Analysis of interactions between rubber tree (Hevea brasiliensis Mull. Arg.) and inter-crop roots in young plantations of NE Thailand

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THESE

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Analyse des interactions entre les racines d'hévéa (*Hevea brasiliensis* Muel. Arg.) et de cultures intercalaires dans les jeunes plantations du Nord-Est de la Thaïlande

par Santimaitree Gonkhamdee

Soutenue le jeudi 1er juillet 2010 devant un jury composé de

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LIST OF ABBREVIATIONS

Acronyms of institutions

EGIDE-PHC programme	Hubert Curien partnership program for bilateral exchanges on science and technology
EMMAH	Mediterranean Environnements and Modelling of Agro-Hydrosystems
INRA	the French National Institute for Agronomic Research
IRD	The French Institute of Research for Development
IWMI	the International Water Management Institute
Lao P.D.R.	The Lao People's Democratic Republic
LDD	The Land Development Department
NAFRI	National Agriculture and Forestry Research Institute
NIH	the National Institutes of Health of the United States of America
PSH	Horticultural Plants and Systems
RRIC	the Rubber Research Institute of Cambodia
RRIM	the Rubber Research Institute of Malaysia
UMR	Mixed Research Unit
UR	Research Unit

Units and symbols

°C	Degree Celcius or centigrade
µS/cm	Micro Siemens per centrimeter
a.s.l.	above sea level
ABA	abscisic acid
C	carbon
Ca	calcium
cm	centimeters
cm^3	cubic centimeters
CO_2	carbon dioxide

DNA	deoxyribonucleic acid
dpi	dot per inch
DRB	dry root biomass
E	evaporation
EC	electrical conductivity
ET	evapo-transpiration
ETo	crop evapo-transpiration (mm/day)
g	gram
H_2O	dihydrogen monoxide - water
ha	hectares
K	potassium
KCL	potassium chloride
kg	kilograms
l	liter
m	meters
m^2	square meter
mg	miligram
Mg	magnesium
ml	milliliters
mm	millimeters
mm^2	square millimeter
MPa	Mega pascal
N	nitrogen
n	number
N_2	di-nitrogen
NH_4OAC	ammonium acetate
P	phosphorus
p	p-value
P	precipitation,
p	mean daily percentage of annual daytime hours
PAR	photo synthetically active radiation

pH	per hydronium ion or pondus Hydrogenii
ppm	part per million
PVC	polyvinyl chloride
Q	runoff
sp.	species
SRL	specific root length
T	transpiration
T mean	mean daily temperature (°C)
TSP	triple sigle phophate
WAS	weeks after sowing
ΔS	change in soil water storage
$\mu mol\ m^{-2}s^{-1}$	micromole per square meter per second

Physical and root architecture parameters

E(0)	exploration index
E(phi)	root exploitation index
$E(\Phi)$	exploitation index
ET	evapo-transpiration
P	physical pressure
q	rate of flow
r	resistance to flow
RAD	Root surface Area Density
RL	root length
RLD	Root Length Density
r_p	resistance to flow in plant
r_s	resistance to flow in soil
RSA	root system architecture
Ψ_l	leaf water potential
Ψ_s	soil water potential
Ψ_w	water potential

Abbreviations related to plants

L.	Linnaeus
DAS	days after sowing
E.	*Eucalyptus*
Glycine max (L.) Merr.	*Glycine max* (Linnaeus) Merril
GN	groundnut
H.	*Hevea*
Hevea brasiliensis Mull. Arg.	*Hevea brasiliensis* Muller Argoviensis
IC	intercrop
IPH	Intercropped + Pruning + High density
P.	*Paraserianthes*
RT	rubber tree
TPN	Trunk Phloem Necrosis
var.	variety

Other abbreviations

3-D	Three dimensions or three dimensional
c.	circa
DART	Data Analysis of Root Tracings (software package)
EDM	Euclidean Distance Map
JAVA	Joint Academic Virtual Application
l	leaf
NIRS	near-infrared reflectance spectroscopy
Mac OS X	Macintosh Operating System version 10 developed, marketed, and sold by Apple Computers Inc.
min	minimum
Msk	Maha Sarakham soil series
NE	Northeast
p	plant
RMB or CNY	Chinese Yuan Renminbi
s	soil
SED	standard error of the difference

Résumé détaillé de la thèse en français

Introduction

Pour d'évidentes raisons agronomiques et environnementales, être en mesure de concevoir et mettre en place des systèmes de culture dans lesquels les plantes accèdent aux ressources de manière optimale revêt une importance cruciale pour tous les intervenants impliqués dans la production agricole. Dans les écosystèmes naturels et cultivés, les plantes déploient, par le biais de leurs systèmes racinaires, des stratégies d'accès aux ressources qui varient et/ou sont modulées en fonction d'un déterminisme génétique, mais aussi selon la disponibilité et la distribution spatiale des ressources (Doussan et al., 2003; Hodge, 2004). En outre, au sein d'une même espèce, les stratégies d'acquisition des ressources varient selon le stade de développement de la plante et/ou des contraintes environnementales (structure et densité apparente du sol, aération, toxicité, etc...). Ces contraintes d'accès aux ressources sont susceptibles d'induire des phénomènes de compétition ou de facilitation au sein du système racinaire et/ou entre systèmes racinaires d'individus voisins.

Les techniques d'intensification telles que la mise en place de cultures d'inter-rang et l'agro-foresterie visent à accroître la productivité globale des terres tout en assurant la durabilité des agro-écosystèmes, via une optimisation de l'utilisation des ressources environnementales (lumière, eau et nutriments) par les plantes, tout en préservant les cycles géochimiques. En théorie, les moyens d'atteindre ces objectif incluent: (i) une réduction de la compétition souterraine via la mise en place d'une complémentarité spatiale et temporelle des systèmes racinaires, (ii) une amélioration de l'accès aux ressources par les divers composants de l'agro-écosystème, par le biais de phénomènes de facilitation, et (iii) une minimisation des pertes par drainage/lessivage au-delà de la zone racinaire. Dans la pratique, les interactions souterraines entre plantes sont complexes et difficiles à mesurer, de sorte que les progrès réalisés dans la conception d'agro-écosystèmes améliorés et durables demeurent modestes, bien que certains principes généraux commencent à émerger (Gregory, 2006).

