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Vegetative propagation of Amazonian trees

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Declaration

I hereby declare that the M.Sc. thesis entitled "Vegetative propagation of Amazonian trees" has been written by me independently and all the sources have been quoted and stated in the references.

In Prague 19.4.2014

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Michaela Jedličková

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Abstract

Domestication, breeding and reforestation in Peruvian Amazon depend on the successful propagation of the trees. Vegetative propagation serves as an alternative to generative propagation. But in case of many tropical species, the successful pathway of asexual propagation has not been developed yet. Therefore, this thesis is focused on vegetative propagation of two important species. We studied the influence of IBA (indole-3-butyric acid) on basal semi-hardwood leafy stem cuttings of *Dipteryx alata* or shihuahuaco (Fabaceae). The groups of cuttings treated with 0, 0.1, 0.2, 0.3 or 0.4 % of IBA were tested on rooting ability. The best rooting ability of shihuahuaco of 23.33 % was observed with the enhancement of 0.4 % of IBA. This study is focused on influence of IBA and IAA (indole-3-acetic acid) and cutting position on rooting ability of softwood cuttings of *Guazuma crinita* or bolaina blanca (Sterculiaceae), too. These cuttings tested on rooting ability were treated with concentrations 0, 0.1, 0.2 or 0.3 % of IBA or IAA. The best results in case of bolaina blanca was achieved by treatment of apical cuttings with 0.1 % of IBA. The final rooting rate was 46.67 %.

Keywords: Cutting, *Dipteryx alata*, domestication, *Guazuma crinita*, vegetative propagation

Abstrakt

Domestikace, šlechtění a reforestace peruánské Amazonie závisí na úspěšném rozmnožování stromů. Vegetativní rozmnožování slouží jako alternativa generativního, ale u mnoha tropických druhů, úspěšný způsob asexuálního rozmnožování ještě nebyl vyvinut. Tudíž se tato práce zaměřuje na vegetativní rozmnožování dvou důležitých druhů. Studovali jsme vliv hormonu IBA (kyselina indolyl-3-máselná) na bazální polodřevité řízky druhu *Dipteryx alata* neboli shihuahuaco z čeledi Fabaceae. Skupiny řízků ošetřeny 0; 0,1; 0,2; 0,3 nebo 0.4% roztokem hormonu IBA a byly testována jejich schopnost zakořenění. Největší procento zakořeněných řízků druhu shihuahuaco bylo pozorováno při použití 0,4% roztoku hormonu IBA. Zakořenilo 23,33 %. Tato studie se také zaměřila na vliv hormonů IBA a IAA (kyselina indol-3-octová) v interakci s vrcholovými a bazálními bylinnými řízky druhu *Guazuma crinita* neboli bolaina blanca z čeledi Sterculiaceae. Tyto řízky testované na zakořenění byly ošetřené 0; 0,1; 0,2 nebo 0,3% roztokem hormonu IBA nebo IAA. Nejlepší výsledek zakořenění druhu bolaina blanca byl dosažen ve skupině vrcholových řízků s použitím 0,1% roztoku IBA. Zakořenilo 46,67 % řízků.

Klíčová slova: *Dipteryx alata,* domestikace, *Guazuma crinita,* vegetativní rozmnožování, řízkování

Contents

List of abbreviations

IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
IIAP	Instituto de Investigaciones de la Amazonía Peruana
INIA	Instituto Nacional de Innovacion Agraria
NAA	α-naphthaleneacetic acid
PVC	Polyvinyl chloride
WPM	Woody plant medium

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1. Introduction

Most forest products from the Amazon region sold locally, nationally and even internationally are extracted from natural forests. During the last decade, the destruction of primary forests has reduced round timber production. The natural forests are not able to meet the growing demand for timber. Consequently, growing of trees outside the natural forests becomes more and more important as supply and provision of environment in the timber producing regions. Growing trees can generate income and rehabilitate degraded land. In the tropical forest regions, smallholders usually produce by management of natural single-species stands or tend to transplant trees in homegardens, fields and secondary forests, too (Hoch *et al.*, 2009). Many tropical trees are propagated by seeds as well. But to improve the timber quality, meet the requirements of local farmers and market, the species must be domesticated and bred. It can be done easily by vegetative propagation. Another benefit of vegetative propagation is higher multiplication rate. But a pathway of cheap vegetative propagation applicable by local farmers is not developed in case of many species (Leakey and Akinnifesi, 2008).

In 2011, Huml conducted research on preferred tree species by farmers in Ucayali region. He found out, that bolaina blanca and shihuahuaco are one of the priority trees in this area. But there are problems with seeds of these species and vegetative propagation of them was not fully examined, yet. So, we decided to experiment with them.

2. Literature review

2.1 Peruvian Amazon

Amazon is spread over parts of seven nations. This region is far from uniform by differences in-between animal and plant life. It also differs due to human activity that causes the variation of conditions of the natural resources. In some areas remain primary forests, whereas others contains of degraded agricultural land (Díaz Gonzáles, 2007).

Nearly 60 % of the national territory of Peru is considered part of the Amazon. It is the second largest part of the tropical forests to be found in Latin America behind Brazil. Peruvian rainforests contain an exceptionally high number of endemic plants. They host 23 % of the known tropical plant species located in the tropics. The varied topography (100-2 500 m asl) and the associated annual rainfall, ranging from 1 100-5 000 mm, provide the conditions necessary for numerous species to thrive. But forests are exploited for timber and used for cultivation and pastures (Díaz Gonzáles, 2007).

2.1.1 City of Pucallpa

Fig. 1: Map of Peru (Anonymous, 2005)

Two seasons can be marked in the region (fig. 1). Dry season begins in June and ends in August. Intense rainy season occurs between November and March. The mean annual rainfall is 1568 mm and the annual average temperature is 26.1 °C, as we can see on climatic graph (Fig. 2). The minimum average temperature fall to 18 °C and maximum reaches 36 °C. Daily temperature oscillations are around 5-8 °C, which is higher that yearly variation (1-2 °C). The mean relative humidity is higher than 75 % (Díaz Gonzáles, 2007). According to the Köppen Climate Classification, Pucallpa belongs to tropical monsoon climate. The warmest month, on average, is January with an average temperature of 26.7°C and the coolest month on average is July, with an average temperature of 25°C. The month with the most precipitation on average is March with 190.5 mm and July is the month with the least precipitation on average with 50.8 mm of precipitation (CantyMedia, 2014). Pucallpa is the capital city of the Ucayali Department. It is located 85 km west of the Brazilian border, in the middle of the Peruvian lowland rainforest. It is connected with Lima by 800 km paved road, which crosses the Andes Mountains. Pucallpa is the second largest and fastest growing city in the Peruvian Amazon. In Pucallpa (founded in 1883), the land use changed dramatically with the arrival of settlers. During the first 10 years, the principal change was from primary forest to agricultural production. After 1-3 years of cultivation, land parcels were left fallow and secondary forests began to regenerate naturally. The intensity of pasturing was initially relatively low but after 10 years, the conversion of primary forest in pastures continued with made increase. This land use change was not direct, but a result of the prevention of the regeneration of secondary forest after the cultivation of annual crops. The shifting cultivation technique was used by farmers, because of the low input involved. After repeated cycles of shifting cultivation, soil fertility decreased and farmers rather changed their land use system to pasture and perennial crops then moved to new holdings. The colonists later directed their economic activities towards forest production, but have exhibited no interest in managing forests, protecting them from damage or in reforesting (Díaz Gonzáles, 2007).

