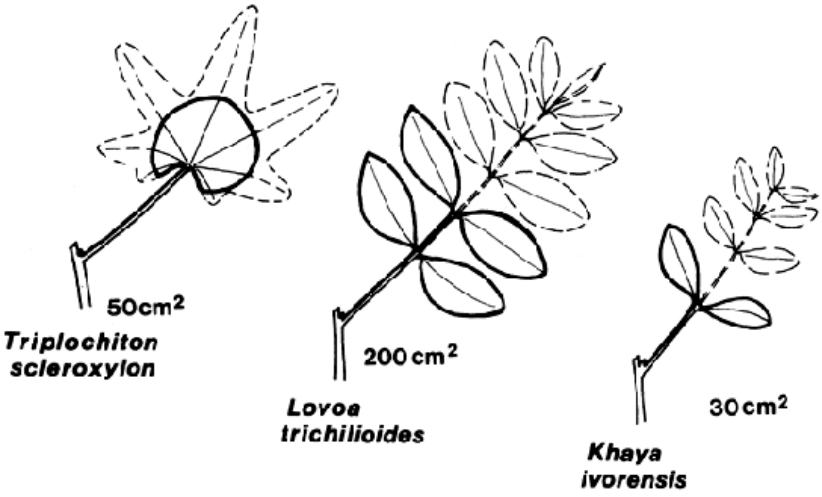

Fig. 14. Optimal leaf area of some species (Longman, 1993).

2.1.9 The plant material collection and cuttings preparation

The cuttings collection is best early in the morning in misty weather or just after rain. The leaves of donor plant should be already trimmed to the optimum size, and the polypropagator, workplace, tools, record sheets and polyethylene bags (white on the outside and black on the inside) have to be prepared. The rooting medium is moist, but not too wet. After taking the shoots, it is necessery to keep the labeled bags in shade, and take them carefully and quickly to the propagation area (Longman, 1993).

The shoots first have to be divided up into cuttings, unwanted material is removed (Fig. 15). The each cutting base have to be recutted by very sharp knife or scalpel blade. Recomended length is 2.5-12 cm, with a stem diameter at the base of about 4-8 mm. Long cuttings usually root best (Longman, 1993).

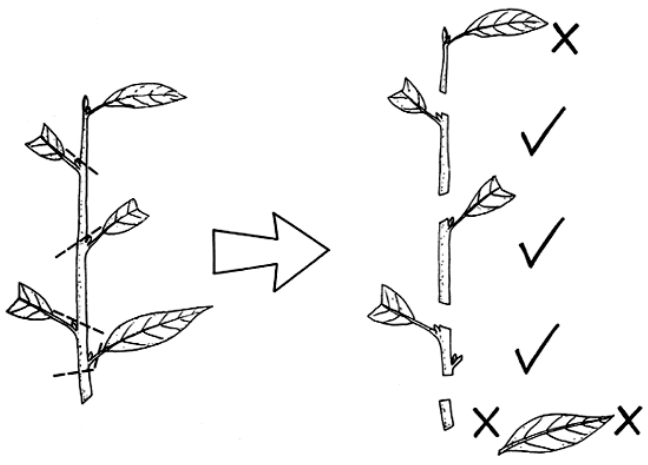

Fig. 15. Dividing the plant material into cuttings (Longman, 1993).

Single-node cuttings give larger numbers of cuttings from limited amounts of material. Two or three node cuttings are suitable for shoots with shorter internodes, those which lack leaves or buds and for species that are harder to root. Many-node cuttings are best for species with minute leaves and very short internodes. Split cuttings are occasionally used where there are two buds at each node (Fig. 16). In robusta coffee *e.g.*, two cuttings are often made out of one by splitting them down the middle. If there is plenty of material, it can be chosen standard size and type of cuttings, otherwise the cuttings can be graded into different size classes (Longman, 1993).

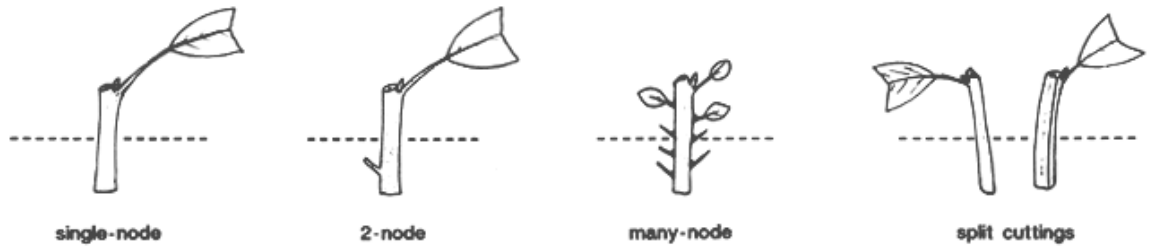

Fig. 16. Types of cuttings nodes (Longman, 1993).

2.1.10 Growth regulators and treatment

Auxins generally make cuttings root more rapidly, stimulate more roots on each cutting and lengh then the zone in which roots are formed, giving a better root system (Fig. 17). They also can turn a very hard-to-root species into a rootable one, or change the type of root produced (Longman, 1993). Tree species and even clones can appear to respond differently to individual and mixed applications of auxin at differing concentrations, even when many other factors are constant (Leakey, 2004). Clones of *T. scleroxylon*, which appeared to have different dose response curves, rooted equally well at 40µg of auxin per cutting (Leakey *et al*., 1982a). Most commonly used artificial auxins are known as indole-3-butyric acid (IBA) and α-naphalene acetic acid (NAA), or combination of IBA/NAA. IBA is usually the most effective (Leakey, 2004).

There are several methods of auxin aplication. *Quick dip* method is dissolving of the auxin in alcohol and treating the base of each cutting for 1-5 secs. *Soak* method is for few species that are intolerant to alcohol. The auxin is dissolved in water, and the prepared cuttings are left standing with the basal part (2-3 cm) in the solution for 4-12 hours before setting them. It is possible to use also auxin rooting powder (*talc).* Dosage of IBA is at a

concentration of 2.5-5.0 grams/litre (= 2500-5000 ppm, or a 0.25-0.5% solution). Solutions of auxins should be kept only for a week, even in the refrigerator (Longman, 1993).

The health status of donor plants and thereby also cuttings is very important aspect of rooting process. Its is important to avoid deseased, or unhealthy plant material. The cuttings can be treated by solution of fungicide, pesticide, or surface sterilant to prevent pests and deseases (Wiesman and Jaenicke, 2002; Longman, 1993).

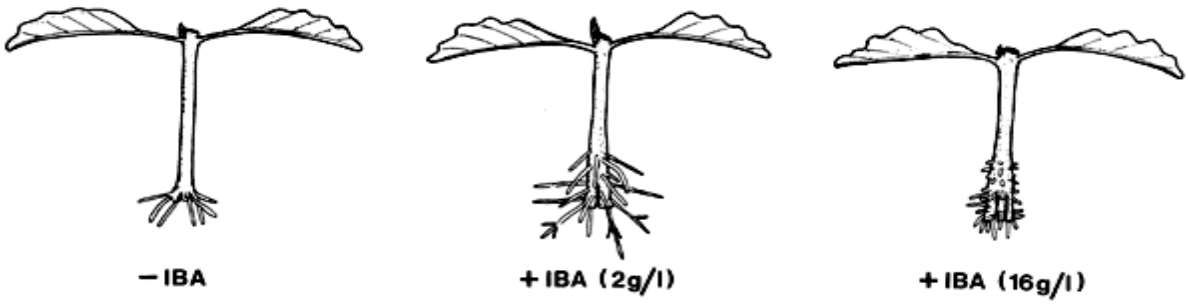

Fig. 17. Different rooting responses to different IBA concentrations (Longman, 1993).

2.1.11 Cuttings instalation, labeling and care

The lid of polypropagator should be closed to maintain air humidity and opened only enough to work. In the substrate, there have to be made holes about the same diametre as the cuttings, arranged in rows, or blocks. The medium is firmed around each cutting after the setting in. Naturally, the cuttings heve to be in shaded, moist environment also during the instalation. It is recomended to use polyethylene bags and fine spray of water (Wiesman and Jaenicke, 2002; Longman, 1993).

The information on labels can include the clone number, the date of setting, the number of cuttings set, the treatment, and the block. and other variables, such as the origin or type of cutting, node number, *etc*. The records are important too (Longman, 1993).

The main features that have to be checked during the propagation process are the water level, air humidity, and the temperature of water, air and rooting medium. The water should be stored in clean barrels in the propagation area, to have similar temperature to the temperature of the rooting medium. The watering is best early in the morning, or late in the afternoon. The health state of the cuttings have to be checked as well (Longman, 1993).

2.2 *Calycophyllum spruceanum* (Bentham) Hooker f. ex Schumann (Rubiaceae) - Capirona

2.2.1 Botanical description

Scientific name *Calycophyllum spruceanum* (Bentham) Hooker f. ex Schumann. origins of the name is from the Greek kalyx (calyx) and phyllon (a leaf) refering to the calyx teeth. Botanical synonyms are *Calycophyllum spruceanum forma brasiliensis* K. Schum. *Calycophyllum spruceanum forma peruvianum* K. Schum., and *Eukylista spruceana* Benth. Indigenous names in Peru are Capirona, Capirona negra and Mulateiro. In Bolivia is *C. spruceanum* called Guayaboji, or Cojesche. Spanish name is Palo amarillo (Quattrocchi, 2000). Indigenous name in Brazil is Pau-mulato-da-varzea (Almeida, 2004).

