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Mémoire de fin d'études d'ingénieur

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Effects of contamination by petroleum hydrocarbons, planting density and clone type on the establishment of European aspens (*Populus tremula* L.) and hybrid aspens (*Populus tremula* L. X P*opulus tremuloides* Michx.)

Camille D'HERVILLY
Agriculture, Environnement, Territoire

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La phytoremédiation par le tremble :

Effets de la pollution par des hydrocarbures pétroliers, de la densité de plantation et du type de clone sur l'établissement du Peuplier tremble (*Populus tremula* L.) et du tremble hybride (*Populus tremula* X *Populus tremuloides* Michx.)

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2015

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Thanks

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Finally, I thank my parents for their support through this internship, even in the distance.

Abstract

An experiment starting in 2013 on the effect of pollution conditions and planting density on establishment characteristic of two clones of European aspens (*Populus tremula* L.) and four clones of hybrid aspen (*Populus tremula* L. X *Populus tremuloides* Michx.) has been carried out in Finland. Soils without contamination, with old creosote contamination and with new diesel contamination were used. The planting densities were of one, two or six trees per buckets. The height of the trees and the survival were followed during 3 years, and chlorophyll fluorescence as additional measure the last year. Statistical univariate analyses were realized. The relative growth rate was used. Results show that the effect of soil contamination is important for survival rate the first year, as old contamination decreases the survival and old contamination improves it. Growth decreases with contamination, and the effect decreases with the time. Transplant choc is thus important to consider. The density has no effect the first year, but its effects increase with the time. The best clones for growth in general and polluted conditions are 191 and R4, however 14 and 27 seem to increase their development in the last year. Clones 27 and 191 have a less important survival than others, and clone 291 is particularly good. Efficiency of photosystem II and performance index are not linked with density or polluted conditions, and are better for the best growing trees. A longer time and some harvest are necessary to conclude, as better knowledge on the evolution of contamination.

Key words: aspen, hybrid aspen, selection, phytoremediation, acclimation, petroleum hydrocarbons, planting density

Résumé

Une expérience commencée en 2013 sur l'effet de conditions de pollution et de densité de plantation sur l'établissement de deux clones de Peuplier tremble (*Populus tremula* L.) et quatre clones d'hybrides (*Populus tremula* L. X *Populus tremuloides* Michx.) a été réalisée en Finlande. Des sols sans pollution, avec ancienne contamination à la créosote et avec contamination récente au diesel ont été utilisés. Les densités de plantation étaient de un, deux et trois arbres par sceau. La taille et la survie ont été suivies durant trois ans, et la fluorescence chlorophyllienne la dernière année. Des analyses statistiques univariées ont été réalisées. La croissance relative a été utilisée. Les résultats montrent que l'effet de la contamination du sol est important pour le taux de survie la première année, une contamination récente le faisant diminuer et une ancienne le faisant augmenter. La croissance diminue avec la contamination, et cet effet diminue dans le temps. Le choc de plantation est donc important. La densité de plantation n'a pas d'effet la première année, mais cela augmente avec le temps. Les meilleurs clones sont 291 et R4, cependant 14 et 27 améliorent leur développement la dernière année. Les clones 27 et 191 ont une survie moins importante que les autres, 291 est plus résistant. L'efficience du photosystème II et l'indice de performance ne sont pas liés à la densité ou à la pollution, et meilleurs pour une croissance plus importante. Une plus longue durée et une récolte sont nécessaires, ainsi qu'une meilleure connaissance de l'évolution de la contamination.

Mots clés : tremble, tremble hybride, sélection, phytoremédiation, acclimatation, hydrocarbures pétrolifères, densité de plantation

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List of acronyms and abbreviations

BTEX: Benzene, Toluene, Ethylbenzene and Xylenes

Chisq: chi-square

Cor: correlation

DBH: Diameter at Breast Height

Df: degrees of freedom

DNAPL: Dense Non-Aqueous Phase Liquid

EU: European Union

HAP : Hydrocarbures Aromatiques Policycliques

Luke: Luonnonvarakeskus, Natural Resources Institute Finland

METLA: Metsäntutkimuslaitos, Finnish Forest Research Institute

NSO: Nitrogen, Sulfur and Oxygen

PAH: Petroleum Aromatic Hydrocarbons

PI: Performance Index

POP: Persistent Organic Pollutants

PS II: photosystem II

RGR: Relative Growth Rate

TPH: Total Petroleum Hydrocarbons

Introduction

The aspen is largely distributed in the North Hemisphere. *Populus tremula* L. is the traditional specie of the European and Asian continent, while the other specie *Populus tremuloides* Michx. is developed in the American continent. They are deciduous trees, and represent only 1% of the forests of Finland as dominant stand (Hannelius and Kuusela, 1995). They have been largely unconsidered all other Europe and particularly in Nordic countries where long rotations until 120 years are common using climax conifer trees and where wood resources are sufficient. The necessity to increase the use of renewable energy has led to a fresh interest in fast growing species as poplars, aspens, alders and willows, what could also allow responding the paper and pulp industry demand (Tullus et al., 2011). In Finland, a recent interest appeared for the aspens as they present the required qualities and as it is possible to select interesting characters due to the high number of clones. Furthermore, crosses between the two species have allowed the creation of different hybrid clones.

Soil contamination has only been studied since the 1980s (Pyy et al., 2013). The emergence of new ecological consideration on soil contamination at the European level has made reconsider not only the pollution problems but also the means used in remediation. The important contamination linked with industrial decades of activity has led to the critics of traditional dig and dump and dig and incinerate techniques, as the number of cases to be remediated is nowadays very important. New methods have been developed. Thermal, chemical and mechanical techniques are useful to quickly remove the risk in case of an important contamination or a danger to populations, but they are expensive and can sometimes include a new risk for the environment as a general non acceptation by the population. Biological techniques have appeared, among them natural attenuation, bioremediation, composting and phytoremediation. This last one consists in the use of plants to extract, degrade or render harmless contaminants. Due to their characteristics, poplars and in particular aspens seems particularly adapted for this aim.

The tree breeding unit of Haapastensyrjä of the Natural Resources Institute Finland (Luke) deals with traditional tree breeding for improvement of Finland forests, and with the effects of climate change on forests as Nordic countries will be more touched by the future modifications of the weather. It was thus a logical place to study the effect of other factors as soil contamination. In 2013, the establishment of the first large scale phytoremediation case in Finland was a starting point for studies on phytoremediation of organic contaminants.

This traineeship consisted in working in different projects specific to this unit, particularly the phytoremediation one. This report will focus on one experiment on phytoremediation started in 2013 and in which analyses of results were needed. It deals with the study of parameters linked with plantation establishment according to different soil pollutions, planting densities and clones of *Populus tremula* L. and the hybrid *Populus tremula* L. X *Populus tremuloides* Michx. To develop this case study, the context of soil contamination, phytoremediation and aspens use will be detailed, and the precise context of the experiment defined. Then, the material and methods will be presented, and the results obtained described. Finally, a discussion on these results and some critics will allow proposing future steps on the subject.

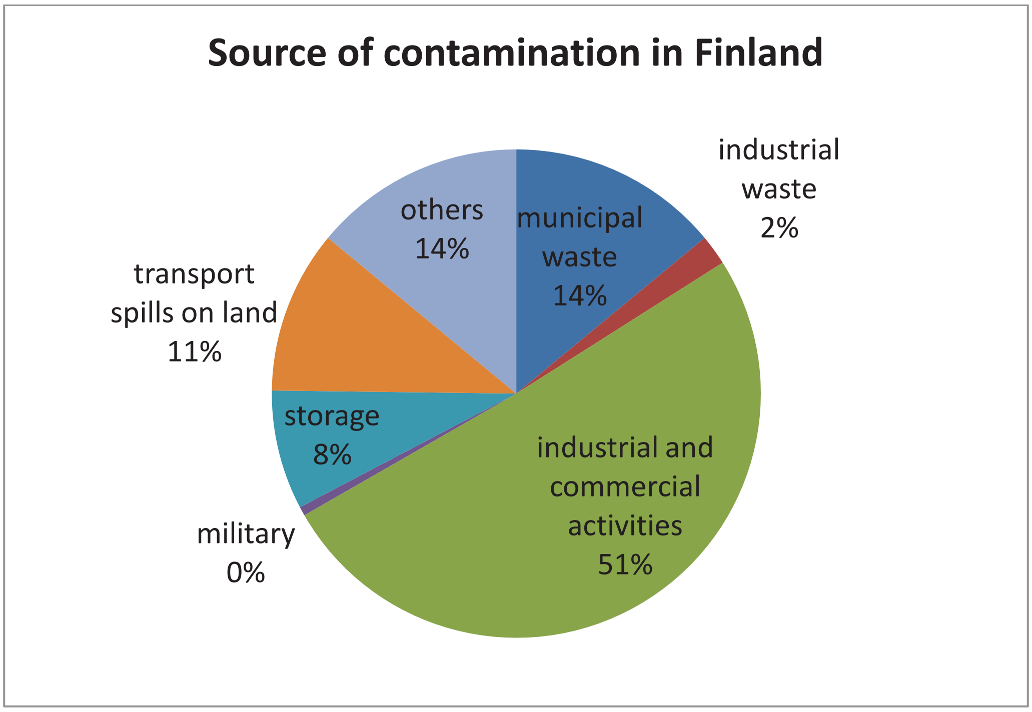


Figure 1: Representation of the percentages of the main local sources of contamination in Finland / Représentation du pourcentage associé aux principales sources de contamination en Finlande, source: EEA, 2011, personal realization / réalisation personelle

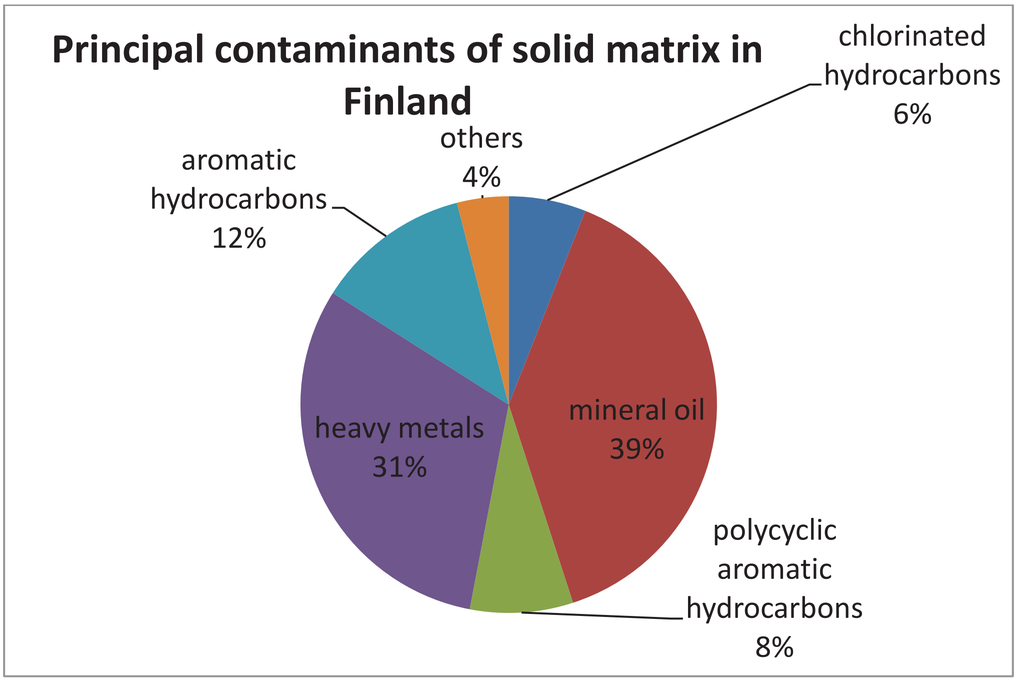


Figure 2: Representation of the percentages of main contaminants of soil and sediments in Finland / Représentation du pourcentage des majeures contaminants du sol et des sédiments en Finlande, source: EEA , 2011, personal realization / réalisation personelle

I. General context: literature review on the subject

1. The emergence of soil contamination consideration

1.1. In Finland

1. 1. 1 Situation

In 2013, 23,850 cases of contaminated areas were suspected or confirmed in Finland, with some of them already remediated. Recent contamination is due to oil and chemical accidents during transport and activity and waste disposal (*figure 1*). In all the industrial and commercial activities, the ones that cause most contamination are petrol station (34%), car service stations (18%) and wood and paper industries (13%) (Van Liedekerke et al., 2014). Most of the time, it concerns small areas, concentrated in southern Finland and the coast where there is an abundance of industrial and business operations, and a densest population. One fifth of these contaminated areas are classified as groundwater areas, one tenth are conservation areas and 20% are near residential areas. Most of the concerned soils are contaminated with organic compounds (70 %), 10 to 20 % with metals and the rest with a mixture of constituents (Pyy et al., 2013). In term of quantities, 65% of contaminants are organic and 31% are metals (Van Liedekerke et al., 2014) (*figure 2*).

Almost 3,000 contaminated sites have been remediated during the last 15 years and €200 million were necessary for this purpose. 300 to 400 remedial decisions are taken each year (Sorvari et al., 2009), thus it is an important subject recently. Primary reasons for undertaking remediation are changes in land use, excavation and construction work, and not environmental considerations. In addition, the remediation objectives are set on the basis of the future use of the area in 90% of cases. Usually, removing of soils and depositing off site is used, whereas in situ treatments (i.e. treatments for which the soil is not moved) were used for only 10-15 sites and consisted of containment, soil vapor extraction, biological methods and chemical oxidation. Close to 1.5 million tons of soil have been excavated (Pyy et al., 2013). Costs often constitute the major barrier to extensive remedial methods (Sorvari et al., 2009).

1. 1. 2 Legislation

At a political level, soil and groundwater were mainly regulated by the Environment Protection Act and Waste Legislation until recently (Sorvari et al., 2009). A Government Decree on the Assessment of Soil Contamination and Remediation Needs was created in 2007, together with a relative guideline from the Ministry of the Environment about values and concentrations of contaminants allowed (*annex 1*). Two important programs have been conducted (Pyy et al., 2013):

- The State Waste Management Work System, with 370 remediation projects completed at the end of 2012 and expenses of €68 million, from which €30 million came from the state budget.
- The Finnish Oil pollution Compensation Fund, which already allowed the investigation of 715 sites and the remediation of 360 cases with €21 million (SOILI program).

Legislation imposes a generic obligation that when assessing the remediation plan and the alternatives methods, the authority responsible should consider the principles of best available technology, best environmental practice, waste minimization and waste recycling. However, there is no obligation to consider transportation distance to a soil treatment or disposal facility currently (Sorvari et al., 2009). It has been calculated that if the same situation continues, the remediation of all areas would take 100 years, and costs €4 billion (Pyy et al., 2013).

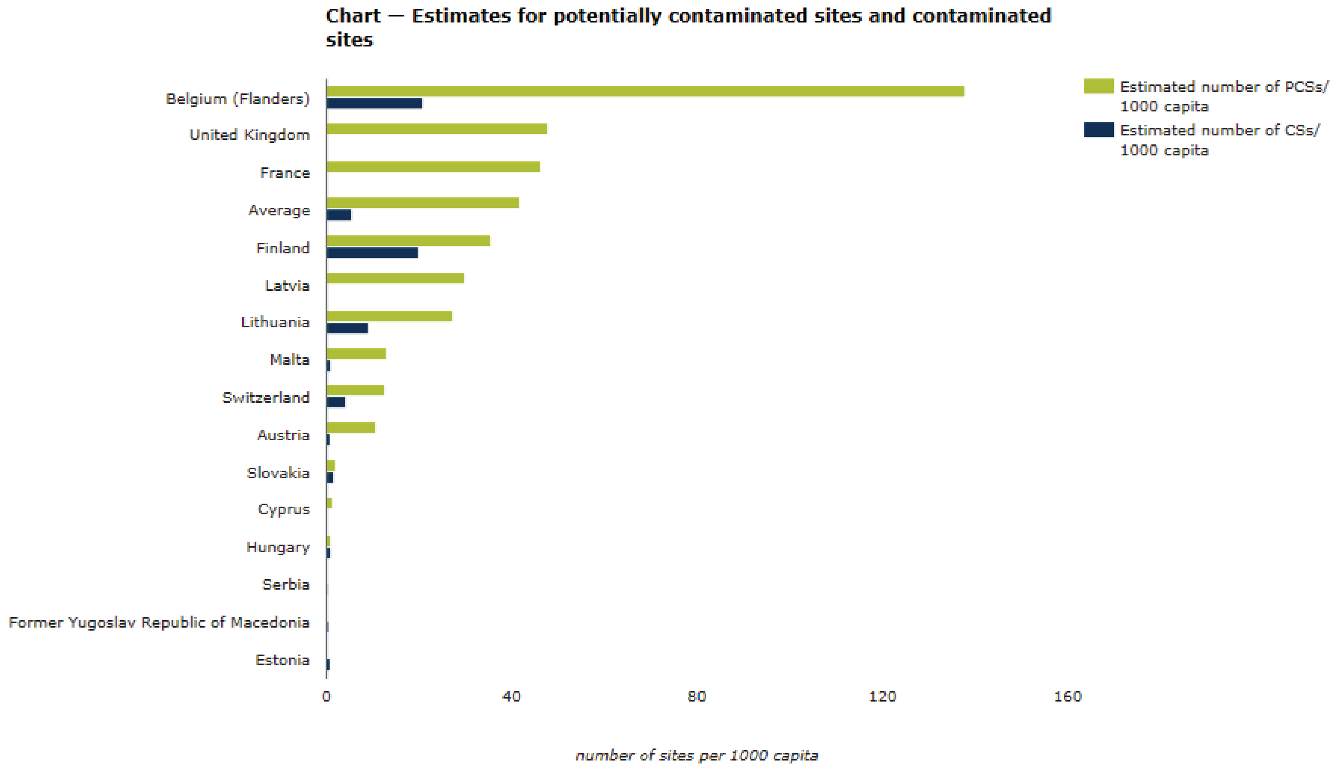


Figure 3: Potentially contaminated sites and contaminated sites estimated by country / Sites potentiellement contaminés et sites contaminés par pays, source: EEA, 2011

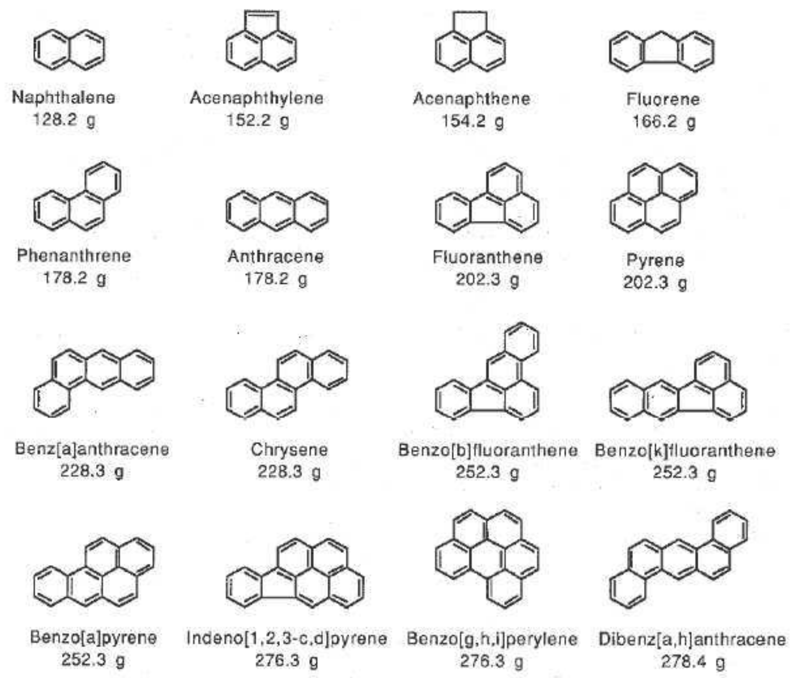


Figure 4: Molecular structure of the 16 PAH selected as priority pollutants by the American Environmental Protection Agency with their molecular weight / Structure moléculaire des 16 HAP (hydrocarbures aromatiques polycycliques) séléctionnés comme principaux polluanrs par l'agence américaine de protection de l'environnement et leur poids moléculaire, source: Henner, 2007

1.2. In Europe

At the European Union scale, a Soil Thematic Strategy was adopted in 2006, but there is no common legislation to protect the soil. This strategy has allowed to realize some inventories at the European Union (EU) level, and to promote the soil protection, but Member States still apply their own legislation when they have one. 2.5 million sites are potentially affected by soil contamination, in which 340,000 sites are considered to require cleanup actions. Inventories have allowed identifying one third of these sites according to estimations (Van Liedekerke et al., 2014). Finland is situated under the average when considering its potentially contaminated sites per capita, but above the average for confirmed contamination cases (*figure 3*). The potentially contaminated sites are the ones in which there is an evidence of contaminating activity whereas the contaminated sites are the ones in which there is contamination. This could be due to the low population of Finland compared to the importance of its industry, and not only to an important contamination. It could be also because it is one of the countries to have best developed its inventory of concerned sites. A proposal has been made for a Soil Framework directive, to draw a better inventory of the situation and identify the problems. The EU level was chosen because of the crucial functions of the soil and the transboundary effects of contamination. However, environment ministers have still not agreed on it (Jones et al., 2012).

2. Pollution by petroleum hydrocarbons

2.1. Characteristics

Organic contaminants represent at least 55% of all contaminants in The European Union (Van Liedekerke et al., 2014). Among them, the widespread class is the one of the petroleum hydrocarbons. Some of their compounds are extremely toxic due to mutagenic and carcinogenic properties (Cook and Hesterberg, 2013). They are the main constituents of oily products such as gasoline, diesel, solvents and penetrating oils (Exponent Inc, 2010). They belong to four classes: the saturates, the aromatics, the asphaltenes (phenols, fatty acids, ketones, esters and porphyrins) ant the resins (pyridines, quinolines, carbazoles, sulfoxides and amides) (Macek et al., 2000). Petroleum hydrocarbons compounds can bind to soil components in a process called aging or weathering, reducing their bioavailability. They can thus become difficult to remove or degrade. Their susceptibility to microbial degradation ranks from linear alkanes to cyclic alkanes, the last being the most difficult to degrade (Macek et al., 2000). In the denomination total petroleum hydrocarbons (TPH), volatile monoaromatic compound (BTEX for benzene, toluene, ethylbenzene and xylene), polycyclic aromatic hydrocarbons (PAH) and aliphatic compounds are included (Cook and Hesterberg, 2013).

2.2. Polycyclic Aromatic Hydrocarbons

The aromatic hydrocarbons are considered for health risk at most contaminated sites. Benzene is classified by the U.S. Environmental Protection Agency, which is a common reference, as a “known human carcinogen” (Exponent Inc, 2010). However, most of the problems are related to polycyclic aromatic hydrocarbons. 16 of them are priority pollutants according to the same Agency (Henner et al., 2007), and six are followed by the World Health Organization. They are molecules relatively neutral and stable, with one or more benzenic rings (*figure 4*).

They are highly hydrophobic and thus have low solubility. They have also low volatilities and long half-lives in geological media (Henner et al., 2007). They are classified as persistent organic pollutants (POP) because of the toxicity for human health, persistence and bioaccumulation in alive

Table 1: Threshold and guideline values defined by Finnish government for aromatic hydrocarbons contents / Valeurs de menace et de référence définies par le gouvernement finnois pour les concentrations en hydrocarbures aromatiques, source: Ministry of the Environment of Finland, 2007

substance	threshold value ($mg.kg^{-1}$)	lower guideline value ($mg.kg^{-1}$)	upper guideline value (mg.kg-1)
Aromatic hydrocarbons			
Benzene (p)	0,02	0,2 t	1 t
Toluene (p)		5 t	25 t
Ethylbenzene (p)		10 t	50 t
Xylenes (p) (1)		10 t	50 t
TEX (2)	1		
Polycyclic aromatic hydrocarbons			
Anthracene	1	5 e	15 e
Benzo(a)anthracene	1	5 e	15 e
Benzo(a)pyrene	0,2	2 t	15 e
Benzo(k)fluoranthene	1	5 e	15 e
Phenanthrene	1	5 e	15 e
Fluoranthene	1	5 e	15 e
Naphtalene	1	5 e	15 e
PAH (3)	15	15 e	100 e

t: based on health risk, e: based on ecological risk, (p): risk under threshold value for ground water, (1): include the structural isomers, (2): toluene, ethylbenzene and xylene, (3): total concentration of the 16 PAH registered for toxicity

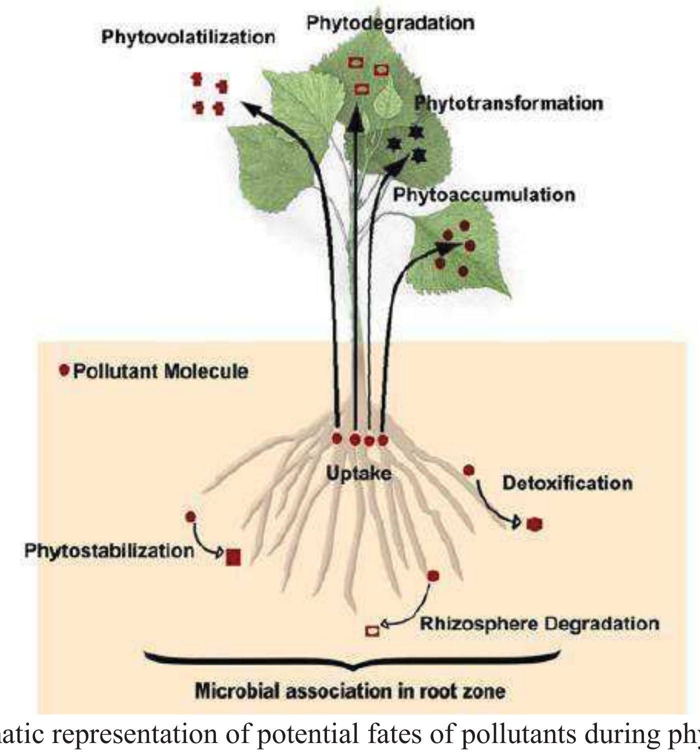


Figure 5: Schematic representation of potential fates of pollutants during phytoremediation. The contaminant can be degraded or stabilized in the rhizosphere, or translocated in the aboveground tissues and then stocked or degraded. / Représentation schématique de la destinée potentielle des polluants pendant la phytoremédiation. Les contaminants peuvent être dégradés ou stabilisés dans la rhizosphère, ou transferés sans les tissus aériens et stoqués ou dégradés. Source: Yadav et al., 2010

tissues due to their high solubility in lipids (INERIS, 2006). Their degree of impact increases with the ring number and the condensation degree, as the solubility in water decreases. The most dangerous are mutagenic and carcinogenic, have immunodepressive effects, and present a risk for the environment. Their biodegradation is slow and is a function of environmental parameters such as oxygen, water and nutrient contents and bioavailability (Henner et al., 2007). They occur as colorless or white or pale yellow solids (Gerhardt et al., 2008).

The authorized values are described in the guideline values defined by the Finnish government (*table 1*). The threshold value is the one from which remediation needs must be assessed. The soil must then be considered contaminated when the upper guideline value is exceeded for one or more contaminants in an area of industrial activity, storage or transport and when the lower guideline value is exceeded for other areas. The threshold value for total PAH is 15 $mg.kg^{-1}$ in dry matter, and the higher guideline value to consider is 100 $mg.kg^{-1}$, based on ecological risk (Ministry of the Environment, Finland, 2007).

3. Phytoremediation

The term phytoremediation comes from the Greek “phyto” or plant and “remedium” that means correct or remove an evil. It consists of the use of green plants and their associated microbiota, soil amendment and agronomic techniques to remove, contain, or render harmless environmental contaminants. It is considered as a new soil remediation technology, but the first plant based system for remediation was installed in Germany more than 300 years ago for treatment of municipal sewage. Research in this domain is more advanced for inorganic contaminants, due to the difficulty to implement easy analysis tools for organic contaminants (Cunningham et al., 1995). The contaminants that can be treated by this way currently are petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides, and landfill leachates (Susarla et al., 1999). Two main techniques can be used. The phytodecontamination consists in the reduction of the contaminant concentration to an acceptable level, whereas phytostabilization is the sequestration of the contaminant in the soil while reducing its bioavailability and possible migration (Cunningham et al., 1995).

3.1. Different mechanisms

3. 1. 1 Phytodecontamination

Different mechanisms are responsible of phytodecontamination (*figure 5*). Phytoextraction consists in the removal of a contaminant from the soil. If the contaminant is not degraded rapidly or completely, phytoaccumulation occurs with accumulation of the pollutant in the plant. Some plants can hyperaccumulate contaminants, in particular heavy metals, whereas others will die or experienced severe stress conditions. In the case of accumulation, it is desirable that contaminants are translocated from roots into the aboveground tissues, in order to remove the contaminant by harvesting. Plants can also eliminate the contaminants with the action of their enzymes or enzyme co-factors by phytodegradation or phytotransformation. Enzymes of interest are in this case deshalogenase, peroxidase, nitroreductase, nitrilase and phosphatase. Phytovolatilization happens when a contaminant is converted into a volatile form that can be less toxic (Susarla et al., 1999).

3. 1. 2 Contaminant route

Contaminants are generally obtained from the liquid phase or sometimes the vapor phase. The compounds more water soluble are more available to transport and diffusion and so to remediation, as the negatively charged ones which are excluded from negatively charged soils. Water and

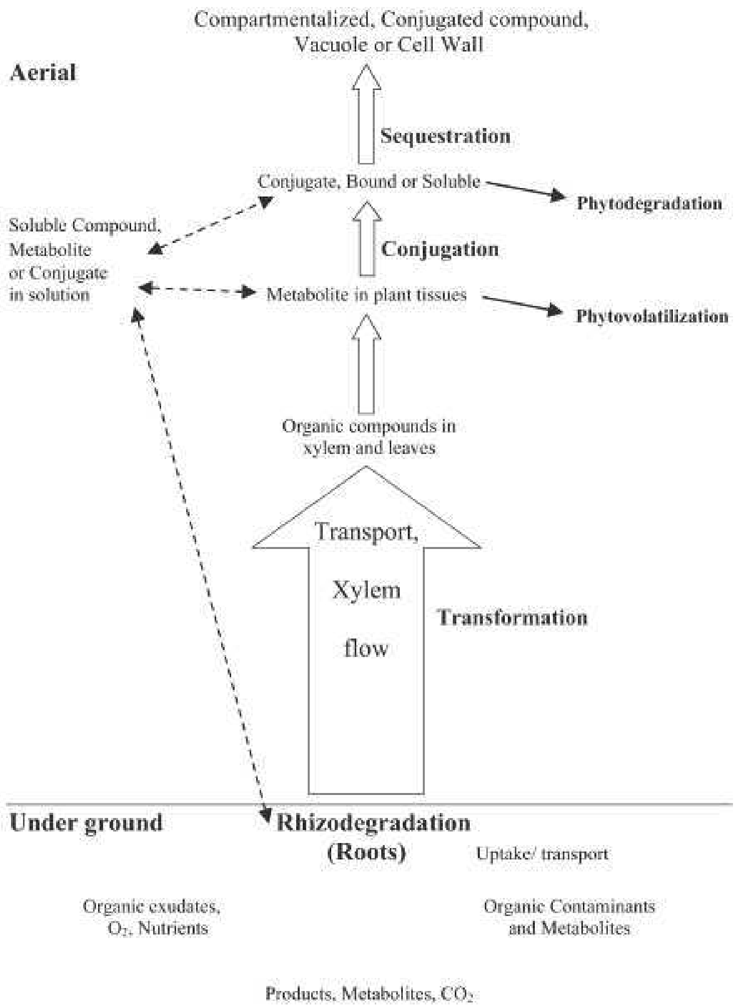


Figure 6: Contaminant route and transformations/ Trajet et transformation des polluants, source: Singh et al., 2003

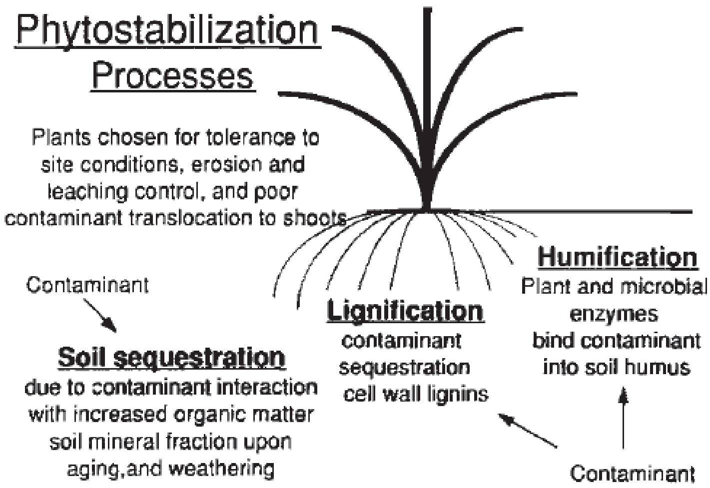


Figure 7: Naturally occuring processes involved in phytostabilization / Processus naturels impliqués dans la phytostabilisation, source: Cunningham et al., 1995

dissolved constituents can then move easily into the roots apparent free space, but have to cross at least twice the cell membrane to pass the Casparian strip in the endotermis and then to gain access to the xylem. The apoplasmic pathway only account for an insignificant amount on the total flux. The relatively lipophilic materials are then subject to enzymatic attack, creating more water soluble compounds, the major enzyme systems being cytochrome P450 oxygenase and glutathione S-transferase. The processes are transformation, conjugation and final disposition, as the metabolized products are transported into vacuoles, intracellular space or various cell wall components (*figure 6*) (Cunningham et al., 1995).