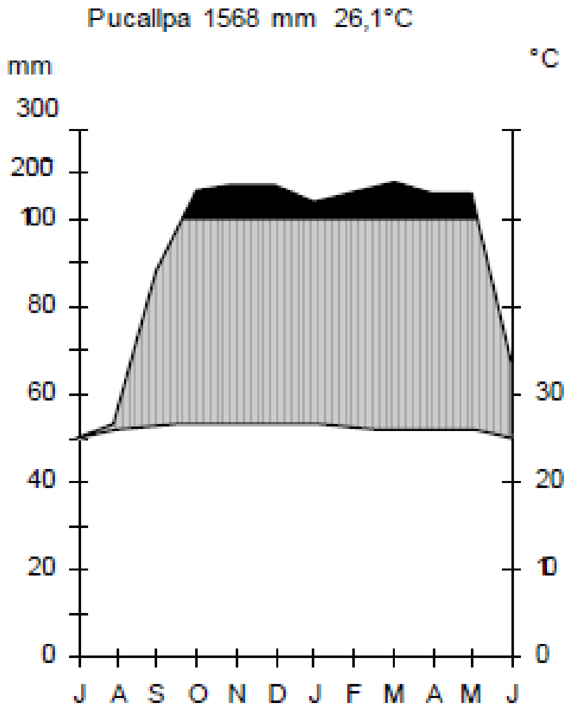

Fig. 2: Mean monthly temperature and rainfall in Pucallpa (Díaz Gonzáles, 2007)

2.2 Domestication

Domestication can be defined as the production of individual genotypes or varieties exhibiting traits that are desirable to humans through the exchange of genetic material by generative or vegetative processes (Harfouche at al., 2012). Trees have been planted for thousands of years and the natural forests and woodlands were cleared for purposes, like to obtain building material and fuel wood and to provide lands for animals and crops. In contrast to most agriculture crops, the principal domestication of trees has occurred in the last one hundred years. Exotic forest species have been used increasingly to produce fast-growing species for fuel wood, timber or pulpwood. However, they are mostly wild populations little changed by people (Turnbull, 2002). Increasingly, products from trees are derived from plantation rather than natural forests. In near future, high demand for forest products will require higher productivity. So, now is the time for tree breeding and biotechnology to improve productivity, sustainability and wood quality (Harfouche *et al.*, 2012)

Tree is considered domesticated when it has been selected for specific genetic characteristics and when it is propagated and cultivated in managed ecosystems. On the other hand, „wild“ tree grows spontaneously in self-maintaining populations in natural or semi-natural ecosystems and can exist independently on human action. However, there exist several stages between wild and fully domesticated trees. Domestication involves changes in exploitation systems as well as changes of tree properties (Wiersum, 2008). Market-driven domestication is a process used to improve quality and productivity of agroforestry trees. It is based on strategies considering needs of farmers (their priorities for domestication); an inventory of the natural resource; the suitable production of tree product; restoration of degraded land and reduction of deforestation; and the wise use and conservation of genetic resources. In practice, plantings are often constrained by the lack of genetically superior seeds that is the traditional source of planting stock. So, one of the first decisions in developing domestication strategy has to be decision between generative and vegetative propagation to achieve genetic improvements. Several economic and biological situations favour a clonal approach. Such as (Leakey and Akinnifesi, 2008):

- the occurrence of individual trees in a wild with rare combination of traits
- the need to combine many desirable traits
- the need for high product uniformity to meet market specifications and ensure profitability (heterogeneity is characteristic for seedlings)
- the species is a shy seeder (does not flower and fruit every year or produces only very small amount of seeds
- the propagation material is limited
- the timescale is insufficient to allow breeding through the slower generative propagation
- the seeds of the chosen species are recalcitrant
- Generative propagation is preferable when large quantities of genetically diverse, low-value plants with unlimited seed supplies are required

A range of vegetative propagation techniques is used as macropropagation (grafting, softwood cuttings, hardwood cuttings, air-layering, suckering) and micropropagation (*in vitro* techniques) (Leakey and Akinnifesi, 2008).

2.3 Vegetative propagation

Vegetative propagation is becoming increasingly important in forestry and agroforestry for the multiplication of limited seed material. But the main reason for cloning is to take advantage of its ability to capture traits in new plants that are exact copies of the genetic code of the mother plant. There is number of factors that need to be considered before formulation of strategy. These factors are the effects of using juvenile or mature tissues; and the methods of propagation. Choose of methods is closely connected with the level of technology that is appropriate (Leakey and Akinnifesi, 2008).

2.3.1 Effects of juvenile and mature tissue

An advantage of propagagation from mature tissue is that by the time the tree is mature it has demonstrated whether or not it has superior qualities. However, it is not easy to take advantage of this superiority, because propagation of many species by mature stem cuttings is difficult. Another important advantage of propagation from mature tissue is that this tissue is already capable of reproductive process and so it will flower and fruit within few years. But woody plants propagated from mature tissue will have a lower stature. This is unacceptable for timber production, which requires the vigour and form from juvenile tissue. For timber trees, propagation from mature trees is limited to the establishment of clonal seed orchards within breeding programmes (Leakey and Akinnifesi, 2008) and to propagation from stump sprouts (Danthu *et al*., 2008).

The sources of juvenile tissue are seedlings, coppice shoots and root suckers. For domestication purpose, stump sprouts of felled trees have the advantage of already shown phenotype. But there are still reasons, why the use seedlings may be preferred over coppicing from trees of known phenotype (Leakey and Akinnifesi, 2008):

- The felling of large number of mature trees for the purpose of generating cultivars may not be acceptable to the owners and may be environmentally damaging.

- The use of seedlings allows the screening of larger population, with more diverse origins. This will maintain genetic diversity among the cultivars
- The elite specimens may have already been removed by loggers, which mean that the seedling population offers better genetic variation.

2.3.2 Micropropagation

Micropropagation offers high multiplication rates; it is also appropriate to free tree from viral diseases. But the cost of the supply and requirements on laboratory conditions is causing problems to the use of micropropagation (Ezekiel, 2010).

2.3.3 Macropropagation

Cuttings

It has become clear that cuttings taken from different parts of the same shoot differ in their rooting capacity. It is also influenced by the cutting length or volume. There are also factors that affect rooting capacity between shoots on the same plant. The shading is one of them, because it determines the amount and the quality of light received by lower shoots. Other factor is nutrient status of the shoots. Stock plant, from which we take cuttings, should be healthy, free from serious insects or diseases, vigorous and of a known identity. We should ensure that all used equipment is sharp and make clean cuts. Cuttings can be made from many parts of plant: true stems, modified stems, leaves, leaf-buds or roots. Stem cuttings can be categorized by various parameters, like the part of the plant from which they are taken, the time of the harvest and the physiological condition of the tissue at the time of harvest. There are four most commonly used types of cuttings: (i) softwood, (ii) semi hardwood and (iii) hardwood cuttings (Leakey and Akinnifesi, 2008).

(i) It is suggested that more that 90 % of tropical trees can be propagated by juvenile stem cuttings (softwood cuttings) by macropropagation, which is more cost effective (Leakey and Akinnifesi, 2008). Softwood cuttings consist of actively growing tissue, which means tissue from current season's growth at the terminal end of stems. They are removed relatively early in the growing season. Immature softwood tissue can be bent into a “U” shape without breaking, but the optimum stage of maturity is when it snaps when bent sharply. Softwood cutting limitation is its high perishability and easy damage. That is why they are cut early in the morning in cool hours. During handling, temporary storage and transport, they must be under cool, moist conditions. Cuttings should be handled as quickly as possible (John *et al.*, 2010). Adventitious root formation is essential for the successful propagation. Generally, ease of adventitious root formation decrease with increasing age of parent stock. This is very important problem, because the desirable characteristics are frequently expressed after plants reach maturity (Davies, 1984). Factors affecting rooting capacity have not been entirely enlightened. However, it is known, that juvenile characteristics, which preserve at the base of trees (in ontogenetically younger tissue) decrease in the tissue laid down later during the tree's development and therefore chronologically younger. This means that the choice of initial material for propagation is a determining factor. The rooting ability of cuttings from stump sprouts is higher that from the crown of adult trees (Danthu *et al.*, 2008; Leakey, 2004). The season in which cuttings are taken is also important factor, which may influence root formation. This theory was tested in the forest of Madagascar, where the average annual rainfall is about 1 600 mm, concentrated during the hot season (October – May), but with no ecologically dry months. Five sampling dates (May, August, October, December and March) of four species were tested and the cuttings were cultivated in polypropagators. Rooting capacity of species varied according to season of collection. The best results occurred during the hot, rainy season. In August, during the cold season, no roots were obtained. The seasonal dependence also varies with propagated species. Several factors could account for seasonal variations, such as physiological condition of cuttings, carbohydrate content or the nutritional richness of the substrate or the temperature (Danthu *et al.*, 2008).

(ii) Semi hardwood cuttings are taken from actively growing and only partially matured tissue of woody plants. Semi hardwood cutting breaks when bent into a “U” shape, in contrast to softwood cutting that will bend without breaking or must be bent more severely to cause breakage. Semi hardwood cuttings tend to root better than hardwood cuttings of the same species, but require careful handling, transport and temporary storage. They should also be cut early in the morning, in cool hour and kept out of direct sun. Length of cuttings should be from 7 to 13 cm and contain two or more nodes. Semi hardwood and softwood cuttings are usually shorter and thinner than hardwood cuttings, because they consist only of the current season's growth. For temporary storage, water and ice in shade location is used to create cool environment with high humidity (John *et al.*, 2010).