It is the fast growing tree of up to 180 cm diametre and 20-35 m high with very straight, regular cylindrical stem (Fig. 18a); (Almeida, 2004; Sears, 2003). In the non-dense canopy in the last upper third may occur partial leaf abscission during the dry season (Sears, 2003).

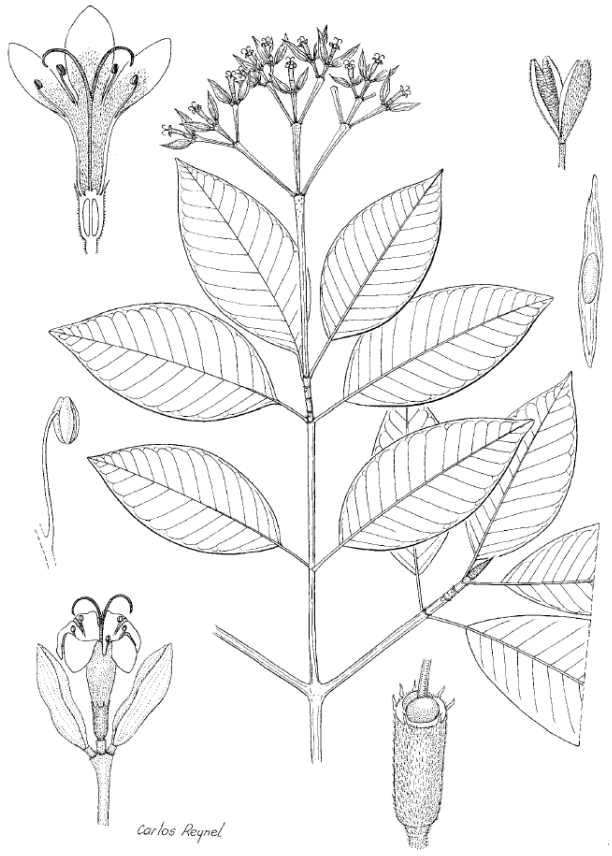

Fig. 18. *Calycophyllum spruceanum.* (a) Growth habit, (b) branch with leaves and flowers, (c) flowers, the two sides in bud, (d) flower, longitudinal section, (e) stamen, (f) hypanthium with removed corolla, (g) fruit, and (h) seed; (Reynel *et al.*, 2005).

Outer bark (Fig. 19) is smooth green, very characteristic, homogeneous, shiny, giving the impression of a well polished pole, covered with red-brown papyraceous rhytidome which is separated in large plates, irregular, revealing the greenish surface of the cortex (Almeida, 2004; Reynel *et al.*, 2005). Homogeneous inner bark is creamy-green and 1-2 mm thin (Reynel *et al.*, 2005).

Fig. 19. Bark of *C. spruceanum.*

Branches have round or flat section in the terminal areas (diameter 5-6 mm), reddish brown when dry, smooth, shiny, with white lenticels. *C. spruceanum* has opposite decussate simple leave arrangement (Fig. 20, 18b and 21), with elliptic or oblong 5-10 cm long and 3-5 cm wide leaves. The non winged petiole (1,5-2,5 cm long) with grooved blades entire is provided with the apex acute coarsely acuminate, base obtuse. The venation is pinnate, secondary nerves (12-15 pairs) are lightly printed on upper and embossed on the underside, the axils of secondary veins with small domatia on the underside (Reynel *et al.*, 2005).

Fig. 20. Leaf arrangemetnt of *C. spruceanum.*

Terminal inflorescences (Fig. 18b and 21) are 10-15 cm long, equipped with lots of hermaphrodite flowers with presence of calyx and corolla (Fig. 18c, 18d, and 21). Flowers (1-1.5 cm long) are entirely wrapped in a deciduous bract, which is normally removed from the central flower as the first. The pedicels are 2-3 mm long, calyx is 1 mm long, white tubular corolla is campanulate (5-6 lobed), with 5-6 equal stamens (Fig. 18e, and 21) at the corners of the lobes. Anthers are fixed exserted dorsal. Pistil have inferior ovary, ellipsoid-truncated, filiform arranged and bifid opened stigma. Each flower mature in 2 to 3 years in open-grown conditions (Reynel *et al.*, 2005).

Fruits are small elipsoid oblong capsules (5-8 mm long), opening in two carpels when splits (Fig. 18g, and 21). Small winged elongated seeds are provided with the embryo in central position (Fig. 18h, and 21); (Reynel *et al.*, 2005).

Fig. 21. *Calycophyllum spruceanum* (von Martius *et al.*, 1889).

Other species of the genus *Calycophyllum* have similar bark and wood and can have a similar folk name, but can be distinguished from *C. spruceanum* by these characteristics: *C. Multiflorum* is a smaller tree (10-25 m high), it has smaller leaves (5-8 cm in length and 3-4 cm wide); *C. acreanum* has larger leaves (up to 20cm long), the calyx has very small sepals. *C. obovatum* has one of its sepals many times larger than the others with format obovate, narrowed at the base (Almeida, 2004).

2.2.2 Origin and distribution

The origin and distribution of *C. spruceanum* is in the Amazon Basin, up to the elevation of 1200 m asl (Reynel *et al.*, 2005). It is a pioneer tree species that colonizes the floodplains, natural disturbed forests and and slash-and-burn agriculture fallows (Linares *et al.*, 1992). It is common in secondery forest areas and naturally also in primary forests. *C. spruceanum* occures areas with high and constant rainfall, but also areas with notable dry seasons. It is stone tolerate tree prioritizing loamy to sandy alluvial fertile soils (Reynel *et al.*, 2005).

C. spruceanum is heliophyte (Reynel *et al.*, 2005; Almeida, 2004) and hygrophyte species (Almeida, 2004). It is a typical tree well adapted to growth in riparian forests temporarily flooded by clear water ("Várzeas"); (Linares *et al.*, 1992; Sears *et al.*, 2003; Reynel *et al.*, 2005; Wightman *et al.*, 2006; Almeida, 2004). Homogeneous natural and semi-natural stands of *C. spruceanum* can often be observed along riverbanks (Linares *et al.* 1992, De Jong, 2001). It is shade-tolerant species and it can survive beneath the fast growing trees (Díaz, 2009).

2.2.3 Silvicultural management

C. spruceanum grows relatively slowly initially. It is shade tolerant, and grows beneath the fast growing species. Its chances of survival amongst the short-lived herbs and grasses are good (Díaz, 2009). The spacing of the plantation is usually 2,5 × 2,5 m, or 3 × 3 m, but it can be higher, depending the system used (100-1100 seedlings/ha); (Wightman *et al.*, 2006).

Study of this species with seeds of different origins in the Peruvian Amazon reported the increase of height from 1.4 to1.6 m at six months and from 3.5 to 4.7 m at one year of age (Sotelo-Montes *et al.*, 2000).

The prunning is recomended from the third or fourth year. It is possible regularly

cut branches for firewood and charcoal production. Stems can be harvested for construction poles and charcoal/firewood after 2 to 3 years, or sawn timber after 15 to 20 years, and then coppiced for successive harvests (Wightman *et al.*, 2006).

There have been recorded six phytophagous insects on *C. spruceanum*: *Aphis gosspyii, A. spiraecola* (Aphididae), *Cyphonia clavata* (Membracidae), *Leuronota calycophylli* (Psyllidae), *Perigona inferrupta* (Sphingidae) and one unidentified species of Dalceridae (Lepidoptera). *L. calycophylli* is the only species that damages the plant (Couturier and Gonzales, 1994).

2.2.4 Uses

C. spruceanum can be incorporated in agroforestry plantations (Sotelo-Montes and Weber, 1997). The most used parts of the tree are wood and bark.

The wood is widely used for constructions (*e.g.*, beams, poles), and further is suitable for production of firewood and charcoal because of the high calorific value and flame quality (Sotelo-Montes and Weber, 1997; Sears, 2003; Wightman *et al.*, 2006). It is smooth to the touch, hard, heavy, straight to curly grained with fine texture and excellent durability and woodworking qualities (Reynel *et al.*, 2005). *C. spruceannum* has diffuse porous wood, very dense when mature (0.65 g/cm^3). Luster is moderate, smell and taste imperceptible. Axial parenchyma is indistinct, with small very numerous, solitary and multiple empty pores. Visible, thin rays are not stratified. Growth rings are visible and demarcated by fibrous zones (Gomez *et al.*, 2006). Genetic correlations indicate that, in general, selection of faster growing trees and/or trees with denser wood would have little effect on wood color and its uniformity (Sotelo-Montes *et al.*, 2008).