3. 1. 3 Rhizodegradation, a particular case of bioremediation

The bioremediation is the biological remediation technique most used currently. It consists in the use of bacteria and other microorganisms to degrade organic contaminants. Indeed, microorganisms present interesting enzymes that can degrade a wider range of contaminants than plants. In particular, hydrocarbons are food for microbes. The concentration in microorganisms is thus susceptible to increase in the presence of organic contaminants, and the degradation will be more efficient (Mukherjee, 2014).

Rhizodegradation is a mechanism subject to important consideration currently. It happens in the rhizosphere, the zone of increased microbial density and activity closest to the roots of the plants (Susarla et al., 1999). The rhizosphere effect is defined as the ratio of microorganisms in rhizosphere soil to the number of microorganisms in non-rhizosphere soil. It is usually from 5 to 20 and can even reach 100 (Cunningham et al., 1995) . This mechanism could be considered as a form of bioremediation favored by the presence of trees. Roots exudate carbon, energy, nutrients, enzymes and oxygen which are delivered in the rhizosphere and can be used by microbial populations (Ndimele, 2010). It stimulates the growth and metabolism of soil microorganisms. Some compounds can also serve as growth substrate (Macek et al., 2000). It can also facilitate the passage of the contaminant in the plant, by increasing the solubility with the help of biosurfactants, enhancing the ability of microbial cells to attach to oil droplets. Mineralization is then the major fate for the contaminants (Martin et al., 2013). Roots have also a physical effect on the soil, important for all remediation types.

New researches also focus on endophytes, which are microbes living within the plant with effects ranging from detrimental to commensual or beneficial. The phyllospheric microorganisms are the ones leaving in the leaves. Both could have an interest in remediation (Cunningham et al., 1995). Furthermore, the interaction of the plants with fungi is a subject of interest currently (Macek et al., 2000).

3. 1. 4 Phytostabilization

Phytostabilization (*figure 7*) consists in the ability of plants to alter soil environmental conditions like pH and moisture content (Conesa et al., 2011). The increase in organic matter and the exudates released by root or associated microorganisms provoke humification or incorporation in the soil humus. This reduces the bioavailability of the contaminants. Furthermore, the binding of the contaminants with the soil or aging phenomenon is increased. Phytostabilization can also happen when contaminants enter the plant, if they are trapped in plant cell walls in the lignification process (Cunningham et al., 1995) . Phytopumping is an efficient mechanism by which plants are used as organic pumps to pull-in large volumes of the contaminated water in the transpiration process. It allows reducing the migration of the contaminant, in particular reducing the risk to reach groundwater (Conesa et al., 2011).

3. 1. 5 Other techniques

All these mechanisms consist in in situ remediation, where plants are in contact with contaminated ground. However, where the contaminant is not accessible to plants, it can be extracted with mechanical means and transferred to a temporary treatment area to be exposed to plants: it is in vivo phytoremediation. In vitro phytoremediation also exists, when components of plants are used such as extracted enzymes. It could be applied in situ in theory, but more likely to material relocated (Susarla et al., 1999).

3.2. Advantages and drawbacks

3. 2. 1 Observations

Most of common remediation technologies need the excavation of the soil and its disposal in another place. Phytoremediation, however, can be realized directly in situ. Furthermore, the cost of plants is lower than most other technologies (\$162 $.m^{-3}$ compared to \$810 $.m^{-3}$ for excavation and incineration), and is relatively environmentally safe as it doesn't include the use of chemicals (Ndimele, 2010). It also reduces soil erosion, increases biomass production and creates a pleasing environment (Cook and Hesterberg, 2013), elements not allowed by a simple bioremediation. Furthermore, there is a general high public acceptance (Macek et al., 2000). The protocols are easy to implement, the maintenance costs minimal and organic material is added to the soil, improving its quality and texture (Gerhardt et al., 2008).

On the other hand, this technology is still new and not completely understood, and there is a possible dissolution and migration of contaminants. It is also limited by the toxicity of the pollutant, which can kill the plants used. The response is slow and can take several years. In the case of phytoaccumulation, the best hyperaccumulators found today are very small plants, and so do not produce a high biomass (Ndimele, 2010). Furthermore, if the accumulated compound is very toxic and not transformed, the biomass needs to be harvested and destroyed to prevent health and ecological risks. Phytoremediation is limited by the climatic and geological conditions of the site as temperature, altitude, soil type and accessibility by agricultural equipment. Some pollutants can become more soluble, increasing their migration (Macek et al., 2000). Physical challenges can appear when the contaminant goes deeper than the roots. The uneven distribution of the contaminant can also cause some problems in the design of the project. When it supposes the inclusion of non-native species, there is also a risk of dissemination (Gerhardt et al., 2008). It must be added than the regulation can create some important constraints (Cunningham et al., 1995), as it is difficult to draw precise plans. Finally, the aging of the contaminants in soils can make the use of biological techniques inefficient if it is too important (Cook and Hesterberg, 2013).

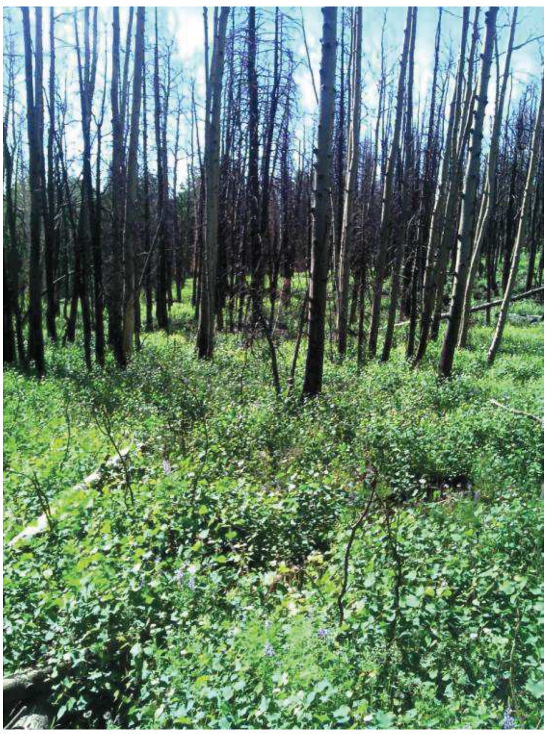
3. 2. 2 Conclusion

The use of phytoremediation could finally be advantageous in the places where pollutants cover a large area, too important for the excavation of the totality of the soil, and where the concentrations are low enough and do not present an immediate risk to human health (Cook and Hesterberg, 2013). To be commercialized and more used, this technology also needs a standard norm, as too many scientific terms are used for the various processes and mechanisms, leading to confusion once in the marketplace. The use of the biomass produced to obtain outputs is a mean of making this process more commercial, with the production of biofuels, wood, biochar, and non-consumable agricultural products, but the contamination level of this biomass has to be regarded (Conesa et al., 2011).

Table 2: Species with a potential for phytoremediation of petroleum hydrocarbons / Espèces présentant un potentiel pour la phytoremédiation des hydrocarbures, source: Frick et al., 1999, used in Ndimele, 2010

Western wheatgrass (Agropyron smithii)
Big bluestern (Andropogon gerardi)
Side oats grama (Bouteloua curtipendula)
Blue grama (Bouteloua gracilis)
Common buffalograss (Buchloe dactyloides)
Prairie buffalograss (Buchloe dactyloides var. Prairie)
Bell rhodesgrasss (Chloris gayana)
Bermuda grass (Cynodon dactylon L.)
Carrot (Daucus carota)
Canada wild-rye (Elymus canadensis)
Tall fescue (Festuca arundinacea Schreb))
Arctared red fescue (Festuca rubra var. Arctared)
Soybean (Glycine max)
Duckweed (Lemna gibba)
Annual ryegrass (Lolium multiflorum)
Ryegrass or perennial ryegrass (Lolium perenne L.)
Alfalfa (Medicago sativa L.)
Verde kleingrass (Panicum coloratum var. Verde)
Switchgrass (Panicum virgatum)
Bush bean (Phaseolus vulgaris L.)
Poplar trees (Populus deltoids x nigra)
Winter rye (Secale cereale L.)
Little bluestern (Schizachyrium scoparius)
Indiangrass (Sorghastrum nutans)
Sorghum (Sorghum bicolor) or sudangrass (Sorghum vulgare L.)
Meyer zoysiagrass (Zoysia japonica var. Meyer)

Figure 8: Regeneration of aspens after a forest fire / Régéneration de Trembles après un feu de forêt, source: Brigham Young University, 2015



3.3. Choice of the species

Trees and perennial grasses are nowadays of common use in phytoremediation, as they do not require yearly planting (Cook and Hesterberg, 2013). The species with a potential for organic contaminants are grasses, leguminous and trees (*table 2*). In general, trees are more planted for BTEX remediation and grasses for PAH and TPH remediation (Cook and Hesterberg, 2013).

3. 3. 1 Grasses and leguminous

Grasses used in prairies have an extensive and fibrous root system that maximizes the root surface area and may penetrate the soil up to 3 m. It increases the contact with the pollutant. Legumes are for their part able to fix nitrogen, so do not have to compete to grow and can produce enough biomass (Ndimele, 2010). Into the herbaceous plants, Poaceae family is the most effective in the degradation of PAH. The more common are tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) (Cook and Hesterberg, 2013).

3. 3. 2 Trees

The important growth and high biomass presence of tissues accumulating contaminants are important characteristics in the choice of species for phytoremediation (Marmiroli et al., 2011). Trees have in general a greater root biomass and a deeper root system. The most used in phytoremediation are willows (*Salix* spp) and hybrid poplars (*Populus* spp.). Their advantages are easy propagation, fast growth, deep roots that can be phreatophytic, high water uptake rates, high absorption surface areas, perennial growth and tolerance to contaminants and flooding. They are interesting in the cases of contaminated groundwater in order to prevent off-site migration. Other trees can be used due to their resistance to stressful conditions and their aptitude to colonize nutrient-depleted soils, as birch (*Betula pendula*) and red mulberry (*Morus rubra*) (Cook and Hesterberg, 2013). Trees can transpire more soil water; it creates greater fluctuations what bring more oxygen to soil. Their ability to extract water reduces water limitations, not only for them but also for the microbes present in the roots zone. This ability is also of use to destabilize soil aggregates. In nutrient poor conditions, trees have the ability to allocate a greater percentage of total biomass to roots to explore the soil for nutrients. As fungi degraders of lignin are usually found in the forest, the use of trees should be good to promote them. However, their use of nitrogen and phosphorous could impede PAH degradation (Cook and Hesterberg, 2013).

4. Aspens and hybrid aspens

Forest in Finland is mostly composed of Scots pine (*Pinus sylvestris*), due to its ability to develop even on poor soils and Norway spruce (*Picea albie*). Broadleaves represent 20% of the growing stock, but most of them are birches (*Betula pendula* and *Betula pubescens*). Aspens and other non-dominant species represent only 3 % of the growing stock (METLA, 2014).

4.1. Characteristics

4. 1. 1 Physiology

The aspens pertain to the Salicaceae family, genus Populus and section Populus (Tullus et al., 2011). Aspens are broadleaves trees, dioecious, what means that female and male flowers are on different trees. They are medium size trees but can reach 35 m to 40 m in height and 1 m in stem diameter at breast height (DBH). There is a great possible genetic variation. They are diploid

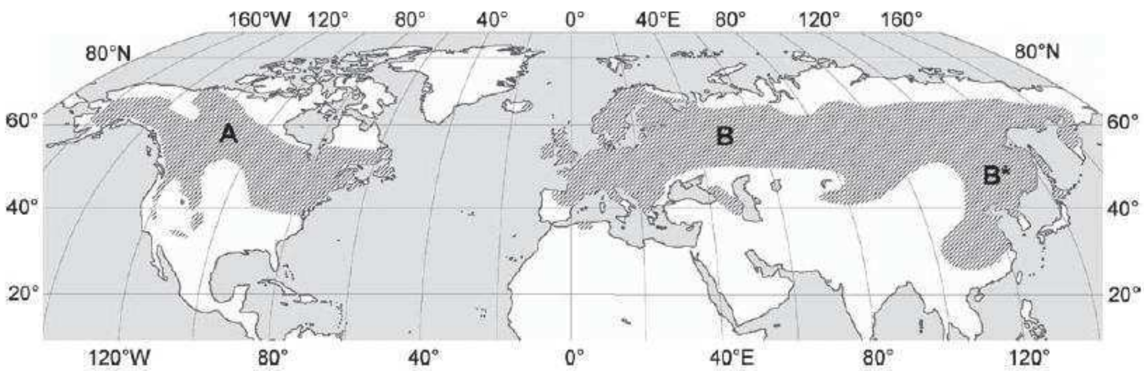


Figure 9: Distribution of *Populus tremuloides* (A) and *Populus tremula* (B and B*) in the world / Distribution de *Populus tremuloides* (A) et *Populus tremula* (B et B*) dans le monde, source: Tullus et al, 2011

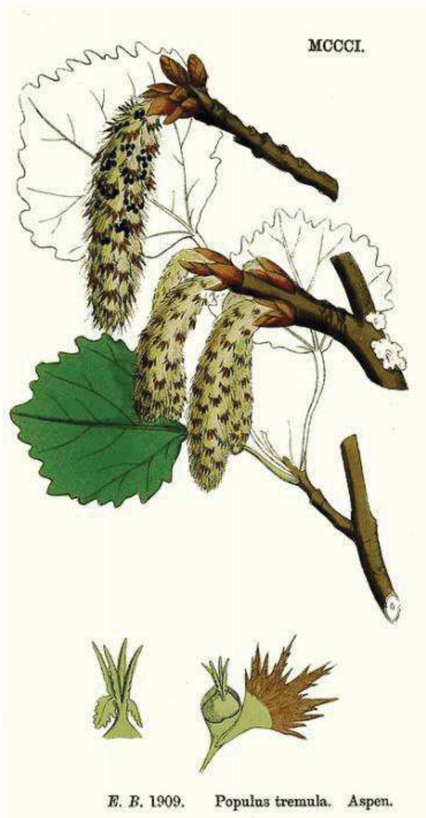


Figure 11: Morphology of flowers and seeds of *Populus tremula* / Morphologie des fleurs et semences de *Populus tremula*, source: Eng. Bot., 1909

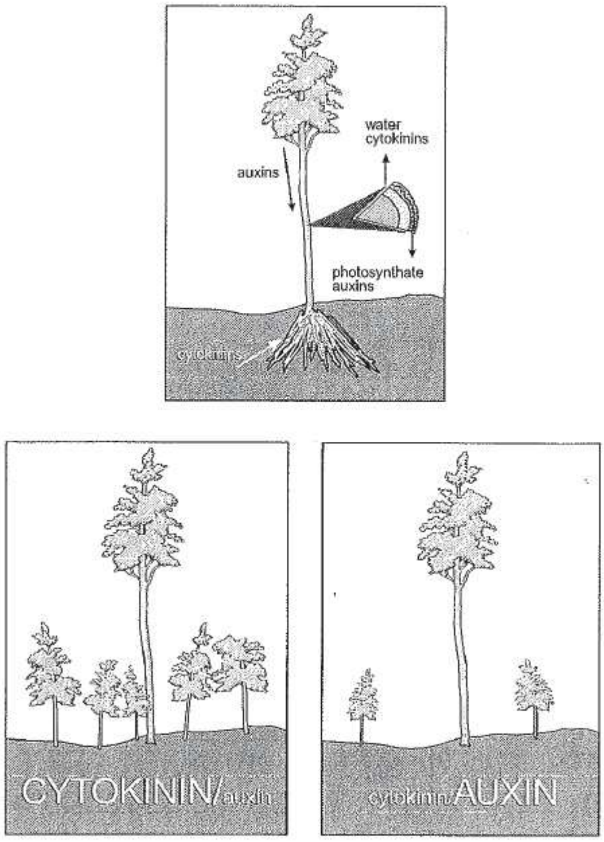


Figure 10: Effect of the flow of hormones on the suckering within an aspen tree / Effet de la variation hormonale sur le drageonnement, source: Bancroft, 1989

(2n=38) as all trees of genus Populus, but it is possible to find triploid or tetraploid individuals (Tullus et al., 2011). Aspens are disturbance-adapted species, and so often first colonizers of areas, in particular after forest fires (*figure 8*). They are light demanding trees and prefer fertile sites, well drained and with an aerated and rich nutrient soil. The better texture for them is medium (sandy loam or loamy sand). There is a thermophilic preference in the boreal climate. The pH more adapted can vary between 5.5 and 7.5, and aspens are more dependent to nitrogen than coniferous stands (Tullus et al., 2011).

Populus tremula L. is the European or Asian aspen. It is one of the most widely distributed trees in the world (Tullus et al., 2011) (*figure 9*). It is commonly found in Finland in mixed stands with pine, spruce and birch (Yu and Pulkkinen, 2001), in which case the aspen doesn't reach its maximal size. It can be used for high quality paper (Qibin et al., 2001). *Populus tremuloides* Michx. is the quaking or trembling aspen, indigenous and widely distributed in North America (figure 9). It is genetically very close to Populus tremula L. (Tullus et al., 2011).

4. 1. 2 Reproduction

They can propagate by seedling or root suckering (Tullus et al., 2011). Aspen reach their maturity in producing flowers at 10-20 years (McDonough, n.d.), but high production only starts at 30-40 years (Hannelius and Kuusela, 1995). Each shoot can produce two to ten inflorescences, in which there are from 20 to 100 flowers of two to ten seeds. A high number of seeds is thus produced, very small and light with a tuft of dispersal hairs at the basal hand (*figure 11*). They are carried by the wind (McDonough, n.d.). However, successive sexual reproduction is rare as the germination conditions are important moisture conditions and because the viability of seeds decreases with time (Stenvall, 2006). Reproduction by root suckers is more common but requires a disturbance that alters the ratio of cytokinin and auxin hormones. When the tree is stressed, the flow of auxin that suppressed root suckers presence from the crown to the root system decreases, and cytokinins in the roots produce then suckering (Bartos, 2000) (*figure 10*).

4. 1. 3 Plantation and degradations

Industrial planting densities are around 1,100 and 1,600 plants $.ha^{-1}$. Higher planting densities, around 4,000 trees ha^{-1} could be used for energy wood but it is not applied in northern Europe. 1 to 3 thinnings are usually carried out (Tullus et al., 2011).

The general degradations of the aspen are cankers by fungal diseases. Stem cankers come from *Neofabrae populi* and *Entoleuca mammata*, and branch cankers from *Leucostoma niveum* and *Venturia tremulae*. One bacterial canker from *Xanthomonas populi* could also lead in growth reduction. Other fungi, *Melampsora*, *Septora* and *Marsonia* may have negative effects. The insects more likely to cause damages are the small poplar borer and large poplar borer, and the poplar leaf beetle may defoliate young aspens. But major damages come from herbivores who feed on aspen, due to the nutritive composition of new shoots. Roe deer, red deer and moose make essential the fencing of the areas in some cases (Tullus et al., 2011).

4.2. Propagation

The propagation of aspens by man can be realized by micropropagation or with cuttings (Stenvall, 2006), but it is difficult with woody cuttings (Peternel et al., 2009). In a commercial way, tissue cultures are used. This is rapid, but the cost for these in vitro techniques is high as frequent transplanting, use of antibiotics and variation in medium nutrient and hormone composition are necessary. Micropropagation with callus culture is common for aspens, with root sprouts, cambium

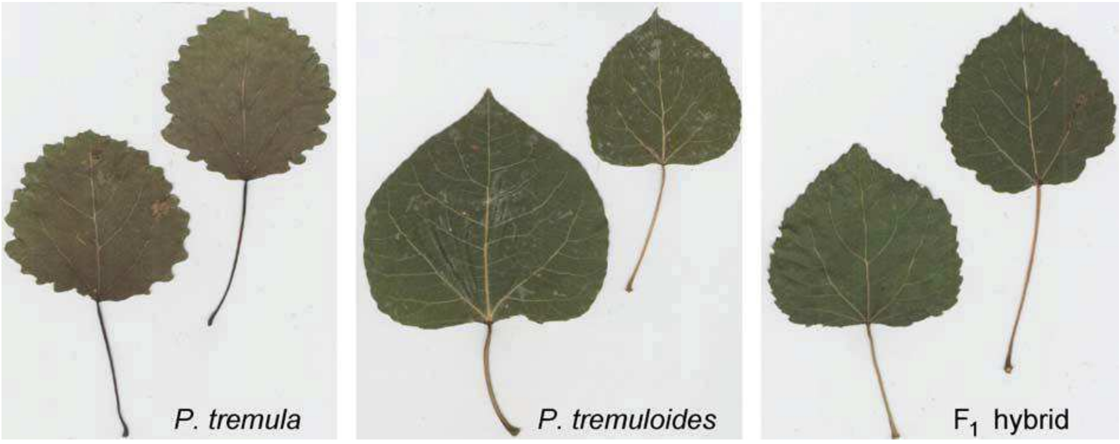


Figure 12: Differences in leaf form between *Populus tremula*, *Populus tremuloides* and the hybrid F1 born by crosses between the two first / Différences dans la forme des feuilles entre *Populus tremula*, *Populus tremuloides* et l'hybride F1 issu de croisements entre les deux précédents, source: Tullus et at, 2011

tissue from terminal branches, shoot tips and root explants, and axillary buds have proved to be efficient too (Peternel et al., 2009).

4.3. Hybrid aspen

4. 3. 1 Crosses

Artificial crosses between *Populus tremula* L. and *Populus tremuloides* Michx. have been first described at the beginning of the 1920s in Germany. Hybrids grow faster and have higher biomass productivity than their parents during the first 20-30 years. They are fertile, and can be crossed and produce viable seeds with their parents. The differences between hybrids and parents can be seen with the leaf form (Tullus et al., 2011) (*figure 12*). The first crosses in Finland are from 1950, due to a wood demand for match industry. A first breeding program was implemented by the foundation for forest tree breeding and the Finnish forest research institute. Then, in the 1990s, aspen wood was thought interesting for the production of high quality paper, and 12 different clones were selected. Between 1997 and 2003, 1,000 ha of hybrid aspen were planted in Finland. New crosses have recently been made, with 200 outstanding selected and currently tested in clone trials (Tullus et al., 2011). Indeed, hybrid aspen allows producing uniformly short fibers and so a significant increase in demand is expected (Yu and Pulkkinen, 2001).

4. 3. 2 Differences with European aspen

Research projects have shown than there are two peaks in growth rate for the hybrid clones, mid-July and August. *Populus tremula* L. presents for its part only one growth peak mid-June. However, growth patterns can be very different between different clones. Hybrids flush earlier and grow longer in general, with 158 to 143 days compared to the 112 days of the European aspen. Hybrid presents consequently an increase in stem volume. However, the sensitivity of the hybrid to the climate is important, and can lead to frost damage (Qibin et al., 2001). The superiority in growth of the hybrid has been explained by the heterosis effect, also called hybrid vigor. Indeed, there is a superiority of the F1 hybrids over the mean value of the two parents. The exact genetic cause of the heterosis effect of the hybrid is unknown, but it is due to the overdominance interaction of two alleles, each from one parental specie, at a homological locus. The most important traits that differ between parents and hybrid are the elongation of stem units and the leaf production. There is an importance of maternal control over phenological traits (Li et al., 1998), so the mother in the North Europe should be the European aspen *Populus tremula* L. (Tullus et al., 2011).

The mean annual increment is expected to reach up to 20 $m^3.ha^{-1}.year^{-1}$ in the southern part of the Nordic region. It is double of what can be expected from the native species. For merchantable timber, rotations of 20-30 years are enough, and the production expected is of 300-450 $m^3 \cdot ha^{-1}$ of pulpwood and aspen logs. However, the risk of environmental impacts is higher. The wood of hybrid aspens is high in carbohydrates and low in lignin, with concentrations of cellulose in ranges of 50-60% and in acid-insoluble lignin of 11-20%. This wood is amenable to different chemical, mechanical and chemithermomechanical treatments for pulping. It bleaches easily. However, the density is only 350-370 $kg.m^{-3}$ compared to the 370-380 $kg.m^{-3}$ of *P. tremula*. Hybrid aspens are more resistant than *P. tremula* to *Venturia tremulae* and *Phellinus tremula*e (Tullus et al., 2011).

There is a high level of variability in the different properties between the different clones of hybrid aspen, so attractive gains might be obtained through selection. It is important to note than the clone X site interaction is highly significant, even greater than the clonal effect. The question of selecting stable clones, due to the broad range of sites in which it will be necessary to plant aspens, has been raised (Yu and Pulkkinen, 2001).

Figure 13: Photography of the site of Somerharju in summer 2013 (after plantation of aspens) / Photographie du site de Somerharju pendant l'été 2013 (après la plantation des Trembles), source: Théo Le Dantec, 2013

II. Context of the establishment of the experiment

1. An experiment of the Natural Resources Institute Finland

The Natural Resources Institute Finland (Luke) was created at the beginning of 2015 by the union of the state research institutes MTT Agrifood Research Finland, the Finnish Forest Research Institute (METLA), the Finnish Game and Fisheries Research Institute and the statistical services of the Information Center of the Ministry of Agriculture and Forestry.

The Finnish Forest Research Institute was the main forest research institution in Finland, and a big center in Europe. It was established in 1917. The current network of research units covers the whole country nowadays. It is a governmental and sectorial research institute, subordinate to the Ministry of Agriculture and Forestry. The aims of the institute are, according to the law, to promote the ecological, economical and socially sustainable management and use of forests. The influence of the institute is on entrepreneurial and business activity based on forests, forests and community, sustainable forestry chains and forest economy and environment (Luke, 2015). The Haapastensyrjä unit is a central tree breeding unit in Finland, and the results of its work serve both practical forestry and research. It manages 40 hectares of land, in which there are 10 hectares of plant nurseries and 2 hectares of greenhouses. It is located 60 km north to Helsinki.

The general cycles of selection in tree breeding consist in intermating individuals to develop progenies, evaluating the progenies according to the traits of interests and selecting and intermating again the favorable genotypes. It allows improving the mean of the new population (Zalesny and Bauer, 2007).

2. The first phytoremediation project of Finland

Phytoremediation is only an emerging technology in Finland, due to the particularly cold conditions, and is not usually accepted as a current remediation method. However, it was recently authorized for two sites contaminated with organic compounds. These initiatives are experimental, and were so implemented in a partnership with the forestry part of the Natural Resources Institute Finland.

2.1. Remediation decision

The Somerharju site is a 7 ha field located in the municipality of Luumäki, in the south-east of Finland (*figure 13*). It was contaminated due to an activity of wood impregnation by creosote carried out between 1947 and 1958 (*annex 2*). The site is limited in the north by the interstate road 6 and in the south by the railway. It belongs to a state company, the Senate Estates since 2007. In 2009, the South East Finland's Industry, traffic and environmental central and the environmental authorities of the municipality of Luumäki decided to start a remediation of this site, as analysis of soil and groundwater had shown important values of toxic components (*annex 3*). The general plan of rehabilitation was created in 2010 and accepted by the Regional State Administrative Agency for Southern Finland (Etelä-Suomen aluehallintovirasto) (decision 176/2012/1) in 2012 (*annex 4*). This plan detailed the choice of the phytoremediation as a method, being the best ecological solution. The aim is to prevent the migration of the contaminants, and to make the place safe for human health, as collection of mushrooms and berries is a common activity in the area. Furthermore, the first houses of the village are situated only 150 m to 200 m south. The soil is sand and refined sand, with many layers. The thickness of the soil is from 2 meters to 13 meters depending on the position.

Table 3: Values of PAH concentration in the soil analyses of Somerharju site / Valeurs des concentrations en HAP dans les analyses de sol du site de Somerharju, source: METLA, 2009, personal realization/réalisation personelle

element analyzed	unit	minimal values	mean	median	maximal value	threshold value	lower guideline value	upper guideline value	toxic waste limiting value
C5-C10 hydrocarbons	mg/kg						100	500	
C10-C21 hydrocarbons	mg/kg	< 0,2	89,9	20	1620		300	1000	
C22-C40 hydrocarbons	mg/kg	< 10	265,9	40	2490		600	2000	
C10-C40 hydrocarbons	mg/kg	< 20	349,5	46	3020	300			10000
Naphtalene	mg/kg	< 0,01	0,16	0,02	5,42	1	5	15	2500
Asenaphthylene	mg/kg	< 0,01	0,35	0,13	10,1				
Acenaphthene	mg/kg	< 0,01	3,58	0,03	248				
Fluorene	mg/kg	< 0,01	2,51	0,09	172				
Phenanthrene	mg/kg	0,01	5,55	0,20	367	1	5	15	1000
Anthracene	mg/kg	0,01	6,36	0,20	199	1	5	15	1000
Fluoranthene	mg/kg	< 0,01	26,40	0,26	1020	1	5	15	1000
Pyrene	mg/kg	< 0,01	25,22	0,26	680				
Benz(a)anthracene	mg/kg	< 0,01	8,52	0,20	376	1	5	15	1000
Chrysene	mg/kg	< 0,01	8,39	0,22	114				
Benzo(b)fluoranthene	mg/kg	< 0,01	5,84	0,21	48,3				
Benzo(k)fluoranthene	mg/kg	< 0,01	2,93	0,20	22,9	1	5	15	1000
Benzo(a)pyrene	mg/kg	< 0,01	2,49	0,20	30,4	0,2	2	15	100
Indeno(1,2,3-cd)-pyrene	mg/kg	< 0,01	0,75	0,20	6,53				
Benzo(ghi)perylene	mg/kg	< 0,01	0,52	0,20	5,61				
Dibenz(a,h)-antracene	mg/kg	< 0,01	0,34	0,20	2,26				
total PAH	mg/kg	< 0,16	100,03	3,00	3030	15	30	100	1000

The site is in a class two water area, and there is a water supply 1.3 km from the subject. The groundwater is 9 to 11 m depth from the surface (Metla, 2012).

2.2. Contamination

The soil analyses of the zone have shown no problems with metal concentrations except for lead, and only in rare cases. However, the hydrocarbons were found problematic. Hydrocarbons having between 10 and 40 carbons exceed the upper guideline values in some cases, and their mean exceeds the threshold value. In details, this comes from the PAH. Exceeding levels were found in soil for phenanthrene, naphthalene, fluoranthene, anthracene, benzo(a)anthracene, benzo(k)fluoranthrene, benzo(a)pyrene and for the sum concentration of the 16 PAH compounds classified as highly toxic. For total PAH, the mean of the analysis exceeded the upper guideline value, and the maximal value found was more than the toxic waste limiting value (*table 3*). The biggest deposit is about 10 to 11 m depth, near the groundwater. Until 580,000 $\mu g.L^{-1}$ of PAH were found in groundwater. Elevated PAH concentrations were found in 1.3 ha in soil and in 4 ha in groundwater. It is known that in the site, one leakage has happened, with 10,000 L of creosote spilled in the soil (Metla, 2012).

2.3. First steps

The Environmental Technique supervisor is Golder Associates Corp., and the research project is led by the Natural Resources Institute Finland and the University of Helsinki. According to their decision, the previous wood land of Scots Pine (*Pinus sylvestris*) has been cut off and replaced by aspens in the years 2013 and 2014. 15 hybrid aspen and 5 European aspen clones have been chosen for the phytoremediation. The end of the project is for 2022 (Metla, 2012).

3. The choice of aspens and the clone selection

3.1. Choice of the specie

The current lack of knowledge in the exact phytoremediation mechanisms associated to each case lead to the current choice of species that have advantages for all possible mechanisms: high uptake of contaminant, water, and developed root system (Marmiroli et al., 2011). Due to the importance of forest in Finland and the possible partnership with the Finnish forest research institute, the use of trees has been selected. The conifers have not been proven to be good at phytoremediation for now, in particular due to their slow growth. Interesting trees being of the Salicaceae family, possible trees could have been poplars, aspens and willows. Poplars are not very present in Finland, and aspen are more common and more studied that willows in the country. Aspens grow quickly, have a high transpiration rate and an important root system. Hybrid aspens have been shown to be the fastest growing tree in Finland (Hassinen et al., 2008). It is furthermore an appropriate tree for regeneration areas.