Dans ce contexte, l'objectif central de ce travail est d'évaluer, par le biais d'une étude détaillée des interactions souterraines entre plantes, si l'introduction de cultures d'inter-rang est susceptible d’apporter des améliorations au fonctionnement des jeunes plantations d'hévéas, dans le contexte biophysique particulier du Nord Est de la Thaïlande.

L'industrie de l'hévéa dans le monde et en Thaïlande

L'hévéa (*Hevea brasiliensis* Mull. Arg.) est la source du caoutchouc naturel, ou latex, qui provient de la sève produite par cet arbre. Le caoutchouc naturel est utilisé pour la production de nombreux produits domestiques et industriels. L'utilisation industrielle du caoutchouc a débuté en Europe dans les années 1750 avec la production de tubes flexibles et de seringues à partir de solutions de latex (Baulkwill, 1989). À l'heure actuelle, les pneus et chambres à air produits pour le secteur automobile consomment plus de la moitié de la production mondiale de caoutchouc naturel. On estime qu'en 2007, mondialement, l'hévéa était cultivé sur 8,95 millions d'hectares (Office of Agricultural Economics, 2009). En Thaïlande, l'hévéa est l'un des arbres cultivés les plus importants et il domine le secteur des cultures commerciales: en 2008, le caoutchouc était cultivé sur environ 2,67 millions d'hectares pour un rendement annuel moyen de 1738 kg de latex par hectare (Office of Agricultural Economics, 2009). En 2008, l'exportation de caoutchouc et produits dérivés a atteint 2,8 millions de tonnes, générant plus de 223 milliards de baths de recettes (environ 5 milliards d'euros) (Office of Agricultural Economics, 2009).

La politique actuelle du gouvernement thaïlandais est d'augmenter les surfaces plantées en hévéa dans le Nord-Est de la Thaïlande, avec pour double objectif d'augmenter les revenus des petits agriculteurs et d'améliorer la durabilité de l'agriculture. La zone couverte par les plantations d'hévéa dans le Nord-Est de la Thaïlande a connu une augmentation constante et soutenue au cours des dernières années et a presque doublé entre 2001 et 2005, passant de 76 238 à 152 890 hectares (Office of Agricultural Economics, 2006). Dans cette region, l'hévéa est planté avec un espacement de 7 à 8 mètres entre les rangs et de 2 à 3 m le long des rangs (Office of Agricultural Economics, 2007). En général, les arbres atteignent une circonférence de 50 cm, à partir de laquelle les arbres peuvent commencer à être saignés (équivalent à un diamètre de ~ 15 cm à hauteur de poitrine), après une période initiale de croissance de 7 ans. Au cours de cette phase initiale de croissance des arbres, dont la durée varie en fonction du matériel clonal utilisé, ainsi que des conditions climatiques et édaphiques (Vinod et al., 1996), l'inter-rang est souvent laissé en jachère. En dépit du fait que la jachère puisse jouer un rôle de maintien ou d'amélioration de la fertilité du sols et de protection contre l'érosion, elle constitue une pratique peu attrayante pour les petits exploitants, du fait que la terre ne génère pas de revenus immédiats durant cette période de 7 ans. En outre, la jachère peut aussi induire une augmentation de la pression des adventices. Une alternative à la jachère est d'introduire, entre les rangs d'hévéa, des cultures d'inter-rang, dans le but de fournir des revenus complémentaires ou de subvenir aux besoins alimentaires domestiques. En effet, certains auteurs ont rapporté que, pendant la phase immature, en particulier au cours des 2-3 premières années, une grande proportion de la superficie totale de la plantation est sous-utilisée par les jeunes hévéas (Laosuwan et al., 1988).

Les principaux types de cultures d'inter-rang utilisées dans les jeunes plantations d'hévéa

Les cultures d'inter-rang représentent une forme particulière d'intensification agraire. En effet, l'intensification agraire porte à la fois sur la temporalité et l'arrangement spatial des cultures dans un système donné. Du fait de la présence d'au moins deux espèces différentes dans un système avec cultures d'inter-rang, compétition ou facilitation entre les espèces en présence vont potentiellement s'exprimer pendant tout ou partie du cycle cultural (Francis, 1986).

Dans les jeunes plantations d'hévéa du Nord Est de la Thaïlande, les agriculteurs utilisent couramment deux types de cultures d'inter-rang. Une première option consiste à planter une légumineuse, qui est alors utilisée pour améliorer la teneur en azote du sol; dans ce cas de figure, l'attente est que la légumineuse améliore la croissance des jeunes hévéas, du fait de la fixation d'azote atmosphérique que ces plantes permettent. Parmi les légumineuses couramment utilisées comme cultures d'inter-rang dans les jeunes plantations d'hévéa du Nord Est de la Thaïlande, figurent: *Vigna ungiculata* (niébé), *Calopogonium caeruleum*, *Calopogonium mucunoides*, *Centrosema pubescens*, *Pueraria phaseoloides* et *Mucuna cochinchinensis* (Office of Agricultural Economics, 2007; Rubber research Institute of Thailand, 2006; Watson, 1989). Par ailleurs, quand des légumineuses arbustives sont utilisées, le feuillage peut être la source d'un fourrage riche en protéines ou d'un mulch riche en éléments nutritifs (Craswell et al., 1998). Le deuxième type de cultures d'inter-rang couramment mis en place dans le Nord Est de la Thaïlande inclut les cultures qui ont une valeur commerciale, tels que, par exemple, le manioc, les arachides, le maïs, le piment et les aubergines (Rubber research Institute of Thailand, 2007).