C. spruceanum is noted for its ability to completely shed and regenerate its bark on a yearly basis, making harvesting the bark a totally renewable and sustainable enterprise. Indigenous people of the Amazon use a bark decoction internal and external, as an admixture in the *Ayahuasca* rituals, and in folk medicine and cosmetics. The decoction is ethnomedically used for treating abscesses, age spots, burns, cancer, cuts, diabetes, eye infections, fibromas, fungal infections, insect bites, liver problems, malaria, ovarian problems, pellegra, rashes, scabies, skin fungi, skin parasites, swelling, uterine cancer, wounds and wrinkles. The traditional remedy is 1 cup of decoction two to three times daily. It can be also applied externally directly to the affected area several times daily and allowed to dry before covering (Taylor, 2005).

The properties documented by research are: antibacterial, anticandidal, antifungal, antioxidantive and insecticidal (Taylor, 2005). The bark contains tannin substances which give it an astringent or drying effect, and a high content of phenols and organic acids. Three seco-iridoids isolated from the wood bark of *C. spruceanum* showed in vitro activity against the tropical parasite, *Trypanosoma cruzi* (Zuleta *et al.*, 2003).

2.2.5 Natural reproduction

C. spruceanum sexually mature in 2-3 years in open-grown conditions. In the study of fallow land realized in Peruvian Amazon near the city Pucallpa (Fig. 22), was the flowering period three months long, lasting from June to August. Flowering peaked in July 80 % of individuals (Díaz, 2009). Another flowering records are since the beginning of the dry season until its end, between April to September (Reynel *et al.*, 2005).

Fruit formation takes four months and occurres almost parallel to anemochoric or hydrochoric seed dispersal. The seed is very small, flat and has long wings (Díaz, 2009). A quantity of seed weighing one gram contains 6 000 seeds (Wightman *et al.*, 2006).

The seed may be dispersed by wind some kilometres (Almeida, 2004; Wightman *et al.*, 2006). The period of seed dispersal near Pucallpa was three months long, beginning in mid September and ending in mid December. The peak in seed dispersal coincided with the beginning of the rainy season. Frequently the crown is either partially or entirely defoliated during the period of seed dispersal. The dry fruits usually remain on the branches for a few weeks after dehiscence (Díaz, 2009).

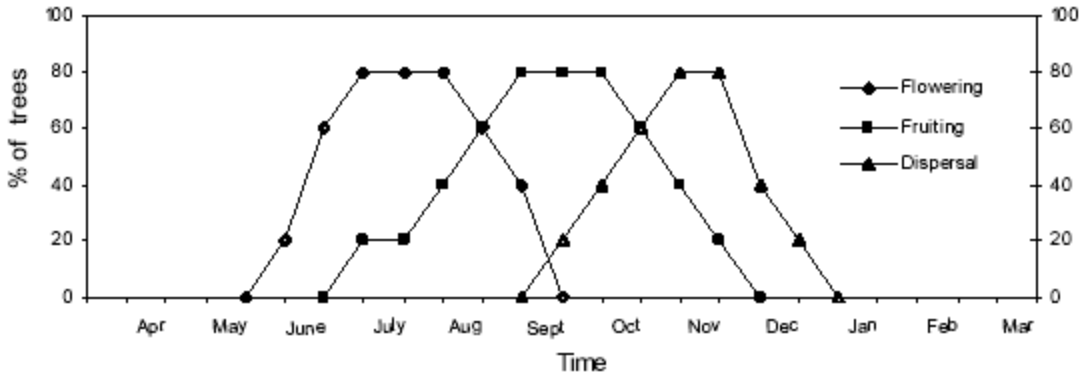

Fig. 22. Phenological behaviour of *C. spruceanum* in the Neshuya-Curimana sector, 1999-2000 (Díaz, 2009).

2.2.6 Artificial propagation

C. spruceanum is usually propagated by seeds. The cotyledon phase begins by epigeal germination (6-60 days), which requires sandy substrate, water, sun and the temperature between 15-33 °C. The seeds can be treated by vater during 12 hours before sowing (Almeida, 2004), The gemination rate is 87 % with fresh seeds in nursery conditions, in open growth conditions with rice plantations is 46 % and without any plantation is 37 % (Díaz, 2001). The small seed envelope opens completely. The equal, foliaceous, opposite cotyledons are horizontally arranged face to face in the interior of the seed (Fig. 23a). Cylindrical hypocotyl is 8-10 mm long. Epicotyl is not yet developed, showing only the beginning of the terminal sprout. Radicle is white, tapered of cylindrical form, 3-5 mm long (Díaz, 2009).

At the photophyllous phase (Fig. 23b), stipules remain for the first weeks, but later are shed, leaving transverse scars (Díaz, 2009).

The last two or three leaf pairs are shed at the metaphyllous phase (Fig. 23c), leaving elongated scars. Seedling axis has quadrangular cross section, without secretions (Díaz, 2009). The seedlings have to be transplanted to plastic bags (Fig. 24), in which they are kept until they reach about size about 50 cm (in the age of 5-6 months) after that can be taken to the final spot (Sotelo-Montes *et al.*, 2000).

Vegetative propagation of *C. spruceanum* is not developed yet. There wasn´t archieved good rooting success without any treatment (Escalante, 1993; cited in Sotelo *et.al.*, 2000). Another study of vegetative propagation of very juvenile material taken from coppice shoots was done in Brazil. It showed 100 % rooting success after 60 days in greenhouse and misty conditions. This research did not prove any significant effect of growth regulators on rooting success, but the root length was greater at concentrations 1000 and 2000 ppm of IBA as well as of NAA (Gatti, 2002).

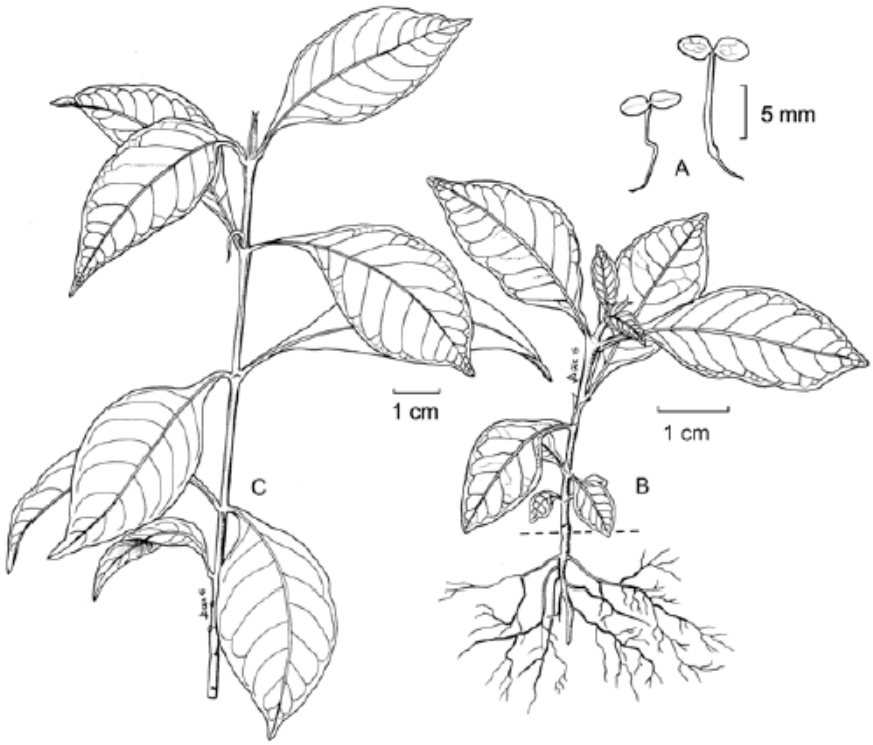

Fig. 23. Morphology of the phases of the initial stage of seedling development of *C. spruceanum*. (a) Cotyledon phase (day 4), (b) protophyllous phase (day 32), and (c) metaphyllous phase (day 70); (Díaz, 2009).

Fig. 24. *C. spruceanum* seedlings.

2.3 *Simarouba amara* Aublet (Simaroubaceae) – Marupa

2.3.1 Botanical description

Scientific name the species is *Simarouba amara* (Aublet, 1775). Some botanical synonyms are *Quassia simarouba* L. f., and *Zwingera amara* (Aubl.) Willd. Indigenous names are: Aceituno (Central America), Chiriguaná (Bolivia), Marupá (Brazil), Simaruba (Colombia), Cedro amargo (Ecuador), Marupa (Peru), and Cedro blanco (Venezuela); (Gutierres and Silva, 2002; Gérard *et. al.* 2004). Other local names are: Marapauba, Paraiba and Tamanqueria (Brazil), Cuna and Guitarro (Ecuador), Simarupa (Guyana), and Soemaroeba (Surinam). In the United Kingdom is *S. amara* called Bitterwood (Gérard *et. al.* 2004)

Simarouba amara is the big, insect pollinated dioceous tree, which can grow up to 35 m in height (Fig. 25a); (Croat, 1978). The trees show good bole form and may be free of branches to 27 m. Common diameters vary from 50 to 60 cm (Cronquist, 1944).

Fig. 25. *Simarouba amara.* (a) Growth habit, (b) inflorescenced twig - female; (c) part of the inflorescence - male (Spichiger *et. al.*, 1989).