The main reason for its choice was the increase of studies concerning this specie and in particular the hybrids between the European and American aspen currently. The Natural Resources Institute Finland has been developing an important selection of aspen clones due to the interest for paper industry. An important clone material is thus available. Selection is important as in general for Populus the variability can be huge between clones (Zalesny and Bauer, 2007).

Type	Chemicals Treated
Phytoaccumulation/ phytoextraction,	Cadmium, chromium, lead, nickel, zinc and other heavy metals, selenium, radionuclides; BTEX (benzene, ethyl benzene, toluene and xylenes), pentachlorophenol, short-chained aliphatic compounds, and other organic compounds
Phytodegradation/ phytotrans- formation	Munitions (DNT, HMX, nitrobenzene, nitroethane, nitromethane, nitrotoluene, picric acid, RDX, TNT), atrazine; chlorinated solvents (chloroform, carbon tetrachloride, hexachloroethane, tetrachloroethene, trichloroethene, dichloroethene, vinyl chloride, trichloroethanol, dichloroethanol, trichloroacetic acid, dichloroacetic acid, monochloroacetic acid, tetrachloromethane, trichloromethane), DDT; dichloroethene; methyl bromide; tetrabromoethene; tetrachloroethane; other chlorine and phosphorus based pesticides; polychlorinated biphenols, other phenols, and nitriles
Phytostabilization	Proven for heavy metals in mine tailings ponds and expected for phenols and chlorinated solvents (tetrachloromethane and trichloromethane)
Phytostimulation	Polycyclicaromatic hydrocarbons; BTEX (benzene, ethylbenzene, toluene, and xylenes); other petroleum hydrocarbons; atrazine; alachlor; polychlorinated biphenyl (PCB); tetrachloroethane, trichloroethane and other organic compounds
Phytovolatilization	Chlorinated solvents (tetrachloroethane, trichloromethane and tetrachloromethane); mercury and selenium
Rhizofiltration	Heavy metals, organic chemicals; and radionuclides

Figure 14: Contaminants known to be treated efficiently accordind to the phytoremediation mechanisms concerned / Polluants connus pour être traités efficacement pour chaque mécanisme de phytoremédiation, source: Susarla et al., 1999

3.2. Previous use of aspens in phytoremediation purposes and mechanisms expected

The most numerous studies for aspen of genus Populus consist in remediation of air contamination and heavy metals. The growth of poplars and aspens is increased by high amount of CO2 (Keller, 1987). They have been found interesting for heavy metals as they can accumulate them in a moderated way that impede their death due to the presence of metal binding molecules and the important transpiration, in particular for cadmium. However, the majority of the metals accumulate in roots, what lead to a more difficult harvest of the contaminants (Hassinen et al., 2008). Populus have also been used for leachate and wastewater treatment and salt (Zupancic Justin et al., 2010). They showed in general better growth and resistance than Salix species. For remediation of oil polluted with organic contaminants, far less results are present. However, benzene, toluene, ethylbenzene, xylene and some chlorinated compounds have been found to be effectively transported through the plant and volatilized into the environment (Yadav et al., 2010). Aspen wood could be a potential sorbent for PAH according to one study, and fungi complexes with the roots could increase the sequestration of total petroleum hydrocarbons (Gunderson et al., 2007). However, most research is nowadays focalized on aspen improvement by genetic engineering, especially in United States. It mainly aims to improve the uptake of metals or add bacterial genes susceptible to pollutants degradation.

Due to the characteristics of aspen and mechanisms concerned for contamination with organic contaminants and particularly PAH, mechanisms expected are phytotransformation, rhizosphere bioremediation and phytostabilization (*figure 14*). Phytoextraction is less expected than for metals and should be less problematic. The material born should not be considered for destruction.

3.3. Material used

The clones used at Somerharju are part of the clone production of the 1990's, progressively reduced to around 20 genotypes. However, these clones were until now not chosen on potential remediation capacities but on short fiber production due to the interest for paper industry and on easy propagation for an efficient production. The possibility of having special capacity on remediation is totally unstudied for these clones.

4. Objectives of the experiment

4.1. A need for more research

To increase the phytoremediation as a technology, it is necessary to realized additional experiments in laboratory and greenhouses. Different experiments with aspen and pollution are carried out at the Haapastensyrjä unit. They deal with organic contamination and salt resistance. Another field trial has also been established in southern Finland, in a case of oil contamination. Furthermore, the clone selection is not the only subject of importance to establish an efficient phytoremediation field, the density of trees to be planted is also important. Indeed, the knowledge of planting practices in contamination cases is very low, due to the low amount of real field experiments. The research has focused until know on the determination of best species.

4.2. Objectives

These report deals with an experiment set up in the year 2013. It focuses on the growth, survival and phytosynthesis of aspen for different clones of European aspen (*Populus tremula* L.)

and hybrid aspen (*Populus tremula* L. x *Populus tremuloides* Michx.) to different petroleum hydrocarbons polluted levels and to different planting densities in first years. The aim is to determine the impact of clone and density on growth in contaminated soils, the best clones and densities if there is an impact, and in a larger way to develop methods for the selection of clones, together with other experiments. The improvement of the knowledge of hybrid aspen behavior in stressing environment will allow optimizing the implementation of real-scale phytoremediation field trials.

5. Particularities of contaminants used

The experiment deals with 2 oily contaminants, both with a wide proportion of PAH: creosote and diesel.

- Creosote is obtained by the distillation of coal tar. It is a thin oily liquid used as an industrial wood preservative and water resistant product. Its density is slightly greater than water, and it is composed of hydrophobic compounds. In the classification of substances, it is a dense non aqueous liquid (DNAPL), which can contain up to 200 chemicals (Haapea and Tuhkanen, 2006). By mass, its composition is 75 to 85% of PAH, 5 to 10 % of phenols, 5 % of heterocyclic NSO and less than 1 % of BTEX (benzene, toluene, ethylbenzene and xylene) (Metla, 2012). The solubility of the different components varies a lot (Haapea and Tuhkanen, 2006).
- Diesel oil is a middle distillation fraction of crude oil. It is a major pollutant near the gas stations. It is semi volatile, whereas gasoline is volatile. The volatilization is linked with the initial content of diesel in the soil and the presence of water (40). Diesel oil is a mixture of hydrocarbons with different physical and chemical properties. It has alkanes between C8 and C26, and alkanes more suitable to microbial attack are between C10 and C25. It has also the highest content of environmentally persistent PAHs and total aromatics of medium distillate fuels (Tesar et al., 2002). It also contains alkanes, alkylbenzenes and alkanoic acids (61% of alkanes and 7% of PAH for low sulfur diesel) (Bartos, 2000).

The migration of the oily liquids being limited due to the absorption in the organic rich surface soil, the contaminants are expected to stay in the rooting zone in a field trial (Adam and Duncan, 2002). This makes this experiment reliable, even if in greenhouse the dispersion of the contaminant in the ground is limited.

6. General hypotheses and variables considered

The parameters studied in this experiment are the growth, the survival, the roots suckers presence and the photosynthesis performance of the trees. As the exact mechanisms happening are not known, some hypotheses have to be done to interpret the results:

- The efficiency of phytoremediation is linked with an important growth, as more pollutants will be removed improving phytodegradation, and more water utilized, improving phytostabilization. Biomass improvement is indeed one of the most important factor of choice for phytoremediation in the present situation (Cook and Hesterberg, 2013).
- The development of the aerial part is correlated with the development of the roots. The uptake of contaminants and rhizoremediation is indeed linked with the development of the roots, what cannot be measured without harvesting the plant.
- An increase in height is correlated with an increase in biomass as the aspens grow in height before growing in diameter (Louisiana Pacific Canada ltée and CERFO, 2002), which is a better indicator of the global development of the tree.
- The older the contamination is, the more difficult it will be for the contamination to be removed (Ndimele, 2010).

The best clones and densities will correspond to the ones allowing a better remediation, but also in first stages to a better adaptation to the conditions. Indeed, trees non adapted and not able to develop are expected to spend their energy on their growth and survival and not on the remediation.

The variables chosen for the study are in agreement with the common variables used until now in the experiments on poplars for phytoremediation. Indeed, the common allometric variables considered are height, diameter, number and surface of leaves, roots length and total biomass, as a better remediation is expected for individuals having the better general growth in biomass (Zalesny and Bauer, 2007). One physiological variable will be used, the chlorophyll fluorescence. Others physiological variables are possible however (Zalesny and Bauer, 2007), it will be part of the discussion.

Trees in best conditions should present a better growth, higher survival rate and higher photosynthesis efficiency in response to the contamination. They are also more interesting if they produce root suckers.

Figure 15: Photography of the experiment in 2015 / photographie de l'expérience en 2015, personal source / source personelle, 2015

Table 4: Clones of aspen used in the experiment / Clones de Tremble utilisés dans l'expérience, source: Luke, 2013, personal realization / réalisation personnelle

Aspen type	Clones used
European aspen	R3, R4
Hybrid aspen	14, 27, 191, 291

III. Material and methods

1. Experimental design

Location: It is located in a plastic greenhouse, in the Haapastensyrjä tree breeding center in Southern Finland (N60°36'56.516'', E24°25'53.396'').

Plantation: The trees are planted in buckets of 10L drained by bottom holes. Each bucket is placed on a basin, in order to avoid loss of contaminant when they are watered (*figure 15*).

Plant material: Four clones of hybrid aspens (*Populus tremula* L. x *Populus tremuloides* Michx.) and two of European aspen (*Populus tremul*a L.) have been used (*table 4*). The seedlings were produced by micro-propagation at the unit laboratory in the end of August 2012. After multiplication the clones started rooting in mid-January 2013. They were transplanted in boxes in February 2013. They grew inside until the beginning of June 2013. Then, they were planted in the buckets for the experiment, their height ranging from about 0.5 cm to 15.5 cm depending on the clone type.

Soil material: The substrate has been previously sampled from the Luke´s field trial of Somerharju, south-eastern Finland. This soil is of the type sandy with gross elements and it hasn't been sorted. Different treatments of the soil are present. As control, non-polluted soil of the Somerharju area has been employed. It was harvested in a place supposed to be free of contamination due to its position far from the contamination zones (*annex 5*). The, two pollution treatments have been set.

- The first one consists in Somerhaju original polluted soil, which is a soil contaminated with old creosote. This soil has been harvested in the area of Somerharju presenting the most important contamination by PAH. Analyses have shown for these zone concentrations in total PAH ranging between 100 and 1,000 $mg.kg^{-1}$, so between the upper guideline value and the maximal toxic value (*annex 5*). This soil has to be remediated according to the law. It represents a soil with old contamination.
- The other one has been created with clean soil from Somerharju in order to study the reaction to a new contamination with PAH. The soil used is the same as in the control treatment without contamination. Diesel was used for this purpose. The contamination has been made by injection of 50 mL of diesel in the substrate of each bucket with a syringe. The contamination level is thus about 0.5 % of oil. It represents a soil with new contamination.

Planting density: Three planting densities were tested for each clone and each contaminated soil. The densities were 1 plant, 2 plants and 6 plants per buckets.

Experimental design: The factors to vary are the clone, the soil contamination and the planting density. The design chosen consists is a design in complete random blocks, or replicates, without repetitions inside the blocks.

Three blocks were created. In each block, every combination possible of the six clones at the three densities in the three soil treatments is present once, a combination being represented by a bucket. In addition, one plant less buckets is present for each soil contamination type as control in each block. Finally, each block contains 54 buckets or combinations, more the three plants less. They are disposed in three columns of 19 buckets each one. In each block, there were 27 individuals per clone, so there were 162 plants per blocks at the beginning of the experiment. It makes a total of 486 trees for the whole experiment. The placement of each bucket has been randomized inside each

N

outside

greenhouse boarder

19	27 d1 old	R3 d6 old	291 d2 old
18	R3 d6 new	R3 d2 new	27 d6 new
17	R3 d2 old	R4 d2 new	R4 d2 old
16	R3 d2 clean	R4 d1 old	R3 d6 clean
15	R4 d1 clean	R4 d6 clean	291 d2 new
14	R3 d1 old	27 d1 new	291 d6 new
13	291 d2 clean	27 d6 clean	191 d2 new
12	14 d1 old	291 d1 new	27 d1 clean
11	R4 d6 old	14 d2 old	R4 d6 new
10	191 d6 new	14d2 clean	clean
9	new	191 d6 old	R4 d1 new
8	291 d6 old	291 d1 clean	191 d6 clean
7	191 d2 old	R3 d1 clean	191 d1 old
6	27 d6 old	R4 d2 clean	191 d2 clean
5	14 d1 clean	14 d6 new	14 d1 new
4	14 d2 new	291 d6 clean	14 d6 clean
3	191 d1 new	27 d2 old	R3 d1 new
2	27 d2 clean	old	191 d1 clean
1	291 d1 old	27 d2 new	14 d6 old
	1	2	3

Block 1

19	14 d2 old	14 d1 clean	R4 d6 old
18	R4 d1 new	R4 d2 new	R4 1 clean
17	R4 d2 old	R4 d1 old	R3 d1 clean
16	R3 d1 new	14 d1 new	R3d2 old
15	191 d2 new	291 d6 new	R3d1 old
14	R4 d6 clean	R3 d2 new	191 d6 old
13	191 d6 clean	291 d1 old	14 d6 clean
12	clean	R3 d6 clean	191 d2 clean
11	14 d6 new	191 d2 old	291 d2 new
10	191 d1 new	R3 d6 old	27 d2 new
9	27 d2 new	R4 d2 clean	27 d6 clean
8	14 d2 clean	27 d1 old	27d1 new
7	R4 d6 new	291 d1 clean	291 d2 clean
6	27 d6 new	27 d6 old	191 d6 new
5	14 d1 old	R3 d2 clean	old
4	14 d2 new	191 d1 clean	27 d1 clean
3	R3 d6 new	291 d6 old	291 d6 clean
2	new	291 d2 old	27 d2 old
1	291 d1 new	191 d1 new	14 d6 old
	1	2	3

Block 2

19	14 d1 clean	27 d2 new	27 d1 clean
18	14 d2 old	27 d6 old	291 d1 new
17	27 d1 old	14 d6 new	14 d2 clean
16	27 d2 clean	291 d2 new	R4 d1 new
15	R3 d2 clean	191 d6 old	27 d6 new
14	191 d1 old	R4 d1 old	R3 d1 new
13	27 d2 old	191 d2 clean	old
12	191 d1 new	R4 d2 new	291 d2 old
11	14 d6 clean	R4 d6 clean	14 d1 new
10	191 d2 new	R3 d1 clean	291 d6 old
9	14 d1 old	191 d2 old	R4 d1 clean
8	291 d6 new	27 d6 clean	new
7	14 d6 old	R4 d2 old	R3 d6 old
6	27 d1 new	191 d6 clean	R3 d1 old
5	191 d1 clean	14 d2 new	291 d6 clean
4	R3 d6 new	R3 d6 clean	R4 d6 new
3	291 d1 clean	R4 d2 clean	291 d2 clean
2	R4 d6 old	R3 d2 old	191 d6 new
1	291 d1 old	clean	R3 d2 new
	1	2	3

Block 3

central alley of the greenhouse

Figure 16: Representation of the experiment layout: « clean » means without contamination, « new » with new diesel contamination and « old » with old creosote contamination, « d » is for density / Représentation de la disposition de l'expérience: « clean » veut dire sans contamination, « new » nouvelle contamination au diesel et « old » contamination ancienne à la créosote, « d » est pour densité, personal realization / réalisation personelle

block in order to overcome the micro-climate effect induced by the location in the greenhouse: indeed the location of each combination is not the same for the different blocks (*figure 16*).

Watering regime: Plants were watered about 0.5 L in each bucket. Watering was realized once a week most of the time, and two to three times a week in the warmer months (June, July and August). Due to the weather conditions of Finland, it was almost never necessary to water them three times a week. Watering was performed with a showerhead hose the first year and a graduated bucket the second and third year.

Dormancy period: The plants were stored in a reserve during winter. They were transported inside when there was no more sign of biologic activity, i.e. no more leaves and that the buds were dried. It happened in October. Trees were implemented in the greenhouse again when the risk of frost was very limited, thus in May. It was the moment when buds were presenting new activity.

2. Previous measurements

Initial height of the trees has been measured just after the plantation on the 7th of June 2013. Then, the trees were measured once a month until September in 2013, and from May to September in 2014, by different trainees. The height has been recorded from the top soil to the last bud with an accuracy of 0,1cm on 2013 and of 1 cm in 2015. The survival of the individuals was determined at every height measurements. Trees were considered as dead when the stem had no more green leaves and was dry with no signs of recovering such as new buds or stem suckers. It has led to some confusion in the first year. Dead trees could have been replaced by root suckers in some cases.

3. Methodology for 2015

3.1. Allometric measurements

It is important, in order to be able to analyze the three years results, to apply the same general methodology than the years before. However, some additional measures can be done.

Height measurements: Height has been measured once in May at the beginning of this year experiment, and then twice a month. The measure was made from the top soil next to the base of the tree until the last bud alive. The same stiff meter was used every time. The accuracy was 0.5 cm. Indeed, due to possible differences in the choice of the soil surface point and the fact that trees can be bent, some trials at first measurements have shown that it was impossible to be precise at less than 0.5 cm (*annex 6*).

Diameter: Diameter has been measured at the end of the growing season for each tree, at approximately 5 cm of height. The measure was done at 0.01 mm of accuracy with a sliding rule measurement tool (Vernier Caliper of Storm instruments).

Survival and root suckers: The survival has been determined at the same time that the height measurement. A tree was considered as dead in the absence of this tree, or the absence of new leaves and green buds. When death was not certain, the tree was still measured and death was verified in the following observations.

The root suckers have been counted at the beginning of the growing season (May) and at the end in August, with the hypotheses that no new root suckers will appear in September. A new stem was considered as a root suckers when it was impossible to see any connection to the tree next to it until the ground level.

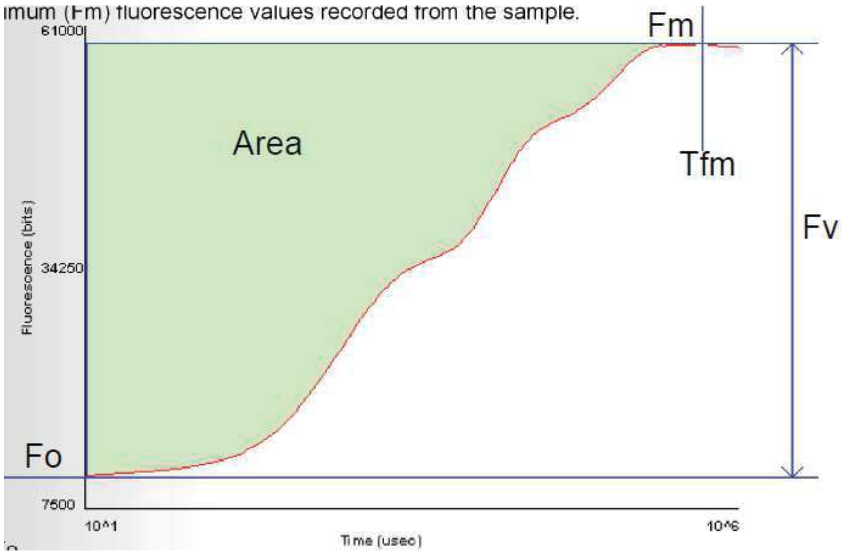


Figure 17: Graphical representation of the general parameters measured by the fluorometer / Représentation graphique des principaux paramètres mesurés par le fluorimètre, source: Hansatech Instruments Ltd, 2006

Figure 18: Correct placement of the clip on the leaf / Placement correct du cache sur la feuille, source: Hansatech Instruments Ltd, 2006

Other observations: The apparition of degradations and diseases was observed and marked, as the state of the trees at the beginning of the experiment. The number of leaves was counted for two replications at the end of the growing season (*annex 7*).

3.2. Physiological measurements: chlorophyll fluorescence

The emission of chlorophyll fluorescence is linked with the photosynthesis process and the stress of the plant. Solar energy leads to a short time change in electronic configuration. The energy is indeed dissipated quickly by different processes. The photosynthesis is one of these processes, but energy can also be lost in heat or fluorescence emission (red/far red radiation). Photosynthesis and fluorescence emission are linked: an increase in one of this phenomenon will provoke the decrease of the other.

A decrease in the photosynthesis efficiency is mainly linked with a decrease in the electron acceptance of photosystem II (PS II). This is due to stress conditions that can be heat, cold, drought, herbicide damage, diseases, pollutant, nutrient deficiency, etc. When a sample is dark adapted, PSII is totally re-oxidized. When the sample is illuminated, a rapid increase in fluorescence followed by a slower decrease is observed (*figure 17*). It is called the Kautsky Induction. Different parameters can be calculated on this curve, and are used to analyze fluorescence measurements (Hansatech Instruments Ltd, 2006). The data calculated are:

- Fv, variable fluorescence, variation between the fluorescence origin at t0 and the maximum fluorescence Fm.
- Fv/Fm, that represents the maximum quantum efficiency of the photosystem II.
- The Performance Index (PI), an indicator of the sample vitality and of its internal force to resist the constraints from outside. PI is calculated using the following formula: PI=(Vj/(dV/dto))*(Fv/Fm)*(Fv/Fo)*(Fm-Fj)/(Fj-Fo), where Vj is the relative variable fluorescence at the step j, to the time at the origin and Fj the fluorescence at j.

Best trees should have an Fv/Fm value of 0.85. Trees are however considered in an acceptable health state from values of 0.75. Lower values are linked with a less efficient photosynthesis, and thus stressed conditions that have an impact on the tree. For the performance index, the more the value is important the best the tree resists the constraints.

A fluorometer has been used: the Pocket PEA Chlorophyll Fluorometer of Hansatech instruments. The fluorescence was measured on the leaves. After a dark adaptation of the samples with clips (*figure 18*), they received a light emission of known intensity and the emission of chlorophyll fluorescence was recorded. The protocol was the following:

- Put the clip on the leaf, ensuring that a living part of the leaf is visible in the central clip opening.
- Close the small shutter plate on the opening to ensure dark adaptation and wait the necessary time for complete re-oxydation of the photosystem II in the cells concerned.
- Turn on the pocket fluorometer.
- Adjust the pocket fluorometer on the leafclip. No exterior light must be able to reach the part of the leaf to measure.
- Open the shutter plate, and then start the measurement.
- Wait for a sound before removing the fluorometer.

The light source intensity used was 3,500 µmols.m^{-2}, and the duration was 1 second. The fluorescence has been measured in the months of supposed biggest activity when the leaves were fully developed, so in June, July and August. The measurements were done once a month, for every tree except the ones dead or not presenting leaves at this moment. One leave has been used for each tree, selected randomly in the ones in good condition on the top of the tree. The leaves were marked

Table 5: Names used to define the contamination and density levels during the analysis / Noms utilisés pour décrir le niveau de contamination et de densité pendant l’expérience, personal realization / réalisation personelle

Name used for analysis	Meaning
clean	Soil without contamination
new	Soil with new diesel contamination
old	Soil with old creosote contamination
low	Planting density of 1 tree per bucket
medium	Planting density of 2 trees per bucket
high	Planting density of 6 trees per bucket

in order to use the same every time if possible. To know the dark adaptation time necessary, four trees in different conditions were chosen and the values of Fv/Fm noted for different times increasing by 5 minutes. The last sample reached its maximal value at 25 minutes, but as only four samples were used, the time of 45 minutes of dark adaptation was chosen in order to ensure a decrease in possible mistakes.

3.3. Observations

To analyze correctly the results, some details are important to know:

- The growing season of 2013 is considered from the beginning of the experiment, so only since June 7th.
- The last measurement of 2013 has been realized in August.
- In 2014, the experiment was set up again in May and measured, but the exact day of this measurement is not known.
- In 2014, measurements have been realized until the end of September.
- The experiment was set up again on May 22nd on 2015. It was a little late, as except in 2 buckets, trees were already presenting undeveloped leaves.

4. Hypotheses

The general hypotheses for the results are that growth and survival will be better in soil with no contamination, and better with old contamination than new one due to the weathering phenomenon. It will be better in less important planting densities as stress will be less important. For the same reason, photosynthesis will be more efficient in these same conditions. The root suckers should develop better in stress conditions (contamination, high planting density), however it is expected that the stress for new contamination will be too important and so the root sucker number less important in this specific case.

5. Statistical analysis

Statistical analyses were performed using the R 3.1.1 project software.

5.1. General methodology

5. 1. 1 General data preparation

Clone, soil treatment and planting density were considered as factor with fixed effect, as they were chosen in the experimental design. Planting density and soil treatment are ordinal: planting density can be ordered from low (1 tree in the bucket) to high (6 trees in the bucket). Soil treatment can be ordered according to the degree of contamination: from clean soil (without contamination), to new contamination (diesel) until old contamination (creosote). The names used for each degree during the analysis are resumed in *table 5*.

The individuals considered are the different trees of the experiment, as specific data is available for each one. Tree number and buckets allow knowing which individual is considered. The replications or blocks have been implemented to improve the reliability of the results. It is interesting to consider that situations are repeated, to decrease false results due to non-valid values. The factor block will thus be considered as a random factor.

Table 6: Details on models used for the analysis of survival, block is considered as random factor / Détails des modèles utilsés pour l'analyse de la survie, "block" est considéré comme un facteur aléatoire, personal realization / réalisation personelle

model number	formula	AIC	deviance	Degrees freedom residuals
1	survival~clone+treatment+density+(1|block)	347.1	325.1	475
2	survival~clone*density*treatment+(1|block)	380.8	270.8	431
3	survival~clone*treatment+(1|block)	340.4	302.4	467

The missing values or evident outliers have been replaced with the nearest neighbor method. Missing values were almost only present in the height measurement of 2013, due to errors linked with death determination.

5. 1. 2 General data analysis

Data were treated separately before allowing general conclusions. Thus only one variable had to be explained each time according to the influence of the factors clone, soil treatment and planting density. Univariate analyses were applied. Descriptive and interferential analysis was realized in each case. The presence of the random factor block induces the presence of matched series. The validity of the different models was realized graphically for the equivariance, independence and normality of the residuals. The graphics used were first the quantile residuals according to fitted values for the hypotheses of equivariance and independence, and then the sample quantiles according to theoretical quantiles for the normality. Reliability of significance was fixed at 95%, so for p-values of less than 0.05. In the models formula, “density” means planting density and “treatment” soil treatment.

5.2. Survival analysis

Specific hypotheses concerned

- The presence of contaminant decreases the survival rate. It is more important for fresh contamination.
- Some clones will be more resistant than others.
- High planting densities will decrease survival rate, and it will be more important in contaminated soils.
- Most of the trees will die at the beginning of the experiment, as the effect of contaminants should then decrease with aging.

Methodology

Due to mistakes in death determining in 2013, the survival had to be determined again for this year. It has been done following these indications: if a significant increase in height was observed in the following measure (more than 0.5 cm due to the precision of this year), the tree was considered as still alive. The tree was considered as dead when no significant increase in height could be noticed, that means the measure after the biggest size. If the death was only noticed as the last measure of the season, the tree was considered as dead only at the beginning of the next season, as it was impossible to assure it by a direct following measure. This was possible because trees were still measured even when considered dead in 2013.

The survival, coded 0 (death) or 1 (alive), follows a binomial law. The probability of survival was analyzed. Generalized linear mixed logistic models fit by maximal likelihood (Laplace approximation) were used (*table 6*). The separate effect of factors was first studied, and then different models with interactions according to the first results. All trees were concerned by the analysis.

All models fit the data (*annex 8, 9 and 10*). The model with the lowest AIC is the model 3, less information is lost by the model in this case. The type II Wald chi-square test was realized in order to determine the significant influence of the fixed factors for the first model. Comparisons were then realized using the least squares means method. The p-value was adjusted using a Tukey method to compare the number of estimates included in the model.

Table 7: Details on models used for the analysis of relative growth rate (RGR) at the end of 2014 and 2015, block is used as random factor / Détails des modèles utilisés pour l'analyse du taux de croissance relative (RGR) fin 2014 et fin 2015, "block" est considéré comme facteur aléatoire, personal realization / réalisation personelle

model number	formula	AIC	deviance	residual deviance	Degrees freedom residuals
1	RGR end 2014 ~clone+density+treatment+(1|block)	1376.2	1354.2	233.8	360
2	RGR end 2015 ~clone+density+treatment+(1|block)	1352	1330	205.0	357
3	RGR end 2015 ~clone*density*treatment+(1|block)	1372.4	1264.4	146.5	314

5.3. Height analysis

Specific hypotheses concerned

- The growth will reduce with the contamination, especially the fresh one.
- The growth will reduce with an increase in planting density, as a competition for resources as water and nutrients appears.
- Some clones will have a better growth, and in general growth will be better for hybrid aspens than for European aspens.

Methodology

The last measurement considered in this report has been done the 21st of August. Another analysis will have to be carried out with the data of the end of September to ensure the results, but the reliability can already be determined by the observation of differences in growth between months.

Only alive individuals were studied. The trees having being planted at 5 months old after first transplantation, they already had begun to grow independently of the conditions tested in that experiment. Furthermore they didn't started rooting at the same time. The analysis of the initial height (at plantation) showed not only differences between clones, what could be normal, but differences for a same clone varying from 3.5 cm to 13 cm. In order to consider this initial growth, relative growth rate has been used. As the time was not a studied factor and was the same for all trees, it was not used in the calculation of this rate.

The relative growth rate (RGR) was calculated as follow: RGR=final height / initial height. The initial height was the measure at implantation of the experiment in 2013 (June 7th). It consists in fact in the multiplication rate as it was found more interesting due to the high multiplication of size. The absolute growth was also used to determine significant growth. It was calculated as follows: absolute growth=final growth - initial growth. Significant growth was determined to be for more than 1 cm, as 1 cm was the largest accuracy used.

The final relative growth rate has been considered, but also the one at the end of 2014 and 2013 to know if results could be determined before the third year. Analyses were also realized for each growing season. When data of 2014 were concerned, calculations were made with growth at a precision of 1 cm as it was the case of the measures of this year. In other cases, 0.5 cm of accuracy was used. The variable studied for interferential analysis was not directly the relative growth rate, as its repartition was not following any common law, but a table containing the initial height and final height considered for each individual. This allowed the realization of general linear logistic mixed model fit by maximum likelihood (Laplace approximation) on binomial law (*annex 11*). These models compare the two heights and thus analyze the relative growth rate as defined in this experiment. The test realized to know the influence of factor was a type II Wald chi-square test, and then comparisons with the least squares means method were realized.

For results of end of 2014 and 2015, models with and without interactions were created (*table 7*). However, for 2014 only the model without interactions fit the data (*annex 12*). The residual deviance being smaller than the degrees of freedom of residuals, the residuals are not over dispersed. For 2015, the two models fit the data (*annex 13 and 14*), and residuals are not over dispersed in both cases.

For 2013 data, the residuals were over dispersed with this type of model. A random factor was added and allowed to solve this problem, but the observation of the comportment of the residuals showed that the model didn't fit the data. Kruskal-Wallis tests on the variable RGR were thus realized to analyze the data of this year, followed by Wilcoxon comparison tests.

Table 8: Details on models used for the analysis of root sucker presence, block is used as random factor / Détails des modèles utilisés pour l'analyse de la présence de nouveaux individus produits par drageonnement, "block" est considéré comme facteur aléatoire, personal realization / réalisation personelle

model number	formula	AIC	deviance	Degrees freedom residuals
1	Root sucker presence ~clone+treatment+density+(1|block)	169.3	147.3	151
2	Root sucker presence ~clone*density*treatment+(1|block)	186.2	76.2	107
3	Root sucker presence~clone*density+(1|block)	182.0	144.0	143

For the analysis of growth during the growing season of 2014 and 2015, as in 2014 the first date of the measurement was not known and in 2015 the growth had already begun at the first measurement, the original size was in this case the last height measurement of the previous growing season. The same model as for the global RGR was applied but couldn't be validated as it didn't fit the data. However, it was important to analyze the influence of the factors due to the results, and thus a Kruskal-Wallis test was applied followed by the Wilcoxon comparison test.

5.4. Diameter

0.1 mm of accuracy has been kept for the analysis, due to the lack of precision in the measurement height. No previous measurement had been realized, thus comparisons were not possible. However, if the sizes were different at the beginning of the experiment, we can suppose that each diameter was very limited, as it mainly increases in lignification processes that happened after the beginning of the experiment. It will be checked with the correlations between the RGR and the final growth. The same general hypotheses than for height can be done.