Effets des cultures d'inter-rang sur la croissance de l'hévéa

Entre 1981 et 1986, Laosuwan et al. (1988) ont testé différentes combinaisons de cultures d'inter-rang dans de jeunes plantations d'hévéas, dans plusieurs régions de la Thaïlande. Leurs résultats ont montré que la croissance radiale des jeunes hévéas, peut, à certains stades de leur développement être affectée par la présence de cultures d'inter-rang. Globalement, ces auteurs n'ont pourtant observé aucun effet négatif des espèces qu'ils ont utilisées comme cultures d'inter-rang (céréales, banane mais aussi certaines légumineuses), sur la croissance des jeunes hévéas (Laosuwan et al., 1988). Par ailleurs, Laosuwan et al. (1988) rapportent que certaines des cultures d'inter-rang qu'ils ont testées, notamment certaines légumineuses et l'ananas, ont permis une amélioration des conditions d'humidité du sol bénéfique aux jeunes hévéas. Plus récemment, Wibawa et al. (2006) ont testé plusieurs systèmes avec *Paraserianthes falcataria* comme culture d'inter-rang et deux types d'écartement entre les rangs d'hévéas, dont un écartement double (16 m). Les résultats de ces travaux montrent que le large inter-rang du système à écartement double donne accès à une bande cultivable de 14 m qui demeure exploitable pendant une plus longue période que l'inter-rang standard. Wibawa et al. (2006) ont également démontré qu'une couverture de légumineuses, en limitant le développement des adventices, peut améliorer indirectement la croissance des jeunes hévéas. C'est le cas en particulier des légumineuses rampantes qui se sont révélées très efficaces pour contrôler *Imperata*. Il a également été rapporté qu'un couvert mixte de légumineuses (*Pueraria phaseoloides* et *Centrosema pubescens)* pouvait avoir un effet positif plus marqué sur la croissance des jeunes hévéas qu'une fertilisation azotée classique (Watson, 1989).Toutefois, il convient de noter qu'en dépit de leurs effets positifs sur la teneur en azote du sol ou la protection de la surface du sol contre l'érosion, l'utilisation de légumineuses comme cultures d'inter-rang peut avoir des effets indésirables: en particulier, du fait que les racines de ces plantes absorbent, selon l’environnement édaphique, de plus grandes quantités de cations que d'anions, elles tendent à avoir un effet acidifiant sur le sol (Bolan et al., 1991; Tang et al., 1998). La profondeur de sol affectée par cette acidification peut atteindre un mètre sous *Stylosanthes hamata* (Noble et Palmer, 1998).

Contexte de la recherche et hypothèses de travail

Les sols du Nord-Est de la Thaïlande sont généralement de qualité agronomique très médiocre,avec notamment de faibles teneurs en matière organiques, en phosphore et en azote et sont considérés comme les terres les moins propices à l'agriculture de l'ensemble de l'Asie du Sud-Est (Parnwell 1988). Le climat de cette region se caractérise par ailleurs par une saison sèche longue et le plus souvent très marquée au cours de laquelle les hévéas doivent très probablement dépendre fortement de leur système racinaire profond pour acquérir l'eau et les ressources minérales essentielles à leur survie. Cette région est une zone marginale pour la culture de l'hévéa en raison de la pluviométrie moyenne annuelle qui y est le plus souvent souvent inférieure au minimum recommandé de 1400 mm et systématiquement inférieure à l'optimum de plus de 1800 mm requis pour cet arbre (Jacob, 2009). Les sols du Nord-Est de la Thaïlande présentent fréquemment un horizon d'impédance mécanique élevée, immédiatement au-dessous de la couche arable (généralement située entre 20 et 40 cm). Selon les conditions d'humidité du sol, cet horizon se comporte comme une barrière physique qui limite ou empêche la croissance racinaire de la plupart des plantes cultivées (Hartmann et al., 1999). Au-delà de consequences immédiates sur le développement racinaire des jeunes hévéas, ces conditions biophysiques adverses sont également susceptibles d'induire, sur le long terme, un stress complexe favorisant une réaction nécrotique du phloème du tronc (Hartmann et al., 2006; Na Ayutthaya Isarangkool et al. , 2007; Do et al. 2010). Cette pathologie couramment désignée en tant que *syndrome de nécrose de l'écorce* ou TPN, affecte de nombreuses plantations d'hévéa dans le monde et touche de manière particulièrement aiguë le Nord-Est de la Thaïlande (Nandris et Chrestin, 1991).

Attendus du projet de recherche

Le principal résultat attendu de cette recherche est la production d'un corpus de données expérimentales sur les modes d'enracinement des jeunes hévéas, en association ou non avec des cultures d'inter-rang. L'approche choisie s'appuie à la fois sur des expérimentations au laboratoire et au terrain. Cet ensemble de données expérimentales comprend des indicateurs quantitatifs, telles que la densité de longueur racinaire, la densité de surface racinaire, l'occupation du volume de sol par les racines, les diamètres racinaires, la densité de la biomasse sèche des racines, la longueur racinaire spécifique, les taux de croissance des racines, etc...

Une première retombée scientifique escomptée est la production de connaissances nouvelles quant aux interactions souterraines entre hévéa et cultures d'inter-rang. En particulier, le travail tente de décrire certains des mécanismes qui influencent la croissance des racines et le développement du système racinaire de l'hévéa en présence de cultures d'inter-rang, via la quantification de paramètres tels que les densités de longueur racinaire, la distribution spatiale des racines au sein du profil, ou encore les variations de taux d'élongation. Il est un fait bien établi que les interactions souterraines entre plantes peuvent induire des réponses plastiques qui ont pour conséquence de modifier sensiblement leur développement racinaire (Weaver et Clements, 1938; Schenk et al., 1999). Ainsi, des études récentes ont montré que tant les associations de deux annuelles ou celles d'une pérenne avec une annuelle pouvaient induire des modifications importantes des profils d'enracinement des cultures principale et d'inter-rang (Li et al., 2006; Mulia et Dupraz, 2006).