(iii) Hardwood cuttings are preferred type, because they are easily prepared, stored, transported and they are less perishable than cuttings from active tissue. Dormant, hardwood cuttings are usually taken in the late fall to late winter from non-active stems after leaves have fallen off and before bud break in the spring. This type of cutting is useful especially for species with high concentration of preformed, dormant root primordia located throughout the length of the stems. Cutting diameter should be generally around 2 cm or larger. It depends on the species. Larger diameter cuttings have stored more reserves than smaller diameter, but the larger the cutting diameter, the longer the cutting should be and the deeper the cutting should be set for support. Larger diameter and longer cuttings are useful for eroded sites, where the water table is deeper. If we are planting into rock riprap, cuttings should be at least 8 cm in diameter, than they will not break or bend. Length of the cuttings should be around 20 cm. They are taken from the terminal end of branches and should contain at least two internodes. The basal cut is made 1 to 2 cm below node. Old wood should not be used; the best is 2 to 7 years old wood with smooth bark. The apical bud plus several centimetres of the cutting should be removed, because the apical part draws too much energy from stored reserves and that reduce the chance to survival. If this part is cut off, the energy is redirected to the root and branch primordia in older part of stem. All side branches are trimmed off too, so the cutting is a single stem (John *et al.*, 2010).

Systems for rooting cuttings

Two most common macropropagation systems for rooting cuttings are (i) mist propagator and (ii) non-mist propagator. The basic principle is that the cutting bases are well supplied with water while the leaves are in moist shady environment to minimize the water stress (Ezekiel, 2010). Minimizing the shock enhance rooting (Leakey, 2004). It is critical to maintain an optimal level of ambient humidity, so the cuttings will not wilt and dry out or become diseased because of a too high humidity (Jaenicke and Beniest, 2002) and the medium has to allow respiration (Leakey, 2004). Both systems are very effective but differ in cost. Mist propagator system involves a lot of cost in material needed for construction, electricity and water (Ezekiel, 2010). Low-cost non-mist propagator has been developed for rooting of leafy stem cuttings. It does not require electricity or running water and can be used in both the moist and dry tropics (Leakey and Akinnifesi, 2008).

(i) Misting is a technique for minimizing plant water loss by controlled periodic wetting of leaves. It is helpful for rooting cuttings. With few plants, constant misting has been successful, but generally, it is better with intermitted misting. Constant misting leads to water waste, nutrient leach from leaves and reduction of soil temperature. Consequence is a restriction of root development. The interval for misting depends upon how quickly water dries from the leaves. Ideally, the mist should be turned on just after the leaves become dry and it should remain on until it wets all leaf surfaces. Death of cuttings is quick, if they become dry and are exposed to bright sunlight. So it is important to keep the cuttings wet at all times when the sun is shining. Over-misting is generally considered less damaging than over-watering. Misting reduces leaf transpiration in two ways: It decrease leaf temperature due to evaporative cooling as the water on the leaf evaporates and it maintains high humidity conditions at the leaf surface. The disease development under mist may be a serious problem. Botrytis and bacterial soft rot can be problem, especially if sanitary conditions are not maintained (Schnelle *et al*., 2003).

Mist propagator is done by adapting a bench for the propagation area or by using a ground bed. Since the area will be constantly wet, the bottom and sides should be of highly resistant material. Construction of steel wire and metal frames also provides a proper air circulation. Then, bottom is cover by freely draining material as gravels (size about 0.6 cm) to a depth of 2.5 cm and 5-7.5 cm of sand. The rooting medium (with or without flats) is placed to a depth of 5 cm on top of the sand. The best results in rooting are obtained when the soil medium is warm. It means that in cool conditions, electric heating cable is placed slightly above the gravel layer in the sand. For most plants, 24 °C is considered optimum. Mist benches (fig. 3) are usually placed in inexpensive Quonsets (Schnelle *et al*., 2003).

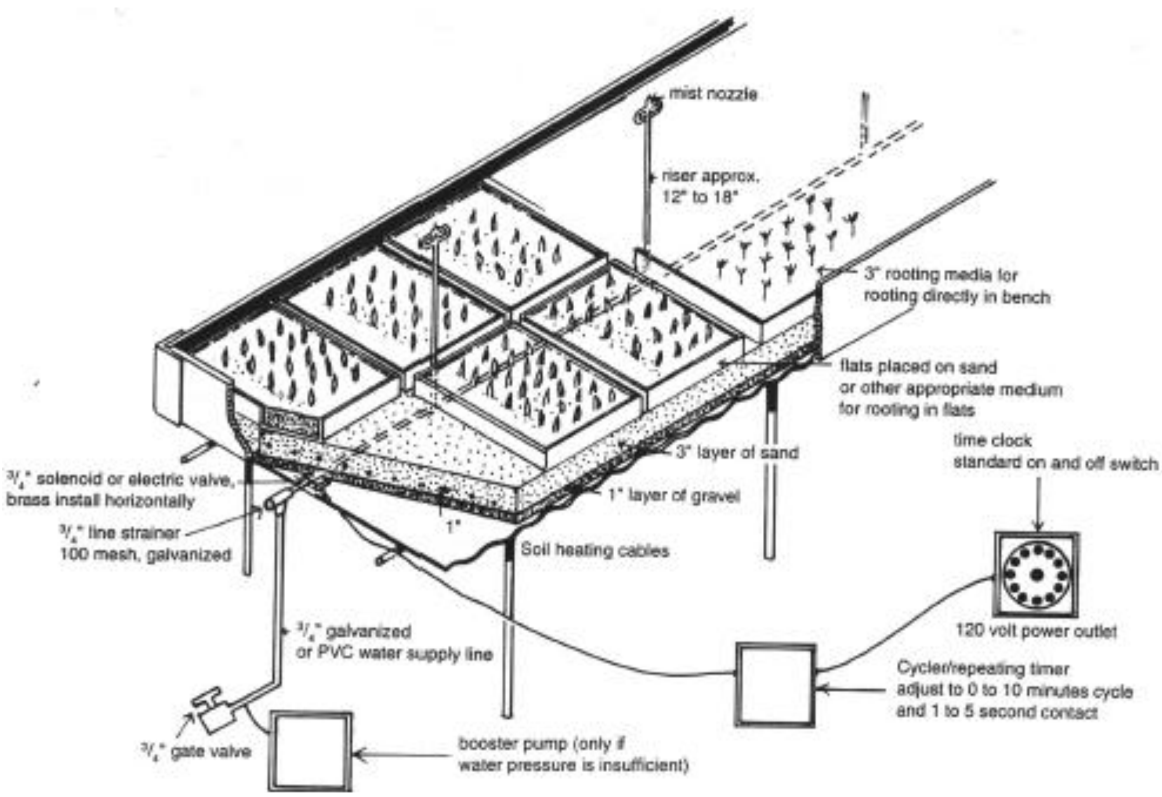

Fig. 3: Typical mist bench (Schnelle *et al.*, 2003)

(ii) The non-mist polypropagator (fig. 4) is a simple wooden frame enclosed by clear polyethylene sheeting, filled with a moist rooting medium and contains a reserve of water. The wooden frame needs to be protected with wood preservatives. If possible, polypropagator should be placed under 60 % shade cloth for protection (Jaenicke and Beniest, 2002). The use of partial shading reduces the rate of photosynthesis which indirectly restricts the rate of root development. It is undesirable but partial shading is sometimes appropriate according to the environment (Schnelle *et al.*, 2003). Temperature usually varies between 28 and 30°C and level of humidity within the propagator is about 90 % after watering, but it can drops to 40 % after opening of the propagator. Temperature and humidity are the main monitored factors during the process of propagation. Non-mist polypropagator is maintained by regular checking of water level (every week), patching up any holes in the polyethylene sheet (Jaenicke and Beniest, 2002).

Comparative studies between mist and non-mist propagators found that non-mist system provides as good an environment for rooting as mist and found lower air and leaf temperatures and vapour pressure deficit under non-mist propagator, which is beneficial to minimise water stress (Leakey, 2004).