5.5. Root sucker analysis

Specific hypotheses concerned

- There will be more root suckers in stressed conditions (high density and contamination), except for the new diesel contamination
- There will be differences between clones

Methodology

It is impossible to know from which of the trees of the buckets a root sucker is created without harvesting the trees, so in this case the individuals are not the trees but the buckets. The analysis has been made for the 162 buckets in which trees were planted at the beginning. Two types of data were analyzed: first, the presence or no of roots suckers at the end of the experiment. In this case, individuals with root suckers were coded 1 and individuals without any root suckers were coded 0, according to a binomial law. The probability of response, responses being presence of root suckers or not, was studied with the use of generalized linear logistic mixed models fit by maximum likelihood (Laplace approximation) using binomial law (*table 8*).

The first model, without interactions, has the lowest AIC so the lowest loss of information. The model 3 was tested because of the results obtained with tests on model 1. It presents a lower AIC than model 2.All models fit the data (*annex 15, 16 and 17*).The effect of factors was studied in an analysis of deviance table with a type II Wald chi-square test and comparisons by least square means method.

The number of root suckers was then studied. No model was verified to analyze these data.

5.6. Chlorophyll fluorescence analysis

Specific hypotheses concerned

- Fv/Fm and PI will be lower in soil with contamination and particularly new contamination, and with an important planting density. Fv/Fm will be under 0.75 for contamination and high density cases.
- Some clones will resist better.

Methodology

The means of the three measures of Fv/Fm and PI were used for each individual. For the interferential analysis of the results, the use of linear mixed models was not possible as the analysis of the residuals didn't allow verifying the models (on all possible laws). This is due to extreme values, that can't be taken away as they concern at least 20 individuals and so cannot be all due to experimental mistakes. For that reason, and as the experimental design is no complete anymore due to the death of individuals, a non-parametric Kruskal-Wallis rank sum test has been used to see the influence of factors, followed by pairwise comparisons using Wilcoxon rank sum test .

The three measures have also been considered separately. Their correlations (Spearman) and variations have been analyzed. The values of June have been analyzed with a Kruskal-Wallis test as they were different of the other months.

5.7. Damage observations and leaves number

The percentage of leaves damaged in each buckets was more precisely reported once on July 24th and August 14th. A proportional odds model was used to determine if some factors could have an influence. When it was the case, the percentage touched in each category was observed for this factor.

No model fit the data for analysis of leaves number when taking into account the two blocks concerned. Thus, Kruskal-Wallis test and comparisons with Wilcoxon test were used.

Table 10: Significance of the different factors for the model 1 on survival without interaction between factors / Significativité des différentes facteurs pour le modèle 1 de la survie sans intéraction entres les facteurs, personal realization/réalisation personelle

factors	chi-square value	degrees freedom	Pr(>Chisq)
clone	12.3	5	<0.05
soil treatment	93.2	2	<1e-15
planting density	7.2	2	<0.05

Table 9: Significance of comparisons on model 1 for survival / Significativité des comparaisons pour le modèle 1 sur la survie, personal realization/réalisation personelle

contrast	estimate	p.value
clean-new	2.65	<0.0001
clean-old	-3.10	<0.01
new-old	-5.76	<0.0001
191-291	-1.55	<0.05
27-291	-1.55	<0.05

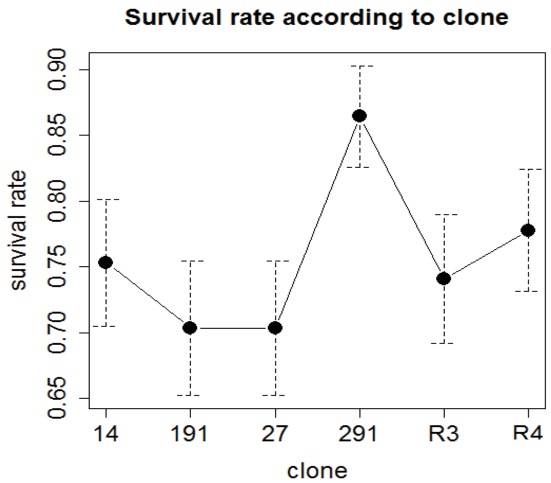


Figure 19: Graphical representation of survival rate by clone / Représentation graphique du taux de survie par clone, personal realization / réalisation personelle

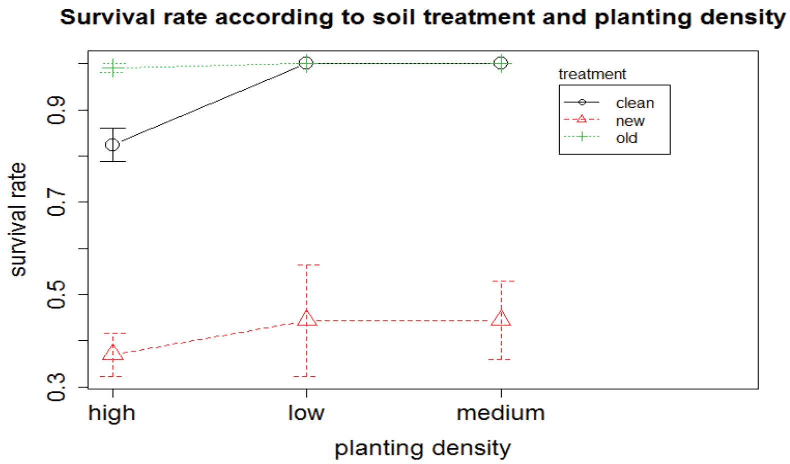


Figure 20: Graphical representation of survival rate by soil treatment and planting density / Représentation graphique du taux de survie par type de pollution et densité de plantation, personal realization / réalisation personelle

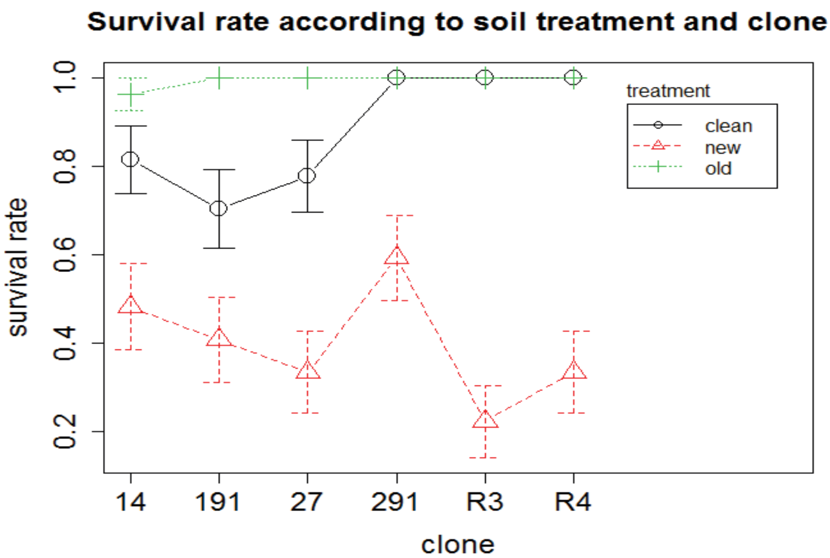


Figure 21: Graphical representation of survival rate by soil treatment and clone / Représentation graphique du taux de survie par type de pollution et clone, personal realization / réalisation personelle

IV. Results

1. Survival

At the end of the experiment, the survival rate is 76%, with 368 trees alive. At the end of the first growing season, this rate was of 92%, but between the end of this growing season and the beginning of the next one, a lot of trees have died or not recovered and so the final survival rate at the end of first year is 78%. It means that 92 % of the deaths happened during the first year, while only one tree died during the last growing season.

The analysis of deviance table on model 1 (without interactions) (*table 10*) shows a significant influence of the three factors, but the chi-square value is much higher for the soil treatment whose influence is stronger than the others. There are significant differences between all soil treatments (table 9). The survival rate in a new diesel soil contamination is very low (40%), whereas there is almost no death in a soil with old creosote contamination (99% of survival). The survival in soil exempt of contamination is of 88%, thus higher than the average. For the clones, only two comparisons are significant (*table 9*). The clone 291 has a significant better survival rate (86%) than 191 and 27 (71 % in both cases) (*figure 19*). The other clones have survival rates close to the average, with 78% for R4, 75% for 14 and 74% for R3. Even if the factor planting density is significant, differences between levels are not. Indeed, highest survival rates of 81% are reached for planting densities of 1 and 2 trees, but the planting density of 6 trees only decreases this rate to 73%.

The analysis of deviance table of model 2 (with interactions) shows a significance only for soil treatment alone (Pr<0.01). The model 3, tested because of the absence of significance between the different density levels in the first model, also presents significance only for the factor soil treatment alone (Pr<1e^{-5}). It confirms the importance of soil treatment on survival.

As the important is to know differences according to soil treatment, it is interesting to have an idea of its effect on other factors, even if it is not significant. The low importance of planting density compared to soil treatment can be seen in figure 20. The better survival rate of 291 is due to its better survival rate in soil with new diesel contamination. The non-hybrids R3 and R4 have bad survival rate for this type of soil but compensate in clean soils (*figure 21*).

Answer to hypotheses:

- The presence of fresh contaminant decreases survival rate, but not the presence of old contaminant which increases it.
- Clone 291 seems to have a good resistance, but it is not superior to all clones.
- Planting density is not of a great influence on survival rate.
- Most of the deaths happen in the first year.

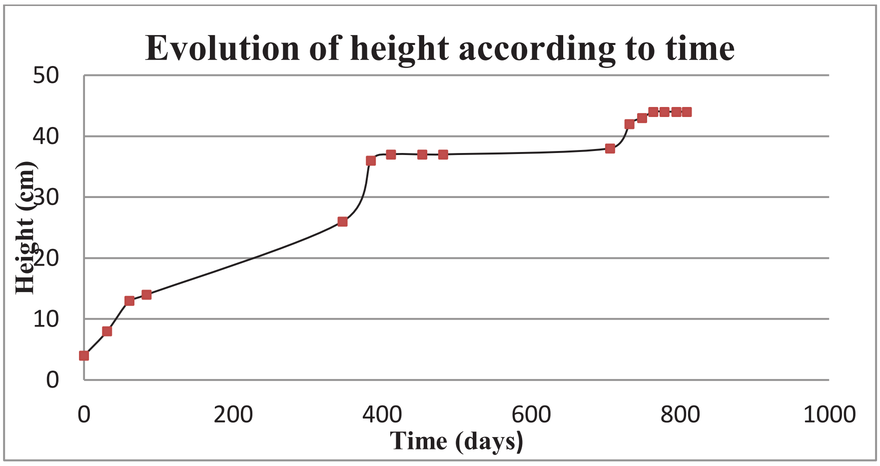


Figure 22: Changes in height of trees in the time / Evolution de la taille des arbres dans le temps, personal realization/ réalisation personelle

Table 11: Significance of factors with the Kruskal-Wallis test for RGR of 2013 / Significativité des facteurs avec les test de Kruskal-Wallis pour le RGR de 2013, personal realization/réalisation personelle

factor	chi-square value	Degree freedom	p-value
clone	27.0	5	<1e-4
density	3.8	2	>0.05
treatment	32.3	2	<1e-7

Table 12: Significance of comparisons by Wilcoxon test on RGR of 2013, Significativité des comparaisons après un test de Wilcoxon pour le RGR en 2013, personal realisation / réalisation personelle

comparison	p-value
291/14	<0.001
291/27	<0.01
291/R3	<0.001
clean/new	<1e-7
clean/old	<0.01
new/old	<0.01

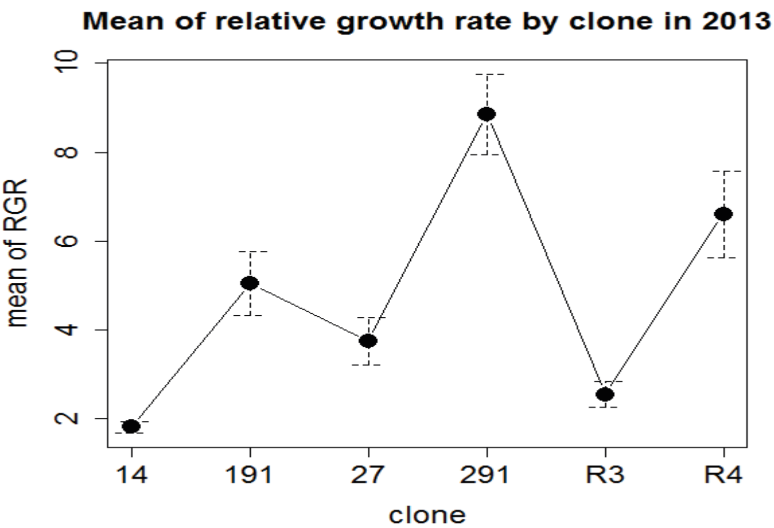


Figure 23: Mean of relative growth rate by clone in 2013 / Moyenne du RGR par clone en 2013, personal realisation / réalisation personelle

2. Height

2.1. General results

The *figure 22* shows the comportment of growth during the three years. For each growing season except 2013, height presents a logarithmic distribution, with an important growth in May and June that strongly decreases in July. The growth mean is close to zero in August and September. All trees included in the three first quartiles didn't present a growth in August in 2014 and 2015. In 2015, a longer growth period can be observed for clone 14 compared to others. A small growth can still be observed in September; however, it is unlikely to have any effect on the results. The difference in 2013 can be due to the realization of the first measurement in June, thus half of the best growth period is not taking into account this year. The absolute growth decreases with the years.

2.2. Growing season of 2013

446 trees were considered. They were measuring between 0.5 cm and 54.5 cm at the end of the growing season, with an absolute growth ranging from 0 to 48 cm. For all individuals, the relative growth rate mean is 4.7, smaller than the standard deviation of 6.2, and the median is only 1.4. That means that half of the trees had a growth of less than 40 % of the original size. It is particularly low when considered that the size of the trees was very limited at the beginning of the experiment, and that the tree that grew more increased its original size more than 40 times. Only 49% of the trees have had an absolute growth of more than 1 cm during this year (219 trees). If only trees with absolute growth of more than 1 cm are considered, the relative growth rate mean is 8.7 and the median 7.0.

The Kruskal-Wallis test gives significant p-values for the factors clone and soil treatment (*table 11*). The clone 291 produces a significant better RGR than all clones except R4 and 191, with a mean of 8.9 and a median of 8.2 (*table 12, figure 23*). Considering the mean, R4 seems interesting too with 6.6, but its median is of only 1.5. 191 presents a mean of 5.0, lower than R4, but a higher median of 2.3. The three other clones present means of less than 4.0 and medians of less than 1.5. All treatments have significant effects on RGR. The growth is better in a soil without contamination (mean of 6.6 and median of 3.9), and worse in a soil with fresh diesel contamination (mean of 2.8 and median of 1.1) (*table 12*). The soil with old contamination presents a mean of 4.5, and a median close to the previous one of 1.3.

A single descriptive interpretation of the characteristics of the individuals allows drawing the same conclusions, as there are twice as many individuals having an absolute growth of more than 1 cm in a clean soil (64%) than in a soil with new diesel contamination (34%). In the soil with old creosote contamination, there are 45% of individuals concerned.

Table 13: Significance of the factors on models without interaction for RGR at the end of 2014 – end of 2015, Significativité des facteurs pour les modèles sans intéraction pour le RGR fin 2014 – fin 2015, personal realisation / réalisation personelle

factor	Chi-square value	Degree freedom	Pr (>Chisq)
clone	359.7 - 325.0	5	<1e-15 - <1e-15
treatment	8.4 - 7.7	2	<0.05 - <0.05
density	20.9 - 29.5	2	<1e-4 - <1e-6

Table 14: Significance of the comparisons on models without interaction for RGR at the end of 2014 – end of 2015, Significativité des comparaisons pour les modèles sans intéraction pour le RGR fin 2014 – fin 2015, personal realisation / réalisation personelle

contrast	estimate	p-value
14-191	0.9 / 0.8	<0.0001 /<0.0001
14-27	0.7 / 0.8	<0.0001 /<0.0001
14-291	1.4 / 1.3	<0.0001 /<0.0001
14-R4	1.2 / 1.0	<0.0001 /<0.0001
191-291	0.5 / 0.4	<0.001 /<0.01
191-R3	-0.8 / -0.8	<0.0001 /<0.0001
27-291	0.6 / 0.5	<0.0001 /<0.001
27-R3	-0.6 / -0.8	<0.0001 /<0.0001
27-R4	0.4 / -	<0.01 / >0.05
291-R3	-1.2 / -1.2	<0.0001 /<0.0001
R3-R4	1.1 / 1.0	<0.0001 /<0.0001
high-low	0.4 / 0.4	<0.001 /<0.001
high-medium	0.2 / 0.2	<0.05 /<0.01
clean-old	-0.2 / -0.2	<0.05 /<0.05

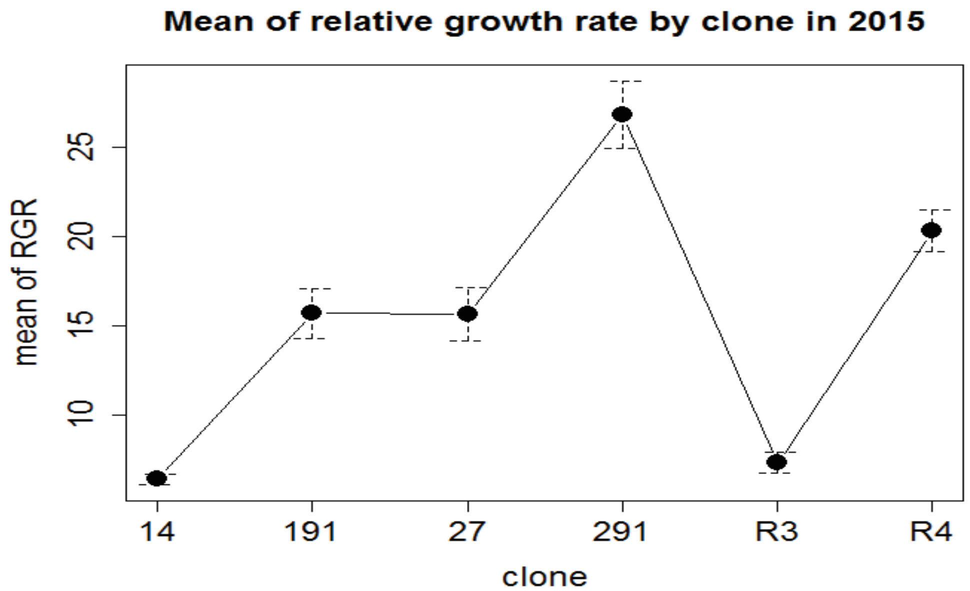


Figure 24: Mean of relative growth rate by clone at the end of 2015 / Moyenne du taux de croissance relative par clone fin 2015, personal realisation / réalisation personelle

2.3. End of 2014 and 2015

371 individuals are concerned at the end of 2014, and 368 at the end of 2015. At the end of 2014 the mean of RGR is 12.9, and the median 10. The RGR varies from 1 to 66, but three quarters of trees have a RGR smaller than 17. The value 1 is due to the only one individual who didn't have a significant growth. At the end of 2015, the mean of RGR is 15.7, and the median 12.8. It ranges from 2.6 to 74. Three quarters of the trees have a RGR of less than 20.

At the end of 2014, clone 291 presents a RGR mean of 22.7, followed by R4 with a RGR mean of 18.0. As for the previous year, clones 191 and 27 follow with respectively 13.4 and 11.1. The clones R3 and 14 have only 6.3 and 4.8. The highest RGR mean is for a planting density of 1 tree, with 15.7, whereas it is 12.9 and 12.5 for 2 and 6 trees. However, the median difference is only of 1 between each category. The mean for soil without contamination is 15.4 whereas it is around 11.4 for the other two soil treatments. The soil treatments are better distinguished with their median: 12.0 for clean soil, 10.0 for old contamination and 8.5 for new contamination. The medians are still lower than the means in general but the order established by medians is the same this time, what was not the case in 2013.

At the end of 2015, clone 291 still appears as the best one (mean of 26.8, median of 21.4), and the second one is still R4 (mean of 20.3, median of 18.9). Then come 191 and 27 (respectively means of 15.7 and 15.6 and medians of 13.7 and 13.1, so very close). The two worst are R3 and 14 (means of 7.3 and 6.4, medians of 5.9 and 6.2). The planting density of one tree is the better (mean of 19.6, median of 13.9), followed by 2 trees (mean of 16.1, median of 13.3) and 6 trees (mean of 14.8, median of 11.7). However, medians are very close between 1 and 2 trees. The best growth is in a clean soil (mean 20.0, median14.7), then almost the same for new diesel contamination and old creosote contamination (means of 13.7 and 13.5, medians of 11.1 and 11.4).

For models without interaction (1 and 2), the three factors have a significant effect this time, but the effect is more important for clone and planting density than soil treatment (*table 13*). The effect of planting density is more significant at the end of 2015 than at the end of 2014. In both cases, the use of clone 14 decreases significantly the RGR than all clones except R3 (table *14, figure 24*). R3 reduces the RGR compared to 191, 27, R4 and 291. 291 increases this rate compared to all except R4. R4 is better compared to 14, R3 and 27 in 2014, but only better compared to 14 and R3 in 2015. Clone 27 has increased in interest. For density plantation, only the planting density of 6 trees can be significantly compared to others, and is worst for the RGR (*table 14*). Only the soil with no contamination and the one with old creosote contamination can be significantly compared. The old contamination has a negative impact on growth (*table 14*).

Table 15: Significance of factors for the model 3 on RGR with interactions at the end of 2015 / Significativité des facteurs pour le modèle 3 sur RGR avec intéractions fin 2015, personal realisation / réalisation personelle

factor	chisquare value	df	Pr (>Chisq)
clone:treatment	30.7	10	<0.001
density	26.7	2	<1e-5

Table 16: Significance of comparisons for the model 3 on RGR with interactions at the end of 2015 / Significativité des comparaisons pour le modèle 3 sur RGR avec intéractions fin 2015, personal realisation / réalisation personelle

soil	contrast	estimate	p-value
clean	14-191	0.59	<0.01
clean	14-27	1.02	<0.0001
clean	14-291	1.55	<0.0001
clean	14-R4	1.07	<0.0001
clean	191-291	0.97	<0.01
clean	27-R3	-0.82	<0.01
clean	291-R3	-1.35	<0.0001
clean	R3-R4	0.87	<0.01
soil	contrast	estimate	p-value
old	14-191	1.00	<0.0001
old	14-291	0.97	<0.0001
old	14-R4	1.11	<0.0001
old	191-R3	-0.89	<0.01
old	291-R3	-0.87	<0.01
old	R3-R4	1.01	<0.001

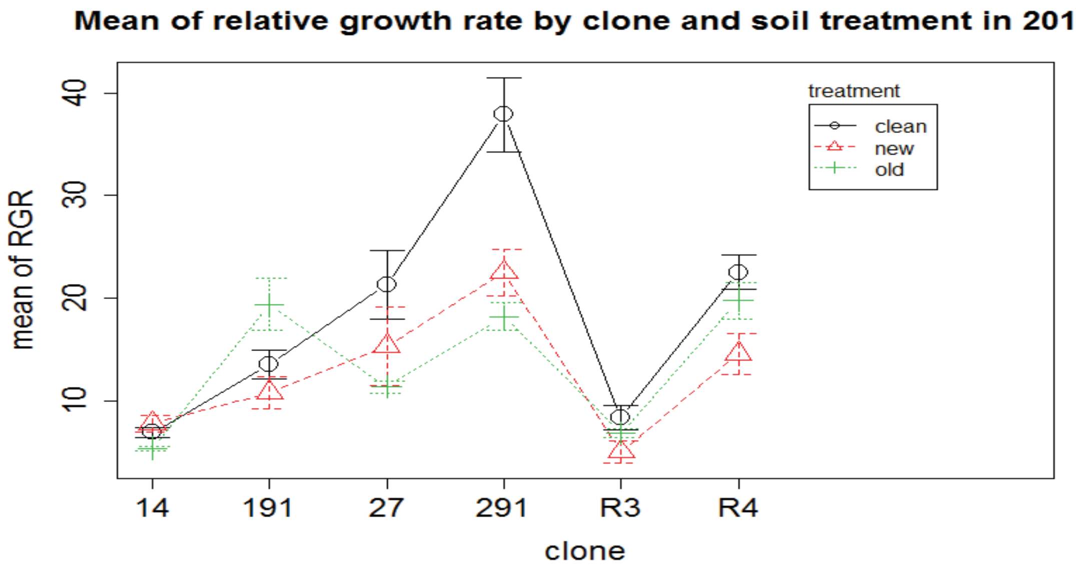


Figure 25: Mean of relative growth rate by clone and soil treatment at the end of 2015 / Moyenne du taux de croissance relative par clones et type de pollution fin 2015, personal realisation / réalisation personelle

At the end of 2015, according to the Wald test on the model 3 with interactions, the interaction between clone and soil treatment influences significantly the RGR (*table 15*). It is then not useful to compare separately clone and treatment due to the type of the test. Density is significant too, but alone. However, the different planting densities cannot be compared then.

In a soil without contamination, clone 291 appears as the best, as it has a significant superior RGR than 14,191 and R3. However, it is not significantly superior to 27 in this case. R4 is only significantly superior to 14 and R3. These two clones are inferior in general, as 14 is inferior to all clones except R3 and R3 inferior to R4, 291 and 27. In this case, 27 can be considered at the same level than R4 (*table 16, figure 25*).

In a soil with old contamination, 291 can only be compared to the two trees with less important RGR (14 and R3), as 191 and R4 only to 14. Differences are not as important as in clean soil (*table 16, figure 25*).

No comparisons are significant for new soil contamination. The graphic shows than the main effects on clone are the same that with an old contamination but clone 191 is less efficient. In general, the RGR mean of 14 and R3 doesn't vary a lot with the contamination type, while it varies in an important way for 291.

A general classification of interactions in groups can finally be established (*annex 18*). General conclusions are that clone R3 and 14 never belong to the first and second group, while clone 291 never belongs to the fourth group. The clone 14 presents a low RGR in all treatments compared to others, as R3. However the variability of good clones can be important according to treatments.

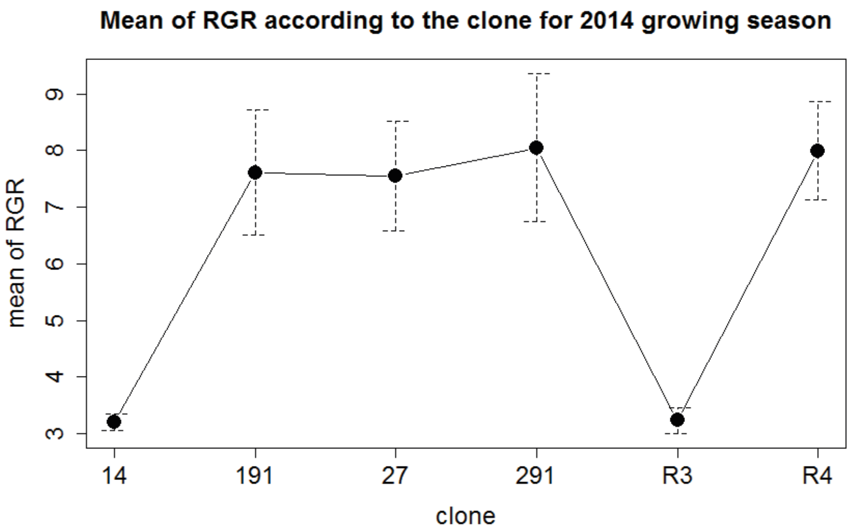


Figure 26: Mean of relative growth rate by clone for 2014 growing season / Moyenne du taux de croissance relative par clones et type de pollution pour la saison de croissance de 2014, personal realisation / réalisation personelle

Table 17: Significance of factors according to Kruskal-Wallis test on RGR for 2015 growing season, Siginificativité des facteurs pour le RGR pour la saison de croissance de 2015 après un test de Kruskal-Wallis, personal realisation / réalisation personelle

factor	chi-square value	Degrees freedom	p-value
clone	55.1	5	<1e-9
density	22.0	2	<1e-4
treatment	1.4	2	>0.05

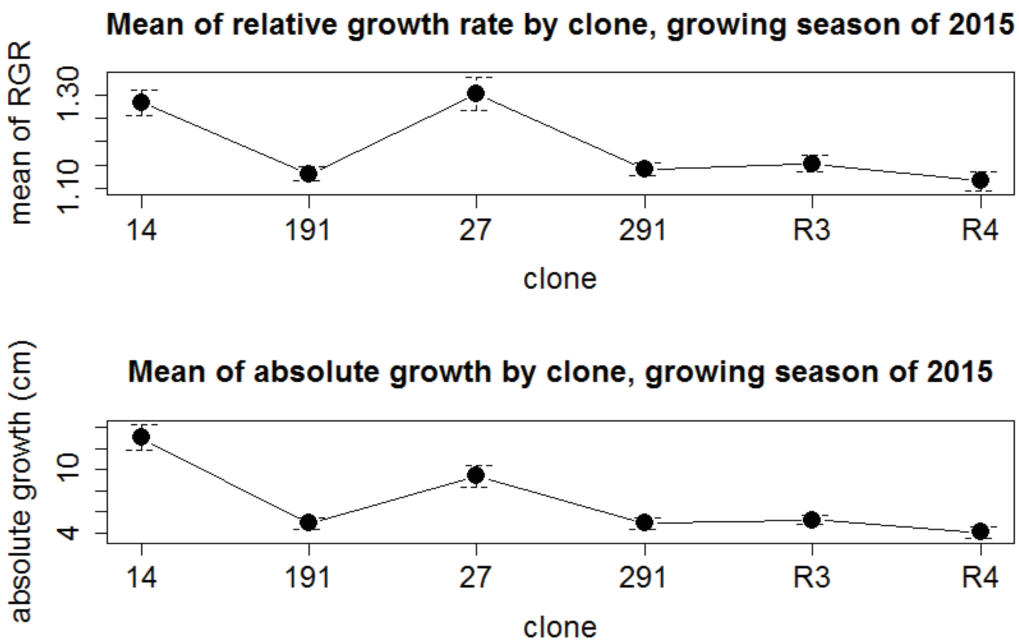


Figure 27: Mean of relative growth rate and absolute growth by clone for 2015 growing season / Moyenne du taux de croissance relative et de la croissance relative par clone pour la saison 2015, personal realisation / réalisation personelle

2.4. Growth during growing season of 2014

The results are similar to the general results. A Kruskal-Wallis test does not allow to conclude on the influence of soil treatment and planting density for this growing season, but the clone is significant (chi-squared=13.8, df=2, p-value<0.05). The graphical observation of the clone for the relative growth rate is similar to the general one, but the differences between the best clones are less relevant: only clones 14 and R3 have a sensibly less important RGR (*figure 26*).

2.5. Growth during growing season of 2015

The Kruskal-Wallis test shows indeed the absence of influence of the treatment (*table 17*). The comparisons show similar results to general analysis for planting density, but not for clone. 14 is significantly better than every clone except 27 (p-value<1e-4 at least), and the same happens for 27 (p-value<0.05 at least). An important observation is that the RGR is strongly correlated to the absolute growth in this case, what was not the case before (Spearman, rho=0.88, p-value<1e-15). This is clearly visible on the graphic (*figure 27*).

Response to hypotheses:

- The growth is reduced in a soil with old contamination compared to no contamination, but nothing significant can be said for new contamination, except that it is worse in this case for the first year.
- A planting density of 6 trees reduces the growth compared to 1 and 2 trees, but only from the second year.
- Differences can be determined between clones, but no clone is significantly better than all the others. Clone 291 is interesting, as clone R4, 27 and 191 in some conditions. It means than even a European aspen is among the best. However, some changes happen in the last year and could drive to a change in clone order in the future.
- Only the interaction of clone with soil treatment is significant. The soil contamination decreases the differences between clones, but the best is still 291 and the worst are still 14 and R3 in general.