Un autre attendu de ce travail est de documenter, au moins indirectement, si, et comment les cultures d'inter-rang influencent la manière dont les hévéas accèdent aux ressources essentielles à leur développement. À cette fin, un accent particulier est mis sur l'interprétation des mesures racinaires en termes, soit de compétition, qui a été identifiée comme un déterminant important du développement de l'hévéa (Harja1 et al., 2005), soit de facilitation qui est actuellement estimée comme étant le processus dominant dans les conditions de stress élevé, par opposition à la compétition qui correspondrait aux situations de moindre stress (Li et al. 2007; Raynaud et al., 2008).

Un attendu plus appliqué de ce travail est la production de connaissances sur les stratégies d'exploration racinaires des jeunes hévéas et de cultures d'inter-rang communément utilisées, afin d'assister les petits exploitants dans le choix et la mise en place de cultures d'inter-rang. La diversité des systèmes racinaires correspondant à une couverture végétale multi-spécifique offre des options pour utiliser les ressources du sol et du sous-sol sur une large gamme de distances latérales et verticales (Stone et Kalisz, 1991; Calder et al., 1997; Jackson et al. 2000; Gonkhamdee et al., 2009).

Une meilleure connaissance des modes d'enracinements dans les jeunes plantations d'hévéa, en fonction de l'introduction ou non de culture d'inter-rang et des caractéristiques souterraines de ces cultures participe donc à un effort de rationalisation des options prises par les agriculteurs en fonction de leurs objectifs de productions, et des contraintes biophysiques liées aux espèces végétales utilisées, d’une part, et au milieu dans lequel elles sont implantées, d’autre part. Dans cette perspective, la connaissance des dynamiques d'enracinement des différentes composantes envisagées d'un agro-écosystème, alimente l'effort de minimisation de la compétition entre hévéas et cultures d'inter-rang, d'optimisation de l'accès aux ressources, de maintenance de services écosystémiques, et de maximisation de la rentabilité économique des exploitations.

Résultats principaux

Etude de la dynamique de la croissance racinaire en rhizotron

Bien que le littérature comprenne un vaste corpus de références sur les interactions souterraines entre plantes, la plupart des travaux sur ce sujet sont basés, soit sur des observations statiques de systèmes racinaires entiers (Schenk et al., 1999), soit sur une analyse de la dynamique de croissance de racines individuelles qui entrent en contact physique direct (Mahall et Callaway, 1991). Dans ce travail, nous avons examiné la dynamique des interactions souterraines dans des associations hévéa-culture d'inter-rang, tant au niveau de la racine individuelle qu'à celui du système racinaire entier, sur la base, notamment, de descriptions numériques détaillées des systèmes racinaires, obtenues à l'aide du logiciel DART (Le Bot et al. 2009).

La numérisation d'architectures racinaires complexes telle que celle étudiées dans ce travail demeure un tache particulièrement fastidieuse: du fait que les systèmes racinaires comprennent, en moyenne, plus de 4000 liens individuels (plus de 8000 dans le cas d'un système racinaire de maïs), l'enregistrement d'une architecture racinaire complète a nécessité en moyenne, 40 heures de travail. Cet inconvénient est néanmoins largement contrebalancé par le fait qu'une fois numérisées, ces descriptions architecturales offrent la possibilité de calculer une très vaste gamme d'indicateurs de croissance et de développement racinaires. Ainsi, ce travail a permis de proposer une approche novatrice de l'analyse de la dynamique racinaire à l'échelle du système racinaire entier, à savoir l'analyse des trajectoires de croissance.

Une différence importante entre le dispositif expérimental utilisé dans ce travail et ceux utilisés dans les études antérieures réside dans le fait qu'aucune disposition particulière n’a été prise pour contraindre les racines d'une plante à rencontrer celles de l'individu voisin. Toutefois, il convient de noter que, de par leur conception, les rhizotrons induisent un développement dans un espace virtuellement bidimensionnel, ce qui doit être considéré comme un facteur qui maximise la probabilité des contacts racinaires. Néanmoins, l'espacement entre les plantes (50 cm), bien que relativement faible, était du même ordre de grandeur que les espacements communément utilisés au champ.

Dans le cas de l'association maïs-hévéa en rhizotron, la croissance et les caractéristiques architecturales des deux plantes, en particulier les longueurs totales de racines, les proportions des différents ordres de ramification ainsi que les taux d'élongation racinaires étaient compatibles avec les valeurs rapportées antérieurement dans la littérature. Ceci indique que, malgré un nombre limité de répétitions, nos conditions expérimentales n'ont pas introduit de biais de nature à invalider les résultats. Par exemple, en conformité avec les conclusions de Pagès et Pellerin (1994), la distribution des longueurs des racines latérales de maïs était du même ordre de grandeur et très asymétrique, pour tous les phytomères (moyenne: 32 mm; médiane: 10 mm). De même, les taux de croissance des racines de maïs et des hévéas étaient comparables à ceux rapportés précédemment (Le Roux, 1994; Pellerin et Pagès, 1994).

Les résultats des expériences en rhizotrons suggèrent également que, dans le cas de l'association maïs-hévéa, les plantes ont ajusté le développement et la direction de l'expansion de leurs systèmes racinaires respectifs en fonction de ceux de leur voisin.