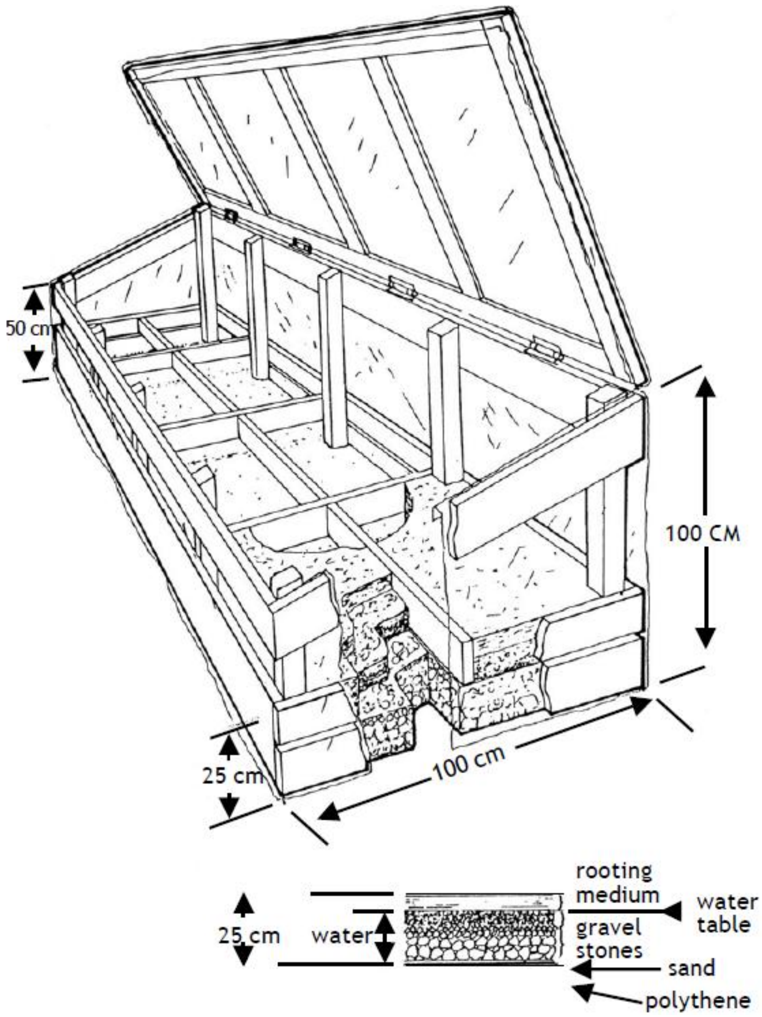

Fig. 4: Schematic representation of a non-mist polypropagator (Longman, 1993)

2.3.4 Adventitious root formation in cuttings

Many plants have two root systems, the primary root system with origin in the radicle and adventitious root system which rises on plant parts not originating from embryo. It means that roots arise on parts of shoot. Adventitious roots usually initiate endogenously from parent plant tissue, but few cases of exogenous origin are known. They help adapt to plant characteristic environment. In some species, they developed a structure and function different from underground roots. Adventitious roots may function as additional support for shoot and permit further shoot enlargement by forming pillars or buttresses. This property is important for tropical woody plants (Barlow, 1986).

Formation of adventitious root system can depend on the environment. For example, adventitious roots are unknown on *Eucalyptus robusta* growing in Australia, but they grow freely in the moister climate in Hawaii (Barlow, 1986). Adventitious roots can develop spontaneously or plant can possess preformed primordia. In that case, roots develop as a response to damage when part of the plant has been severed from the existing root system. Therefore, cutting removed from plant under appropriate conditions may produce a new root system and entire individual (Lovell and White, 1986). But a cutting is in a thermodynamically unfavourable state since the preparation. Only a new root system can restore plant thermodynamics and sustain life. Altered metabolism in a cutting regenerates the root system (Haissig, 1986). Roots of some species are formed rapidly and vigorously under appropriate conditions, but much less readily in others. Their formation varies between species, even between cultivars, with age and nature of the plant part. Some species root quickly and easily when cuttings are taken from seedlings, but they root with difficulty when cuttings are taken from older plants, mainly in case of woody plants. So, some species cannot be propagated by this method. Woody plants often possess preformed primordia which are initiated when the plant is intact. At that time, rate of its development is slow. However, if the plant part is isolated from the root system, development of preformed primordia is accelerated. That is why later primordial stages require wounding. But only few cells of the rooting zone of a cutting initiate primordia. Some of plants also require growth regulators or other chemicals (Lovell and White, 1986).

Induced primordia

Induced primordia form as a result of some manipulation or treatment. The most common treatment is taking of a cutting. Primordia may arise at nodes or internodes. Relative frequency of primordia in these locations is probably influenced by the length of internode present below the lowest node on the cutting. Although, root primordia are occasionally induced very close to the basal end of cutting, roots tend to form a few millimetres above the base of a cutting. The distance of the node from the cut base is an important factor determining the frequency of rooting at the node. But information about the position of cut is rarely given, so it is difficult to evaluate the relative importance of the two locations for most species. Despite the large number of species, there are few locations at which primordia arise. Usual sites are rays (close to the phloem and cambium), bud or leaf gaps, pericycle, callus produced at the base of the cutting or internal callus. There are some unusual locations for primordia rise, as few cell layers below the surface or lenticels (Lovell and White, 1986). Adventitious root formation is complex and number of factors influences the growth, but generally adventitious roots share developmental origin and growth progression with lateral roots (Sukumar, 2010).

Regulation of root formation

The influence of plant hormones on adventitious root formation has been examined in numerous studies with a range of species, doses, growth, and treatment conditions. But the effect of different hormones is in many cases contradictory. Compounds, for which the data are the most clear are auxins and ethylene. In most species, ethylene has a positive effect, but in few cases, ethylene inhibited growth of adventitious roots or had no effect (Sukumar, 2010). Auxin is the most effective treatment to enhance successful propagation. It promotes cell differentiation, starch hydrolysis and mobilization of sugars and nutrient to the cutting base (Leakey, 2004). Most commonly, the auxin IBA (indole-3-butyric acid) is used. It has higher stability and success than IAA (indole-3-acetic acid), although IAA is more abundant in plants (Sukumar, 2010). It is produced in the leaf primordia, young leaves and developing seeds. IAA moves basipetally. (Jaenicke and Beniest, 2002) Capability to induce roots was also discovered in case of NAA (α-naphthaleneacetic acid). Many chemical analogues have been synthesized for auxin-like activity, but none of them is used on a large scale for rooting (De Klerk *et al*., 1999). Tree species and clones can appear to react differently to applications of auxin at differing concentration, even when many factors are constant (Leakey, 2004). There are observed differences in affectivity between various auxins, that may lie in the nature of the compounds, like the affinity to the auxin receptor involved in rooting, the concentration of free auxin that reaches the targeted cell or the amount of auxin synthesized by the plant itself. Auxin enters cuttings by the cut surface, even if the epidermis functions poorly. The growth regulator is rapidly taken up in cells by pH trapping and by influx carriers. But there exist two major pathways of conversion: oxidation and conjugation. IAA and IBA (to a lesser extend) may be inactivated irreversibly by oxidation. NAA is not oxidized. All three mentioned auxins are conjugated which is reversible inactivation. Because of oxidation and conjugation, only 1 % and less of auxin taken up by the tissue occurs in the free form. At concentrations that are favourable for rooting, auxin blocks the growth of roots. Induction of root primordia is also inhibited by auxin at concentration optimal for rooting. It shows that various phases of the process acquire different concentration or opposite hormone (De Klerk *et al*., 1999). In general, hardwood cuttings require the highest concentration of growth regulator (0.3 - 1.0 %), semi-hardwood an intermediate concentration (0.3 - 0.5 %) and softwood cuttings the lowest (0.1 - 0.3 %). Some cuttings do not need the treatment at all. (Scianna *et al*., 1998)

Rooting ability is not affected only by growth regulators, but by other factors, too (fig. 5). The theory of cellular totipotency suggests, that all higher plant cells have the genetic ability to form root, but rooting capacity is frequently unexpressed. This indicates the lack or ineffectivity of one or more factors needed for adventitious rooting. It was suggested, that genetic mutations has affected rooting ability (Haissig, 1986). But more formerly unrootable species and clones are now rooting relatively easily thanks to better understanding. Genetic differences influence the variance relatively little. Analyzed data commonly finds that the factors explaining much of the variance are length of the cutting, leaf abscission and leaf area (Leakey, 2004). Water stress reacting on the stock plant may influence the subsequent rooting of cuttings, too. For example, pea cuttings from non-stressed, high-irradiance stock plants rooted poorly, compared to non-stressed, low-irradiance stock plants. Brief water stress of stock plants increased rooting from the high-irradiance treatment but not from low irradiance treatment. But there exists evidence of opposite effect on other species. Tests indicated that leaf temperatures may differ by several degrees between irradiance treatments, which would result in different levels of water stress. Increased irradiance might increase or decrease leaf temperature, depending upon the amount of evaporative cooling that occurs. Stock plant irradiance and tissue temperature also influence auxin transport, therefore, it influences rooting of cuttings. Water stress influence rooting, but the type of response depends on general environmental factors to which stock plants and cuttings are exposed. And level of irradiance is a key factor in determining the amount of water stress in a cutting. In addition, CO_2 level, to which cuttings are exposed, may influence water relations through effects on stomata and stomata closure reduce rates of net photosynthesis in cuttings of various species (Haissig, 1986). Leafy cuttings need enough light to photosynthesise. However, the highest values of photosynthesis in severed cuttings have been found at relatively low levels of irradiance (Leakey, 2004). Inorganic ion uptake effects water relation in cuttings through osmoregulation (Haissig, 1986).