The bark of *S. amara* (Fig. 26) is in long pieces, from 4 to 12 cm wide and 2 to 5 mm thick, folded lengthwise, light, flexible, tenacious, very fibrous, externally of a light brownish-yellow color, rough, warty, and marked with transverse ridges, internally of a pale yellow. It is without odor, and of a bitter taste (Remington *et al.*, 1918).

Fig. 26. Bark of *S. amara*.

S. amara has alternate, imparipinnate compound leaves (22.5-30 cm long); (Fig. 25b, 27, and 30). The petioluled leaflets are oblong (5-12 cm long), obtuse and apiculate blades at the the apex, with a glossy green surface and a white underside. The very fine lateral nerves are united near the margin. The secondary and tertiary veins are very indistinct (Franceschinelli *et al.*, 1998).

Fig. 27. Compound leaf of *S. amara.*

The unisexual yellow sessile flowers are relatively small (up to 11 mm long) and densely arranged in large panicles (usually 30 cm long); (Fig. 25b, and 28). The calyx is connate along its basal half and has (4) 5 (-6) sepals. The dialipetalous corolla also has (4-) 5 (-6) petals. The staminate flowers have (8-) 10 (-12) stamens with a scalelike appendage at the base of the filament. The pistilate flowers have a pseudo-apocarpous gynoecium comprised of a gynophore topped by (4-) 5 (-6) individual, uniovulate carpels connate only along their ventral sutures and the single gynobasic style. The gynophore is present in staminate flowers, but the ovary is absent or vestigial. Stamens are absent or vestigial in pistilate flowers (Franceschinelli *et al.*, 1998).

The large, olive shaped fruits are a carpophores with up to five drupaceous mericarps, almost black when ripe (Franceschinelli *et al.*, 1998). The fruits are produced 1-3 months after pollination, and are 17 mm in length, with the 10-14 mm long seeds which do not experince dormancy. The primary dispersals of *S. amara* are primates and birds (Croat, 1978).

Fig. 28. *Simarouba amara*, printed as *Quassia Simaruba* (Woodville, 1792).

Simarouba amara is frequently confused with two other continental species of the neotropical genus, *S. glauca* and *S. versicolor.* Researches sometimes treated these species as conspecific. *S. glauca* and *S. versicolor* were found to be morphologically closer to each other than to *S. amara.* Geographical and morphological data, with leaf features are useful for species identification. *S. amara* is restricted to wet forests and *S. glauca* to dry forests. A character often noted in this species is the extremely bitter bark (Franceschinelli *et al.*, 1998).

2.3.2 Origin and distribution

Simarouba amara is a widespread tropical tree species of upper Mesoamerica, Central America, the Amazon basin and Carrebean region. It is low-area species of very humid, rainy conditions and altitudinal range from the sea-level to 850 m (Chudnoff, 1980). It attains its best development in Brazil and northern South America, while in Central America it occurs infrequently and in smaller size. *S. amara* occur in the seasonal forest on sandy soils and in the rain forest (Cronquist, 1944).

2.3.3 Silviculture management

The best development of the seedling of *S. amara* height and diameter in Jenaro Herrera in Peru, was observed in spacing of 10 × 10 and 15 × 15 cm. After 3.5 months in nursery beds *S. amara* reached a higher survival rate and increase in diameter in full sun conditions. By contrast in low light conditions was the greater increase in height (Vargas and Portocarrero, 1992).

The survival rate, diameter and hight increasement after the transplantation in Jenaro Herrera was better with „soil cake“ in open grown conditions than with bare root, in hedges (Vargas and Portocarrero, 1992).

S. amara is the host plant for the *Fulgora laternaria* (Machaca or Peanut-headed Lanternfly), (Janzen and Hogue, 1983).

2.3.4 Uses

S. amara can be used in agroforestry plantations. In the study focused in the Atlantic region of Costa Rica was *S. amara* one of 16 species (of 62 studied) classified as the most suitable for incorporation in coffee plantations (Linkimer *et al.*, 2002). The most

used parts of the tree are wood, bark and leaves.

The wood of *S. amara* is whitish or light cream-colored, sometimes with a yellowish cast, and shows no distinction between heartwood and sapwood. It is without odor, but it has a mild to decidedly bitter taste. The wood has medium coarse texture, with straight grains, without interlocked grains. It is porous without figure and possesses a high luster. The wood floats and durability in forest is low. Physical and mechanical properties can vary greatly depending of origin and growth conditions (Gérard *et. al.* 2004). The wood of *S. amara* is used for interieror exterior purposes if properly treated. It is used locally as a general utility species. Its is also used in furniture and cabinet work, music instruments, millwork, patterns, core stock, matches and core for paper (Loureiro, 1968).

The leaves and bark of *S. amara* are used in the natural medicine in the tropics. The indigenous tribes of the Amazon rainforest use bark as a treatement for fevers, malaria, and dysentery, as a hemostatic agent to stop bleeding, and as a tonic. In Cuba is an infusion of *S. amara* leaves and bark used as astringent, a digestion and menstrual stimulant, and as antiparasitic remedy. It is further taken internally for diarrhea, colitis, stomach and bowel disorders, hemorrhages, indigestion, anemia and internal bleeding and used externally for wounds and sores. The traditional remedy for diarrhea or dysentery is 2-3 cups of decoction of bark daily. There are reported some side effects at high dosages (increased perspiration and urination, nausea, and/or vomiting); (Taylor, 2005)

The properties documented by research are: amebicide, antibacterial, anticancerous, antidysenteric, antileukemic, antimalarial, antimutagenic (cellular protector), antiparasitic, antiprotozoal, antitumorous, and antiviral The main active compounds in *S. amara* are called *quassinoids*, which belong to the triterpene chemical family. It include: benzoquinone, canthin, dehydroglaucarubinone, glaucarubine, glaucarubolone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sitosterol, and tirucalla. Ailanthinone, glaucarubinone, and holacanthone are the ones documented to be antiprotozal, anti-amebic, antimalarial, and even toxic to cancer and leukemia cells (Taylor, 2005).

2.3.5 Natural reproduction

In the Adolpho Ducke Forest Reserve, there was observed that the phase of complete flowering occurred in the transition of wet season (Nov-Dec). The phase of mature fruit occurred in the wet season (Feb and Mar). The phenophase new leaves occurred in the dry season (Sep and Oct). Fruit formation got positive and significative

correlation with rainfall, negative and significative correlation with solar radiation and evaporation. New leaves phenophase showed positive and significative correlationship with solar radiation, evaporation and maximum temperature; negative and significative with rainfall. It didn`t present linear correlation with the phenophase complete flowering (Pinto *et al.*, 2005).

2.3.6 Artificial propagation

S. amara is usually propagated generatively by seeds (Fig. 29). The weight of thousand seeds was estimated to 365 g. In germination experiments in Jenaro Herrera in Peru was tested five pre-germination treatments and six substrates. The values of fresh seeds without treatment do not differ statistically from the values obtained from seeds soaked for six, 12 and 24 hours in cold, or five minutes in acetic acid, except the test with organic matter sterilized with formaldehyde. In relation to the different substrates, with non-sterile, silty soil was achieved the highest percentage of germination. Viability test with fresh seeds and those left in the natural conditions for one to three months indicate the average percentages of viable seeds, of 79% to 43.5% and 24.8% respectively. Seeds with insect attacks represent 6.4% and 2.6%. The percentage of rotting by fungi attack increases to 9.2% in fresh seeds, 47.3% left for a month, and 71.5% left for three months (Vargas and Portocarrero, 1992).

Only a few studies were done about the vegetative propagation of *S. amara* till now. In experiments in nursery bed in Costa Rica with soil substrate were used lignified cutings (15 cm long) from different parts of mature (15 m high) tree. After 2.5 months was recorded almost 100 % mortality, even with auxin treatment (García-Villamán, 1974). Also in another experiment from Costa Rica with 20 cm lignified cuttings in greenhouse-mist conditions shows *S. amara* the largest mortality (95,3%) between 8 species studied. Generally 6th week of the experiment, the cuttings started to rot and did not presented any callussing, or rooting (Zanoni-Mendiburu, 1975). This suggests that *S. amara* is difficult to root species.

Fig. 29. *S. amara* seedlings.

3 OBJECTIVES

Vegetative propagation can be important part of domestication of tropical trees. An effective method of vegetative propagation of *Callycophylum spruceanum* (Capirona) and *Simarouba amara* (Marupa) was not developed yet. Some difficult-to-root species require growth regulator treatment for successful rooting. This research aims to develop the methods of vegetative propagation by juvenile leafy stem cuttings of *Callycophylum spruceanum* and juvenile and mature semihardwood leafy stem cuttings of *Simarouba amara* in subirigated polyethylene sheet polypropagators.

The aim is to determine and analyze total root length, length of the longest root, and number of roots and callusses per cutting, percentage of rooting and callussing, mortality, leaf abscission, and vigor of these cuttings using different treatment concentrations of Indole-3-butyric acid (IBA): 1000, 2000, 4000 and 5000 ppm and one control group without any IBA treatment. The rooting medium in these experiments was white fine sand (0,1-0,2 mm).