Cette interprétation est étayée par les faits que: 1. chez le maïs, les trajectoires de croissance des systèmes racinaires étaient initialement orientées en direction les systèmes racinaires des hévéas, 2. le maïs a développé des racines latérales anormalement longues à partir de ses racines axiales les plus proches du système racinaire d'hévéa voisin, 3. tant le maïs que l'hévéa ont vu le taux d'élongation de leurs racines impliquées dans un contact physique direct diminuer de manière significative suite à ce contact, 4. l'expansion totale du système racinaire de l'hévéa était significativement plus élevée en présence qu'en l'absence d'un maïs voisin et, par ailleurs, cette expansion plus élevée chez l'hévéa était synchrone avec la phase de croissance la plus intense du maïs (de 20 jours après le semis environ, jusqu'à la formation des épis), et 5. l'hévéa et le maïs voisin ont montré des taux d'expansion concomitants de leurs systèmes racinaires, selon des cycles d'une dizaine de jours.

Toutefois, une telle coordination du développement racinaire des plantes associées en rhizotrons n'a pas pu être confirmée dans le cas de l'association manioc-hévéa. Dans ce cas, au contraire, aucun effet du contact physique direct de deux racines sur leurs taux d'élongation respectifs n'a pu être détecté, et ce en dépit du fait que le manioc a développé des systèmes racinaires denses qui ont intersecté en de nombreux points celui de l'hévéa voisin. Cette absence de réactivité pourrait être liée au fait que les deux plantes, manioc et hévéa appartiennent à la famille des *Euphorbiacées:* dans l'hypothèse d'une médiation chimique d'une régulation inter-spécifique de la croissance des racines, manioc et hévéa pourraient donc, du fait d'une certaine proximité génétique, secréter des exsudats racinaires suffisamment similaires pour que ces deux espèces ne puisse pas détecter ni réagir à la présence l'une de l'autre. A l'inverse, dans le cas de l'association maïs-hévéa, le maïs (un membre de la famille des *Poaceae*) serait sensible aux exsudats racinaires de l'hévéa et vice-versa, du fait d'une distance génétique plus grande entre ces deux plantes. Malheureusement, le cas de l'association hévéa-arachide (un membre de la famille des *Fabacées*) n'a pas permis de fournir de confirmer l'hypothèse d'une médiation chimique du contrôle du taux d'élongation racinaire d'une espèce par une autre, en raison du faible développement racinaire des plantes d'arachide qui n'a permis d'obtenir des contacts entre racines en quantité suffisante (qui n'ont pu être obtenus que dans moins de 20% des essais (répliques) mis en place.

Dans le cas de l'association maïs-hévéa, compte tenu qu'eau et nutriments étaient disponibles en quantités non-limitantes, la probabilité que les observations réalisées puissent être liées à un phénomène de compétition apparaît très peu probable. Dans ce contexte, il semble légitime de considérer l'existence d'un mécanisme de communication qui permettrait au maïs, et dans une certaine mesure, à l'hévéa, de détecter et d'ajuster leurs développement racinaire respectifs en fonction de la présence de racines d'une autre espèce végétale dans le volume de sol qu'ils explorent. Chez les hévéas, les changements de trajectoires du système racinaire ont été d'une ampleur beaucoup plus faible que chez le maïs; il demeure donc impossible, au vu de ces seuls résultats, de déterminer si ces deux espèces sont en mesure de déployer les mêmes stratégies pour adapter le développement de leur système racinaire à celle de leur voisin. Il paraît en effet plausible que, compte tenu des différences de taux d'expansion du système racinaire moyen entre les deux espèces (le maïs produisant au moins cinq fois plus de longueur racinaire par jour que l'hévéa), l'hévéa ne soit pas en mesure de déployer un comportement préemptif, d'autant qu'un tel comportement n'aurait qu'une utilité très limitée comparativement à celui du maïs, incomparablement plus efficace. Bien que dans le cas de l'hévéa, un mécanisme d'auto-inhibition correspondant à une réduction de l'allocation des ressources vers les parties les moins prometteuses du système racinaire (Falik et al., 2003, 2005) puisse être invoqué, puisque que l'élongation des racines d'hévéa est réduite dans le cas des contacts intra-spécifiques, un tel mécanisme ne peut pas être intervenu pour le maïs qui ne montre pas de signes de ralentissement de la croissance racinaire suite aux contacts intra-spécifiques.

Des données récentes suggèrent que les racines sont capables de détecter et d'éviter la présence de racines voisines (Krannitz et Caldwell 1995), et de délimiter l'espace en «territoires» (Schenk et al. 1999). La ségrégation racinaire semble être particulièrement fréquente dans les environnements où l'accès aux ressources est limité (Schenk et al., 1999); au niveau du système racinaire, la ségrégation des racines peut fournir des avantages concurrentiels en termes d'accès à l'eau et pour l'absorption des nutriments (Casper et Jackson, 1997) ainsi que des avantages au niveau de l'occupation de l'espace en limitant le chevauchement entre les systèmes racinaires individuels (Brisson et

Reynolds, 1994). Une telle ségrégation pourrait, au moins en partie, résulter d'un mécanisme d'évitement des volumes de sol sous l'influence des exsudats racinaires d'autres plantes (Krannitz et Caldwell, 1995). Cependant, certains auteurs ont rapporté qu'une médiation chimique des interactions souterraines entre plantes est peu probable en raison de la décomposition rapide de composés organiques utilisés comme "molécules d'identification" (Falik et al., 2003). D'autres mécanismes ont été suggérés, comme une combinaison d'oscillations hormonales et électriques (Souda et al. 1990) qui pourraient être perçus par les racines voisines, en l'absence de contact direct.

En plus du développement racinaire, nous avons également examiné l'influence des associations entre hévéa d'une part et maïs, manioc et arachide d'autre, sur la croissance des parties aériennes des plantes. Dans l'ensemble, le maïs et le manioc ont produit beaucoup plus de longueur de tiges, de surface foliaire, de biomasse aérienne sèche (mais aussi plus de biomasse racinaire sèche), que les hévéas, que ces derniers aient été cultivé seuls ou en association avec une des trois espèces testées. En revanche, seule la surface foliaire de l'arachide, était plus importante que celle des jeunes hévéas. En raison des caractéristiques du manioc et du maïs, à l'issue de la période expérimentale (11 semaines), ces deux plantes avaient atteint une taille très supérieure à celle des jeunes hévéas (bien que ces derniers aient été âgés de 8-12 mois). Ceci a des implications importantes pour la compétition pour la lumière entre les jeunes hévéas et ces deux cultures d’inter-rang.