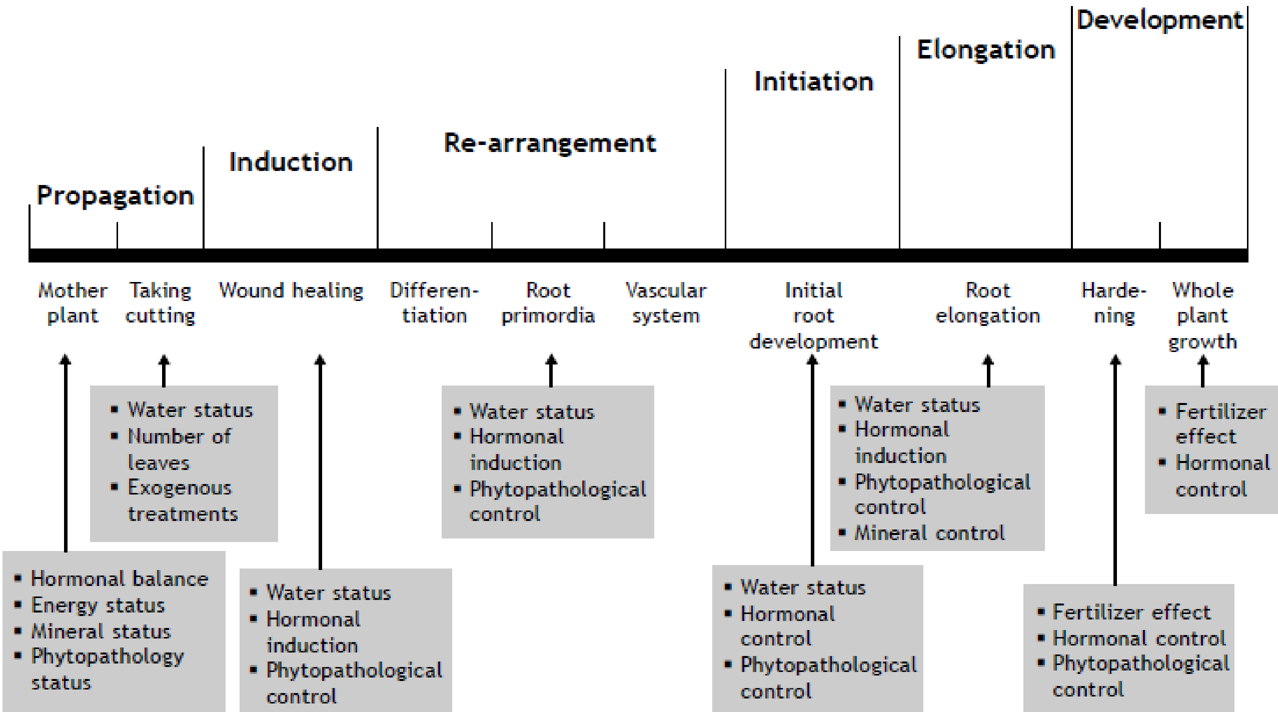

Fig. 5: Different stages in the rooting process and the some factors influencing them (Jaenicke and Beniest, 2002)

It is important that motherplants and cuttings are in optimal condition in the point of view of nutrient and energy status is concerned. The metabolic activity in cuttings takes place in the remaining leaves and initiation of roots relies on photosynthetic activity of the leaf area (Jaenicke and Beniest, 2002). Assimilates have to be produced faster than they are lose by cuttings through respiration (Leakey, 2004). Therefore, it is important to maintain a sufficiently large leaf area on a softwood and semi-softwood cuttings. The leaves will continue in the production of necessary metabolites, but they will lose water through transpiration of this leaf area. So, the leaves are trimmed to the optimum balanced leaf area. It vary between species but it is usually maintained at 50 cm^2 on a cutting (Jaenicke and Beniest, 2002). The optimal size of leaf area depends on leaf thickness, stomatal density, leaf morphology and the age of the leaf (Leakey, 2004). Another important factors affecting rooting ability are cutting length and diameter (in reality probably volume). Cuttings can either be cut to a constant length, so they vary in the number of nodes, or can be cut to a predetermined number of nodes. The number of rooted cuttings is in maximum by using constant length, close to optimum. There was found negative relation between leaf area and cutting length. It suggest, that short cutting cannot provide the storage capacity for assimilates coming from large leaf (Leakey, 2004).

To develop a practical rooting protocol for leafy stem cuttings of an unstudied species, firstly, it is desirable to experiment with optimal auxin application, optimal leaf area and optimal cutting length and experimentally investigate their interaction within propagation environment, but we need to understand that no two cuttings have the same rooting capacity (Leakey, 2004).

2.4. Characteristics of Dipteryx species

Dipteryx spp. trees are canopy-emergents widespread in neotropics, which reach 35-60 m in height and up to 150 cm in dbh (diameter at breast height). They belong to family Fabaceae. Several of these species are collectively known in Peru as shihuahuaco and internationally traded as cumarú or Brazilian teak. They are supported by broad buttresses. At least in natural forest conditions, they are extremely slow growing. Maximum adult age of *Dipteryx odorata* has been estimated to 1000-1200 years and adult age of *Dipteryx panamensis* has been estimated to 330 years. Large seeds (3-4 cm) mature in the dry season, providing an important source of food for animal, like bats, agoutis, peccaries, deers and tapirs. Frugivorous bats feed on fleshy mesocarp and disperse its seeds on location away from parent tree. *Dipteryx* trees provide important nesting sites for parrots and macaws. Bird communities may use individual trees for decades or centuries. The seedlings are shade-tolerant and regenerate in both late-successional and mature forest and occupy variety of habitats from floodplain to upland forests. While seedlings and saplings are shade tolerant, they require light to grow into the large size-classes. Medium sized trees (juveniles) have been found to occur in very low densities in natural unlogged conditions compared to other size classes (Putzel *et al.*, 2011).

Shihuahuaco have been targeted for past several years by extraction, logging. After fall, they leave large gaps, facilitating successional forest growth. *Dipteryx* timber is valued for its high wood density and resistance to rot. It is ideal for outdoor application. In North America and Europe, it is used for decking and patio furniture. The majority of shihuahuaco is shipped to China due to the growing dominance of Chinese timber companies and exporters in Peru and plays major role in flooring market. From the point of uses, *Dipteryx* timber could be considered as alternative to teak, but its supply is not supported by extensive plantation production (Putzel *et al*., 2011).

According to Putzel *et al.* (2011) there are two species prevalent around Pucallpa, Ucayali: *D. micrantha* and *D. alata.* A third species, *D odorata* which occurs over a large area of Amazonia and has been identified in northern and southern Peruvian Amazon, does not appear to be present in this study region, despite the common use of the name in local commercial forest inventories and export documents. This confusion is also mention by Arajuo (2012) working in Campo Verde, Ucayali and she confirmed, that *D. micrantha* and *D. alata* are the most abundant *Dipteryx* species in Ucayali region. *D. alata* and *D. odorata* are very taxonomically close and difficult to distinguish. Systematic work on *Dipteryx* is needed, the genus has not been revised since 1940, Ducke's revision (Putzel *et al*., 2011).

Seedlings that I worked with were identified by IIAP (Instituto de Investigaciones de la Amazonía Peruana) as *Dipterix alata* Vogel. It is a native species of Peru (Putzel *et al*., 2011) and Brazilian Savanna (Czeder *et al*., 2012) known as shihuahuaco amarillo in Peru (Putzel *et al*., 2011) or as baru, combaru or cumaru in Brazil. The plant has economic potential thanks to its multiple uses. Fruit, rich in proteins, fibres and unsaturated fatty acids (Puebla *et al*., 2010), especially oleic acid (Czeder *et al*., 2012), is used for human nutrition, animal feed and in cosmetics. The oil extracted from the almond is traditionally used by local inhabitants to treat fever and snakebites (Puebla *et al*., 2010). The roasted baru almond is also consumed by the Brazilian Savanna population and used in regional gastronomy, too. Its taste resembles to the taste of peanuts, but the texture is softer. High physical diversity of fruits and almonds was reported from trees from the same region. That could indicate a high potential for plant breeding (Czeder *et al*., 2012). Shihuahuaco wood is used for building as other species from genus *Dipteryx*. It is widely distributed in the dry forests. Its flowers are hermaphrodite and pollination is performed by insects (Soarez *et al*., 2008). The fruit is a drupe and 35 pieces or 215 seeds weight around 1 kg

(Arajuo, 2012). Shihuahuaco seeds have germination rate 96 % under controlled conditions and germinate after 6 days (Saboya and Borghetti, 2012), but they are recalcitrant. It is semi-deciduous tree with odd-pinnate compound leaves. The wood density is 0.9-1.2 g/cm^3. It is resistant to xylophages, but resists penetration of wood preservatives (Araujo, 2012). The species suffered from habitat conversion for agriculture. It is also exploited for its excellent quality of timber and medicinal seeds. All these reasons have led to massive declines in population numbers (World Conservation Monitoring Centre, 1998). There was not published any scientific article about vegetative propagation of this species, but macropropagation by cuttings was described in diploma thesis by Rollo in 2009.