4 MATERIALS AND METHODS

4.1 Experimental site description

The research was done in august 2009, near the city Pucallpa, which is situated in Peruvian Amazon basin in Ucayali region. Both experiments were established in nursery school (Fig. 30a) at Peruvian Amazon Research Institute *(Instituto de Investigaciones de la Amazonía Peruana - IIAP).* The geografical position of the nursery is latitude 8°23'55.5" S, longitude 74°38'25" W, and altitude 152 m asl.

Fig. 30. (a) Nursery school at Peruvian Amazon Research Institute near the city Pucallpa; (b) The construction of subirigated non-mist polyethylene polypropagator.

4.2 Propagator and rooting medium

In the experiments low technology subirigated polyethylene polypropagators were used, which are suitable for developing countries with less o no availibility of water and electrocity. These polypropagators are also relatively cheap and easy to construct. The polypropagator was constructed following a design as described by Leakey *et al.*, (1990). The wooden frame was three meters long, one metre wide and one metre high with a sloping cover (Fig. 30b). The construction was joined together by nails and enclosed in a single sheet of polyethylene. The base of the propagator was completely water tight and the lid was airtight (Fig. 32b). The bamboo stick for watering was installed. As the

drainage, stones (Fig. 31a,b), gravel, and coarse sand (Fig. 31c) were used (see Tab. 1).

Tab. 1. Material for the construction of one poly-propagator used in the experiments.

Material	Quantity	Unit
Wood (250 × 25 mm)	8	m
Wood (50 × 50 mm)	10	m
Wood (50 × 25 mm)	32	m
Polyethylene sheeting (2 m wide)	10	m
Stones (30-120 mm)	0.5	m^3
Gravel (5-10 mm)	0.25	m^3
Coarse sand	0.25	m^3
Rooting medium	0.5	m^3
Nails	1	kg
Drawing pins	0.5	kg
Hinges	4	pieces
Screws	0.5	kg

Fig. 31. (a) (b) Stones, (c) Gravel, and coarse sand; (d) Fine sand rooting medium.

As the rooting medium (propagating substrate), white fine sand (0,1-0,2 mm) was used (Fig. 31d). The drainage material and rooting medium was througly washed before use. In addition, the rooting medium was sterilizated for two hours in hot steam in enclosed iron barrel (Fig. 32a). The water level inside the barrel was under the grate, so that the sand after the sterilization wasn´t wet. The polyethylene sheeting at the base of the propagator was carefully filled in by a thin layer of sand to protect it. The drainage consisted of a thick layer (10-15 cm) of large stones, covered by successive layers of small stones and gravel adding up to a total depth of 20 cm. The spaces between stones and gravel were further filled with water. The level where the drainage layer was fully saturated was marked at the bamboo stick. The rooting medium was slicked down by smoothing trowel (Fig. 32c). The holes were made in the rooting media according the experimental design to a depth of 15-25 mm (Fig. 32d).

Fig. 32. (a) Sterilization of the rooting medium, (b) Subirigated non-mist polypropagator, (c) The preparated rooting medium, and (d) Making holes in the rooting medium.

4.3 Plant material source and cuttings preparation

The cuttings of *C. Spruceanum* were taken from 15 ten months old seedlings (100-120 cm tall), and of *S. amara* from 30 one year old ortets (150-180 cm tall). These donor trees were grown in the nursery near the propagation area. The seedlings were from open-polinated family. Proper physiological conditions and absence of pests and deseases of the ortets were considered. Only vigorous and healthy trees were selected. The material were taken from the same branch position at the ortets in early morning and transported to the propagation area in moistured polystyrene boxes (Fig. 33a). The cuttings were separated by sterile garden scissors (Fig. 33b). The parametres of the cuttings were single node, semihardwood one-leaf stem cutting of *C. spruceanum* and many node semihardwood one-leaf stem cutting of *S amara*. At the petiole of compound leaf of *S. amara* were left one to four basal leaflets. After that the leaf of each propagule of both species was trimmed to suitable size of 50 cm². The cuttings were then selected to be as uniform as possible (Fig. 34a,b). Vigorosity, lignification level, and stem diametre was considered. Length of the cutting of *C. spruceanum* was 7 cm, diameter ranged between 2-4 mm. Length of the stem part of *S. amara* without the petiole was 3 cm, diameter ranged between 1-1.5 cm (measured at the central part). All the cuttings were treated for 15 minutes in a solution of fungicide *Cupravit.*

Fig. 33. *C. spruceanum* (a) Collected plant material in polystyrene boxes, (b) Dividing the plant material into cutttings.

Fig. 34. (a,b) Selection of the cuttings of *C. spruceanum.*

4.4 Experimental design

Both experiments were established in randomised blocks, where cuttings of both species were randomly divided into 5 groups of 10 cuttings with 3 replications to give a total of 150 cuttings (10 cuttings × 5 treatments × 3 replicates) of *C spruceanum* and 150 cuttings (10 cuttings × 5 treatments × 3 replicates) of *S. amara*. The stem base of each cutting from each group was treated by one of the alcohol solutions *1000, 2000, 4000,* and *5000* ppm of IBA (Fig. 37a), except the control group without any IBA treatment. The time of each treatment took 3 secs. Treated basal ends of each stem cutting were air dried briefly for 5 min before carefull insertation to a depth of 15-25 mm (Fig. 37b,c). The sand around each propagule was slightly firmed (Fig. 37c,d), and the cuttings were sprayed before each closing of the lid. The distributions of the treatments of each species are shown in (Fig. 35, and 36).

$T_{1.4}$ (4000 ppm)	$T_{1.2}$ (1000 ppm)	$T_{1.3}$ (2000 ppm)	$T_{1.5}$ (5000 ppm)	$T_{1.1}$(control)
$T_{2.4}$ (4000 ppm)	$T_{2.5}$ (5000 ppm)	$T_{2.2}$ (1000 ppm)	$T_{2.3}$ (2000 ppm)	$T_{2.1}$ (control)
$T_{3.3}$ (2000 ppm)	$T_{3.4}$ (4000 ppm)	$T_{3.1}$(control)	$T_{3.5}$ (5000 ppm)	$T_{3.2}$ (1000 ppm)

Fig. 35. IBA hormonal treatment distribution of *C. spruceanum*

$T_{1.2}$ (1000 ppm)	$T_{1.1}$ (control)	$T_{1..3}$ (2000 ppm)	$T_{1.5}$ (5000 ppm)	$T_{1.5}$ (4000 ppm)
$T_{2.1}$ (control)	$T_{2.5}$ (5000 ppm)	$T_{2.2}$ (1000 ppm)	$T_{2.3}$ (4000 ppm)	$T_{2.3}$ (2000 ppm)
$T_{3.3}$ (2000 ppm)	$T_{3.4}$ (4000 ppm)	$T_{3.4}$ (5000 ppm)	$T_{3.5}$ (1000 ppm)	$T_{3.1}$ (control)

Fig. 36. IBA hormonal treatment distribution of *S. amara.*

Fig. 37. *C. spruceanum* (a) IBA alcohol solution treatment of the cuttings, (b) Instalation of the cuttings, (c) Cuttings at the beginning of the experiment, (d) Detail of the cutting.

4.5 Experimental conditions

Relative humidity (RH) and temperature (°C) data conditions inside the polypropagator were collected through simple USB temperature and moist datalogger (Fig. 38). The experiments were done at relatively hot season (August). The mean relative humidity inside the propagator was maintained at 95 % and maximum and minimum day-night temperature ranged between 37,7 °C and 21,6 °C respectively with mean value of 27.95 °C.

Whenever the propagator was opened for observation, fine jet of water spray was applied to raise the relative humidity inside the propagator. The water level inside the propagator was checked and maintained at that marked on the bamboo stick.

The propagator was kept under polyethylene shadecloth to avoid excessive heat accumulation. The first week of propagation, the shadecloth was doubled.