Les paramètres de développement de la partie aérienne des jeunes hévéas mesurés dans le cadre de ce travail était systématiquement plus élevés (bien qu’à des niveaux statistiquement non-significatifs) lorsque les arbres étaient associes à une culture d’inter-rang (qu’il s’agisse du maïs, du manioc ou de l'arachide) que lorsque qu’ils étaient cultives seuls (contrôle).

L’absence d’effet significatif des cultures d’inter-rang sur le développement de l’hévéa lors de ces expérimentations pourrait être liée au fait que la période d'essai n’a correspondu qu’à un seul cycle de culture d’inter-rang. Il demeure possible que, dans un contexte de plantation, les tendances observées en rhizotrons se transforment en différences statistiquement significatives, du fait de l’effet cumulatif des cycles successifs des cultures d’inter-rang.

Expérimentations au terrain

Bien que les expériences en rhizotron aient apporté des éléments concluants, au moins dans le cas la combinaison maïs - hévéa, quant au fait que les interactions souterraines entre ces deux plantes peuvent induire des modifications de la croissance des racines, à la fois l'échelle de la racine individuelle et à celle du système racinaire entier, les expérimentations au terrain ont fourni, de manière assez prévisible, une image plus complexe des interactions souterraines entre hévéa et cultures d’inter-rang. La grande variabilité de l'enracinement observée au terrain était, en premier lieu, très probablement liée au fait que variabilité des facteurs biophysiques, celle du sol et du climat particulier, ont pu induire chez l'hévéa et les cultures d’inter-rang des réponses plastiques qui ont modulé le développement racinaire (Hodge 2004; Pierret et al., 2007). Néanmoins, un premier résultat obtenu en 2006, par le biais de la mise en place de 'pièges à racines' dans le traitement niébé - hévéa a été de montrer que, dans les conditions de cette expérimentation, rien n'indiquait que ces deux plantes avait un comportement compétitif marqué l'une vis-à-vis de l'autre. En ce qui concerne les caractéristiques des racines, au cours des deux années 2007 et 2008, il a pu être vérifié que les racines des jeunes hévéas présentaient systématiquement des diamètres plus élevés que celles des cultures intercalaires (ou des mauvaises herbes dans le cas du contrôle). Il a pu être également observé que l’hévéa développait systématiquement des racines de faible longueur spécifique (dans une fourchette de 5 à 10 m/g de biomasse racinaire sèche) mais qui occupaient une fraction relativement élevée du volume du sol (par comparaison aux cultures d'inter-rang tout au moins), en particulier aux profondeurs supérieures à 50 cm. Les racines des cultures d’inter-rang et d’adventices avaient en moyenne un diamètre au moins deux fois plus faible que les racines d’hévéa, étaient caractérisées par une longueur spécifique de l’ordre de 20 à 40 m/g de biomasse racinaire sèche et occupaient une fraction relativement faible du volume du sol. Ces caractéristiques correspondent à des stratégies d'exploration du sol contrastées : d’une part, les hévéas paraissent ‘investir’ dans des racines ‘coûteuses’, car de faible longueur spécifique (Bouma et al., 2001; Fitter et al., 1991), probablement pour assurer une certaine durabilité de ces organes; d’autre part, les cultures d’inter-rang favorisent l'allocation des assimilâts vers des racines de longueur spécifique élevée, de construction moins ‘coûteuses’, probablement en réponse à un impératif de croissance plus rapide (suggéré par les taux d’élongation racinaire mesurés au cours des expérimentations en rhizotron).

Bien que les densités de longueur racinaire (RLD) des hévéas aient augmenté de façon marquée de 2007 à 2008 dans les traitements avec arachide et maïs, ce paramètre ne s’est pas révèle être un indicateur fiable de la concurrence / complémentarité entre hévéa et cultures d’inter-rang. En 2008, il a toutefois été observé que, dans tous les traitements, la RLD des cultures d’inter-rang chutait aux profondeurs supérieures à 50 cm, ce qui indique que la plupart des interactions souterraines entre hévéa et cultures d’inter-rang était très certainement restreinte aux horizons de sol peu profonds, au moins dans le contexte biophysique de l’agro-écosystème étudié. Par ailleurs, en conformité avec ce qui a été observé au cours des expérimentations en rhizotron, il y a eu en 2008, une augmentation marquée de la RLD des hévéas cultivés en association avec le maïs, par rapport à celle observée dans les autres traitements.

Enfin, excepté le cas du manioc (mais il doit être noté que les observations correspondant à cette culture ont été effectuées sur un site différent de celui où ont été effectuées les autres observations de terrain), l’introduction de cultures d’inter-rang telles que le maïs et l'arachide n'a pas eu d'impact significatif sur le développement des jeunes hévéas, comme en attestent l'évolution de leur circonférence, hauteur et développement foliaire. Ce résultat de terrain est compatible avec les résultats des expérimentations en rhizotron qui n'ont démontré aucun effet inhibiteur des cultures d’inter-rang sur le développement de la partie aérienne des hévéas.