2.5 Characteristics of Guazuma species

Guazuma crinita Mart. is considered by farmers in The Peruvian Amazonia as a priority species for tree improvement programs. It is possible to inter-cultivate it with food crops, thanks to its small crown with thin branches and natural self-pruning of the older branches in the lower crown (Weber and Montes, 2008). This species belonging to the Sterculiaceae family provides wood products at an early age, can be coppiced for successive harvests and significantly contributes to farmer's income. It is a fast-growing pioneer species growing on floodplain and disturbed secondary forests in the Peruvian, Brazilian and Ecuadorian Amazon (Rochon *et al.*, 2007). *G. crinita*, is known in Peru as bolaina blanca (Wightman *et al.*, 2006). Until recently, *G. crinita* was mostly traded locally in the Ucayali river area. In low-income neighbourhoods surrounding cities, more than half of houses had walls made of narrow bolaina boards. After the earthquake in 2007, the boards served for emergency prefabricated houses. Since then, the market for bolaina gradually expanded across Andes and according to official production figures, bolaina has become the one of the top 20 species harvested nationally. This species has great potential for sustainable production by smallholder farmers. Therefore, the stock of *G. crinita* in local forests has increased. Smallholders are preserving stands for occurrence of natural regeneration. *G. crinita* has also been used in government and NGO reforestation (Putzel *et al* 2013). It has a fast initial growth rate of up to 3 m in height (Maruyama *et al.*, 1996) and average diameter increase of 4.8 cm per year (Putzel *et al.*, 2013). Naturally, this medium-sized species (Maruyama *et al.*, 1996) occurs up to 1500 m asl and is cultivated up to 900 m asl (Wightman *et al.*, 2006). *G. crinita* grows better on light-textured soils, with relatively high organic matter and available phosphorous, potassium, calcium and magnesium. The poorest growth occurs on very sandy and acidic soils (pH less than 4.5) with low organic matter and available cation content (Weber and Montes, 2008). It is sensitive on aluminium content (Wightman *et al.*, 2006). Bolaina blanca is traditionally propagated by seeds, but obtaining and storage of selected seeds is difficult and do not always permit an adequate production of desirable selected seedlings (Maruyama *et al.*, 1996). Trees can begin flowering after 2-3 years in open-grown conditions (Rochon *et al.*, 2007). Flowering is yearly initiated during rainy moths (Wightman *et al.*, 2006). Fruit is a lightweight feathered capsule dispersed by wind and water. Reproductive characteristics of *G. crinita* probably result in high levels of genetic variation within populations and relatively low genetic differentiation among populations (Rochon *et al.*, 2007). In 1 kg of seeds, there are about 850, 000 pieces. Seeds germinate normally in germination beds using sifted soil and sand without humus after 5-8 days. As I already mentioned, bolaina prunes it-self. It happens when the branches are thick. In market, it is possible to get higher price for bolaina wood with fewer nodes. Therefore, to avoid the formation of large nodes, it is best to prune after 3-4 years. Bolaina regrows vigorously, so it is feasible to produce two or three cuts without the need to replant (Wightman *et al.*, 2006).

Bolaina's timber can have different tonality, dependent on the age of tree and its exposition on light. Its colour goes from white to pale brown. It is not resistant to outdoor conditions (Wightman *et al.*, 2006). The wood is diffuse-porous and of moderate density (400-550 kg/m^3). Wood density is considered the most important factor influencing the wood's physical and mechanical properties. These properties determine if the wood is appropriate for different products. In general, wood with higher density tends to be stronger and stiffer, but it has greater shrinkage compared with lower-density wood. This knowledge allows tree improvement programs to assess the potential usefulness of the species for different wood products. Timber of *G. crinita* is used primarily for wall panels and construction poles, secondarily for matches, cases and for moulding (Maruyama *et al.*, 1996). Construction poles must be strong and stiff and wall panels should not shrink. Wood density is under stronger genetic control than tree growth traits. A negative correlation was observed between tree growth and wood density. There was observed lower wood density on larger trees and in more humid part of the same region and greater density in the lower part of stem. But the strength of these relations depends on the provenance and zone. The recommended rotation age of *G. crinita* is 6-10 years, but younger trees are often harvested to supply the demand for wood (Weber and Montes, 2008).

G. crinita was successfully micropropagated by the subculturing of the shoots from aseptically germinated seedlings. Seeds were germinated on woody plant medium (WPM) supplemented with trans-zeatin and shoots were elongated and rooted on WPM with 1μM of kinetin (Maruyama *et al*., 1996). This species was also propagated by cuttings by Rollo in 2009.

3. AIM OF THE THESIS

The aim of the thesis was to examine vegetative propagation of Amazonian trees, especially species. The thesis was focused on:

- Investigation of rooting ability and influence of exogenous hormones on cuttings of *Dipterix alata* (shihuahuaco) and *Guazuma crinita* (bolaina blanca) in non-mist polypropagator.
- Getting higher rooting rate, than was achieved until now.
- Production of vegetative propagation protocol applicable by farmers in multiplication, domestication and breeding practice. The produced trees can serve as material for reforestation purposes, too.

Our experiment also served to compare two different non-mist polypropagators: one with frame made of wood and other with frame made of PVC. The use of PVC can ensure lower cost of acquisition of parts needed to build the frame and its greater durability in outdoor environment.

4. Material and methods

4.1 Study site description

The experiment was conducted from July to August 2013 in a tree nursery of IIAP (Instituto de Investigationes de la Amazonia Peruana) in region Ucayali, Peru. It is located at km 12.4 carretera Frederico Basadre, close to the city of Pucallpa. The geographical position of the nursery is latitude 8° 23’ 56.1” S and longitude 74° 38’ 24.4” W and altitude 152 m asl.

4.2 Plant material and cuttings preparation

The cuttings of *Dipteryx alata* were taken from saplings from IIAP tree nursery, planted June 4th 2009. The source of plant material for saplings was INIA (Instituto Nacional de Innovacion Agraria). So at time of our experiment, the plants were 4 years old (fig. 6). Approximately 3 month before our harvest, the saplings were trimmed, so at the day of collection, we acquired 150 two-node semi-hardwood basal cuttings. The length of shihuahuaco cuttings varied between 4.5-7.8 cm and their diameter was between 2.74-8.34 mm.

Fig. 6: *Dipteryx alata* saplings before harvest of cuttings

The material for cuttings of *Guazuma crinita* (fig. 7) was taken from the same nursery. The saplings of bolaina blanca were planted in February 3rd 2011. It means that in time of the experiment, the plants were 2 years and about 5 months old. We prepared 105 apical and 140 basal one-node softwood cuttings. The saplings were trimmed one month before the experiment to acquire softwood cuttings. The length of bolaina cuttings varied between 5-8 cm and their diameter was 0.69-9.68 mm.

Fig. 7: *Guazuma crinita* saplings before harvest of cuttings

All cuttings were taken early in the morning to keep them in relatively humid and cool conditions to avoid wither. All cuttings were treated by fungicide Cupravid for 15 minutes.

4.3 Experimental design

The *Dipteryx alata* experiment had one major factor: concentration of IBA treatment. We used five levels of treatment (0, 1000, 2000, 3000 and 4000 ppm). For each group were propagated by 30 cuttings. The *Guazuma crinita* experiment had two major factors: concentration of hormonal treatment (IBA or IAA) and cutting position (apical or basal). That produced 7 different groups. The apicals were treated with IBA or IAA (1000, 2000 and 3000 ppm). The basals were supplemented with IBA or IAA (1000, 2000, and 3000 ppm). And control group of apicals and basals. Each group of apicals was propagated by 15 cuttings and each group of basals was propagated by 20 cuttings. Hormonal treatment was done by submersion of all the cuttings bottom part into an alcoholic solution for three seconds. The treated cuttings were briefly air-dried for 2 minutes before insertion to fine sand used as rooting media.