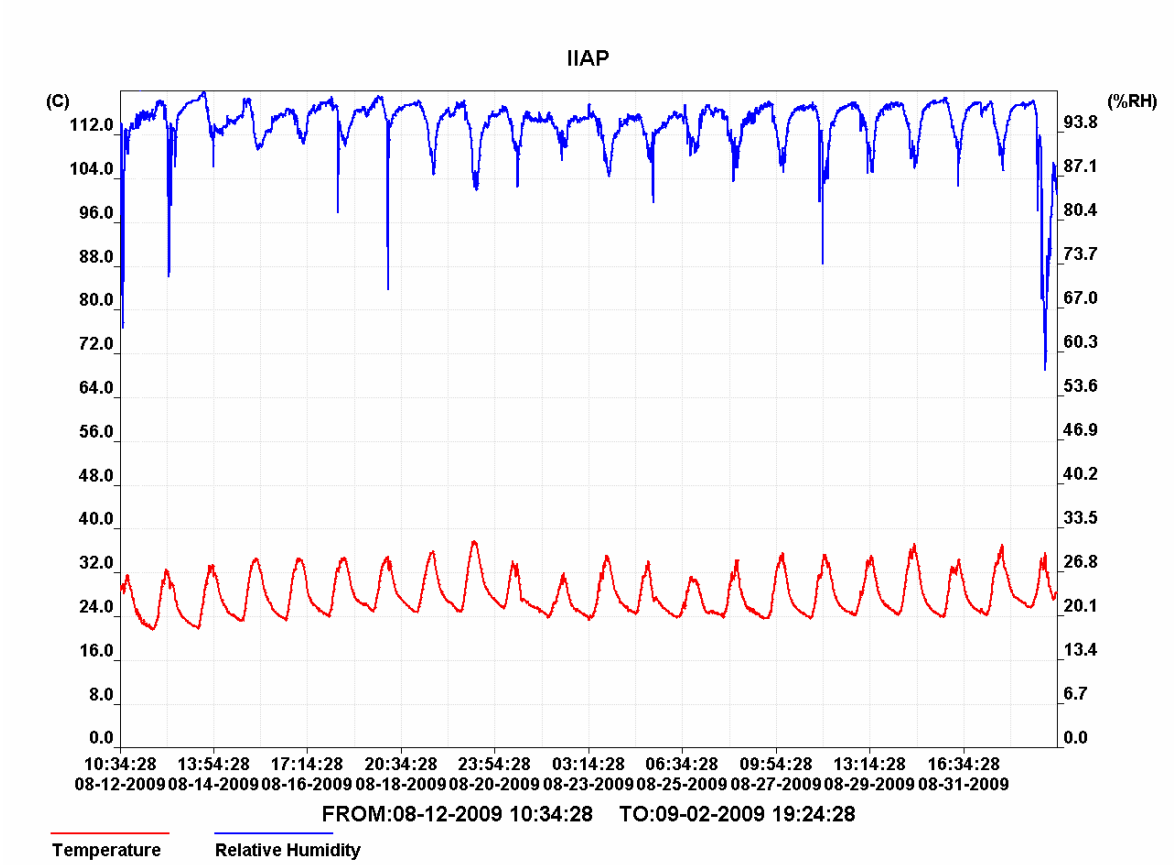

Fig. 38. Temperature and relative humidity inside the polypropagator during the experiment.

4.6 Data collection and evaluation

Every week of the experiment, five cuttings were controlled for the presence of callus, root, or mortality. Final evaluation of both experiments was made after 21 days from the day of planting (Fig. 39a), when the majority of the cuttings rooted. The sand was removed from all propagules and their roots were washed (Fig. 39b), before the rooting parametres were measured (Fig. 39d). The cutting was considered as rooted if it has at least one primary root more than 1 mm long (Fig. 39c). Data was collected in terms rooting parameters as total root length, length of the longest root, and number of roots and callusses per cutting, percentage of rooting and callussing, mortality, leaf abscission, and vigor. The vigor scale was determined as 1-best vigor, 2-good vigor, 3-bad vigor, and 4-mortality of the propagule.

Fig. 39. *C. spruceanum* (a) Cuttings at the end of the experiment, (b) Washing of the cuttings, (c) Succesfuly rooted cutting, (d) Measurement of the rooting parametres.

4.7 Data analysis

The statistical analysis was done using StatSoft CR STATISTICA 2010, version 9.0 computer software package. Because the normality assumptions necessary for parametric tests were not met, the non-parametric tests were used for the analysis.

For the relationship of number and percentage of rooting, calluss formation, mortality, and leaf abscission with IBA concetrations, Pearson´s Chi-square test was conducted. It tests the null hypothesis that there is no significant diference between expected frequencies and observed frequencies of rooting parametres for IBA concetrations among cuttings from all IBA concetrations, against the alternative hypothesis that a significant difference between these frequencies exists at the a = 0.05 (95%) confidence level.

The Kruskal-Wallis ANOVA (KW ANOVA) one-way analysis of variance tests were used to analyze these rooting parametres: number of roots, total root length, length of

the longest root, number of calluses, and vigor. The KW ANOVA tests the null hypothesis of no difference between five group medians, against the alternative hypothesis that a significant difference exists between the medians at the a = 0.05 (95%) confidence level.

Two sided tests for relative frequency and multiple tests were done to find out relations between each group of IBA treatment and measured rooting parametres.

The correlation matrix was used to find out correlation coefficients of the measured rooting parametres.

5 RESULTS AND DISCUSSION

5.1 The effect of IBA treatment on rooting parametres of *C. spruceanum* leafy stem cuttings

5.1.1 The effect of IBA treatment on number of rooted cutting

In the case of number of rooted cutting, the null hypothesis of Pearson´s Chi Square test was rejected in favor of the alternative. The results indicate that there is a significant difference in distribution of rooted cutting of *C. spruceanum*, among the five groups tested ($P < 0.05$).

The two sided test for relative frequency showed that the number (and percentage) of rooted cutting for treatment 2000 ppm of IBA was significantly higher (80 %), than for control group without any IBA treatment and group 1000 ppm of IBA (10 % and 43.3 % respectively). Number of rooted cuttings for group with treatment 4000 ppm of IBA was significantly higher (70 % rooted), than for control group without any IBA treatment and group 1000 ppm of IBA as well (10 % and 43.3 % respectively). Number of roots per cutting for treatment 5000 ppm of IBA was significantly higher (66.6 % rooted), only than for control group without any IBA treatment (10 % cuttings rooted); ($P < 0.05$). These results are shown in Table2.

There is no significant difference in number of rooted cutting between the group treated with 5000 ppm of IBA and groups treated with 4000, 2000, and 1000 ppm of IBA, and also between the groups treated with 2000 ppm and 4000 ppm.

The cuttings treated with auxins usually root with a higher percentage of cuttings rooted (Leakey, 2004; Swami *et al.,* 2003; Wiesman and Lavee, 1995). Tree species and even clones can appear to respond differently to individual and mixed applications of auxin at differing concentrations, even when many other factors are constant (Leakey, 2004). The study of vegetative propagation of coppice shoots of *C. spruceanum* showed 100 % rooting success after 60 days. This research did not prove any significant effect of growth regulators on rooting success (Gatti, 2002). This fact can be explained by very juvenile material used in this research and different propagating conditions (greenhouse, mist system).

Tab. 2. Effect of IBA concenration treatment on number and percentage of rooting, calluss formation, mortality, and leaf abscission of *C. spruceanum* semihardwood cuttings (day 21).

IBA concentration (ppm)	No.	No. of rooted cuttings n [%]	No. of cuttings with callus n [%]	No. of cuttings with mortality n [%]	No. of cuttings with leaf abscission n [%]
Control	30	3 [10]^{a}	18 [60]^{a}	11 [36.6]^{a}	15 [50]^{ab}
1000	30	13 [43.3]^{b}	16 [53.3]^{a}	9 [30]^{ab}	17 [56.6]^{b}
2000	30	24 [80]^{c}	24 [80]^{b}	3 [10]^{b}	10 [33.3]^{a}
4000	30	21 [70]^{c}	22 [73,3]^{ab}	6 [20]^{ab}	20 [66.6]^{b}
5000	30	20 [66.6]^{cb}	20 [66.6]^{ab}	7 [23.3]^{ab}	18 [60]^{b}
Total	150	81 [54]	100 [66.6]	36 [24]	80 [53.3]

Means indicated by the same leter on the column are not statistically different from each other ($P < 0.05$); (Two sided test for relative frequency).

5.1.2 The effect of IBA treatment on number of cuttings with callus

In the case of the number of cuttings with presence of at least one callus, the null hypothesis of Pearson´s Chi Square Test was not rejected. The results indicate that there is no significant difference between the distribution of number of callused cutting of *C. spruceanum* among five groups tested ($P > 0.05$).

The two sided test for relative frequency showed that the number (and percentage) of cuttings with presence of callus for treatment 2000 ppm of IBA was significantly higher (80 %), than for control group without any IBA treatment and group treated with 1000 ppm of IBA (60 % and 53.3 % respectively); ($P < 0.05$). These results are shown in Table 2.

There is no significant difference in number of callused cutting between all the other groups tested.

5.1.3 The effect of IBA treatment on number of cuttings with mortality and on number of cuttings with leaf abscission

In the case of the number of cuttings with mortality, the null hypothesis of Pearson´s Chi Square Test was not rejected. The results indicate that there is no significant difference between the distribution of death cuttings of *C. spruceanum* among five groups tested ($P > 0.05$).

The two sided test for relative frequency showed that the number (and percentage) of death cuttings for treatment 2000 ppm of IBA was significantly lower (10 %), than for

control group without any IBA treatment (36.6 %); ($P < 0.05$). These results are shown in Table 2.

There is no significant difference in number of death cuttings cutting between all the other groups tested.

In the case of the number of cuttings with leaf abscission, the null hypothesis of Pearson´s Chi Square Test was not rejected. The results indicate that there is no significant difference between the distribution of number of cuttings with leaf abscission of *C. spruceanum* among five groups tested ($P > 0.05$).

The two sided test for relative frequency showed that the number (and percentage) of cuttings with leaf abscission for treatment 2000 ppm of IBA was significantly lower (33.3 %), than for groups treated with 1000, 4000, and 5000 ppm of IBA (56.6 %, 66.6 %, and 60 % respectively); ($P < 0.05$). These results are shown in Table 2.