Perspectives de recherches ouvertes par cette étude

Certains des résultats obtenus dans le cadre de cette thèse ouvrent des perspectives pour des recherches plus approfondies, avec une finalité agronomique appliquée. En premier lieu, comme on a pu le voir dans ce qui précède, ni la croissance des parties aériennes, ni celle des parties souterraines des hévéas n’ont été affectées négativement par la présence d’une autre plante, d’espèce différente, dans leur voisinage immédiat, et ce, tant en rhizotron qu'au terrain. Cependant, ce résultat correspond toujours à une période d'observation courte, et il y serait maintenant nécessaire, dans l'optique de la formulation de recommandations agronomiques, de confirmer le caractère générique de cette observation, pour un large éventail de conditions biophysiques et sur de plus longues périodes de temps. Par ailleurs, les questions relatives à la chronologie de mise en place et d'expansion des systèmes racinaires apparaît être un point à élucider. En effet, les expérimentations en rhizotron, notamment dans le cas de l'association maïs – hévéa, ont clairement indiqué que les phases d'intense expansion et de potentielle compétition/facilitation entre culture d'inter-rang et hévéa sont circonscrites dans un temps assez bref. Il semble donc important de clarifier les rôles et impacts respectifs des cultures d'inter-rang saisonnières de celle des couvertures plus permanentes et de leurs successions dans le temps. A titre d'exemple, on peut citer le travail de Collet et al. (2006) qui montre que la taille du système racinaire du jeune chêne peut se trouver considérablement réduite par la compétition exercée par un couvert herbacé permanent (et ce bien que d’autre caractéristiques racinaires, telles que la densité de ramification ne soit globalement pas affectée par cette compétition).

Un autre résultat présenté dans ce mémoire, dont une étude plus approfondie mériterait d'être entreprise dans le futur, consiste à déterminer si et comment certains comportements 'territoriaux' mis en évidence au cours des expérimentations en rhizotrons se manifestent en fonction des conditions biophysiques rencontrées au terrain, et comment de tels comportements pourraient éventuellement être manipulés pour façonner l'architecture racinaire des hévéas. De récents travaux sur l'écologie des interactions souterraines entre plantes indiquent que la compétition pour les nutriments bio-disponibles est gouvernée par une variété de mécanismes étroitement dépendant des propriétés du sol, ainsi que des nutriments et des plantes considérées (Raynaud et al. 2008): la facilitation paraît ainsi être le mécanisme dominant sous conditions de stress élevé (Li et al. 2007), tandis que la compétition dominerait dans les environnements moins stressants. Dans cet esprit, utiliser des techniques basées sur l'introduction de cultures d'inter-rang pour stimuler la croissance des racines d'hévéa dans certaines parties du profil, notamment les couches profondes et humides du sol, pourrait, sous certaines conditions d'environnement, se révéler bénéfique pour la productivité à long terme d'une plantation. Cet aspect revêt un intérêt particulier dans les zones telles que le Nord-Est de la Thaïlande, où les précipitations annuelles sont marginales pour l'hévéa et où une sécheresse saisonnière marquée prévaut.

Un effort de recherche serait finalement nécessaire pour comprendre comment la coordination de la croissance racinaire de plantes cultivées en association, comme par exemple celle observée en rhizotron dans le cas des cycles d'expansion synchrones des systèmes racinaires de l'hévéa et du maïs, influence les rendements des cultures. Il a été rapporté que l'introduction de cultures d'inter-rang peuvent être préjudiciables aux rendements de l'une des espèces cultivées en association, en raison de la compétition entre systèmes racinaires pour une ou plusieurs ressources (Celette et al., 2005; Collet et al. , 2006; Li et al., 2006). Toutefois, et en contradiction avec ce qui précède, d'autres recherches montrent que la combinaison d'espèces cultivées simultanément peut aboutir à des rendements améliorés, supposément en raison d'une exploration améliorée du sol et par voie de conséquence, une utilisation plus efficace de ses ressources (Li et al., 2006; Mulia et Dupraz, 2006; Malezieux et al. 2009). Bien que les travaux présentés dans ce rapport, ne permettent pas, à eux seuls de conclure de manière assurée comment les espèces cultivées en association peuvent se compléter mutuellement sur le plan fonctionnel, ils apportent des éléments de réponse préliminaires à cette question complexe ainsi que des méthodes permettant de les obtenir. Au total, ce travail représente donc une contribution à la conception des agro-écosystèmes durables qui deviennent de plus en plus indispensables dans le contexte d'une demande mondiale croissante en produits alimentaires et en matières premières.

CHAPTER I - INTRODUCTION: General Background

1. Role of Plant Roots

1.1 Main processes involved in water and nutrient uptake

Roots serve several important functions in plants. They anchor the plant in the substratum and are the principal organ for water and mineral uptake from the surrounding environment. Roots are also important for the storage of food reserves. The roots produced in the soil by a plant are collectively called the root system. The root system begins its development from the embryonic radicle that grows out of the seed after the seed has absorbed sufficient amounts of water; it then continues to grow as the radially symmetrical primary root of the new plant. Secondary lateral roots develop from the primary root, and each of these will in turn form new lateral roots of tertiary rank, The depth, degree of branching, and type of root spreading vary across species. Two morphological types of root systems are commonly distinguished: fibrous and tap root systems (Figure 1). Fibrous root systems are composed of large numbers of roots that are nearly equal in size. Root systems of this type are found in grasses. A tap root system is one in which the primary root, remains the largest root and a number of smaller roots are formed from it (Dickison, 2000).

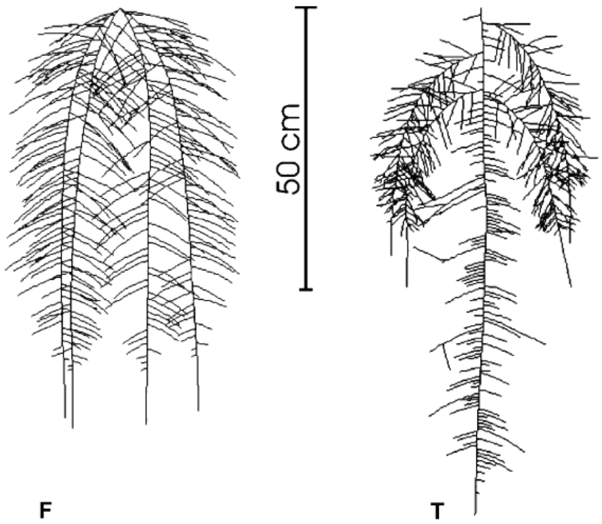


Figure 1 Examples of contrasted root system architectures. The F root system is a fibrous root system and the T root system is the naturally occurring taprooted system. (from Doussan et al., 2006)

Lateral root can arise in a manner that makes water and nutrients more accessible to the plant, this aspect of plant growth has considerable economic importance because it can alter the vigor of crop and horticultural plants under changing growing conditions (Dickison, 2000). The path of water and ion flow through the plant begins at the root hair zone of actively growing roots. Most of the water is absorbed by the plant through delicate root hairs that extend from the epidermal cells. Water uptake decreases with root age (Dickison, 2000).