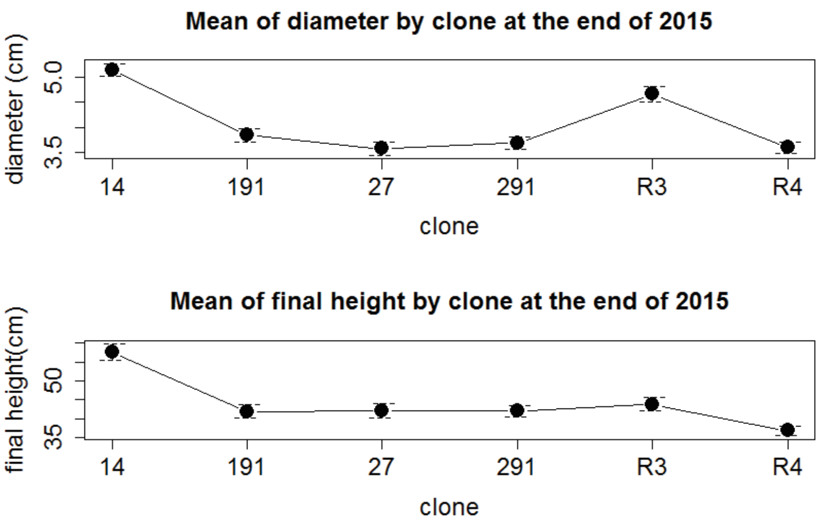


Figure 28: Mean of diameter and final height by clone at the end of 2015 growing season / Moyenne du diamètre et de la taille finale par clone fin 2015, personal realisation / réalisation personelle

Table 19: Significance of factors for model 1 without interactions for root sucker presence / Significativité des différents facteurs pour le modèle 1 sans intéraction pour la présence de tiges par drageonnement, personal realisation /

factors	Chi-square	df	Pr(>Chisq)
clone	21.97	5	<0.001
density	14.37	2	<0.001
treatment	2.99	2	>0.05

Table 18: Significance of comparisons for model 1 without interactions for root sucker presence / Significativité des comparaisons pour le modèle 1 sans intéraction pour la présence de tiges par drageonnement, personal realisation / réalisation personelle

contrast	estimate	p-value
14 - R4	-1.92	<0.05
27 - R4	-1.92	<0.05
291 - R4	-2.83	<0.01
R3 - R4	-2.83	<0.01
High-low	2.19	<0.001
Low-medium	-1.43	<0.05

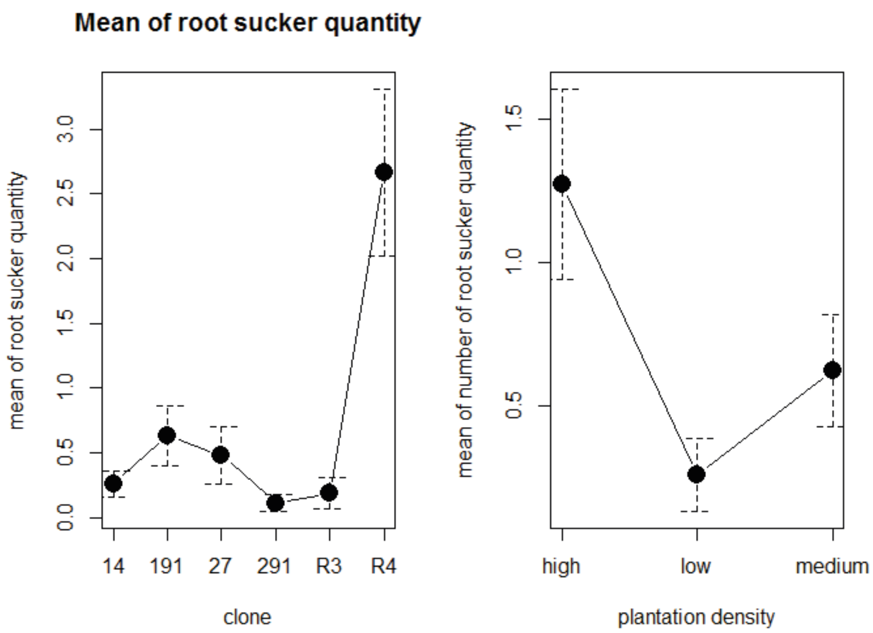


Figure 29: Mean of root sucker number by clone and planting density at the end of 2015 / Moyenne du nombre de tiges par drageonement par clone et densité de plantation fin 2015, personal realisation / réalisation personelle

3. Diameter

It is first important to know if the non-consideration of the original diameter doesn't lead to confusions. The graphics representing diameter according to general RGR and then general relative growth rate show that the diameter is not correlated with RGR (Spearman, rho=-0.14, p-value<0.01) whereas diameter and final height have a good correlation (Pearson, cor=0.83, p-value<1e-15). Thus, diameter is linked with absolute growth and cannot be analyzed with a strong reliability without knowing the original diameter. However, it can be interesting to see where are the differences between the final height and the diameter, to know in which cases the diameter is more independent of the height.

The only situation in which differences can be graphically observed is when diameter is observed according to the clones. The global disposition is the same, but in the case of clone R3, the diameter seems to increase in a more important way than the height (*figure 28*).

4. Root suckers

At the end of the experiment, there is at least one root sucker in 45 buckets, what represents only 28% of the buckets. According to the analysis of the deviance table of model 1 (without interactions), clone and density significantly influence the presence of root suckers, but not the treatment (*table 18*). This influence comes from the clone R4, which produces significantly root sucker in more cases than all the clones except the 191(*table 19*). Indeed R4 presents root suckers in 59 % of the buckets, and 191 presents root suckers in 41 % of the buckets, while for the others it varies between 11 % (R3 and 291) and 22 % (14 and 27). For density, the initial presence of 6 trees or 2 trees per bucket favors the presence of root suckers when compared to 1 tree per bucket. There are 44% of buckets with root suckers for a planting density of 6 trees, 28 % for 2 trees and 11 % for one tree.

There is no influence of interaction between factors showed by deviance table on model 2 (with interaction), but deviance table on model 3 "event~clone*density" confirms the influence of clone (chisq=15.4, Pr<0.01) and density (chisq=9.5, Pr<0.01) alone. However, no comparisons are then significant.

The number of root suckers goes from 0 to 12. 47% of the individuals having root suckers present more than 2, which represents 13% of the total individuals. Root suckers presence is thus a limited phenomenon. The median of root sucker number is 2 for clone R4, whereas it is 0 for all the other clones. It also presents a higher number of root suckers in average (2.7) (*figure 29*). The median is 0 for all densities, and means have only small variations (from 0.3 to 1.3), so density doesn't seem to be as significant for the number of root suckers as for their presence.

Response to hypotheses:

- The presence of root suckers is significantly more important for a planting density of 6 or 2 trees compared to 1 tree.
- Clone R4 is the best for root sucker presence significantly except when compared to 191. It seems also better for root sucker number.

Table 20: Significance of factors for Fv/Fm - PI with Kruskal-Wallis test in 2015 / Significativité des différents facteurs pour Fv/Fm - PI avec le test de Kruskal-Wallis en 2015, personal realisation / réalisation personelle

factor	Chi-square	df	p-value
Clone	26.45 - 95.2	5	<1e-4 - <1e-15
Density	1.14 - 0.078	2	>0.05 - >0.05
Treatment	2.35 - 4.94	2	>0.05 - >0.05

Table 21: Significance of comparisons for Fv/Fm and PI with Kruskal-Wallis test in 2015 / Significativité des comparaisons pour Fv/Fm et PI avec le test de Kruskal-Wallis en 2015, personal realisation / réalisation personelle

comparison	p-value on Fv/Fm	p-value on Pi
R3-191	<0.05	<1e-5
R3-27	<0.05	
R3-291	<1e-4	<1e-4
R3-R4	<1.01	<1e-7
291-14	<0.05	<1e-7
14-191		<1e-9
14-R4		<1e-12
27-191		<0.001
291-27		<0.01
R4-27		<1e-4

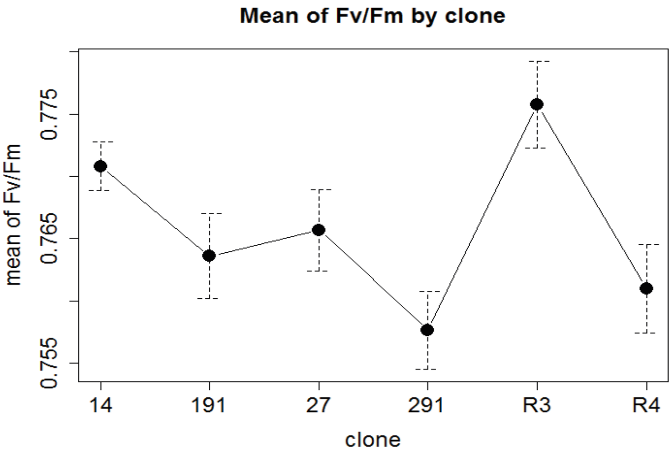


Figure 30: Mean of Fv/Fm by clone in 2015 / Moyenne de Fv/Fm par clone en 2015

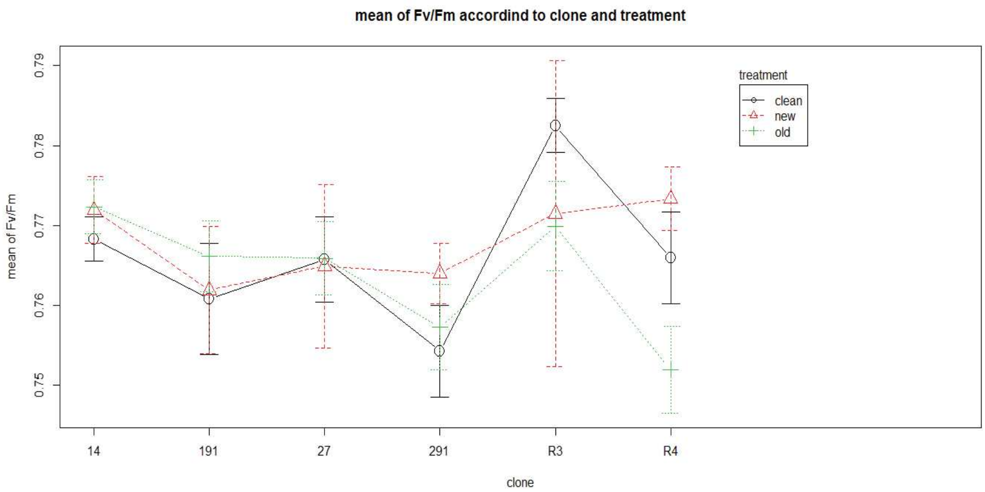


Figure 31: Mean of Fv/Fm by clone and soil treatment in 2015 / Moyenne de Fv/Fm par clone et type de contamination en 2015, personal realisation / réalisation personelle

5. Chlorophyll fluorescence

367 trees are concerned, what means all trees without the dead ones and an extra one who lose its leaves in July. The value of maximum quantum yield of PSII photochemistry Fv/Fm, is always lower than 0.85, which is the value of perfect health. However, plants are considered to be healthy from a value of 0.75 (Maxwell and Johnson, 2000). There are 292 trees for which the value is at least 0.75, so 80% of the trees are in relatively good health. The lowest value is 0.65, but an observation of the histogram shows that only isolated cases have values lower than 0.7. The distribution is not normal due to the lowest values.

The performance index is representative of the vitality of the sample; it is so interesting to see its repartition. Most of the trees have a performance index between 0 and 3: indeed only 4 individuals have a PI higher than 3. For both variables, an influence seems to appear only concerning clone.

According to the Kruskal-Wallis tests, only the variable clone has a significant p-value for the variable Fv/Fm (table 20). It is the same for the variable PI. For the variable Fv/Fm, the clone R3 presents a better value than all the others except 14 (*table 21, figure 30*). The value of the clone 14 is significantly superior only to 291. For the variable PI, the main general distribution can be seen, but this time two groups can be formed. The group with the better means contains 14, 27 and R3, while the other is composed of 191, 291 and R4. For planting densities, means of Fv/Fm varies from 0.761 to 0.766, and for soil treatment from 0.763 to 0.766.

When comparing the data of the three months, we can see than the values of Fv/Fm and PI are lower in July and August than in June. Moderate correlation between the values of each month (between 0.4 and 0.79) can be found in both cases, except between June and August in the case of Fv/Fm values (correlation under 0.4).

Comparisons by soil treatment have been realized, even if it is not significant (*figure 31*). The values of Fv/Fm are very close for each treatment. The soil without contamination only seems to increase the contrasts between the best, R3, and the worst, 291. Clone R4 has a particular comportment. Differences can’t be observed for PI.

The analysis of the data of June shows slight difference. Clone was still the only factor having an influence, but the significant differences between clones changed a little. The clone 27 is this time the one with the lowest value of Fv/Fm compared to R3, R4, and 191. Differences are not so important except for this clone, and R4 is this time among the best. There is no clone under the value 0.75 of good state. For the performance index, 14, R3 and 27 are still significantly the best. However, only clone 14 is graphically really better.

Figure 33: Example of degradation / Exemple de dégradation, personal source / source personelle

Figure 32: Example of degradation / Exemple de dégradation, personal source / source personelle

Table 22: Significance of factors for leave number with Kruskal-Wallis test / Significativité des différents facteurs pour le nombre de feuilles avec le test de Kruskal-Wallis

factor	Chi-square	Degrees freedom	p-value
clone	37.1	5	<1e-6
density	57.1	2	<1e-12
treatment	17.8	2	<0.001

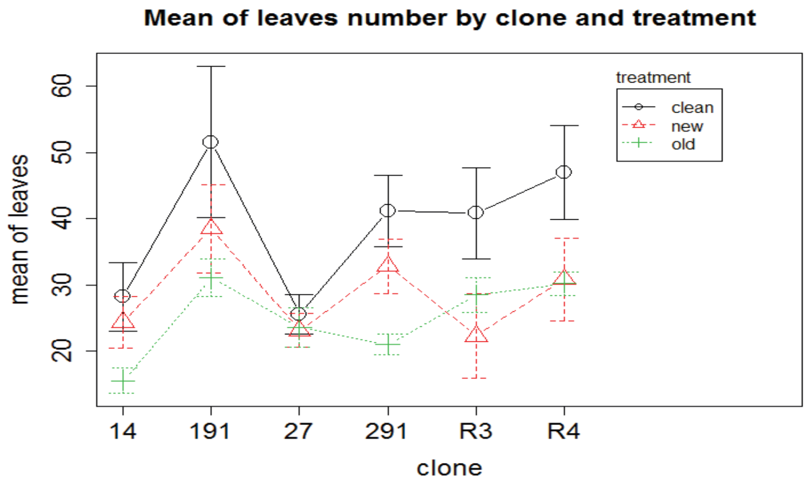


Figure 34: Mean of leaves number by clone and soil treatment / Moyenne du nombre de feuilles par clone et type de pollution, personal realisation / réalisation personelle

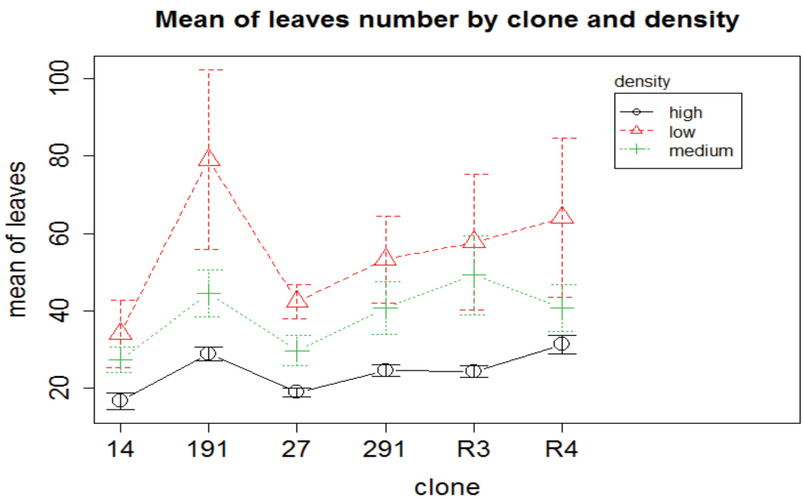


Figure 35: Mean of leaves number by clone and planting density / Moyenne du nombre de feuilles par clone et densité de plantation, personal realisation / réalisation personelle

6. Conditions of leaves

Leaves of trees were touched by insect and fungi degradations since the end of June, with the most important effects on leave surface degradation in July and August, before a progressive recovery (figures 32 and 33). For most of the trees, a quarter maximum of the plant was touched: 78% of the trees presented less than a quarter of their foliage touched in July, and 74% in August. These degradations were thus limited. However, it could have been a source of stress for the trees, and some dried leaves could be due to contamination. It is denied as there is no main difference between the different treatments, and even less foliage degradation in the case of new diesel contamination. In the worst moment, clone 14 was the most touched with more than a quarter of its foliage touched in 52% of the buckets, and a high planting density double the percentage of degradations of more than one quarter of the foliage (from 14% for 1 tree and 16% for 2 trees to 35% for 6 trees). However, clone 14 is a clone with good efficiency for photosynthesis.

For general leaves number, the Kruskal-Wallis test gives a significant influence of all factors (*table 22*). All densities are significantly different (6 trees/1tree: <1e-9, 6 trees/2trees: <1e-6, 2 trees/1tree: <0.05). For treatment, only clean soil and old contamination are significantly different (p-value<1e-4). For clone, 14 is significantly different for all except 27 (p-value<0.05 at least) and 27 is significantly different of 191 and R4 (p-value<0.01). The effect of density and treatment are expected: the number of leaves increases when density decreases, and it is higher when there is no contamination. Clones 14 and 27 have a less important number of leaves than others (*figures 34 and 35*).

The observation of the graphics of leaves number according to RGR and growth in 2015 shows very bad correlations. The growth is thus not dependent of the number of leaves.

Figure 36: Soil with new diesel contamination / Sol contaminé au diesel (récent), personal source / source personelle

Figure 37: Soil with old creosote / Sol contaminé à la créosote (ancien), personal source / source personelle

Figure 38: Soil without contamination / Sol sans pollution, personal source / source personelle

V. Discussion

1. Interpretation of the results

1.1. Transplant shock

The transplant shock or transplant stress is an explanation to negative impacts on growth and survival on early stages when trees are planted into a new environment. The symptoms are generally a decrease in growth rate compared to naturally regenerated trees of this new environment, leaf abscission and mortality. It is associated to the acclimation of the seedlings to new environmental conditions and are due to within-plant characteristics, as the plant is acclimated to its previous environment, can have a deficiency in carbohydrates and nutrient reserves and has a generally too high root/shoot ratio. Other factors are environmental stress, like drought, frost or mechanical damage, and interactions with surrounding environment linked with physical and nutritional conditions of the soil and a possible competition with surrounding vegetation (Close et al., 2004).

In this experiment, the young trees were transferred from indoor to outdoor conditions. The plantation in buckets and presence of the greenhouse protection diminishes some risks as frost and wind damages, as competition with vegetation. However, the important death and the 51% of trees that didn't have a significant growth the first year can be related to this transplant shock. Indeed, trees were acclimated to the indoor conditions they were in during five months. As they grew in boxes, they were also acclimated to high stand density and had probably developed a shade tolerant foliage, things that changed in the experiment. They could have experimented drought, as watering decreases. Furthermore, the soil in which they grew until June 2013 was appropriated for growing conditions, whereas the soil in which they were transplanted was poorer, with rocks and not cultivated. This has an important impact as rocky conditions make it difficult for the roots to enter in contact with the soil, and the absence of cultivation practices makes it difficult for roots to extend (Close et al., 2004). Finally, and maybe most important, some of the soils were polluted, offering very different conditions to these trees. It also means than the transplant shock could have been different according to the category of soil treatment. And indeed, it was found that the treatment was significantly influent for survival. This is confirmed by the more important number of significant growth in the clean soil than the others the first year. The differences observed between clones in survival could be due to different capacities of acclimation, as it is common between sub-populations (Close et al., 2004). Finally, survival seems to be principally affected by transplant shock, which is totally overcome in third year. This effect could be decreased by an acclimation of the plant before establishment.

1.2. Soil and contamination

In the results of survival, it was found that old contamination improves survival rate, what is not conform to hypotheses, particularly as fresh contamination actually reduces survival. To understand it, differences observed in soil characteristics according to the contamination need to be evidenced (*figure 36, 37 and 38*). In the buckets with old creosote contamination, the soil is darker, with the presence of moss the third year compared to soils with no contamination. It is less dry and more compact and elastic. The size of the particles is thinner. Clean soil takes a more important volume, dries quicker, is exempt of moss most of the time. The soil with new contamination is in an alternative state, drying easily but darker than the soil without contamination. The soil comes from the same general area. The possibility that the site where contamination occurred was already in a different state before contamination is denied by the fact that soil for clean and new contamination

treatments were taken exactly in the same location and still a difference can be seen. More certainly, differences are due to contamination. The effect of oil on clay and sandy soils has been studied (Khamehchiyan et al., 2006). An increase in crude oil reduces the maximum dry density and optimum water content. There is also a reduction in permeability and strength. That would explain that the soil is less dry in buckets with old contamination.

The fact that survival in clean soil is less important than in old soil contamination could be due to the best retention in water of the contaminated soil due to the changes in properties. As the contaminants are hydrocarbons, there are also organic and could improve the organic matter rate, what is another explanation. The thinner texture could be a clue too. However, the growth is still better in soil without contamination compared to old contamination, so finally there is a negative effect of this contamination for growth. The best explanation is thus that the better general characteristics of the old contaminated soil for growth have an immediate effect, decreasing the importance of transplant shock, but in the long term the effect of the contaminant itself prevails.

1.3. Trees and contamination

The pollutants are before anything else a factor of stress for the plant. The impact of oil pollution on plants is linked with the type and amount of oil involved, the degree of weathering, the time of year and the species and age of plants concerned. Light oils cause acute injuries (oils with benzene, toluene and xylene) and heavy oils cause chronic injuries (oils with PAH). Fresh crude oil is more toxic than old oil. Oil penetrates in the plant by injuries and then moves into the intercellular spaces. Oil can provoke physical interference with gaseous exchange if stomata are blocked, a loss of energy in heat due to a useless increase in oxygen uptake, penetration and damage in mitochondria, and inhibition of important mechanisms of transport (Baker, 1970). The less important survival in soil with new contamination can be explained by these effects, as the lowest growth in the first year. The aging or weathering, caused by the sequestration of the compounds bound to soils particles, decreases the toxicity (Tang et al., 2011). It could explain the absence of significance of soil with new contamination in the following years, but this is more likely due to the decrease in trees considered for growth due to the important quantity of dead trees in this case. The aging phenomenon also means that the toxicity has diminished in soil with old contamination, what can explain the better survival and growth compared to new diesel contamination in 2013. Another observation is that the general effect of soil contamination on growth decreases with the time.

1.4. Differences between clones according to soil treatment

For survival, a new soil contamination seems to increase the differences between clones. For height, it is different; differences are increased in a soil without contamination. Only clones 14 and R3, the worst for RGR, have not an important variation. These two different comportments could be explained because the survival of the clones is good in general and so important differences are seen only in the most stressful conditions, where an actual risk of death is present. The height seems more different between clone in general, and in that case is better expressed in optimal conditions (without contamination).

1.5. Stress conditions of the plant

The trees with best photosynthesis efficiency, thus a best Fv/Fm value, were supposed to present a better growth as biomass production is linked with photosynthesis efficiency. Furthermore, trees with better internal force to resist outside constraints (best PI) should be more able to resist stress conditions and thus to allocate their resources for growth. In 2015, the best clones for RGR were indeed 14 and 27, which are part of the better for the fluorescence values.

However, clone 27 was not as good during the measurement of June. The importance of the fluorescence values for clone R3 cannot be explained by an important growth in height for the RGR and the absolute growth. However, R3 was the clone for which the diameter was less correlated with the final height: it could have still developed better than others, but in diameter. Another possibility for R3 is that the photosynthesis efficiency is not used for the growth of the shoots, but of the roots. Clones 291 and R4 have the less important values, and indeed presented less growth this year.

The reasons for the more important stress of clones 291, R4 and 191 are not obvious. It could be that they are less able to resist outside constraints, but they were no more touched by insect and fungi degradations than others. A hypothesis is they are effectively remediating the soil compared to others. In that case, they should be the most interesting clones, as it is the final aim. However, the absence of significant influence of the factor treatment and the absence of significant differences between clones in a soil with fresh contamination makes this hypothesis not very realistic. It could only be that they are less efficient compared to other clones.

Oil is supposed to inhibit gaseous exchange and to create damage in the mitochondria (Baker, 1970). Previous experiments have shown than photosynthesis rate was influenced in particular by diesel contamination and stomatal conductance inhibited for the majority of species. However, there was no drastic decrease for most poplar clones. In particular, the chlorophyll fluorescence parameters were less impacted than real photosynthesis in polluted conditions. It is linked with a higher disturbance in the carboxylation part of photosynthesis, and not in the thylakoids. The structure of the thylakoid is indeed very stable and so the electron flow is not affected through the photosystem II (Pajevik et al., 2009). It seems that this phenomenon is happening here. Thus, the chlorophyll fluorescence values could be efficient to indicate a growth and a stress linked with some insect degradation or weather conditions, but not linked to pollution by organic contaminants. It could be also due to a smaller impact on photosynthesis by organic oily contaminants than heavy metals or other contaminants.

The decrease in Fv/Fm and Pi values in July and August compared to June could be linked with the degradations of the leaves observed these two months on the plants, what could be a source of stress. It also shows than these values were higher when the growth was higher (in June), and confirms the theory that the best the values are, the best is the growth.

Leaves number seems to be an indicator of density and soil pollution conditions, but it is limited to determine the degree of contamination. It has been proved to be efficient as indicator of resistance to salt conditions in another experiment of the unit (Vaario et al., 2011), but here the clones with less leaves had also a better growth and a better photosynthesis efficiency. Leaf area seems more promising, as the size of the leaves is different according to clone.

1.6. Case of planting density

The effect of planting density is not as important as expected, and does not present interactions with other factors. Its importance seems however to increase with the time. An interesting element is that if high density reduces growth, it also improves the presence of root suckers. That means that there is still a remaining question about density: if growth is reduced but there are more trees due to the original plantation density and the root suckers, finally the biomass could be still improved and so the decontamination better. If growth is finally a relevant indication, high planting densities of 6 trees per buckets are not advised.

The root suckers generally appear in the first three years for poplars, as the specific roots necessary have an important growth during the first year (from 1 m to 3 m in real conditions) (Louisiana Pacific Canada ltée and CERFO, 2002). It is thus supposed than the presence and

number of root suckers should not increase a lot if no additional stress appears. The root suckers have most of the time a very fast growth in the first years, however the weakest individuals are quickly eliminated (until 80% in the first 5 years) (Louisiana Pacific Canada ltée and CERFO, 2002), they could thus still disappear. Another interesting element is that density and particularly root suckers density is not supposed to have a very important effect on the growth in height of intolerant species as aspens, that doesn't have the ability to wait for better conditions to develop (Louisiana Pacific Canada ltée and CERFO, 2002).

1.7. Choosing the best clone

The different measures and analyses realized had as an aim to choose the best clone and density for phytoremediation purposes. Results for density have already been discussed. For survival, the best clone is 291 while 191 and 27 are not so good in general. However it is verified only for new soil contamination. For old contamination, there is no need is selecting a specific clone for survival as there is an important general survival rate. Without contamination, European clones are very good. For root suckers production, R4 is the best and 191 is interesting, but it could decrease in time.

For height, two clones that cannot be chosen are 14 and R3 according to the general RGR. Without contamination, 291 is the best followed by R4 and 27. In new contamination, the same can be observed, but 191 must this time be considered with R4 and 27. However, it is not significantly confirmed in this case. With old contamination, 291, 191 and R4 have a better growth. However, changes are appearing in the third year. Clone 14, which has one of the best RGR in 2015, is also the biggest clone according to height mean and absolute growth mean. Clone 27, the other better clone of 2015, enter also the better clones in the general results of the end of 2015. 14, 27 and R3 have also the best photosynthesis efficiency, and are thus more susceptible to increase their biomass production. 14 and 27 could thus become the best clones, except in new soil contamination for 27 due to the decrease in survival rate. That means that at least one or two years more are necessary to confirm or not the general results. In general, 3 to 4 years could be enough for clonal selection on phenotype for aspen (Stener and Karlsson, 2004), but in the field and without additional stress due to contaminants. Furthermore, to consider possible cankers apparition and for specific industrial value determination, selection should not be made before 10-15 years old in the field (Stener and Karlsson, 2004). Field trials of 10 years are thus expected for reliable results.

The best clones for establishment are 291, followed by R4, as even if 27 and 191 are good in growth they are not good for survival rate. For development, more research is needed.

1.8. Hybrid and European

One hypothesis rested on the better growth of hybrid aspen. If the European clone R3 is indeed not very good for growth, R4 is among the best. It is not possible to conclude on the superiority of hybrid aspens with so few clones and time.

2. Critical analysis of the experiment

2.1. Critics on the creation of the experiment

It was particularly interesting to create three replications for this experiment. Indeed, due to the low survival rate of the trees in the soil with fresh diesel contamination, the chances to still have one sample of each combination in the analysis of height is higher. If the combination doesn't exist anymore in any of the replications, it only confirms the influence of the factors concerned on

survival rate. However, due to the proximity of the replications it is not very efficient to take into account possible variations of the conditions. For this aim, it could have been interesting to implement these replications in different greenhouses. The presence of other experiments with aspens in this greenhouse could furthermore have increased the degradation by specific insects.

The effect of pollutants must be nuanced as their migration is limited by the buckets limits. Organic pollutants are supposed to bind with the soil particles, however the analyses of the contamination of Somerharju area have shown that these contaminants can go deeply in the ground the first years, what was not possible for the diesel in that experiment. The main problem is that the precise level of contamination is not known, and the characteristics used to describe the contamination are not the same for both contaminants. Diesel is here described by the percentage of general contamination by oil, while creosote is described by an amount of hydrocarbons and particularly PAH found in some general analyses in the original site. Furthermore, contaminants are rarely uniformly distributed in real cases (Conesa et al., 2011), so the exact contamination of the soil with creosote used is not known. Diesel and creosote are both oily compounds, however they have different properties. Differences could thus be explained by the product type or by the age of pollution, and it cannot be confirmed for sure.

Planting density has not been considered in the main studies linked with contamination so far, and one of the main reasons to carry out this experiment was to determine its possible influence compared to the usual factors pollution and clone. However, in real field cases, planting density requires special consideration that could be more important that any result found here. Erosion prevention, allowance of an optimum water uptake as trees are not watered in most of field treatments and faster canopy closure are important elements to consider (Zalesny and Bauer, 2007). Closer trees efficient at the beginning could still be removed when they begin to reduce growth due to their development, but it would increase costs. General tree spacing for populous varies from 2.1 X 3.0 m to 4.0 X 4.0 m (Zalesny and Bauer, 2007). The densities used in that experiment are thus linked with basic research, and cannot be applied directly on the field.

2.2. Critics on the measurements

Measurements were realized by different trainees every year and the soil is not perfectly uniform at the base of the tree. Thus, some mistakes linked with the operator exist. It is increased by the fact that a part of the trees did not grow straight. A lack of information exchange on the previous measurements led to a change in the accuracy between year and the determination of measurement dates by trainees presence conduced to different dates for end of measurements. However, due to the very small growth of September, there should be no impact on the results.

Only survival and height measurements were carried out every year, and thus are really reliable. Fluorescence measurements were realized only one year, so results must be considered carefully as they could be due to particular weather conditions. Each measure could also reflect the conditions of the day, and it was not possible to realize very frequent measurements due to the availability of the fluorometer. As the main aim was to compare the different combinations between themselves and not the evolution in the time, it was however not really problematic for this experiment. The lack of clips conduced to the realization of the measurements in several time. Therefore, the complete protocol had to be applied twice per replication and an entire day was necessary to carry out all the measurements. This should not be a problem as the measure was made after dark adaptation and so not dependent of changes in ambient light, and as the health of the individual cannot change in a few hours. The presence of root suckers in August was different than in May, due to the difficulty to differentiate stems and root suckers.

2.3. Critics on the analysis method and the results

The individuals considered were the trees most of the time, however the individuals implemented in the complete block design were the buckets. It was more logical to analyze every tree as precise information was known for each than to work with means per buckets; however it diminishes the interest of this design. The relative growth rate being used, the importance was focalized on the capacities of each tree to improve its size. It was thus more reliable than to use absolute growth or direct height. However, the trees had been created in the same conditions according to the same protocols. In a real remediation case, the same differences could exist between initial heights, and the important would be the visible growth in centimeters. The absolute growth could be thus a more interesting measure, but the trees implemented should have approximately the same size or having being chosen for beginning rooting at the same time. Another possibility should be to study the micropropagation capacities of the different clones in parallel before drawing conditions.

Significant differences were not so important, particularly for interactions. This experiment principally allows knowing which factors are important among all factors considered, but cannot always allow choosing the best conditions. A transformation of the variables to adjust better the repartition to a law could have been used in cases where no models could be used. It was tried for fluorescence measurements, but no new factor was showing an influence after it. The use of block as a random factor was important to compare the different replications before concluding. Mixing all the values could have allowed increasing the number of individuals in each group, but the first solution seems better for the reliability. The major problem met is the limitation of significant interactions of the treatment with other factors, what doesn't allow drawing precise conclusions for phytoremediation purposes.

2.4. Other aspects

The trees were stored during winter, however to be used in Finland it is interesting to know if they survive. Furthermore, less movement of the trees could limit possible additional damages, as buckets are heavy. The development of the roots could have been limited because of the presence of the bucket. The watering was initially planned to be adapted to the need of every conditions, to give the trees just the water strictly necessary. It has not been realized by simplification, and is finally better because in real conditions the amount of water received will be the same, not depending on the soil contamination.