There is no significant difference in number of death cuttings cutting between all the other groups tested.

5.1.4 The effect of IBA treatment on number of roots, total root length, and length of the longest root per cutting

In the case of number of roots per cutting, the null hypothesis of KW ANOVA was rejected in favor of the alternative. The results indicate that there is a significant difference in number of roots per cutting between the five groups tested. There are at least two statistically different groups of IBA treatments ($P < 0.05$) in relation to number of roots per cutting of *C. spruceanum.* The biggest value of mean number of roots per cutting (4.13) was recorded at group treated with 2000 ppm, followed by group treated with 4000 ppm and 5000 ppm (3.70 and 3.46 respectively); (Tab. 3; Fig. 40).

The multiple test showed that number of roots per cutting for treatment 2000 ppm of IBA was significantly higher than for control group without any IBA treatment, and for group treated with 1000 ppm of IBA. Number of roots per cutting for groups treated with 4000, and 5000 ppm of IBA was significantly higher than for control group without any IBA treatment as well ($P < 0.05$). These results are shown in Table 3.

There is no significant difference in number of roots per cutting between group with treatment 1000 ppm of IBA and control group without any IBA treatment, between group treated with 5000 ppm of IBA and groups treated with 1000, 2000, and 4000 ppm, and between group treated with 2000 ppm of IBA and the group treated with 4000 ppm of

IBA.

Thus, although treatment 2000 ppm showed the most number of roots per cutting gain it was not a significantly greater than the total number of roots per cutting gain for groups treated with 4000, and 5000 ppm of IBA solution.

Tab. 3. Effect of IBA concenration treatment on number of roots, total root length, length of the longest root, number of calluses, and vigor of *C. spruceanum* semihardwood cuttings (day 21).

IBA concentration (ppm)	No.	No. of roots/ cutting	Total root length (cm)	Length of the longest root (cm)	No. of calluses/ cutting	Vigor (1-best-4-death)
Control	30	0.23 ± 0.15^{a}	0.10 ± 0.08^{a}	0.03 ± 0.05^{a}	1.30 ± 0.23^{a}	2.93 ± 0.17^{a}
1000	30	1.36 ± 0.31^{ab}	2.36 ± 0.70^{ab}	1.15 ± 0.35^{ac}	1.30 ± 0.25^{a}	2.66 ± 0.21^{ab}
2000	30	4.13 ± 0.64^{c}	11.84 ± 2.53^{c}	3.01 ± 0.47^{b}	1.73 ± 0.23^{a}	1.93 ± 0.19^{b}
4000	30	3.70 ± 0.66^{bc}	9.97 ± 2.27^{bc}	2.40 ± 0.37^{bc}	1.86 ± 0.27^{a}	2.33 ± 0.21^{ab}
5000	30	3.46 ± 0.70^{bc}	7.99 ± 2^{bc}	1.94 ± 0.36^{bc}	1.63 ± 0.30^{a}	2.46 ± 0.19^{ab}
Total	150	2.58 ± 0.27	6.45 ± 0.86	1.71 ± 0.17	1.56 ± 0.11	2.44 ± 0.09

Means indicated by the same leter on the column are not statistically different from each other at $P < 0.05$ (KW ANOVA).

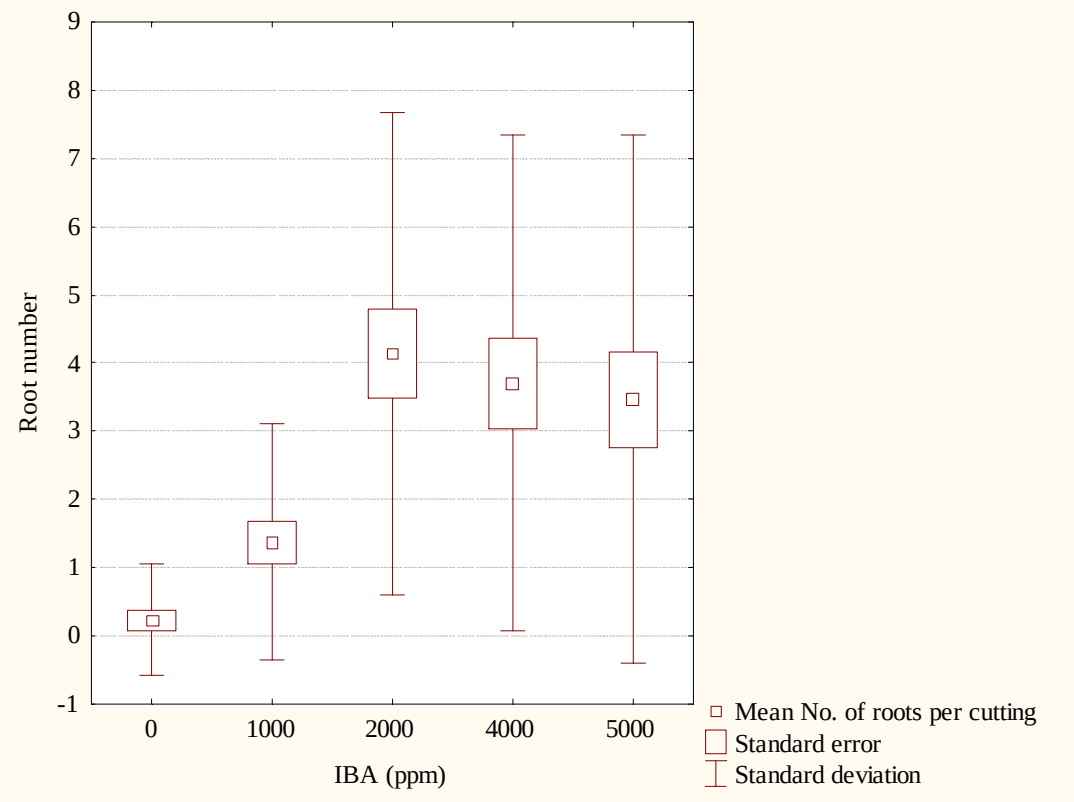

Fig. 40. Mean No. of roots per cutting of *C. spruceanum* leafy stem cuttings.

In the case of total root length per cutting, the null hypothesis of KW ANOVA was rejected in favor of the alternative as well. The results indicate that there is a significant difference in total root length between the five groups tested. There are at least two statistically different groups of IBA treatments ($P < 0.05$) in relation to the total root length of *C. spruceanum.* The biggest value of mean total root length per cutting (11.84 cm) was recorded at group treated with 2000 ppm, followed by group treated with 4000 ppm and 5000 ppm (9.97 cm and 7.99 cm respectively); (Tab. 3; Fig. 41).

The multiple test showed that total root length for group with treatment 2000 ppm of IBA was significantly higher than for control group without any IBA treatment, and for group treated with 1000 ppm of IBA. Total root length for groups treated with 4000, and 5000 ppm of IBA was significantly higher than for control group without any IBA treatment as well ($P < 0.05$).

There is no significant difference in total root length between group with treatment 1000 ppm of IBA and control group without any IBA treatment, between group treated with 5000 ppm of IBA and groups treated with 1000, 2000, and 4000 ppm, and between group treated with 2000 ppm of IBA and the group treated with 4000 ppm of IBA.

Although treatment 2000 ppm showed the most total root length, it was not a significantly greater than the total total root length for groups treated with 4000, and 5000 ppm of IBA solution.

In the case of length of the longest root per cutting, the null hypothesis of KW ANOVA was rejected in favor of the alternative as well. The results indicate that there is a significant difference in length of the longest root between the five groups tested. There are at least two statistically different groups of IBA treatments ($P < 0.05$) in relation to length of the longest root of *C. spruceanum.* The biggest value of mean length of the longest root per cutting (3.01 cm) was recorded at group treated with 2000 ppm, followed by group treated with 4000 ppm and 5000 ppm (2.40 cm and 1.94 cm respectively); (Tab. 3).

The multiple test showed that length of the longest root for groups treated with 2000, 4000, and 5000 ppm of IBA was significantly higher than for control group without any IBA treatment. Length of the longest root for group treated with 2000 ppm of IBA was also significantly higher than for control group trested with 1000 ppm of IBA ($P < 0.05$).

There is no significant difference in length of the longest root between all the other groups.

These results conform to that of number of roots per cutting and total root length:

Thus, although treatment 2000 ppm showed the biggest length of the longest root per cutting, it was not a significantly greater than the length of the longest root per cutting for groups treated with 1000. 4000, and 5000 ppm of IBA solution.

Rooting ability of *C. spruceanum* was sensitive to the IBA application. One study of IBA effects on the length of the main root of *C. spruceanum* cuttings was done in Brazil (Gatti, 2002). The effect after 20 days of this rooting experiment using juvenile coppice shoots cuttings in greengouse conditions was very similar with best value of the longest root at the concentration 2000 ppm as well. At the day 60 of this experiment, the concentrations 1000, 2000, and 3000 ppm of IBA had very similar effect on main root length, while untreated cuttings had the main root slightly shorter (Gatti, 2002). There are many other species where the cuttings treated with IBA produce more roots as well as longer roots than untreated cuttings. In *Milicia excelsa* the mean number of roots per cutting was higher when the cuttings were treated with IBA (Ofori *et al.*, 1996).