4.4 Polypropagators

The non-mist polypropagators used for the experiment were the IIAP's property. The one in which shihuahuaco was, had frame made of PVC tubes and the other in which bolaina blanca was, had frame made of wood as described by Leakey *et al.* (1990). The polypropagators were kept under shading made of double layers of plastic shading net. Whenever the polypropagators were opened for observation, cuttings were water sprayed to increase relative humidity inside. The level of water in spaces between stones and gravels was checked, too and refilled when needed. Temperature and humidity in polypropagators were check with the digital thermometer with indoor/ outdoor hygrometer and written down every hour from 8 o'clock till 17 o'clock except weekends. The pH was measured in both polypropagators with La Motte Precision pH test kit. Both substrates had pH equal to 8.

4.5 Experimental conditions

The experiment of *Dipteryx alata* was conducted in the dry period. It lasted from July 1th till August 13th. During this experiment, 5 days was raining and at 3 days occurred that the outside temperature between 8 and17 o'clock was under 20°C. The temperature inside the polypropagator did not exceeded 31°C and relative humidity was not lower, than 57 %.

The experiment with *Guazuma crinita* was set July 12th and ended August 12th. During this experiment, 2 days was raining and 3 days occurred that the outside temperature between 8 and 17 o'clock was under 20 °C. The relative humidity inside the polypropagator did not decrease below 55 % and temperature did not increase over 32 °C.

4.6 Data collection and evaluation

Data were collected in terms of parameters like diameter and length of a cutting, number of roots, root length, callus formation, number of produced shoots and leaves and mortality. Final evaluation of cuttings was made after 42 days in case of shihuahuaco and in case of bolaina blanca after 30 days. A cutting was considered rooted if it had at least one root longer than 1 mm.

Statistical analysis was done using Microsoft Excel 2010 and software R version 3.0.1. The Chi square test was used to estimate if watched factors are affecting the experiment. The obtained results were evaluated on 95 % level of probability.

5. Results

5.1 Dipteryx alata experiment results

The results are showed in form of graphic (fig. 8). The highest rooting rate was achieved by treatment of 4000 ppm (0.4 %) of IBA. 16.67 % of cuttings formed callus, 60 % of cuttings formed new leaves, 23.33 % of cuttings rooted and 23.33 % died.

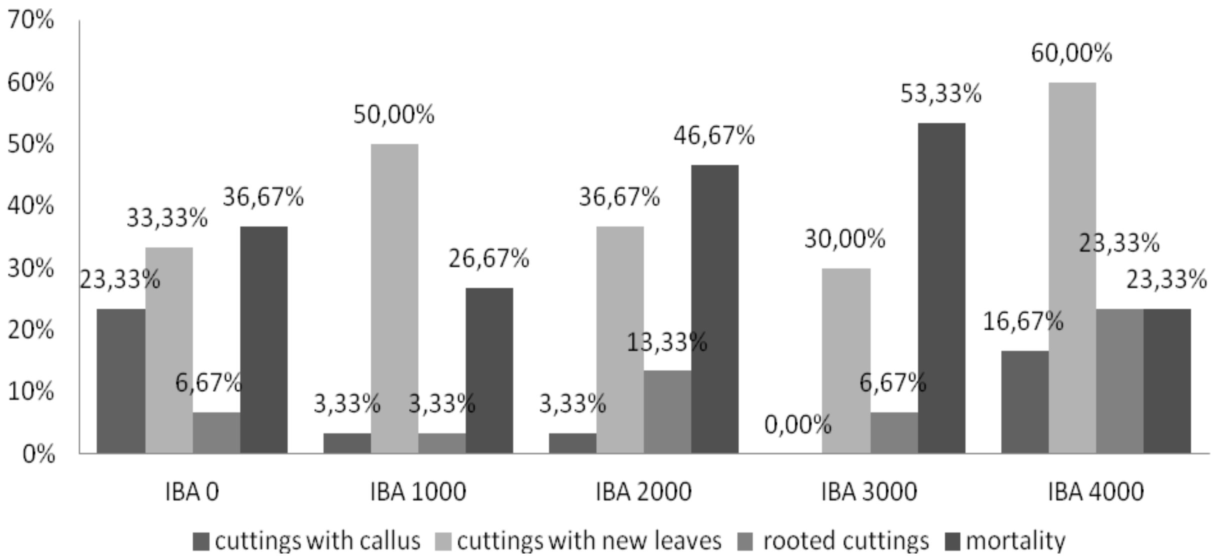

Fig. 8: Shihuahuaco experiment results

The Pearson's Chi square test rejected that there is no significant difference in the distribution of mortality among groups with different treatment. Pearson's Chi square test also rejected that there is no significant difference between groups with different treatment and callusing, production of new leaves and rooting. That means that the mortality of cuttings, callusing, production of new leaves, and rooting of cuttings of *Dipteryx alata* depends on used treatment.

According to ANOVA test, we can say, that the occurrence of callus (fig. 9 and fig. 10) is generally influenced by the diameter of cutting and used treatment. Firstly, we tested it globally. After the use of logistic regression, the model showed that the best chance in callusing has the group with no treatment. But we have to consider, that in case of many cuttings, the root cells were in form of callus before differentiation.

Fig. 9: Callus of *Dipteryx alata*

Fig. 10: Callus of *Guazuma crinita*

When we globally tested the influence of all the variables (hormone concentration, length and diameter) on mortality, the ANOVA test showed that the greater diameter of cutting increases its possibility to survive. The probability to survive is 2.4 times higher with every millimetre.

The Poisson regression showed that diameter strongly effects the formation of new leaves. With every other added millimetre, the probability on formation of new leaf grows up about 35 %. Type of used treatment is a significant factor, too. It cannot be excluded from the model.

We tested the influence of variables on the length of the longest root. The hypothesis was formulated: in case of formation of root, what influence on its length will have hormone concentration. But there was not any significant output.

The results of statistical analyses showed, that the major factors influencing the growth of cuttings (fig. 11) are the used hormone concentration and the diameter. The length of cuttings did not show any significance, which does not support the hypothesis of Leakey and Akinnifesi (2008), that cuttings are influenced by the length.

Fig. 11: Example of rooted cutting of *Dipetryx alata*

5.2 Guazuma crinita experiment

The results of experiment with bolaina blanca are shown in two graphics. Results of apical cuttings with different hormone enhancement are shown in fig. 12 and results of experiment with basal cuttings are in fig. 13. As we can see, the best rooting rate was achieved with the use of apical cuttings treated with 0.1 % of IBA. It is interresting, than any apical cutting did not produce any new leaves and their rooting ability was generally higher than rooting ability of basal cuttings.