Some limitations are the absence of knowledge on root development as on total biomass production, and that the study focalizes on young trees and so on plantation implementation. Indeed, phytoremediation efficiency is highly dependent on root development, as pollutants are uptaken by the roots, and an important part of degradation or stabilization happened in this zone. Furthermore, a good implementation of a tree relies on the development of its roots, what will help to resist the weather constraints and reduce wind damages. In general, this experiment focalizes on allometric traits that are height, diameter, development of root suckers and leaves. It is important for determining establishment success, but doesn't lead to any information on phytoremediation success (Zalesny and Bauer, 2007). The study of physiological and anatomical traits is necessary to really answer the question of phytoremediation efficiency. The most used for Populus are contaminant concentration in roots, stems and leaves, water usage, stomatal conductance, number of stomata per leaf and presence of root exudates (Zalesny and Bauer, 2007). Chlorophyll fluorescence measurements didn't answer to the expectations on phytoremediation efficiency they were supposed to furnish. This may be not the best option to obtain this information. The calculation of leaf area could be a solution; however it is still an allometric parameter that cannot give a phytoremediation level. The leaf level of net photosynthesis could be calculated, but it requires specific material.

Another general remark is that the aspens have been chosen for their short fibers production. That may be not the better conditions for phytoremediation purposes.

3. A proposal for additional methods for this experiments and the others to come

3.1. Proposal for following with this experiment

Three years being a quite limited period to study the growth of a tree, it could be interesting to keep at least one repetition two years more in order to see possible changes. It could allow knowing if the best clones will change, as it seems to be the case in the growing season of 2015. Some other information necessary need however the harvest of some plants.

3. 1. 1 Harvest

At least one entire replication should be harvested to obtain significant results. To realize the harvest without breaking the roots, a shovel can be used to extract the plant and the soil bound to the roots. Then, stem and buds of the aerial parts must be separated from the root part. To also separate the leaves could be interesting to compare the biomass included in the growth and transpiration processes for each plant. However, as one aim of this experiment is to compare differences after a growth season, there will be no leaves left or only some leaves not representative of the real number during the growth.

Then:

- Mains soils particles can be carefully removed. Roots need then to be washed in order to eliminate all the particles closely bound to them.
- The disposition of the roots can be classified. It is important to know if the roots develop on the sides, as it can improve their anchoring.
- The length of the longest main root is measured. The root number can be determined for the main roots, according to a minimal diameter chosen depending of the present situation.
- The roots can be separated between stumps, lateral roots and basal roots to analyze more precisely their disposition. They can also be separated into fine roots and main roots. Indeed, fine roots will be the one increasing root contact with the soil, and thus possible uptake of contaminants.
- The different parts of the plant are dried in a sterilizer at 70°C, the common temperature used for biological tissues, until constant weight (Zalesny et al., 2007). The final weight is recorded.

If the weight before drying operations is recorded, the percentage of water in the plant can be determined. However, the transpiration rate is more important than the water content for phytoremediation.

These measurements will allow knowing the dry biomass of each part of the plant, and thus the total biomass, the percentage of root biomass, and particularly the percentage of fine roots. It will allow determining the effect of factors clone, planting density and soil treatment for total biomass production, root development and root surface. Root surface cannot be determined precisely but can be compared between combinations with comparisons of the percentage of fine roots and number of main roots. However, drawbacks of these measurements are that the initial biomass is not known, and the leaves not taken into account as it will be realized at the end of the growing season. An advantage is that if the total biomass per buckets is considered, it would solve the problem

described with the different effects of planting density. The better situation for implementation will indeed be the one where more biomass is created all trees included.

3. 1. 2 Possible analyses

Soil analyses are not very susceptible to be interesting as the initial knowledge of contamination is not precise, and as three years is not a sufficient time for efficient phytoremediation. As analyses costs are very expensive, it could be better to realize it for an experiment when it is correctly done since the beginning.

Analysis of root constituents could allow knowing if the contaminants are really removed from the soil, and for which clones it is the most important. Analysis of the shoots would allow knowing if contaminants are degraded or stocked. However, these analyses cannot be done if the parts of the plants have also to be harvested for biomass research. One solution is to use one replication for biomass knowledge, and one for analysis. Another solution is to keep only a small part of the plant for analysis. These analyses could be oriented in the determination of PAH concentration in the plant, but as different concentrations are initially present in the contaminants, it could not allow to compare remediation between soil treatments. Analysis of the PAH degrading bacteria presence would be possible as the analyses could be done in the soil bound to roots. It would allow comparing the rhizosphere effect according to the different factors. The main drawback is however that to be really effective it has to be done during the growing season, moment when the phytoremediation is supposed to take place, and in that experiment other measurements are carried out during the growing season. It seems so unlikely to be realized.

3. 1. 3 Measurements on remaining replications

The following of fluorescence measurement would allow confirming or not the previous results, but stomatal conductance and direct photosynthesis efficiency could be used and would be more reliable. Measurement of leaf surface could be useful to compare the percentages of major parts of plants implied in photosynthesis processes. It can be realized by determining leaf area with the help of models on three branches or three leaves chosen randomly in different size levels of the plant, depending on the development of the tree (Zalesny and Bauer, 2007). An approximate area can be determined by dividing the total area found by the number of leaves measured, and then multiplying it by the total number of leaves. However, the calculation of the area will be difficult to realize directly on the tree, and for the correct estimation it is necessary to dry the leaves as the correct formula is: Total leave area= (area of subsampled leaves/dry mass of subsampled leaves) *total tree leaf dry mass. It would thus need a harvest and impede to wait until the end of the growing season.

The simple number of leaves could be used, but it is less reliable to link with photosynthesis efficiency as shown in this experiment. The leaf color is not really reliable as stress indication as it is slightly different for every clone. Height, diameter, survival and root sucker number are always at the base of the experiment.

As a conclusion of the previous part, all measurements cannot be done and an orientation has to be given to this experiment. To keep it as an experiment on allometric parameters could allow obtaining the necessary information for implementation, which is the base of an efficient remediation. The other information on pollutants concentrations and bacteria presence could be studied in other experiments.

Figure 39: Photography taken in Somerharju field trial in May 2015. In the backgound, an important development of birches can be observed. / Photographie prise au champ d'expérimentation de Somerharju en 2015. Au second plan, un important développement de bouleaux peut être observé., personal source/source personelle, 2015

3.2. General considerations and proposal for other experiments

3. 2. 1 General considerations

The results of this experiment have to be nuanced as for every experiment realized in laboratory or greenhouse. The conditions are not real, and field experiments are necessary. Results on field experiments are lacking in general for phytoremdiation purposes, because it is still not very applied and due to the long time necessary to obtain some results. The most important data to analyze are thus the results of Somerharju field trial, which will determine the efficiency of phytoremediation. However, the real conditions make the interpretation of the results more complicated.

Phytoremediation is often cited for its low cost, but maintenance costs can increase in some cases. For example, in the case of Somerharju experiment, birches are more capable to colonize the area than aspens, and a work of cutting has to be carried out (*figure 39*). It is probable than at the end of the phytoremediation the aspens will be the only trees developed in the area, and thus the opposite phenomenon could happen, with difficulties to come back to the first situation of pine dominated forest. Indeed, the original remediation plan was established with a perspective of coming back to the original conditions. The choice of using hybrid aspens could conduce to an invasion of the zone by non-native species. They could also be less adapted to the conditions. In real cases, a combination of clones has to be considered for more variability.

3. 2. 2 Other experiments to implement

Other greenhouse experiments to implement should consider the use of the same contaminant in different stages of weathering, for example comparing the growth on the soil of Somerharju area to a fresh creosote contamination. More relevant, it should be done with different degrees of contamination with the same product. The origin of the effects on plants would thus be clear and possible analysis more relevant. Aging could be taken into account by seeing the changes in time. A possibility should be to:

- Use the same original clean soil and establish different degrees of contamination by creosote or diesel, using the sale original product at different doses.
- Analyze the contamination according to the product, determining the different hydrocarbons present. This would be reliable is the contaminant has been mixed in a homogenized way. It can be in this case realized only once for one type of contamination, and doesn't has to be realized if precise information are known on the products.
- The clones could be also planted at different densities. However it would never correspond to a real density if it is done in a bucket. The realization of the experiment in different pools must be considered, as it has previously been done for saline pollution.
- The same general allometric measures considered previously could be realized.
- Analyses of soil is the most efficient way to know if phytoremediation really occurs, and to which level depending on the case. It would need to carry the experiment a long time, at least 10 years. Analyses in the soil next to the roots, in the roots and other tissues could give previous information and allow determining more precisely the mechanisms used by aspens in the case of pollution by organic contaminants.

Conclusion

This experiment allows drawing some conclusions on the establishment of aspens for phytoremediation purposes. The first year of establishment is decisive as trees as strongly affected by the changes in conditions. A new diesel contamination has an important impact on the decrease of survival rate, and contamination in general delays growth. However, an old creosote contamination provoked some changes in the soil characteristics that limited the negative effects on establishment, and even improved the conditions in the early stages. That means that establishment will be easier in the case of old contamination, and that some previous acclimation of the plant is necessary to remediate fresh contamination The effect of soil contamination decreases then, compared to other factors. Density plantation has no particular effect in the first stages of establishment, but it begins to be influent in the third year. This factor will probably be more relevant in development steps of the plantation, probably reducing growth according to these first results.

The different clones present diverse comportments. The most important for establishment is to select the ones with a good capacity to overcome the initial conditions. In these conditions, there is an importance in selecting clones for the case of fresh contamination. Clone 291 is interesting for these first years due to its high survival rate and important relative growth, as R4. 191, 27, 14 and R3 have less resistance in one or the other cases. However, some changes seems to be happening during the development of the clones, as clones 14 and 27 have the most important relative growth rate the last year, and the best photosynthesis efficiency. 14 and 27 are thus potential future best clones, what could be only confirmed by additional years of measurements. If one objective is to implement higher densities without cost, clone R4 is interesting due to its high production of root suckers.

After this experiment, it is obvious that a lot of information are lacking. Indeed, if initial resistance during establishment is important, the development of the trees will be the main subject for phytoremediation purposes. To determine the best clones for growth, it is necessary to know how the trees develop in the next years. As the increment in size will progressively decrease, it may be determine before trees reach maturity, in 2 years more. However a final decision must not be taken before at least 10 years of experiment in the field. Furthermore, it is the increment in biomass that is important, and thus the total biomass has to be considered, what makes necessary the harvesting of some trees. Finally, the real remediation capacities will not be known without analyses. What is advised is thus to let at least one replicate for further development and to realize some harvesting in order to consider the biomass. It would also be useful to realize other experiments in which analysis of contaminants would be led since the beginning.

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Annexes

Appendix

THRESHOLD AND GUIDELINE VALUES FOR HARMFUL SUBSTANCES IN SOIL

The threshold and guideline values for the concentrations of some common harmful substances in soil as total concentration per dry matter are presented in this Appendix. The threshold and guideline values of inorganic substances are compared with the result measured from a particle size of less than 2 mm. If there is reason to suspect that the soil contains harmful substances other than those presented in this Appendix or inorganic substances with a particle size in excess of 2 mm or in a more harmful form than usual, these shall also be taken into account in the assessment of soil contamination and remediation needs.

The guideline values have been defined on the basis of either ecological risks (e) or health risks (t). If the risk of groundwater contamination is higher than normal in concentrations below the lower guideline value, the substances are marked with the letter p.

In addition to individual measured concentrations, a comparison of concentrations of harmful substances in soil with threshold and guideline values can also be carried out with statistical parameters describing various concentration distributions of the area if there is a sufficient amount of measurement results available for statistical processing and it is otherwise justified with respect to the assessment.

Substance (symbol)	Natural concentration[1] mg/kg	Threshold value mg/kg	Lower guideline value mg/kg	Higher guideline value mg/kg
Metals and semimetals[2]				
Antimony (Sb) (p)	0,02 (0,01-0,2)	2	10 (t)	50 (e)
Arsenic (As) (p)	1 (0,1-25)	5	50 (e)	100 (e)
Mercury (Hg)	0,005 (< 0,005-0,05)	0,5	2 (e)	5 (e)
Cadmium (Cd)	0,03 (0,01-0,15)	1	10 (e)	20 (e)
Cobalt (Co) (p)	8 (1-30)	20	100 (e)	250 (e)
Chrome (Cr)	31 (6-170)	100	200 (e)	300 (e)
Copper (Cu)	22 (5-110)	100	150 (e)	200 (e)
Lead (Pb)	5 (0,1-5)	60	200 (t)	750 (e)
Nickel (Ni)	17 (3-100)	50	100 (e)	150 (e)
Zinc (Zn)	31 (8-110)	200	250 (e)	400 (e)
Vanadium (V)	38 (10-115)	100	150 (e)	250 (e)
Other inorganic				
Cyanide (CN)		1	10	50
Aromatic hydrocarbons				
Benzene (p)		0,02	0.2 (t)	1 (t)
Toluene (p)			5 (t)	25 (t)
Ethylbenzene (p)			10 (t)	50 (t)
Xylenes[3] (p)			10 (t)	50 (t)
TEX[4]		1		
Polycyclic aromatic hydrocarbons				
Anthracene		1	5 (e)	15 (e)
Benzo(a)anthracene		1	5 (e)	15 (e)
Benzo(a)pyrene		0,2	2 (t)	15 (e)
Benzo(k)fluoranthene		1	5 (e)	15 (e)
Phenanthrene		1	5 (e)	15 (e)
Fluoranthene		1	5 (e)	15 (e)
Naphthalene		1	5 (e)	15 (e)
PAH[5]		15	30 (e)	100 (e)
Polychlorinated biphenyls (PCB) and polychlorinated dibenzo-p-dioxins and furans (PCDD/F)				
PCB[6]		0,1	0.5 (t)	5 (e)
PCDD-PCDF-PCB[7]		0,00001	0.0001 (t)	0.0015 (e)

Substance (symbol)	Threshold value mg/kg	Lower guideline value mg/kg	Higher guideline value mg/kg
Chlorinated aliphatic hydrocarbons			
Dichloromethane (p)	0,01	1 (t)	5 (t,e)
Vinyl chloride (p)	0,01	0.01 (t)	0.01 (t)
Dichloroethenes[3] (p)	0,01	0.05 (t)	0.2 (t)
Trichloroethene (p)	0,01	1 (t,e)	5 (e)
Tetrachloroethene (p)	0,01	0.5 (t)	2 (t)
Chlorobenzenes			
Trichlorobenzenes[3]	0,1	5 (t)	20 (e)
Tetrachlorobenzenes[3]	0,1	1 (t)	5 (e)
Pentachlorobenzene	0,1	1 (t)	5 (e)
Hexachlorobenzene	0,01	0.05 (t)	2 (e)
Chlorophenols			
Monochlorophenols[3] (p)	0,5	5 (e,t)	10 (e)
Dichlorophenols[3] (p)	0,5	5 (t)	40 (e)
Trichlorophenols[3] (p)	0,5	10 (e,t)	40 (e)
Tetrachlorophenols[4] (p)	0,5	10 (e,t)	40 (e)
Pentachlorophenol (p)	0,5	10 (e,t)	20 (e)
Pesticides and biocides			
Atrazine (p)	0,05	1 (e)	2 (e)
DDT-DDD-DDE[8]	0,1	1 (e)	2 (e)
Dieldrin	0,05	1 (e)	2 (e)
Endosulphan[9] (p)	0,1	1 (e)	2 (e)
Heptachlorine	0,01	0.2 (t)	1 (e)
Lindane (p)	0,01	0.2 (t)	2 (e)
TBT-TPT[10]	0,1	1 (e)	2 (e)
Petroleum hydrocarbon fractions and oxygenates			
MTBE-TAME[11]	0,1	5 (t)	50 (t)
Petrol fractions (C5-C10[12])		100	500
Middle distillates (>C10-C21[12])		300	1000
Heavy petroleum fractions (>C21-C40[12])		600	2000
Petroleum fractions (>C10-C40[12])	300		

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[1] The median and range of the natural concentration of fines in moraine when defined by extraction with aqua regia, except pyrolytically defined mercury. It must be taken into account in site-specific analyses that especially in clay soils the natural concentrations may be clearly higher than those measured from moraine.

[2] The guideline values for metals and semimetals defined on ecological grounds are derived by adding the average natural concentration of the mineral soil to the calculatory concentration describing the acceptable ecological risk of the substance. Correspondingly, the natural concentration of the soil in the area can be taken into account in site-specific analyses if it has been analysed with a reliable method.

[3] Total concentration including the structural isomers of the substance.

[4] Total concentration including the following compounds: toluene, ethylbenzene and xylene.

[5] Total concentration of PAH compounds including the following compounds: anthracene, acenaphthene, acenaphthylene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, phenanthrene, fluoranthene, fluorene, indeno(1,2,3-c,d)pyrene, chrysene, naphthalene and pyrene.

[6] Total concentration including PCB congeners 28, 52, 101, 118, 138, 153, 180.

[7] Total concentration stated as WHO toxicity equivalent including PCDD/F compounds and dioxin-like PCB compounds.

[8] Total concentration including the following compounds: dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE).

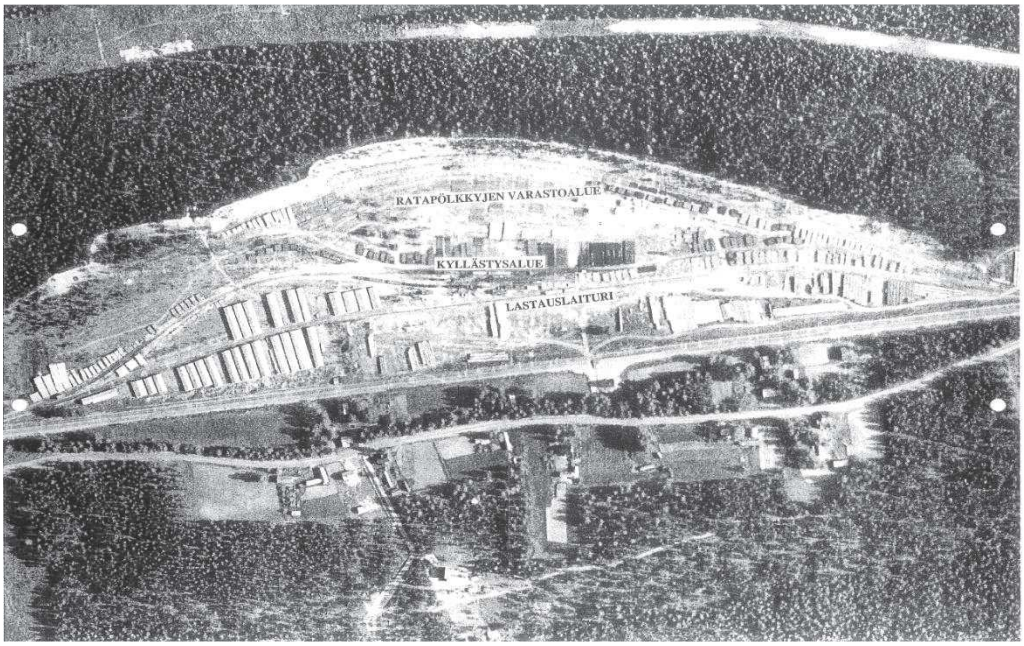
[9] Total concentration including the following compounds: alpha-endosulphane and beta-endosulphane.

[10] Total concentration including the following compounds: tributyl tin (TBT) and triphenyl tin (TPT).

[11] Total concentration including the following compounds: methyl *tert*-butyl ether (MTBE) and *tert*-amyl methyl ether (TAME).

[12] series of n paraffins in gas-chromatographic analysis.

Annex 2: Photography of the site of Somerharju during the industrial activity / Photographie du site de Somerharju durant la période d'activité industrielle, source: METLA



Annex 3: Photography showing layers of cresosote (in black) in the soil of Somerharju site / Photographie montrant des couches de créosote (en noir) dans le sol du site de Somarhaju, source : METLA, 2012

During the observation period the domestic wells (L4, L5, L9 and once also L11) of Arvola and the Senate Estates' ring well (L2) have also been monitored. Low PAH-compound readings have been noted in well L2 four times, in well L5 once and in the well L9 twice. In the regulation 401/2001 laid down by the social and healthcare ministry of Finland, a quality standard requirement was set for three PAH-compounds (benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(g, h, i)perylene) and to their sum concentration (0,1 µg/l). The concentrations of these compounds have not exceeded the quality standards in the domestic wells. PAH-compounds found in the wells are naphthalene, acenaphthylene, acenaphthene, fluorene, and phenanthrene. A request of statement (12.5.2010) was made to Valvira by the Lappeenranta region's environment agency's health inspector Riitta Lehti about the dangers of these products and their concentrations. Valvira has stated that that no probable health risk will come of the compounds or of their concentrations.

5.2.3 Surface water

There is no surface water at the subject location.

5.2.4 Sediment

There is no sediment at the subject location.

6.0 THE NEED OF RISK MANAGEMENT AND ITS GOALS

The contaminated soil found at Somerharju subject location can be found to be a possible health risk through swallowing the soil or through skin contact, or by eating its berries and mushrooms. Creosote has travelled to the groundwater and the present time both at the point of flow to the groundwater and at the impregnation zone there is leakage of PAH-compound contamination spreading to the groundwater. The biggest strain on the groundwater is caused by the soil and creosote phase of the impregnation zone. The PAH-compounds that have made it to the groundwater have caused a spreading, of which no information whether the spreading is expanding or not, is available at this time.

According to the PIMA-regulation and instruction of the environment agency the goal of the risk management in general is to stop the contamination of new environment, and etc. stop the passage of toxic substances from the soil to the groundwater. If toxic substances have already passed into the groundwater, the goal can be the stopping of the expansion of the contaminated groundwater's spreading or guaranteeing the quality of groundwater of the estates next to the site. If toxic substances are found in a large area in the groundwater and possibly as unrelated phases below the surface of the groundwater, a vast rehabilitation of the groundwater is not purposeful or possible.

Contaminated soil and groundwater can be found at the Somerharju's subject, of which the ground soil and the impregnated zone are estimated to need risk management. The goals of the risk management are:

- **To remove the health risk for humans caused by touch or eating mushrooms and berries**
- **To remove possible health risk of groundwater contaminated by the toxic substances in domestic use**
- **To find out if the contamination of the groundwater continues, meaning does the area of groundwater with toxic substances spread**
- **If the contamination of the groundwater continues, trying to end or limiting the future contamination of the groundwater**

7.0 THE PRINCIPLES OF RISK MANAGEMENT

7.1 Choosing the risk management procedure for the surface soil

Fytoremeditation, which is a purifying method done by using plants as the purifiers, covering of the soil and a mass exchange have been considered as rehabilitation methods for the surface soil(review of the rehabilitation possibilities 1, 30.4.2010 and review of rehabilitation possibilities 2, 14.12.2010). The covering method included a covering of clean soil or bog water forming resistant structure. Only a cover on a clean soil is observed, because bog water forming resistant structure was estimated not to achieve any resolve on the reducing of the risk factors. The mass exchange option included different options for handling the masses: delivery to the final destination, stabilizing and burning. A summary is displayed in chart 6 for the comparison of the methods. The comparison is based on an eco-efficiency examination dated 11.8.2011 (supplementary material). In the eco efficiency examination of the surface soil's risk management each method was evaluated for their efficiency to reduce the risk, their effect on the environment, costs and other effects. By comparing these factors together, the best solution as a whole can be evaluated.

Out of the methods mentioned, the mass exchange option was evaluated to be the most expensive and the least environment friendly. In addition the whole flora of the area would be destroyed completely, because of which the area could not be used for mushroom or berry collecting for several decades. On the other hand, with mass exchange, the risks would be removed swiftly and definitely.

The cover option was the cheapest of the methods, but it was estimated to produce a bit more negative effects (etc. environment effects) compared to fytoremeditation. The cover option would also have destroyed the flora at the zone and the progress to the subject's original state would be as slow as with the mass exchange method. To both the cover method and to the mass exchange method some landscaping could be used, which currently is not included in the expense estimates in the chart 6. The structure used for the covering would affect the eco efficiency of the covering method. If the covering would be made lightly by bringing more clean soil into the site, it would be the least expensive method compared to the other two, but there would be a risk of the covering moving from its place in the many years it would be needed. If the risk brought by the surface soil would be removed completely, it would require a covering thick enough to withstand years of erosion. As such the solution might have bigger negative effects on environment, because of resources used and fumes brought by the deliveries.

Fytoremeditation is estimated to be the ecologically best solution out of the three, because with it, decontamination of the soil with the least possible harm and distraction to the environment is possible. During the method the current wood land consisted mainly of scots pine would be replaced with common aspen, with which the wood land form of the area would be preserved. Fytoremeditation isn't widely used as a soil rehabilitation method, but it is estimated to apply well to this subject. The uncertainty of the fytoremeditation is reduced with research done during the rehabilitation. Therefore using fytoremeditation for this subject is making it more trustworthy method of rehabilitation in Finland.

Fytoremeditation is presented as the best possible method for the subject based on the factors mentioned before.

7.2 Option for the risk management procedure of the surface soil – fytoremeditation

In fytoremeditation plants are improving the bio-dissolution of the toxic substances or gathering them on themselves. The first of the two mechanisms is the mechanism applied in the subject area which means microbes living in the plants' root system improving the bio-dissolution process while simultaneously binding soil into the toxic substance, with which for instance loose dust is reduced, thus making it less dangerous for people visiting the area. No toxic plant material is born during the process, since the plants are not binding the toxic substances on themselves.

Plants with lots of root biomass and roots that go deep underground and with a complex bacteria and mushroom root are required factors for making an efficient fytoremeditation. The plants are providing a support network for bacteria and mushroom roots which destroy the toxic-substances. The dissolution of the toxic substances is mainly handled by microbe, but the roots of the plants might partly participate to the process. Both the root system and the microbes however are needed for efficient dissolution.

The forest research institute has developed the fytoremeditation done with trees. Bred seedlings of common aspen are planted to the rehabilitation area in the procedure. The forest research institute has bred different seedling types, of which the most adaptable are cloned and planted at the site. A hybrid aspen is by far the most fast growing tree in South Finland's environment. The hybrid is a mix of the common aspen (*populus tremula*) and American aspen (*p. tremuloides*). This "species" of tree has been researched in Finland since 1950 and have proven to be fast growing and very adaptable to South East Finland's climate. The hybrid aspen is able to grow at very different areas from barren to lush soil. Important to fytoremeditation is the hybrid aspen's ability to grow fast in overall size and in root size, it's ability to form root connections fast and it's root system's ability to puncture deep into the soil, especially in those situations, where soil is barren and the humidity may vary greatly (like the sandy soil of the subject).

7.3 Risk management procedure of the contaminated groundwater

The risk management of the groundwater includes the following:

- Getting clean groundwater for those households who are in a danger area of having contaminated groundwater
- Finding out the size of contamination spreading and whether it is stable or not and managing a possible follow-up procedures based on the results. Preparation for the possibility that the spreading is expanding will be taken during the procedure and if it should expand, possible procedures for reducing the toxic substance mass are looked into.

Because at the current moment or in the future there might be health risks brought about the use of contaminated groundwater in the estates south of the impregnation area, a domestic water supply outside the area will be provided for these estates. A general plan has been made for the management of the domestic water (presented at an inhabitant event 13.12.2010, appendix J), plans for the building of the well are on the works and the well is estimated to be completed during the year 2011.

The size of the spreading of the groundwater is determined and whether the impregnated zone causes additional contamination meaning does the spreading travel with the groundwater to new places. Additional information about the extent of the contamination spreading in the groundwater and about its stability is needed for this.

Because specific flow conditions of the groundwater is not known, it is not possible to estimate precisely to what direction to South of the impregnation plant and to how big an area the toxic substances have traveled with the groundwater, especially in Somerharju's side. Because of this, a study is needed where the first thing to do is to map out the area's rock and soil's geology and with it estimate the flow conditions of the groundwater. The early information is used to decide the locations of the area's new groundwater pipes.

A more described plan has been made from the soil and groundwater researches (appendix J) and the studies have been started in summer 2011. Based on the data of those studies the installation of the groundwater pipes is planned, so that the flow conditions and the extent of the contaminated groundwater can be studied more. When the extent of the contamination is known, the levels of contamination in the groundwater are monitored for a year, in order to find out whether the size of the contamination spread is expanding, stable or shrinking. If the contamination spread is shrinking or stable, the monitoring of the contamination spread might be the only rehabilitation needed. If however the size of the spreading is expanding, possible solutions to either removing the phase or to limit the contamination spreading will be investigated.

Possible solutions for the rehabilitation of the subject area have been evaluated in the Examination of options for rehabilitation 2 –report (Golder Associates Oy, 14.12.2010). Rinsing of the soil and the electrokinetic deconstruction for the phase removal where looked into in the report, as well as biological rehabilitation, natural cleaning and chemical oxidizing for the cleaning of the toxic substances in the groundwater. For the impregnated zone rehabilitation it was decided that there was no sure or fast way to do it, because free and leftover phase is still found at the site, which removal is extremely hard technically. Because a simple decrease of concentration of the substances does not produce definite decrease in the concentrations as long as there is phase left in the starting area, methods for removal of the phase are the primary action. If practical methods are found, the most promising of these can be tested for instance in a laboratory scale, in another location or at the actual subject area within a smaller scale.

8.0 EXECUTION OF THE PROCEDURES

8.1 Rehabilitation of the surface soil with fytoremeditation

8.1.1 Measuring the area of rehabilitation

The rehabilitation of the surface soil in this context refers to the upper most, about 0,5 m layer of soil. The area of effect of the exposure routes of the subject is estimated to reach to depth of 0,5 m at maximum from the ground. Fytoremeditation however reaches to depths of a few metres which is the depth where the roots of the aspen grow.

The rehabilitation is measured to an area displayed in the drawing 12 of the appendix B.

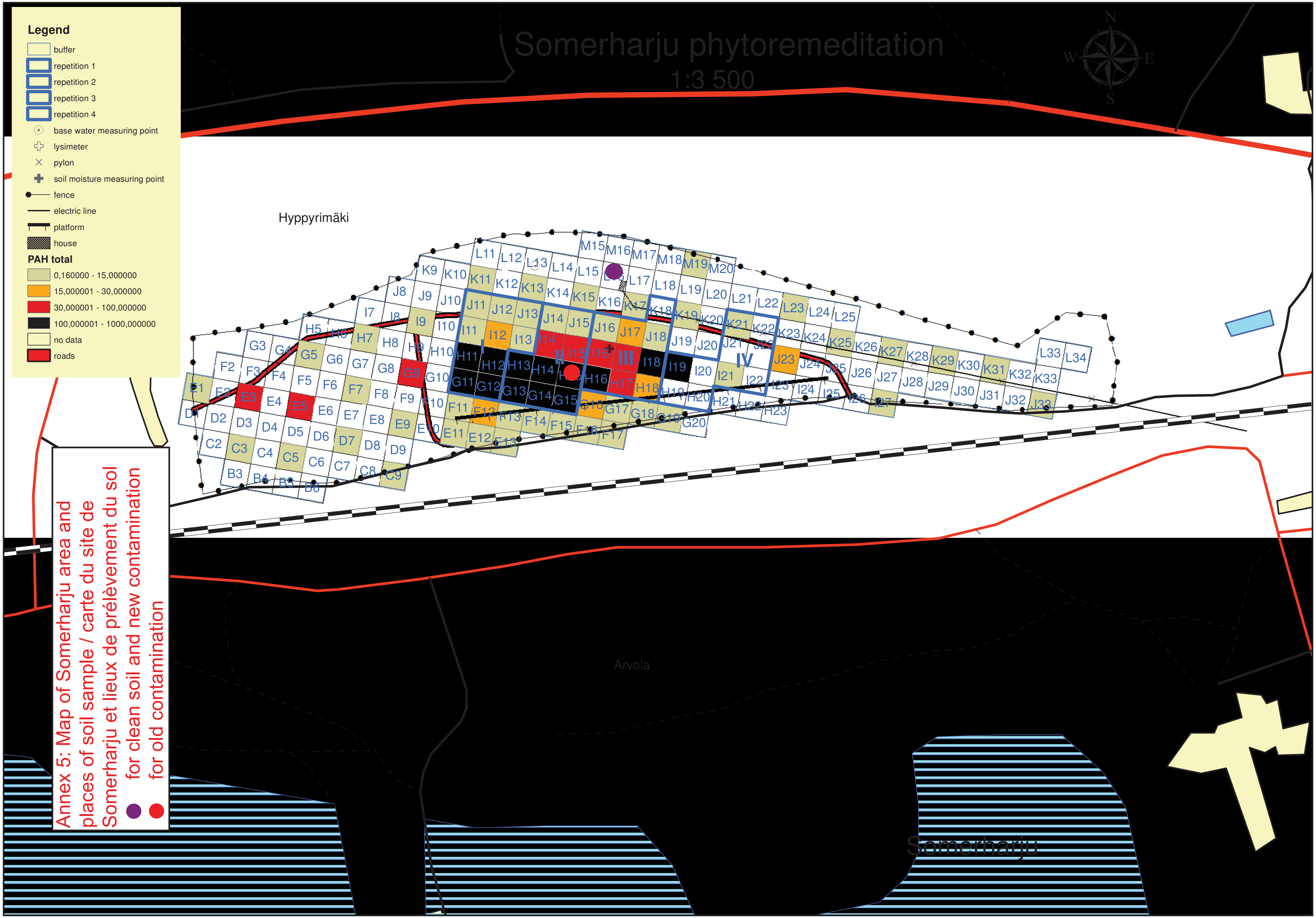
8.1.2 Goals of rehabilitation

The goals of rehabilitation are the risk management goals for the surface soil set in chapter 6. In addition research information is provided with the project to the partners of co-operation of the project (Finnish Forest Research Institute, The University of Helsinki).