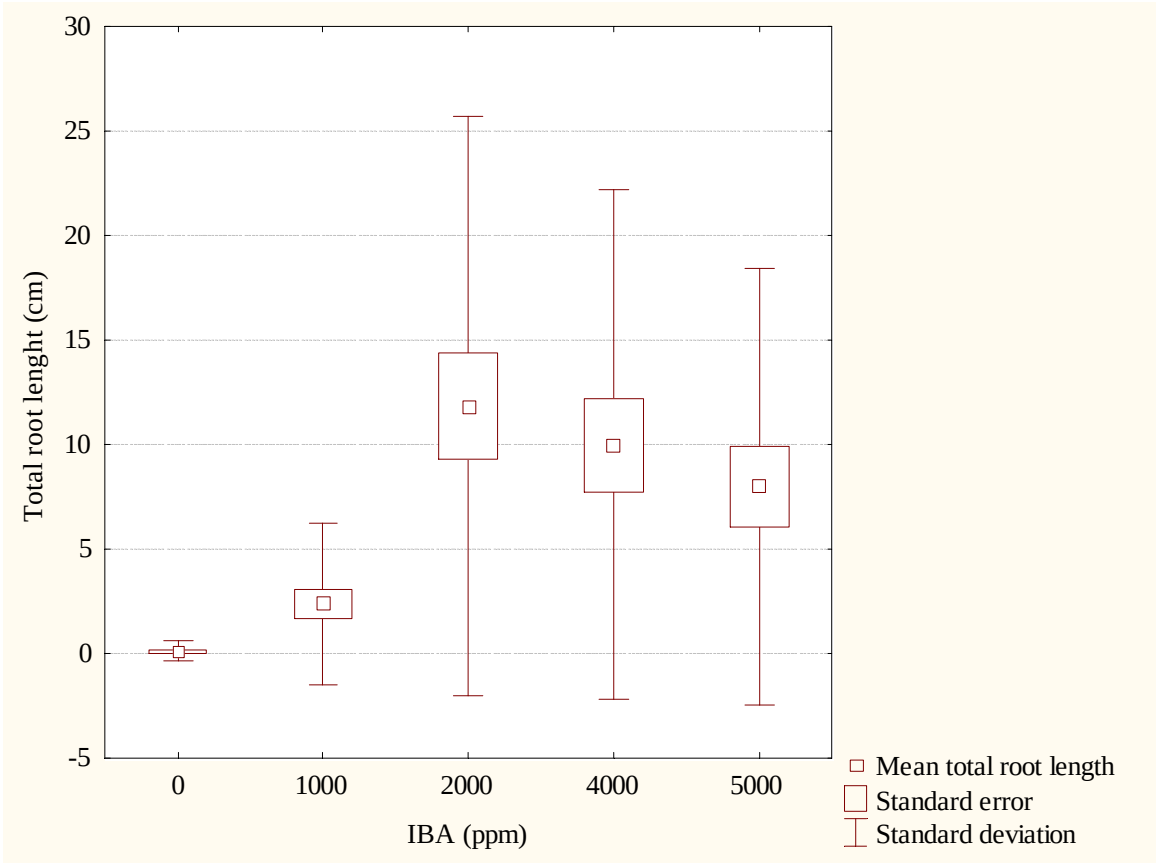

Fig. 41. Mean total root length per cutting of *C. spruceanum* leafy stem cuttings.

5.1.5 The effect of IBA treatment on number of calluses per cutting

The null hypothesis of KW ANOVA is in this case valid. The results indicate that there is no significant difference in number of calluses per cutting between the five groups tested. There are not at least two statistically different groups of IBA treatments ($P > 0.05$) in relation to the number of calluses per cutting. The biggest value of mean number of callusses per cutting (1.86) was recorded at group treated with 4000 ppm, followed by group treated with 2000 ppm and 5000 ppm (1.73 and 1.63 respectively); These results are shown in Table 3.

5.1.6 The effect of IBA treatment on vigor of the cuttings

The null hypothesis of KW ANOVA was rejected in favor of the alternative. The results indicate that there is a significant difference in vigor between the five groups tested. There are at least two statistically different groups of IBA treatments ($P < 0.05$) in relation to the vigor of *C. spruceanum.* The best value of mean vigor per cutting (1.93) was recorded at group treated with 2000 ppm, followed by group treated with 5000 ppm and 4000 ppm (2.44 and 2.33 cm respectively); These results are shown in Table 3.

The multiple test showed that vigor for treatment 2000 ppm of IBA was significantly higher than for control group without any IBA treatment ($P < 0.05$).

There is no significant difference in vigor between all other groups of IBA treatment.

Although treatment 2000 ppm showed the most vigor gain, it was not a significantly greater than the total vigor gain for groups treated with 1000, 4000, and 5000 ppm of IBA solution.

5.1.7 Correlations between rooting parametres

The biggest correlation coeficient showed number of roots with total root length per cutting (0.88), followed by length of the longest root with total root length per cutting (0.86), length of the longest rooot with number of roots per cutting (0.79), vigor with mortality (0.78), and number of callusses per cutting with cuttings with callus (Tab. 4).

Tab. 4. Correlation matrix of rooting parametres of *C. spruceanum* semihardwood cuttings (day 21).

r>=	-1	-0,80	-0,60	-0,40	-0,20	0	0,20	0,40	0,60	0,80	1

Variable	IBA Treatment	Number of roots	Longest root length	Total root lenght	Cuttings with callus	No. of callusses	Leaf abscission	Rooted cuttings	Mortality	Vigor
IBA Treatment	1,00	0,35	0,30	0,29	0,09	0,12	0,11	0,36	-0,10	-0,15
Number of roots	0,35	1,00	0,79	0,88	0,33	0,18	-0,26	0,72	-0,43	-0,65
Longest root length	0,30	0,79	1,00	0,86	0,36	0,20	-0,34	0,73	-0,44	-0,73
Total root lenght	0,29	0,88	0,86	1,00	0,26	0,11	-0,27	0,57	-0,34	-0,58
Cuttings with callus	0,09	0,33	0,36	0,26	1,00	0,77	-0,32	0,48	-0,60	-0,56
No. of callusses	0,12	0,18	0,20	0,11	0,77	1,00	-0,16	0,32	-0,44	-0,34
Leaf abscission	0,11	-0,26	-0,34	-0,27	-0,32	-0,16	1,00	-0,30	0,43	0,68
Rooted cuttings	0,36	0,72	0,73	0,57	0,48	0,32	-0,30	1,00	-0,58	-0,72
Mortality	-0,10	-0,43	-0,44	-0,34	-0,60	-0,44	0,43	-0,58	1,00	0,78
Vigor	-0,15	-0,65	-0,73	-0,58	-0,56	-0,34	0,68	-0,72	0,78	1,00

5.2 Rooting experiment of *Simarouba amara*

Simarouba amara presented (98.5 %) mortality of both, treated and untreated cuttings, and did not present any callussing, or rooting success. All the leaflets were shed after first week of the experiment, and the majority of the propagules started to be succetible to rot after second week of the experiment. The statistical analysis was not proceeded due tu high amounts of measured zero values. These results are similar to that from Costa Rica (García-Villamán, 1974; Zanoni-Mendiburu, 1975), which wasn´t internationally published. There are no more records about the vegetative propagation of *S. amara*. The results suggests that *S. amara* is difficult to root species with the plant material, or type of cutting used in this experiment. The specific leaf arranggement of this species does not offer good quality semihardwood cuttings. According to (Longman, 1993), long cuttings usually root best and the recomended length of the cuttings 2.5-12 cm, with a stem diameter at the base of about 4-8 mm. The plant material used in present study allowed maximum possible longitude of the cuttings 3 cm, and the minimum diametre of one centimetre. It is probable, that these parametres of propagules are not suitable for this species. The reccomendation is to use of younger, thinner, and more vigorous and juvenile plant material, (*e.g.* from coppice shoots) for the future study of vegetative propagation of *S. amara.*

6 CONCLUSION

The present study has revealed that the rooting ability, as well as other rooting parametres of *Calycophyllum spruceanum* was higher with application of Indole-3-butyric acid, thus this species is sensitive to aplication of this auxin. The best concentration for successive vegetative propagation of juvenile leafy stem semihardwood cuttings of this species was 2000 ppm of IBA. The great advantage of this tree is ability of sprouting from stumps and therefore the juvenile shoots can be utilized for the vegetative propagation, as well as the plant material detached from young seedlings by prunning.

In the case of *Simarouba amara*, the future study of vegetative propagation and rooting behavior of this species is needed. This tree can sprout thin shoots from the stumps as well (personal observation), and this juvenile plant material could serve as a good source of succesfully rooting cuttings.

Vegetative propagation of these two species may reduce the rotation time period, which is interesting especially for the farmers in the Amazon Basin. The mass propagation of one or few genotypes at one place is not recomended, because of the risk of genetic diversity decrease. Both species has relatively good viability and germination rates of the seeds, and vegetative propagation by leafy stem cuttings may serve as alternative to sexual propagation of these useful agroforestry species.

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