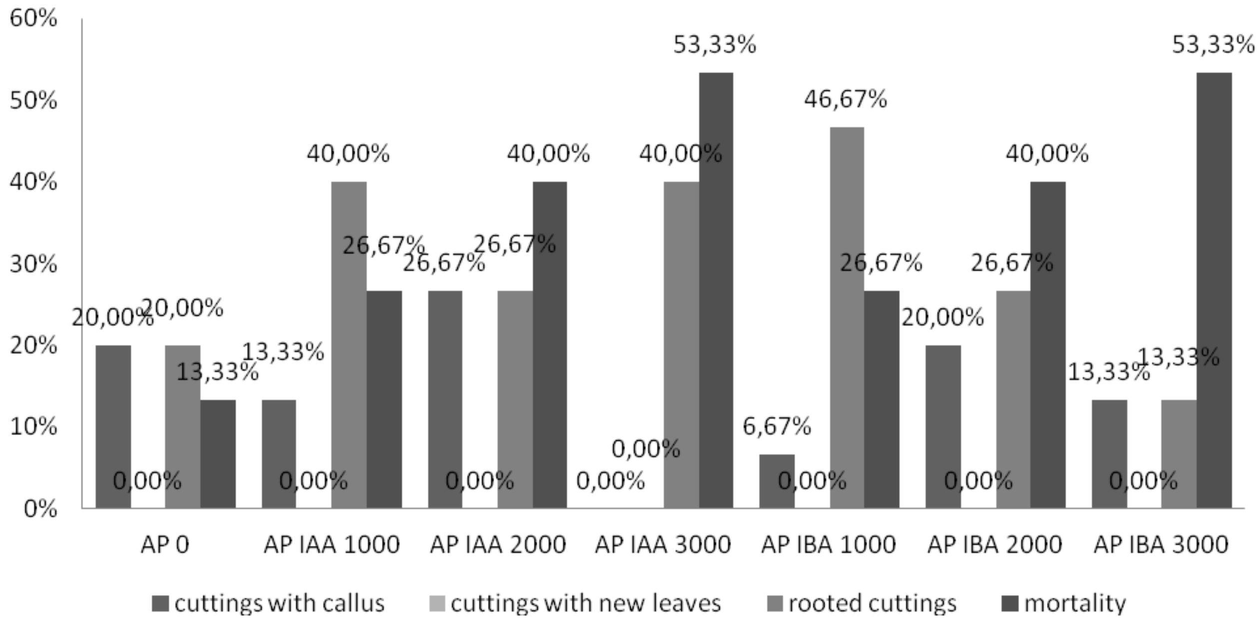

Fig. 12: Bolaina experiment results of apical cuttings

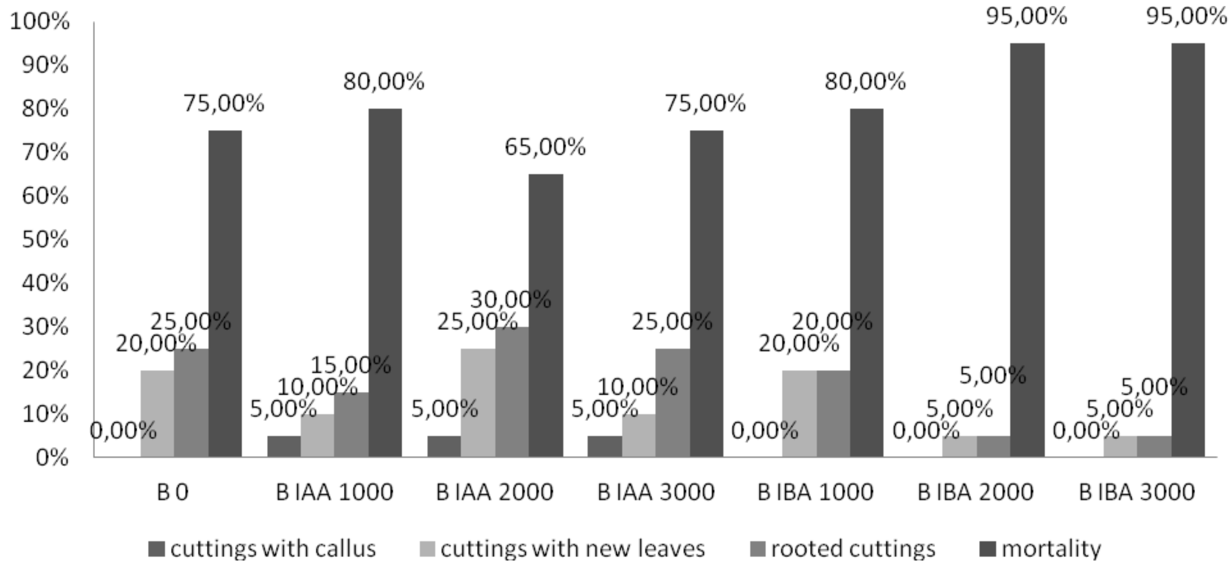

Fig. 13: Bolaina experiment results of basal cuttings

The Pearson's Chi square test denied that the mortality of bolaina cuttings does not depend on type of cutting and the concentration of exogenous hormone. It did not reject that the occurrence of callus, production of new leaves and root formation do not depend on type of cuttings. This means that we can only prove that mortality of cuttings of bolaina blanca depends on used cutting and exogenous hormone.

Then we tested influence of all the variables (cutting type, hormone concentration, length and diameter) on the occurrence of callus with the logistic regression. It showed that the occurrence of callus is significantly influenced by the type of cutting and length or diameter of cuttings does not affect the callus formation. Apical cuttings have higher probability of callus formation than basal cuttings. But there has not been found any significant dependence on the concentration of used hormone.

We also tested a statistical difference among all variables and mortality of cuttings. The variables with major influence on mortality are the type of the cutting and diameter of the cutting. The apical cuttings have higher probability to survive than the basal cuttings and with every added millimetre to the diameter, the cuttings have 1.98 times higher probability to survive, too.

The influence of hormone concentration, length and diameter on the formation of new leaves was tested. The type of cutting surly influence the leaf formation, but it cannot be statistically proved, because none of apical cuttings produced any new leaf. That is why we focused only on the basal cuttings. The Poisson regression showed that significant variable is the cutting diameter but not the length.

ANOVA showed the dependence of the length of the longest root on the type of cutting and on the used hormone concentration. The question was: In case of the root formation, what influence would type of cutting and hormone concentration have on its length. But this test did not show any significant difference.

Generally, the apical cuttings (fig. 14) ended up better than the basal cuttings (fig. 15).

Fig. 14: Example of rooted apical cutting of bolaina blanca

Fig. 15: Example of rooted basal cutting of bolaina blanca

5.3 Polypropagators

If we compare the two used polypropagators, one with frame made from PVC tubes (fig. 16) and the other with frame of wood (fig. 17), the one from PVC brings more benefits. It has greater durability in these tropical conditions. High relative humidity destroys wood, not to mention termites and fungi. The PVC tubes are also cheaper to buy and do not need application of wood preservants. The tubes are also lighter and easy to manipulate with. To sum up, the non-mist polypropagators with the frame made of PVC tubes could become current practise.

Fig. 16: Polypropagator with frame made of PVC

Fig. 17: Polypropagator with frame made of wood

6. Discussion

In 2009, Rollo conducted study on rooting ability of *D. odorata* and *G. crinita* in nursery bed and polypropagator. The seedlings of *D. odorata* were obtained in Macuya experimental forest 86 kilometres from Pucallpa. As I mentioned, Putzel *et al.* (2011) declare, that in that area, *D. odorata* does not occur and it is very similar to *D. alata.* Therefore, I suppose, that he propagated *D. alata.*

6.1 Dipteryx alata

Rollo conducted the experiment with about 8 month old seedling and propagated basal and apical leafy stem cuttings in nursery bed and polypropagator for 59 days. His best rooting rate was 15 % in nursery bed with basal cuttings. In polypropagator, his basal cuttings did not root at all, and only 6.6 % of apicals showed its rooting ability. My best result was that 23.33 % of basal cuttings rooted with the enhancement of 0.4 % of IBA. Without treatment, 6.67 % of basal cuttings rooted in my case. If we consider, that I worked with 4 years old seedlings and as Davies claims (1984), rooting ability decline with age, and duration of my experiment was shorter than Rollo's, 23.33 % of rooted cuttings are an achievement. The mortality of basal stem cuttings in polypropagator without any treatment was in both experiments similar (33 % in Rollo's, 36.67 % in mine). Similar is also percentage of the same cuttings with callus (25 % in Rollo's, 23.33 % in mine). Therefore we can say that results of these two experiments are not contradictory.

6.2 Guazuma crinita

To examine the rooting ability of *G. crinita,* Rollo propagated this species in two different propagation conditions: nursery bed and polypropagator. In each stand, one half of cuttings was treated by coconut water as rooting stimulator. The cuttings were made from about 4 months old seedlings. The best rooting ability achieved was 96.7 % in polypropagator with no stimulation after 27 days. My best result was 46.67 % in case of apical cuttings treated with 0.1 % of IBA after 30 days. I used seedlings 2 years and 5 month old. It is possible, that the rooting ability declines so much with the age of trees, but more probable is, that the environmental conditions during the preparation of cuttings or during the rooting itself somehow influenced the rooting ability of cuttings in my experiment. It is possible, that the cuttings wilted a little before installation, experienced water stress. If that is the case, hormonal treatment can be used to enhance the rooting of cuttings in case of bad conditions. I suggest that, because the time of preparation of all cuttings was the same and they all experienced the same conditions. So if the rooting rate differs between cuttings with different treatment but the same type (apical or basal), the hormonal treatment probably helped the rooting process despite the conditions.

7. Conclusion

Because of the recalcitrant seeds and logging of *Dipteryx alata*, difficulties with seed obtaining and market expansion of *Guazuma crinita*, it is necessary to establish successful and cheap way of vegetative propagation of these species. The important result of this thesis is that both species are able to root. But it is necessary to have the rate of rooting as high as possible and achieve high multiplication rate so farmers would not be limited by plant material. The best result of 23.33 % of rooted cuttings of *Dipteryx alata* was achieved in case of basal cuttings with 0.4 % of IBA. This rooting rate could already be used for tree production and for breeding, too. But the experiment with *Guazuma crinita* was not as successful as it should be. The output of bolaina experiment is that we can fight the unfavourable conditions decreasing the rooting rate of cuttings with hormones, concretely with 0.1 % IBA in case of apical cuttings and 0.2 % IAA in case of basal cuttings. The cuttings of both species are mainly influenced by their diameter. With increasing diameter increases the probability of survival and formation of new leaves.

The knowledge about propagation of these two important species is enough for their multiplication for timber production by local farmers, but to fully reach their potential, more experiments should be conducted not only on the possibilities of propagation. More information is needed about the plants characteristics, distribution and genetics. At least, both species need revision.

8. References

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