The goals of the risk management procedures are to rehabilitate the area so that no risk or harm comes from the surface soil to the environment or health in the subject's current use. No concentration goals are set for the toxic substances during the rehabilitation of the surface soil, because the goals are risk based determined, so that the summed total of risk level of the toxic substances reaches the wanted levels. RISC 5-program is used to determine the risk level (Waterloo Hydrogeologic 2001). The model is based on the ASTM (American Society For Testing and Materials; ASTM 1995)'s RBCA-standard (Risk Based Corrective Action Applied at Petroleum Release Sites) and on the calculation diagrams explained in the instructions and standards of the United States' environment agency's U.S.EPA. The program includes a conceptual model which is tweaked to match the observed situations.

RISC 5 includes widely used, accepted and tested part models for description of, etc. discharge sources of the calculation systems, discharge events, the spreading mechanisms and both supply routes and exposures and for quantification of the risks. In this case the RISC 5 –program is used with determinance, which makes the result themselves as singular number values that describe the risk. The calculation is executed in a conservative manner following the principle of caution as a so called Worst Case –scenario.

In fytoremeditation different toxic substances (PAH-compounds for instance) react in different ways to the rehabilitation. Because the ratios of concentrations of the toxic substances may change from the current ones during the rehabilitation, maximum concentrations are set for them, with which the risk levels maintain the accepted level. The risk levels are set assuming that the toxic substance at hand is the only toxic substance at the subject. There total caused risk value is calculated with a formula: $\Sigma(Ci/Cihyv.) < 1$.



Annex 5: Map of Somerharju area and places of soil sample / carte du site de Somerharju et lieux de prélèvement du sol
● for clean soil and new contamination
● for old contamination

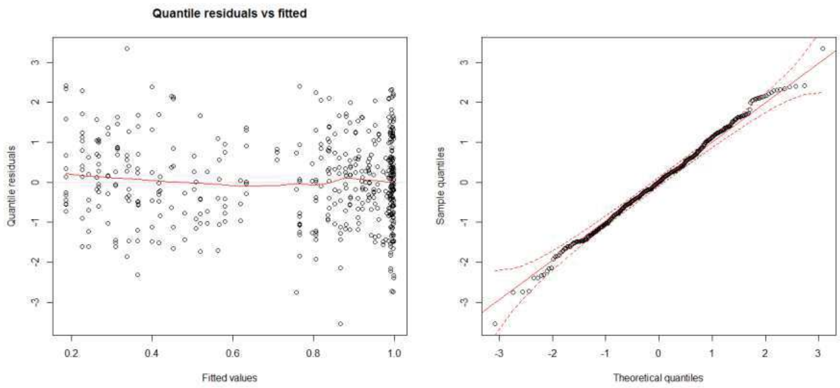
Annex 6: Table used to measure the height of the trees in 2015 (extract) / Tableau utilisé pour mesurer la hauteur des arbres en 2015 (extrait)

Bucket number	Clone	Tree number	Density	Treatment	Height May date: 11	Heigh June date: 5	Height June date: 22	Height July date: 7	Height July date: 22	Height August date: 7	Height August date: 21
1	291	**1**	1	3	26,5	34,5	34	34	34,5	34	34,5
2	27	**1**	2	1	77	85	86	85,5	86	86	86
2	27	**2**	2	1	39	43	43	42,5	43,5	43	43
3	191	**1**	1	2	-	-					
4	14	**1**	2	2	-	-					
4	14	**2**	2	2	-	-					
5	14	**1**	1	1	44	51,5	58,5	67	67,5	67,5	67,5
6	27	**1**	6	3	35	38	37,5	38,5	38,5	38,5	38,5
6	27	**2**	6	3	42	48,5	48	47,5	48	48	47,5
6	27	**3**	6	3	25	25	24,5	24,5	24,5	24,5	25
6	27	**4**	6	3	36	40	43,5	45,5	45,5	46	46
6	27	**5**	6	3	23,5	26	27,5	28	28	28,5	28,5
6	27	**6**	6	3	24	26,5	26,5	26,5	26,5	26,5	26,5

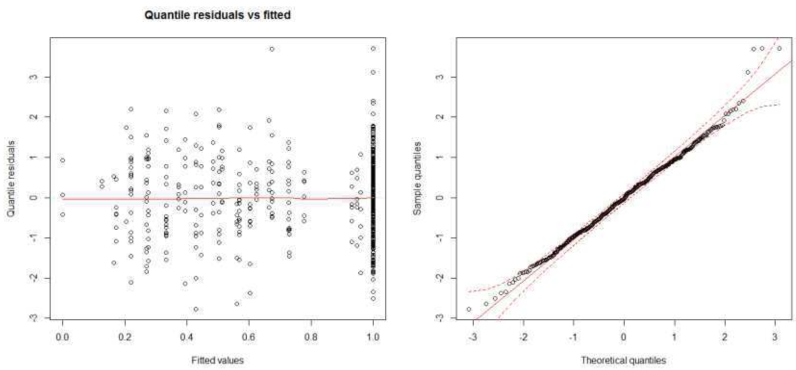
Annex 7: Table used to register degradations in 2015 (extract) / Tableau utilisé pour enregister les dégradations en 2015 (extrait)

bucket	density			29/06/2015	08/07/2015	24/07/2015	perc	14/08/2015	perc
1				insect	insect	insect	-0,25	insect	-0,25
2				insect	insect, leaves dried	insect, fungi	-0,25	insect, fungi	-0,25
3									
4									
5				-0,5	holes, insect	insect	0,25	insect	-0,5
6				insect	holes, insect	insect	-0,25	insect	-0,25
7				insect	insect	fungi	-0,25	fungi	-0,25
8				insect	insect	insect	-0,25	insect	-0,25
9									
10					insect	insect	-0,25	insect	6:0,75, others:-0,25

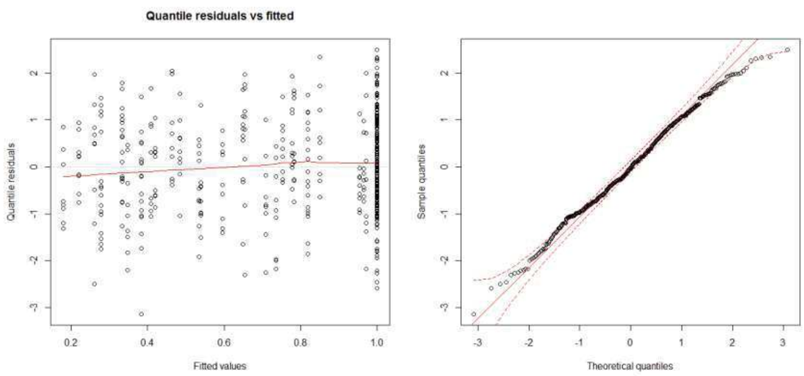
Annex 8: Graphics used to check the validity of model 1 for survival / Graphiques utilisés pour vérifier la validité du modèle 1 pour la survie



Annex 9: Graphics used to check the validity of model 2 for survival / Graphiques utilisés pour vérifier la validité du modèle 2 pour la survie



Annex 10: Graphics used to check the validity of model 3 for survival / Graphiques utilisés pour vérifier la validité du modèle 3 pour la survie

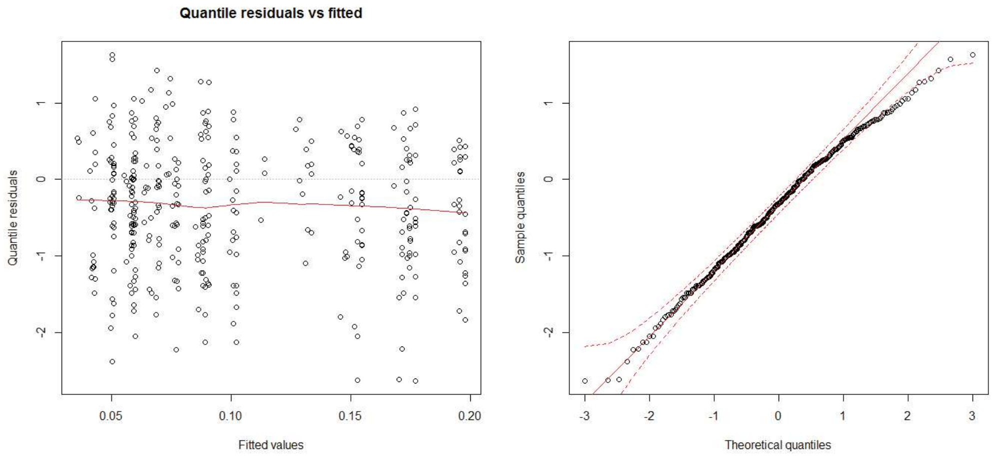


Annex 11: Example of script in the case where the variable of the model used is a table / Exemple de script quand la variable utilisée pour le modèle est un tableau

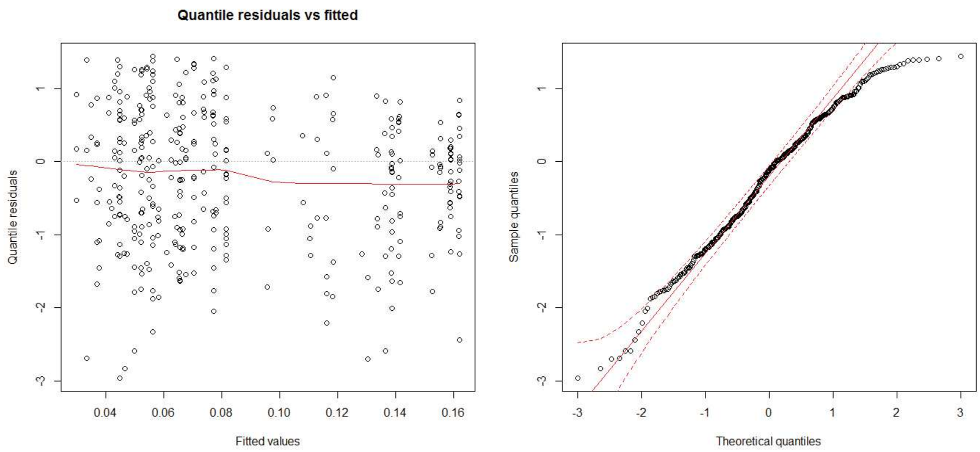
```
reponse15<-cbind(june7_13, august21_15)
model15<-glmer(reponse15~clone+treatment+density+(1|block), family="binomial", data=H2015)
overdisp.glmer(model15)
plotresid(model15)
summary(model15)

Anova(model15)
lsmeans(model15,pairwise~treatment)
lsmeans(model15,pairwise~clone)
lsmeans(model15,pairwise~density)
```

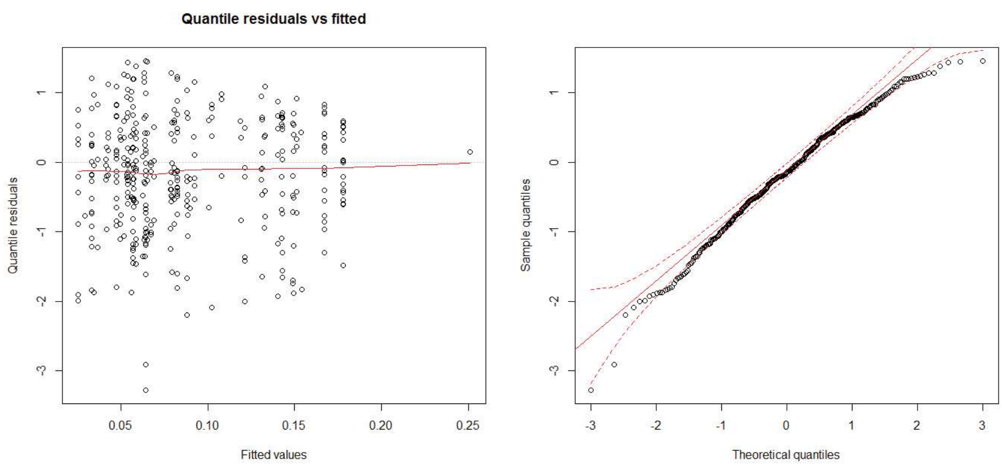
Annex 12: Graphics used to check the validity of model 1 for RGR at the end of 2014/ Graphiques utilisés pour vérifier la validité du modèle 1 pour le taux de croissance absolue fin 2014



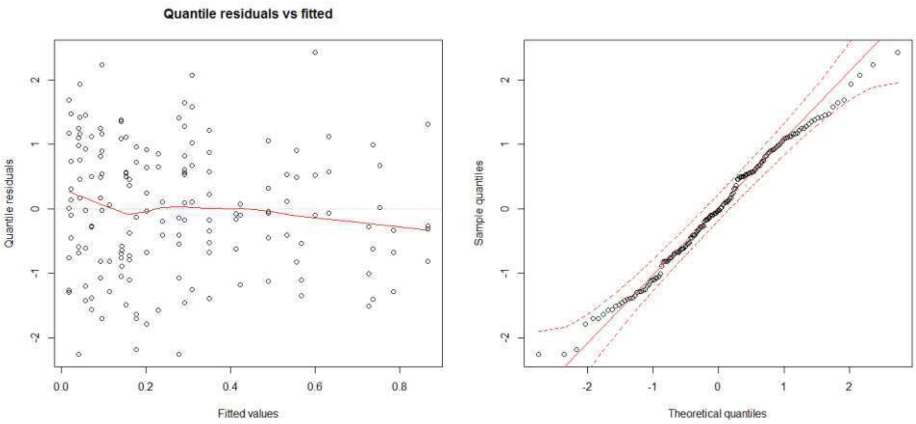
Annex 13: Graphics used to check the validity of model 2 for RGR at the end of 2015/ Graphiques utilisés pour vérifier la validité du modèle 2 pour le taux de croissance absolue fin 2015



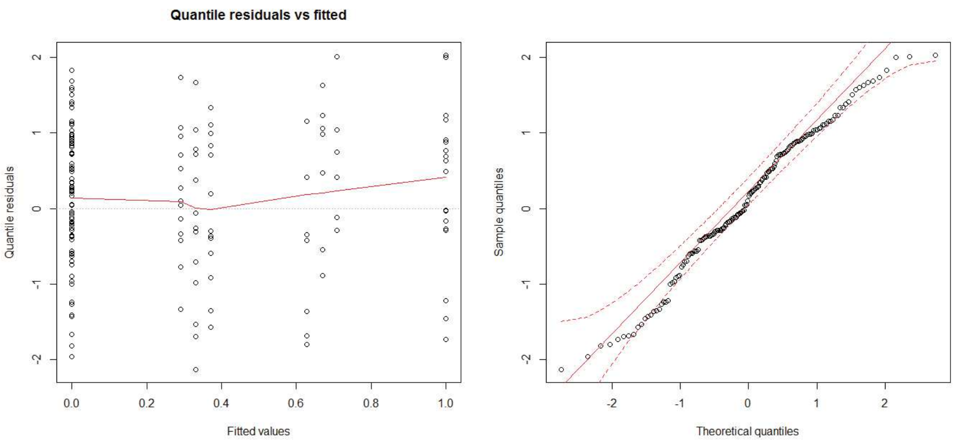
Annex 14: Graphics used to check the validity of model 3 for RGR at the end of 2014/ Graphiques utilisés pour vérifier la validité du modèle 3 pour le taux de croissance absolue fin 2014



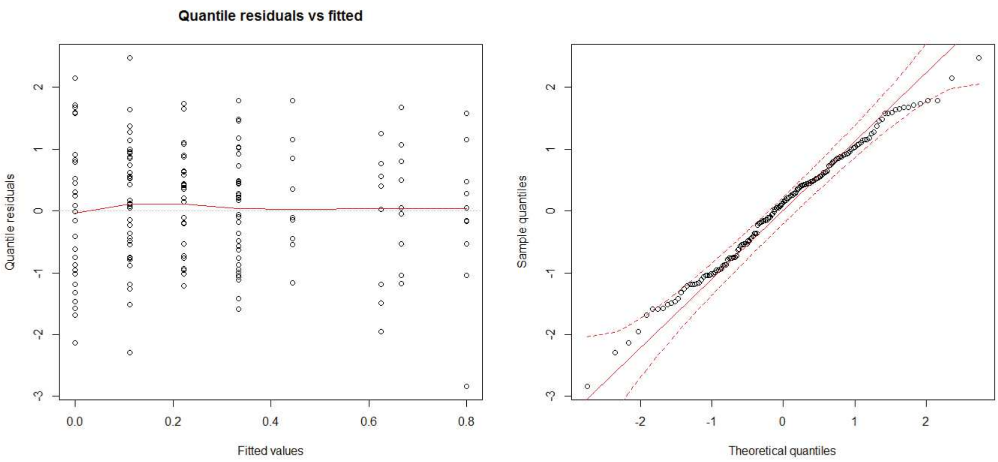
Annex 15: Graphics used to check the validity of model 1 for root suckers presence / Graphiques utilisés pour vérifier la validité du modèle 1 pour la présence de drageonnement



Annex 16: Graphics used to check the validity of model 2 for root suckers presence / Graphiques utilisés pour vérifier la validité du modèle 2 pour la présence de drageonnement



Annex 17: Graphics used to check the validity of model 3 for root suckers presence / Graphiques utilisés pour vérifier la validité du modèle 3 pour la présence de drageonnement



Annex 18: Classification of significant comparisons in the model 3 with interactions between factors for RGR at the end of 2015 / Classification des comparaisons significantes sur le modèle 3 avec intéractions entre facteurs pour le taux de croissance relative fin 2015

group	1	2	3	4
291 clean				
R4 clean				
27 clean				
291 new				
R4 old				
27 new				
191 old				
291 old				
R4 new				
191 clean				
27 old				
191 new				
R3 clean				
14 new				
14 clean				
R3 old				
14 old				
R3 new	NA			

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Version française du mémoire de fin d'études d'ingénieur

La phytoremédiation par le tremble :

Effets de la pollution par des hydrocarbures pétroliers, de la densité de plantation et du type de clone sur l'établissement du Peuplier tremble (*Populus tremula* L.) et du tremble hybride (*Populus tremula* X *Populus tremuloides* Michx.)

TRADUCTION SYNTHETIQUE DU RAPPORT ORIGINEL

***

Rapport originel :

The use of aspens for phytoremediation:
Effect of contamination by petroleum hydrocarbons, planting density and clone type on the establishment of European aspens (*Populus tremula* L.) and hybrid aspens (*Populus tremula* X *Populus tremuloides*

Camille D'HERVILLY
Agriculture, Environnement, Territoire
2015

Luke
NATURAL RESOURCES INSTITUTE FINLAND

VetAgro Sup

Table des matières

Introduction

Les trembles sont largement répartis dans l'hémisphère nord. *Populus tremula* L. ou Peuplier tremble est l'espèce traditionnelle des continents européen et asiatique, tandis que l'autre espèce *Populus tremuloides* Michx. ou Peuplier faux-tremble est développée sur le continent américain. Ce sont des arbres à feuilles caduques, et ils représentent seulement 1 % des forêts finlandaises en tant que peuplement dominant (Hannelius and Kuusela, 1995). Ils n'ont généralement pas été considérés dans les pratiques sylvicultrices en Europe et particulièrement dans les pays nordiques où de longues rotations allant jusqu'à 120 ans sont communes, avec l'utilisation de conifères correspondant au stade climax, et où la ressource en bois est suffisante. La nécessité d'augmenter la part des énergies renouvelables a conduit à un intérêt nouveau dans les espèces à croissance rapide tels les peupliers, trembles, aulnes et saules. Cela devrait également permettre de répondre à la demande de l'industrie du papier et de la pâte à papier (Tullus et al., 2011). En Finlande, un intérêt récent est apparu pour le tremble car il a les qualités requises et qu'il est possible de sélectionner des caractères intéressants du fait de l'important nombre de clones. De plus, des croisements entre les deux espèces ont permis la création de différents clones hybrides.

La contamination des sols est seulement considérée depuis les années 1980 (Pyy et al., 2013). L'émergence de nouvelles considérations écologiques sur la pollution des sols au niveau européen a provoqué la reconsidération non seulement des problèmes de pollution mais aussi des méthodes utilisées pour la décontamination. L'importance de la pollution liée à des décennies d'activités industrielles a conduit à la critique des techniques traditionnelles d'excavation et décharge ou incinération, du fait du nombre élevé de cas de décontamination nécessaires aujourd'hui. De nouvelles méthodes ont été développées. Les techniques thermiques, chimiques et mécaniques, efficientes, sont utiles pour éliminer rapidement le risque en cas de contamination importante ou incluant un danger pour la population. Elles sont cependant chères et peuvent inclure un risque pour l'environnement ainsi qu'un manque d'acceptation par la population. Des techniques biologiques sont apparues, parmi lesquelles l'atténuation naturelle, la bioremédiation, le compostage et la phytoremédiation. Cette dernière consiste en l'utilisation de plantes pour extraire, dégrader ou rendre inoffensifs les contaminants. Du fait de leurs caractéristiques, les peupliers et en particulier les trembles semblent particulièrement adaptés pour cette méthode.

L'unité d'amélioration des arbres Haapastensyrjä de l'institut finlandais des ressources naturelles (Luke) travaille sur l'amélioration traditionnelle des forêts finnoises et sur les effets du changement climatique sur les forêts, du fait de la forte variation du climat attendue pour les pays nordiques dans le futur. Il s'agissait donc d'un lieu idéal pour étudier l'effet d'autres facteurs tels la contamination des sols. En 2013, l'instauration du premier cas à grande échelle de phytoremédiation en Finlande a été l'élément déclencheur d'études sur la phytoremédiation des contaminants organiques.

Ce stage consistait en la participation à différents projets spécifiques à cette unité et particulièrement ceux liés à la phytoremédiation. Ce rapport se propose de se concentrer sur une expérience de phytoremédiation commencée en 2013 et pour laquelle des analyses étaient nécessaires. Elle se focalise sur l'étude de paramètres pour l'établissement de plantations selon différents niveaux de pollution des sols, de densité de plantation et différents clones de *Populus tremula* L. et de l'hybride *Populus tremula* L. X *Populus tremuloides* Michx. Pour développer ce cas d'étude, le contexte de la pollution des sols, de la phytoremédiation et des trembles sera détaillé, et le contexte précis de l'expérience défini. Puis, le matériel et les méthodes nécessaires seront présentés, et les résultats obtenus décrits. Enfin, une discussion sur ces résultats et des critiques permettront de proposer de futures étapes pour ce projet.

I. Synthèse du contexte de l'expérience

Cette expérience est centrée sur la croissance, la survie, le stress et la réponse au stress de différents clones de Peuplier tremble (*Populus tremula* L.) et de tremble hybride (*Populus tremula* L. X *Populus tremuloides* Michx.) pour différents contaminants contenant des hydrocarbures pétroliers et différentes densités de plantation. L'objectif est de déterminer l'impact des facteurs clone et densité de plantation sur ces variables pour des sols contaminés, de manière à déterminer les meilleurs clones et densités à utiliser et à mieux comprendre les phénomènes en jeu. De manière plus générale, le but est de développer des méthodes de sélection efficaces pour les clones, et de permettre la mise en place d'essais à plus grande échelle. Cette expérience est en particulier liée à un cas réel de contamination, où ces mêmes clones sont utilisés (champ d'expérimentation de Somerharju).

Les recherches bibliographiques ont permis de formuler les hypothèses suivantes quand à cette étude :

- L'efficience de la phytoremédiation par les trembles est liée à une croissance importante (Cook and Hesterberg, 2013), car une plus grande quantité de polluants est ainsi extraite ce qui augmente la phytoextraction et la phytodégradation et car plus d'eau est utilisée, améliorant la phytostabilisation.
- Le développement des parties aériennes est lié à celui des racines. L'extraction des contaminants passe en effet par le développement des racines et la rhizoremédiation y est possible. Cependant, ceci ne peut être vérifié sans récolte.
- Une augmentation de la taille est liée à une augmentation de la biomasse, étant donné que la hauteur s'accroit avant le diamètre (Louisiana Pacific Canada ltée and CERFO, 2002).
- Plus la contamination est ancienne, plus il sera dur d'extraire les contaminants à cause du phénomène de vieillissement au cours duquel les contaminants organiques se lient avec des particules de sol (Ndimele, 2010).

La première étape consiste en l'adaptation des arbres aux nouvelles conditions. Les arbres ayant des difficultés à s'acclimater utiliseront plus d'énergie pour leur survie et moins pour la dépollution. Les meilleures conditions seront donc celles permettant un meilleur établissement et sont donc une croissance importante, un taux de survie élevé et une photosynthèse efficiente en réponse à la contamination. Une forte production de nouveaux individus par drageonnement pourrait aussi être intéressante.

II. Matériel et méthodes

1. Dispositif expérimental

L'expérience est réalisée sous une serre à paroi plastique dans l'unité d'Haapastensyrjä (N60°36'56.516'', E24°25'53.396''). Les plantes sont placées dans des sceaux de dix litres drainés grâce à un fond percé et placés dans des cuvettes de façon à éviter la perte de contaminants (*figure 15*). Quatre clones d'hybride (*Populus tremula* L. X *Populus tremuloides* Michx.) et deux clones de Peuplier tremble (*Populus tremula* L.) sont utilisés (*tableau 4*). Les clones ont été produits par micro-propagation fin août 2012, et ont été transplantés une première fois en février 2013. Ils ont grandis à l'intérieur jusqu'à leur transplantation dans les sceaux et le début de l'expérience en juin 2013. Le sol utilisé à été prélevé dans le champ d'expérimentation de Somerharju. Il est de type sableux et comporte des éléments grossiers. Deux cas de pollution sont considérés : le premier consiste en l'utilisation de sol provenant de la zone contaminée du champ d'expérimentation. Il s'agit d'une contamination ancienne à la créosote, contenant entre 100 et 1000 $mg.kg^{-1}$

d'hydrocarbures aromatiques polycycliques. Une pollution nouvelle au diesel à été créée à partir de sol non pollué en provenance du même site, la contamination étant réalisée par injection de 50 mL de diesel dans le substrat de chaque sceau. La pollution y est donc récente. Le même sol sans contamination est utilisé comme témoin (*annexe 5*).

La densité de plantation a été testée pour un, deux et six arbres par sceau. La disposition consiste en un plan aléatoire complet en blocs, sans répétitions dans chaque bloc (*figure 16*). Cela veut dire que dans chacune des trois répétitions créées, chaque combinaison des facteurs clone, traitement du sol et densité de plantation est présente une seule fois. Trois sceaux sans arbres sont également présents dans chaque bloc, pour chaque niveau de contamination. Il y a donc 57 sceaux par répétition, disposés en 19 lignes et trois colonnes. Cela représente 486 arbres au total.

L'arrosage est effectué à raison d'un demi litre d'eau par sceau, de une à deux fois par semaine selon la période, rarement trois fois. Les plantes sont stockées dans une réserve durant l'hiver, et positionnés de nouveau sous tunnel en mai de chaque année.

2. Mesures précédentes

Des mesures de hauteur et la détection de la mort des individus ont été réalisées en 2013 et 2014.

3. Méthodologie pour 2015

Des mesures de la hauteur ont été réalisées deux fois pas mois, depuis la surface du sol jusqu'au dernier bourgeon en vie avec une précision de 0,5 cm (annexe 6). Le diamètre a été mesuré à 5 cm de hauteur environ à la fin de la saison de croissance, avec une précision de 0,01 mm. La survie et la présence de clones par drageonnement a été observée. L'apparition de dégradations et le pourcentage de feuilles touchées ont été notés (annexe 7), ainsi que le nombre total de feuilles sur deux blocs à la fin de la saison de croissance.

Des mesures de la fluorescence chlorophyllienne ont également été réalisées. Cette fluorescence est inversement liée à l'efficience de l'activité photosynthétique, étant donné que l'énergie non utilisée au niveau du photosystème II pour la photosynthèse est perdue sous forme de fluorescence chlorophyllienne. La présence d'un stress diminue la photosynthèse et augmente l'émission de fluorescence (Hansatech Instruments Ltd, 2006). L'utilisation d'un fluorimètre (Pocket PEA Chlorophyll Fluorometer de Hansatech instruments) a permis de déterminer certaines valeurs :

- Fv, la fluorescence variable, fluorescence émise entre un brusque éclairage après une adaptation à l'obscurité et la saturation du photosystème II et Fm, la fluorescence maximale (*figure 17*).
- Fv/Fm, l'efficience quantique maximale du photosystème II. Sa valeur est acceptable à partir de 0,75 (Maxwell and Johnson, 2000).
- L'index de performance PI, indicateur de la vitalité de l'échantillon et donc de la force interne de la plante pour résister aux contraintes externes.

Le protocole est le suivant:

- Placer le cache sur la feuille et fermer l'ouverture (*figure 18*).
- Attendre le temps nécessaire déterminé au préalable (ici 45 minutes).
- Allumer l'appareil, l'ajuster sur le cache.
- Libérer l'ouverture et lancer la mesure. Attendre l'émission d'un son pour être sur que la mesure est prise.

L'intensité lumineuse utilisée était de 3500 µmol.m^{-2}, et la durée une seconde. Les mesures ont été réalisées une fois par mois en juin, juillet et août. Les feuilles ont été marquées de façon à pouvoir utiliser toujours la même si possible.

4. Hypothèses

Les hypothèses formulées sont que la croissance et le taux de survie seront meilleurs dans des sols sans contamination, et meilleurs dans les cas de contamination ancienne que récente dû au vieillissement des contaminants. Ils seront également meilleurs dans les cas de faible densité de plantation. La photosynthèse sera plus importante pour de faibles densités de plantation et sans contamination du sol. Le drageonnement sera plus développé dans les cas de stress (pollution, forte densité de plantation).

5. Analyses statistiques

Les facteurs fixes sont clone, contamination du sol et densité de plantation (*tableau 5*). Le facteur bloc est aléatoire, et induit la présence de séries appariées. Les variables étudiées sont :

- La survie, codée 1 ou 0
- Le taux de croissance relative, calculé par : RGR=taille finale / taille initiale.
- La croissance absolue a pu être utilisée. Elle se défini par : croissance absolue=taille finale – taille initiale.
- Le diamètre.
- La présence de tiges par drageonnement, codée 1 ou 0.
- Le nombre de tiges par drageonnement.
- Fv/Fm ou efficience du photosystème II.
- PI ou l'indice de performance, représentant la résistance aux contraintes externes de l'échantillon.
- Les dégradations.
- Le nombre de feuilles pour deux répétitions.

Un modèle linéaire mixte généralisé sur loi binomiale a été utilisé pour la survie, avec et sans interactions (*tableau 6*). Pour la croissance, des modèles linéaires généralisés mixtes sur loi binomiale pour la variable représentée par un tableau contenant taille initiale et taille finale ont été réalisés, avec et sans interactions, pour fin 2014 et fin 2015 (*tableau 7*). La taille initiale était dans ce cas la taille mesurée au début de l'expérience. Pour les saisons de croissance de 2013, 2014 et 2015 les modèles ne s'ajustaient pas aux données et des tests de Kruskal-Wallis ont été réalisés. Des tests de corrélation ont pu être réalisés suivant les cas. Pour le diamètre, seul le diamètre final est connu. Un test de corrélation entre diamètre et taille a été réalisé, et les différences entre ces deux variables observées. Pour les tiges par drageonnement, des modèles linéaires mixtes sur loi binomiale ont été utilisés (*tableau 8*). Les individus étaient les sceaux dans ce dernier cas, dû à l'impossibilité de savoir de quel arbre provenaient les nouvelles tiges.

Pour la fluorescence chlorophyllienne, une moyenne des trois mesures a été réalisée. Les modèles possibles ne s'ajustaient pas aux données et des tests de Kruskal-Wallis ont donc été effectués. Les différences entre dates ont été observées. Un modèle proportionnel de Odds a été utilisé pour déterminer quels facteurs intervenaient sur les dégradations des plantes, et des observations ont été faites par rapport aux résultats. Pour le nombre de feuilles, aucun modèle ne s'ajustant aux données, des tests de Kruskal-Wallis ont été réalisés.

Le niveau de significativité a été fixé à 95 %. Des comparaisons multiples par la méthode des moindres carrés ont été réalisées dans le cas de modèles, et des comparaisons deux à deux par le test de Wilcoxon dans les autres cas pour les facteurs significatifs.

III. Résultats

1. Survie

A la fin de l'expérience, le taux de survie est de 76%, et il est de 78 % à la toute fin de la première année d'expérience.

Selon le modèle 1 sans interactions, les trois facteurs sont significatifs (*tableau 10*). Tous les types de sols sont significativement différents des autres. Le taux de survie dans le sol avec nouvelle contamination au diesel est très faible (40%), tandis qu'il n'y a presque pas de mort dans le sol à la contamination ancienne à la créosote (99% de survie) (*tableau 9*). Le taux de survie dans un sol exempt de contamination est de 88 %, plus grand que la moyenne.

Seulement deux comparaisons sont significatives pour le clone (*tableau 9*, *figure 19*). Le clone 291 a un taux de survie significativement meilleur (86%) que 191 et 27 (71%). Même si le facteur densité de plantation est significatif, les différences entre niveaux ne le sont pas.

Pour les modèles 2 et 3 avec interactions, seul le facteur contamination du sol est significatif. La faible importance de la densité de plantation par rapport à la contamination est visible graphiquement (*figure 20*). Le meilleur taux de survie de 291 est dû à sa meilleure survie dans le sol contaminé au diesel (*figure 21*). Les clones de *P. tremula* (R3 et R4) ont des bons taux de survie dans un sol sans contamination.

2. Taille

La croissance est plus importante en mai et juin, et diminue à partir de juillet. La croissance absolue diminue avec les années (*figure 22*).

En 2013, le taux de croissance relative est en moyenne de 4,7 et la médiane de 1,4. La moitié des arbres n'ont donc vu leur taille augmenter que de 40 % maximum. Seulement 49 % des arbres ont eu une croissance absolue de plus de 49 %. Le test de Kruskal-Wallis donne des influences significatives pour les facteurs clone et contamination (*tableau 11*). Le clone 291 a un taux de croissance relative plus grand que tous les autres excepté R4 et 191, avec une moyenne de 8,9 (*tableau 12*, *figure 23*). La croissance est meilleure dans un sol sans contamination (moyenne de 6,6) et pire dans un sol à contamination récente au diesel (moyenne de 2,8) (*tableau 12*). La contamination ancienne présente une moyenne de 4,5. Ceci est confirmé par l'observation des pourcentages d'arbres ayant eu une croissance de plus de 1 cm dans les différents sols.

Pour fin 2014 et fin 2015, les résultats sont similaires. Les modèles sans interaction donnent une significativité pour tous les facteurs (*tableau 13*), plus importante pour le clone et la densité de plantation. Dans les deux cas, le clone 14 a un taux de croissance relative significativement inférieur à tous les autres excepté R3. R3 a un taux plus faible comparé à 191, 27, R4 et 291. 291 voit ce taux augmenter comparé à tous sauf R4 (moyenne de 26.8 pour 191 et de 20.3 pour R4 fin 2015). R4 est meilleur comparé à 14, R3 et 27 en 2014 mais seulement comparé à 14 et R3 en 2015. Le taux a en effet pris plus d'importance pour le clone 27 (*tableau 14*, *figure 24*). En ce qui concerne la densité de plantation, seule la densité de 6 arbres par sceau peut être comparée aux deux autres, et est pire pour le taux de croissance relative. Seuls les sols à contamination ancienne et sans contamination peuvent être comparés. La contamination a un impact négatif sur la croissance (*tableau 14*).

Selon le modèle 3 avec interactions, l'interaction du clone avec la pollution du sol est significative fin 2015. La densité est significative seule dans ce cas (*tableau 15*). Dans un sol sans contamination, le clone 291 est significativement le meilleur comparé à 14, 191 et R3. R4 est seulement significativement supérieur à 14 et R3. 14 est inférieur à tous excepté R3 et R3 à R4, 291 et 27. Dans un sol à contamination ancienne à la créosote, 291 ne peut cette fois être comparé significativement qu'à 191 et R4 mais est toujours supérieur. Les différences ne sont pas aussi importantes (*tableau 16*, *figure 25*). Aucune comparaison n'est significative pour une contamination récente, mais le graphique laisse présager le même comportement que pour une contamination ancienne. Le taux de croissance varie moins suivant la contamination pour 14 et R3 qui sont stables, et plus pour 291 (*annexe 18*).

Pour la croissance en 2014 seulement, les résultats sont similaires aux résultats généraux. Le test de Kruskal-Wallis ne permet pas de conclure quant à l'influence de la contamination et densité de plantation, mais le clone présente un effet significatif. Les différences entre clones sont cependant moins évidentes que dans le cas général (*figure 26*).

Pour la saison 2015, le test de Kruskal-Wallis montre l'absence d'influence du facteur pollution du sol (*tableau 17*). Les comparaisons montrent des résultats similaires aux résultats généraux pour la densité de plantation, mais pas pour le clone. Le meilleur clone est cette fois 14 comparé à tous les autres excepté 27, et la même chose se passe pour 27. Le taux de croissance relative est fortement corrélé à la croissance absolue dans ce cas (Spearman, rho=0.88, p-value<1e-15) (*figure 27*).

3. Diamètre

Le diamètre est faiblement corrélé au taux de croissance relative (Spearman, rho=-0.14, p-value<0.01), mais a une bonne corrélation avec la taille finale (Pearson, cor=0.83, p-value<1e-15). Le diamètre est donc lié à la croissance absolue et ne peut être analysé avec certitude sans connaissance du diamètre original. La seule différence observable entre le diamètre et la taille est liée au clone R3, pour lequel le diamètre semble augmenter de manière plus importante que la taille (*figure 28*).

4. Tiges par drageonnement

28 % des sceaux présentent au moins une tige par drageonnement à la fin de l'expérience. Selon l'analyse du modèle 1 sans interaction, le clone et la densité de plantation influencent la présence de drageons significativement, mais pas la contamination (*tableau 19*). Cette influence vient du clone R4 (tiges dans 59 % des sceaux), qui produit plus de tiges que tous excepté 191 (41 % des sceaux). Cette valeur est inferieur à 25 % pour les autres. Pour la densité de plantation, 6 arbres et 2 arbres par sceau augmentent la présence de tiges, avec 44 % des sceaux concernés pour la plus haute densité et 28 % pour 2 arbres (*tableau 18*). Il n'y a pas d'influence de combinaisons de facteurs d'après l'étude du modèle 2 et 3, mais l'influence du clone et de la densité de plantation seuls est confirmée par le modèle 3.

Le nombre de nouvelles tiges varie de 0 à 12. 13% des sceaux seulement présentent au moins 2 tiges. La médiane est de 2 pour R4, alors qu'elle est de 0 pour les autres clones d'où une confirmation de l'effet de ce clone. Les variations en nombre sont minimes selon la densité de plantation, ce facteur ne paraît donc pas si important au final (*figure 29*). Aucun modèle parmi ceux essayés ne s'ajuste aux données dans ce cas.

5. Fluorescence chlorophyllienne

La valeur de Fv/ Fm est toujours en dessous de la valeur optimale de 0,85, mais 85 % des arbres présentent une valeur supérieure à 0,75, qui est la valeur limite pour une définir un bon état général. Seuls des cas isolés présentent des valeurs inférieures à 0,7.

Le test de Kruskal-Wallis montre une influence significative seulement pour le clone, que ce soit pour Fv/Fm ou PI (*tableau 20*). En ce qui concerne Fv/Fm, le clone R3 présente une meilleure moyenne que tous excepté 14. 14 est supérieur seulement à 291. Pour PI, deux groupes peuvent être formés. Les meilleures valeurs sont pour 14, 27 et R3 (*tableau 21*, *figure 30*).

En comparant les valeurs des trois mois de mesure, on remarque que les valeurs sont inférieures en juillet et août par rapport à juin. Quelques différences peuvent être observées en juin. Pour Fv/Fm, le clone 27 est cette fois celui avec la moins bonne valeur comparé à R3, R4 et 191. Cependant les résultats généraux sont les même pour PI.

Les corrélations ne sont jamais bonnes entre les mois. Les valeurs de Fv/Fm sont proches pour chaque cas de contamination (*figure 31*). Les contrastes entre chaque clone semblent accentués dans le cas d'absence de contamination.

6. Conditions des feuilles

Les feuilles ont été touchées par des dégradations de la part d'insectes et champignons dès juin, et particulièrement en juillet et août (*figures 32 et 33*). 78% des arbres présentaient moins d'un quart de leur feuillage touché en juillet, et 74 % en août, cela est donc resté limité. Cela peut cependant avoir été une source de stress pour l'arbre. Il n'y a pas d'influence de la contamination sur ces dégradations, et aucune dégradation ne semble donc due à la pollution. Une forte densité de plantation augmente la présence de dégradations.

Pour le nombre de feuilles, le test de Kruskal-Wallis démontre une influence significative de tous les facteurs (*tableau 22*). Les effets de la densité de plantation et de la contamination sont attendus : le nombre de feuilles augmente quand la densité de plantation diminue (*figure 35*) et en l'absence de contamination (*figure 34*). 14 est significativement différent de tous excepté 27, et 27 de 191 et R4. Les deux ont un nombre de feuilles moins important. La corrélation du nombre de feuille avec le taux de croissance relative est très mauvaise.

IV. Discussion

1. Interprétation des résultats

Le choc de plantation est une explication quant aux impacts négatifs sur la croissance et la survie dans les premières étapes après l'introduction dans un nouvel environnement. La croissance est diminuée, les feuilles tombent et il y a une mortalité importante. Cela est associé au processus d'acclimatation des plantes et est dû à des caractéristiques intra plantes tel des déficiences en réserves et un ratio tige/racine trop grand, et à des facteurs externes comme la sécheresse, le froid, les dommages mécaniques et une possible compétition avec la végétation environnante (Close et al., 2004). Dans cette expérience, les jeunes arbres ont été transplantés et transférés de conditions internes à externes. Certains risques sont diminués par la présence de la serre et la plantation dans des sceaux. Cependant, la mortalité majoritairement observable la première année et la faible croissance en hauteur de cette même année peuvent être attribuées au choc de plantation. Les arbres avaient probablement des feuilles plus adaptées à l'ombre et étaient placées dans des conditions de densité importante. Ils étaient également probablement arrosés plus souvent. Le sol dans lequel ils ont été transférés n'est plus spécifique à la favorisation de la croissance, avec la présence d'éléments grossiers qui rendent plus difficile le contact des racines avec le sol (Close et al., 2004). Enfin et le plus important, certains des sols étaient pollués, offrant des conditions très différente aux arbres. La contamination du sol a effectivement un effet sur la survie et sur l'importance de la croissance. Les différences pour chaque clone pourraient être dues à différentes capacité d'acclimatation. Il est donc important de prendre en compte cet effet lors de la plantation de jeunes plantes dans des terrains pollués, et une préparation à l'acclimatation peut être nécessaire.

Lors de l'analyse de la survie, il a été trouvé que la contamination ancienne améliorait le taux de survie. Des différences observées dans les différents types de sol peuvent aider à interpréter ce résultat (*figures 36, 37 et 38*). Dans les sceaux avec contamination ancienne, le sol est plus sombre, avec la présence de mousses la troisième année. Il est moins sec et plus compact et élastique que le sol sans contamination. Ce dernier sèche plus rapidement. Le sol avec contamination nouvelle est souvent dans un état intermédiaire, séchant rapidement mais plus sombre que le sol sans contamination. Les sols viennent de la même zone générale, et la possibilité d'une différence à l'origine est écartée car le sol sans contamination et avec contamination au diesel ont été pris exactement au même endroit et cependant des différences commencent à apparaître. Une augmentation dans la présence d'huile réduit la densité maximale de matière sèche et la contenance optimale en eau. Il y a aussi une réduction dans la perméabilité (Khamehchiyan et al., 2006). Cela pourrait expliquer pourquoi le sol est moins sec dans le cas d'une contamination ancienne. Finalement, le fait que la survie est plus importante dans le sol à contamination ancienne serait dû à la meilleure rétention en eau de ce sol et aux changements dans ses propriétés. Comme les contaminants sont hydrocarbonés, le taux de matière organique est aussi amélioré, ce qui pourrait être une autre explication. Cependant, la croissance est toujours meilleure sans contamination, donc l'effet négatif du contaminant semble prévaloir dans le long terme.

Les polluants sont avant toute chose un facteur de stress pour la plante. L'impact de l'huile est lié avec le type et la quantité d'huile impliquée, le degré de vieillissement, la période de l'année et l'espèce concernée. Les huiles légères peuvent causer des dommages précis tandis que les huiles lourdes, avec HAP (hydrocarbures aromatiques polycycliques), causent des blessures chroniques. L'huile fraîche est plus toxique que l'ancienne. L'huile peut provoquer des interférences avec les échanges gazeux si les stomates sont bloqués, une perte d'énergie sous forme de chaleur due à une augmentation inutile en oxygène, des dommages dans les mitochondries et l'inhibition des mécanismes de transport (Baker, 1970). La survie et croissance moins importantes dans le cas de contamination récente peuvent être expliquées par ces effets. Le vieillissement, causé par la séquestration des constituants dans le sol, diminue la toxicité (Tang et al., 2011). Cela pourrait expliquer l'absence de significativité pour la contamination récente les années suivantes, mais ceci est plus surement lié à l'important taux de mortalité de la première année. La toxicité ayant diminué pour la contamination ancienne, cela explique par contre la meilleure survie et croissance dans ce sol comparé à la contamination récente. L'effet général de la contamination diminue dans le temps.

En ce qui concerne la survie, une contamination récente augmente les différences entre clones. Pour la croissance, cela est différent : les différences sont augmentées par l'absence de contamination. Ces deux comportements différents peuvent s'expliquer car la survie est en général bonne et donc les différences sont seulement visibles dans des cas de stress extrêmes, où un risque réel est présent. La croissance est plus variable pour chaque clone en général, et dans ce cas la présence de contamination la réduit globalement.

Les arbres avec la meilleure efficience pour la photosynthèse étaient supposés présenter une meilleure croissance étant donné que la production de biomasse est liée à la photosynthèse. Les arbres à meilleures force interne pour résister aux contraintes devraient également être ceux qui résistent le mieux aux conditions de stress. En 2015, les meilleurs arbres pour la croissance sont 14 et 27, qui sont aussi les meilleurs pour les valeurs des paramètres de fluorescence. L'importance de R3 pour l'efficience de la photosynthèse ne peut pas être expliquée par une forte croissance en hauteur. Cependant, R3 est le clone pour lequel le diamètre semble le plus augmenter comparé à la croissance générale. Il pourrait donc s'être également développé, mais en diamètre. Une autre possibilité est que sa croissance soit plus développée pour les racines. Les clones 291 et R4 ayant des valeurs moins importantes présentaient en effet une croissance moins importante cette année là.

Les raisons pour les moins bonnes conditions de 291, R4 et 191 ne sont pas évidentes. Ils pourraient être moins capables de résister aux contraintes externes, mais n'ont pas été plus touchés par des dégradations que les autres. Une hypothèse est qu'ils dépolluent effectivement le sol contrairement aux autres, ce qui crée un stress. Cependant, l'absence de significativité du facteur contamination contredit cette hypothèse. Il est probable qu'ils sont simplement moins efficients que les autres.

L'huile est censée inhiber les échanges gazeux, et créer des dommages au niveau des mitochondries (Baker, 1970). Des expériences précédentes ont montré que le taux de photosynthèse était influencé par la présence de diesel. Cela ne conduisait cependant pas à des effets drastiques dans le cas des peupliers. De plus, les paramètres de fluorescence chlorophyllienne étaient moins impactés que la photosynthèse réelle dans des conditions de pollution. Cela était lié à un plus grand effet de la pollution sur les processus de carboxylation, et non au niveau des thylakoïdes. La structure des thylakoïdes est en effet stable et le flot d'électrons peu affecté à travers les photosystèmes (Pajevik et al., 2009). Les paramètres calculés à partir de la fluorescence chlorophyllienne pourraient au final être de bons indicateurs de croissance et de stress lié aux dégradations, mais pas de la pollution par des constituants organiques. Ceci peut également être dû à un impact moins important de ces constituants sur la photosynthèse par rapport aux métaux lourds par exemple. La diminution des valeurs en juin et juillet pourrait être liée aux dégradations des feuilles, mais montre aussi que ces valeurs sont moins importantes lorsque la croissance est moins importante, confirmant l'indication de la croissance par les mesures de fluorescence chlorophyllienne.

Le nombre de feuilles semble un indicateur de la densité de plantation et des conditions de pollution, mais cela est limité pour déterminer le degré de contamination. Cela a été défini comme un bon indicateur de la toxicité dans le cas de pollution saline (Vaario et al., 2011), mais nécessite plus de recherche pour confirmation dans le cas de pollution organique. Le nombre de feuilles n'explique pas l'importance de la croissance en hauteur, la surface foliaire serait sans doute plus efficace comme la taille des feuilles varie beaucoup entre clones.

L'effet de la densité de plantation n'est pas aussi important qu'attendu, et ne présente pas d'interaction avec d'autres facteurs. Son influence sur la croissance semble augmenter dans le temps. Une augmentation de la densité diminue la croissance mais augmente le drageonnement. Si la croissance est limitée mais qu'il y a plus d'arbres, la décontamination pourrait être meilleure et donc des questions restent en suspens sur l'influence de ce facteur. Les tiges par drageonnement apparaissent généralement dans les trois premières années, ce nombre ne devrait donc pas augmenter si de nouveaux stress n'apparaissent pas. Les nouvelles tiges ne devraient pas avoir un effet très important sur la croissance en hauteur générale, car les espèces intolérantes se développent dès le départ en hauteur sans attendre une situation favorable (Louisiana Pacific Canada ltée and CERFO, 2002).

Pour la survie, 291 est efficace tandis que les clones 191 et 27 ne sont pas les meilleurs. Pour une contamination ancienne, cela n'a pas vraiment d'importance dû au très faible taux de mortalité général. Pour la croissance en hauteur, R3 et 14 semblent ne pas pouvoir être choisis selon les résultats généraux. Sans contamination, 291 est le meilleur suivi par R4 et 27. Avec une contamination nouvelle, 191 doit cette fois être considéré également et les différences sont moins importantes. Cela n'est pas confirmé significativement. Avec une contamination ancienne, 291, 191 et R4 ont une meilleure croissance relative. Cependant, cet ordre semble commencer à changer dans la dernière année. Le clone 14, meilleur en 2015 pour la croissance est aussi le clone présentant la taille et la croissance absolue les plus grandes. L'autre meilleur clone de 2015, 27, entre également dans les meilleurs pour les résultats généraux. 14, 27 et R3 ont aussi les meilleures valeurs pour la photosynthèse. Selon de précédentes recherches, trois à quatre ans sont nécessaire pour réaliser un choix dans les clones. Cependant, ici les conditions sont de plus particulières du fait de la pollution. Un à deux ans sont nécessaires en plus au minimum pour pouvoir tirer des conclusions, et au moins dix ans d'expérimentation en plein champs permettraient de prendre en compte d'autres paramètres comme l'apparition de cancres et la valeur commerciale.

Le meilleur clone pour l'établissement est 291 suivi de R4, 191 et 27 devant aussi être considérés pour une contamination ancienne. De plus amples recherches sont nécessaires en ce qui concerne le développement des arbres.

La supériorité des hybrides sur les clones de *Populus tremula* L. ne peut pas être confirmée ici.

2. Analyse critique de l'expérience

La présence de trois réplications est intéressante car elle permet de conserver des combinaisons dans le cas de la mort de certains arbres, et confirme l'influence des facteurs. Cependant, si le but est de prendre en compte des variations externes, il serait plus intéressant de les placer dans des endroits différentes et non côte à côte. La présence d'autres trembles à proximité pourrait de plus avoir augmenté le risque de dégradations.

Les effets des polluants doivent être nuancés car leur migration est limitée par la présence du sceau. Les polluants organiques sont supposés rester dans la zone des racines, cependant à Somerharju les polluants se sont déplacés en profondeur dans le sol. Le majeur problème est que le niveau précis de contamination n'est pas connu dans chaque cas, et que les caractéristiques utilisées pour la détermination de la pollution ne sont pas les même pour les différents contaminants. Le diesel est décrit par un pourcentage général de pollution, et la créosote par une quantité d'hydrocarbures trouvés dans des analyses générales, alors que la pollution est rarement uniformément répartie dans des cas réels (Conesa et al., 2011). Les deux contaminants ont également des propriétés différentes, et les effets observés pourraient donc provenir du type de polluant ou de l'âge sans certitude.

La densité de plantation n'a jusqu'à présent pas été majoritairement étudiée dans le cas de contamination. Dans des cas réels, des considérations spéciales doivent être prises en compte comme la prévention de l'érosion, l'allocation optimale de la ressource en eau et la fermeture de la canopée (Zalesny and Bauer, 2007). Des arbres proches pourraient être efficaces au départ avant de devoir être éliminés, ce qui pourrait augmenter les coûts. Les espacements généralement utilisés sont beaucoup plus importants que ceux de cette expérience (2.1 X 3.0 m à 4.0 X 4.0 m) (Zalesny and Bauer, 2007), et les résultats ne peuvent donc pas être appliqué directement sur le terrain.

Les mesures ont été réalisées par différents stagiaire chaque année, des erreurs liées à l'opérateur peuvent donc exister. Certains arbres sont de plus courbés, ce qui rend la mesure plus incertaine. Un manque d'échange d'information a conduit à des changements dans la précision et dans la date des dernières mesures.

Seules l'observation de la survie et la mesure de la taille étaient réalisées chaque année et sont donc fiables. Les mesures de fluorescence n'ont été réalisées qu'une seule année, et les résultats pourraient être dus à des conditions particulières cette année là. Elles reflètent également les conditions du jour de la prise de mesures. Comme le but principal était de comparer les combinaisons entre elles et non dans le temps, cela ne devrait cependant pas poser de problèmes. Le manque de caches pour la réalisation des mesures a obligé à réaliser la mesure en deux fois pour chaque bloc, et un jour entier était nécessaire à chaque fois pour la prise de mesures. Cependant, les changements dans les conditions de stress par jour devraient être assez limités pour que les résultats soient fiables. Les tiges par drageonnement étaient parfois difficiles à identifier avec certitude.

Les individus considérés étaient les arbres la plupart du temps, cependant les individus créés avec le plan d'expérience étaient les sceaux. Il était plus logique d'analyser les données au niveau des arbres quand des informations précises étaient disponibles pour chacun d'entre eux, cependant cela diminue l'intérêt du plan initial. Le taux de croissance relative a été utilisé car était plus cohérent. L'utilisation de la croissance absolue pourrait être considérée dans des conditions particulières d'instauration des plantes ou des connaissances associées sur les qualités des clones pour la micro-propagation.

Les différences significatives entre facteurs sont limitées, particulièrement dans le cas des interactions. Cette expérience permet d'avoir une première idée de l'influence de facteurs, mais ne permet pas de sélectionner à un haut pourcentage de fiabilité les meilleures conditions. Une transformation des variables pour obtenir un meilleur ajustement à une loi théorique et améliorer l'utilisation de modèles pourrait être utilisée, mais s'est révélé inefficace dans le cas de la fluorescence chlorophyllienne. Le principal problème est lié au manque d'interactions significatives avec le traitement, qui ne permet de tirer que des conclusions générales.

Les arbres étaient entreposés à l'intérieur pendant l'hiver, cependant ils doivent pouvoir résister aux conditions hivernales en Finlande dans des cas réels de phytoremédiation. Des limitations additionnelles sont le manqué de connaissance sur le développement de la biomasse totale et des racines ainsi que la jeunesse des arbres. La phytoremédiation est en effet fortement dépendante du développement des racines, pour absorber les polluants et à cause des phénomènes liés à la rhizosphère. Un bon développement général des racines permet également de résister à des contraintes externes. Cette expérience se focalise sur des paramètres allométriques. Des données physiologiques sont cependant essentielles pour confirmer la dépollution (Zalesny and Bauer, 2007). Il est important d'ajouter que les arbres concernés n'ont à la base pas été sélectionnés pour la dépollution, mais pour leur production de fibres courtes.

3. Des propositions supplémentaires pour cette expérience et les autres à venir

Trois ans sont assez limités, il pourrait être intéressant de garder au moins une répétition deux ans de plus. Cela permettrait d'observer les changements attendus dans l'importance des clones. Certaines informations nécessitent cependant une récolte de la plante.

Une répétition entière doit être récoltée pour que cela soit significatif. Les tiges et bourgeons seront ensuite séparés des racines. Séparer les feuilles pourrait également être intéressant, maïs si la fin de la saison de croissance est attendue cela ne sera pas possible.

Ensuite:

- Les principales particules de sol sont enlevées. Les racines doivent être nettoyées de façon à éliminer les particules fortement liées.
- La disposition des racines peut être classifiée. Il est important de connaître le type de développement car cela peut améliorer l'ancrage dans le sol.

- La longueur des principales racines sera mesurée. Le nombre de racines principales peut également être déterminé, en accord avec un diamètre choisi dépendant de la situation.
- Les racines peuvent être séparées entre latérale et basales pour étudier plus précisément leur disposition, mais surtout entre racines fines et conséquentes. Les racines fines permettent en effet d'augmenter la surface en contact avec le sol.
- Les différentes parties de la plantes doivent être séchées à 70°C en étuve, la température commune pour les tissus biologiques jusqu'à poids constant (Zalesny and Bauer, 2007). Le poids final est noté.

Ces mesures permettent de connaître la biomasse totale de la plante et donc la croissance totale. Le pourcentage de biomasse attribué aux racines est intéressant. La considération de la biomasse totale du sceau permettrait de résoudre le problème de l'effet final de la densité et des nouvelles tiges.

La réalisation d'analyses du sol n'est très réaliste car la pollution initiale n'est pas connue et cela est cher. De plus, le temps nécessaire pour la phytoremédiation est de plusieurs années. L'analyse des constituants des racines pourrait être intéressante pour savoir si les contaminants sont vraiment extraits du sol. L'analyse des tiges permettrait de savoir si les contaminants se sont déplacés. Cela ne peut pas être réalisé si la biomasse est étudiée comme précédemment décrit, ou alors des échantillons doivent être prélevés. La présence de bactéries dégradant les HAP dans le sol proche des racines serait plus efficiente, mais devrait être réalisée pendant la saison de croissance.

Pour les répétitions restantes, une mesure pouvant être ajoutée est la détermination de la surface foliaire, mais cela nécessite de ramasser les feuilles pour plus d'efficacité (Zalesny and Bauer, 2007).

Cette expérience doit être nuancée, comme toutes les expériences réalisées en laboratoire ou sous serre. Les résultats obtenus en plein champ restent décisifs, et permettent de mettre en avant des éléments non observables à échelle réduite. La phytoremédiation est souvent citée pour ces bas coûts, mais il peut être nécessaire d'entretenir la zone en plein champ (*figure 39*). L'utilisation d'hybrides pourrait l'imiter l'adaptation aux conditions en Finlande.

D'autres expériences en serre pourraient être menées selon un plan légèrement différent. Le même contaminant pourrait être utilisé à différentes stades de vieillissement, ou mieux le même contaminant à différentes niveaux de contamination, cette fois connus. Il est conseillé :

- d'utiliser le même sol d'origine et d'établir différentes niveaux de contamination par diesel et créosote.
- D'analyser les niveaux de contamination.
- De planter des clones à différentes densités. Pour correspondre à des densités réelles, des contenants plus conséquents et proches de la réalité doivent être utilisés.
- Les mêmes mesures allométriques peuvent être réalisées.
- La connaissance de la variation en pollution reste la base, dans la plante pour connaître les mécanismes en jeu et établir des comparaisons ou dans le sol, mais dans ce cas plusieurs années sont nécessaires pour observer un effet.

Conclusion

Cette expérience permet de tirer quelques conclusions sur la mise en place de trembles à des fins de phytoremédiation. La première année de mise en place est décisive car les arbres sont fortement impactés par les changements de conditions. Une nouvelle contamination au diesel a un important impact sur la diminution du taux de survie, et la contamination retarde la croissance en général. Cependant, une ancienne contamination à la créosote provoque des changements dans les caractéristiques du sol qui limitent les effets négatifs sur l'établissement des arbres, et améliorent même les conditions dans les premiers stades. Cela signifie que l'établissement sera facilité dans le cas de pollution ancienne, et qu'une acclimatation préalable est nécessaire dans le cas d'une contamination récente. L'effet de la contamination des sols diminue ensuite, comparé à l'effet des autres facteurs. La densité de plantation n'a pas d'effet particulier dans les premières étapes de l'établissement des plantes, mais commence à être influente dans la troisième année. Ce facteur aura sûrement plus de pertinence dans les étapes de développement de la plantation, réduisant probablement la croissance selon ces premiers résultats.

Les différents clones présentent des comportements variés. Le plus important pour la mise en place est de sélectionner ceux ayant une bonne capacité à surmonter les conditions initiales. Dans ce cadre, il est important de sélectionner les clones dans le cas d'une contamination récente. Le clone 291 est intéressant pour ces premières années en raison de son taux élevé de survie et de son important taux de croissance relative, tout comme R4. 191, 27, 14 et R3 ont moins de résistance dans l'un ou l'autre cas. Toutefois, certains changements semblent se produire au cours du développement des clones, étant donné que les clones 14 et 27 ont le taux de croissance relative le plus élevé la dernière année, et la meilleure efficience photosynthétique. 14 et 27 sont donc de potentiels futurs meilleurs clones, ce qui ne pourrait être confirmé que par des années de mesures additionnelles. Si un des objectifs est de mettre en place des densités plus élevées à moindre frais, le clone R4 est intéressant en raison de sa forte production de nouvelles tiges par drageonnement.

Après cette expérience, il est évident que beaucoup d'informations manquent. En effet, si la résistance initiale lors de l'établissement est importante, le développement des arbres sera le sujet principal dans un cadre de phytoremédiation. Pour déterminer les meilleurs clones pour la croissance, il est nécessaire de savoir comment les arbres vont se développer dans les prochaines années. Etant donné que l'accroissement va progressivement diminuer, cela devrait être déterminé avant que les arbres n'atteignent leur maturité, en deux ans de plus. Cependant, une décision finale ne doit pas être prise avant au moins dix ans d'expérience en plein champ. En outre, c'est l'accroissement de la biomasse qui est important, donc la biomasse totale doit être considérée, ce qui rend nécessaire la récolte de certains arbres. Enfin, les capacités réelles de dépollution ne seront pas connues sans analyses. Il est donc conseillé de laisser au moins une réplication en place pour la poursuite du développement et de réaliser une récolte afin de s'intéresser à la biomasse. Il serait également utile de réaliser d'autres expériences dans lesquelles l'analyse des contaminants serait réalisée dès le départ.

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RESUMÉ

Une expérience commencée en 2013 sur l'effet de conditions de pollution et de densité de plantation sur l'établissement de deux clones de Peuplier tremble (*Populus tremula* L.) et quatre clones d'hybrides (*Populus tremula* L. X *Populus tremuloides* Michx.) a été réalisée en Finlande. Des sols sans pollution, avec ancienne contamination à la créosote et avec contamination récente au diesel ont été utilisés. Les densités de plantation étaient de un, deux et trois arbres par sceau. La taille et la survie ont été suivies durant trois ans, et la fluorescence chlorophyllienne la dernière année. Des analyses statistiques univariées ont été réalisées. La croissance relative a été utilisée. Les résultats montrent que l'effet de la contamination du sol est important pour le taux de survie la première année, une contamination récente le faisant diminuer et une ancienne le faisant augmenter. La croissance diminue avec la contamination, et cet effet diminue dans le temps. Le choc de plantation est donc important. La densité de plantation n'a pas d'effet la première année, mais cela augmente avec le temps. Les meilleurs clones sont 291 et R4, cependant 14 et 27 améliorent leur développement la dernière année. Les clones 27 et 191 ont une survie moins importante que les autres, 291 est plus résistant. L'efficience du photosystème II et l'indice de performance ne sont pas liés à la densité ou à la pollution, et meilleurs une croissance plus importante. Une plus longue durée et une récolte sont nécessaires, ainsi qu'une meilleure connaissance de l'évolution de la contamination.

Mots clés : tremble, tremble hybride, sélection, phytoremédiation, acclimatation, hydrocarbures pétrolifères, densité de plantation