The importance of the mobility of nutrients in soils in relation to availability to plants was emphasized by Barber (1962) and these ideas which were refined and further developed were summarized in a concept of ‘bioavailability of nutrients’ (Barber, 1984). Although this concept is focused on aerated soils, its principles may also be applied to submerged soils and plant species such as lowland rice. In principle this concept may also be applied to forest trees. In mature forest stands, however, the application of this concept for the development of simulation models of nutrient delivery and uptake is considerably restricted by the high spatial heterogeneity of soil and soil solution chemistry in relation to the stem distance (Koch and Matzner, 1993) and the ill-defined absorbing area of ectomycorrhizal root systems.

The concept encompasses three components: root interception, mass flow, and diffusion. As roots proliferate through the soil they also move into spaces previously occupied by soil and containing available nutrients, as, for example, adsorbed to clay surfaces. Root surfaces may thus intercept nutrients during this displacement process (Barber, 1984). Calculations of root interception are based on (a) the amounts of available nutrients in the soil volume occupied by the roots; (b) root volume as a percentage of the total soil volume-on average 1% of the topsoil volume; and (c) the proportion of the total soil volume occupied by pores, on average 50%, but very much dependent on the soil bulk density (Marschner, 1997). In general, only a small part of the total nutrient requirement can be met by root interception.

The second component is the mass flow of water and dissolved nutrients to the root surface, which is driven by transpiration. Estimates of the quantity of nutrients supplied to plants by mass flow are based on the nutrient concentration in the soil solution and the amount of water transpired either per unit weight of shoot tissue or per hectare of a crop. The contribution of diffusion, the third component relating to the supply of nutrients to the root surface can be calculated on the basis of the effective diffusion coefficients (Marschner, 1997).

1.2 Water transport in the soil-plant-atmosphere continuum

Steudle (2001) summarized the understanding of how water moves from soil to atmosphere via plants is the cohesion-tension theory as follow:

1) Water forms a continuous hydraulic system from soil, via plant, to the atmosphere. This system is analogous to an electrical system with several resistors arranged in series and in parallel.
2) Evaporation from leaves reduces their water potential causing water to move from the xylem to the evaporating surfaces; this, in turn, lowers the water potential of the xylem.
3) Gradients of water potential within the plant result in water inflow from the soil into the roots and thence to the leaves.
4) Water has high cohesion and can be subjected to tensions (negative pressure) up to several hundred MPa before the column will break. The pressure in xylem vessels is less than the equilibrium vapour pressure of free water at that temperature.
5) Walls of vessels are the weakest part of the system and can contain air and/or water vapour. When a critical tension is reached in the xylem vessels, air can pass through pits in the walls resulting in cavitation (embolism).

The driving force, then, for the transfer of water from soil to plant to atmosphere is a gradient of water potential, Ψ_w, and most of this water flows in the xylem. Water can also flow in the phloem, often against this gradient in Ψ_w, and is driven by gradients in P (the physical pressure). Typically, within the pores and cells of plants, Ψ_w is in the range 0 to-3 MPa, but in the atmosphere it is much lower, giving a very large gradient of potential between a leaf and the atmosphere. The flux of water at this interface is affected by the change of phase, as water is converted form liquid to vapour. Energy is required to meet the latent heat of vaporization and it is the amount of energy available, together with the vapour pressure gradient between the leaf and the atmosphere and the rate at which the moistened air can be moved away, that govern the potential loss of water from a plant canopy covering the soil surfacem, Monteith and Unsxorth (1990) quote by Gregory (2006). This external "demand" for water distinguishes water uptake by plants from nutrient uptake in which it is the plant itself that determines demand and regulates uptake via membranes in root cells. For water, plants exercise their chief control at the site of water loss to the atmosphere via the stomata.

Despite the numerically small gradients of water potential within the soil-plant system, they are very important in inducing fluxes of water. These gradients of potential within the continuum drive a flux in a manner similar to what occurs in an electrical resistance network,: this "Ohm's law analogy" has been widely adopted as the basis for understanding and quantifying processes of water uptake and loss (Kramer, 1969; Campbell, 1985; Daamen and Simmonds, 1996 cited by Gregory, 2006). Each element of the pathway that water follows on its way from the soil to the atmosphere is characterized by a resistance that can be measured if the flux and potential gradient are known (Figure 2). At its simplest, this analogue assumes a completely inelastic system without storage so that the inflow to a segment in the system equals the outflow, and the potential difference is proportional to the resistance of the segment:

$$q = (\Psi_s - \Psi_l)/(r_p + r_s) \qquad \text{Eq (1)}$$

where q is the rate of flow, Ψ is the water potential, r is the resistance to flow, and subscript s refers to soil, l to leaf, and p to plant. A clear limitation of equation 1 is that it ignores storage, which is likely to be particularly important in the stems of trees. The processes of flow are also more complex than that suggested by this analogue because flow in soil is largely driven by a gradient of matric potential, that in the plant by a gradient in osmotic and matric potentials, and that to the atmosphere by a gradient of vapour pressure. The resistances, too, may not be constant but change with q especially in herbaceous plants (Jarvis et al., 1981 cited by Gregory, 2006). However, the analogy has been useful in highlighting the stage in the continuum and in analyzing the major resistances in the pathway between soil and atmosphere.

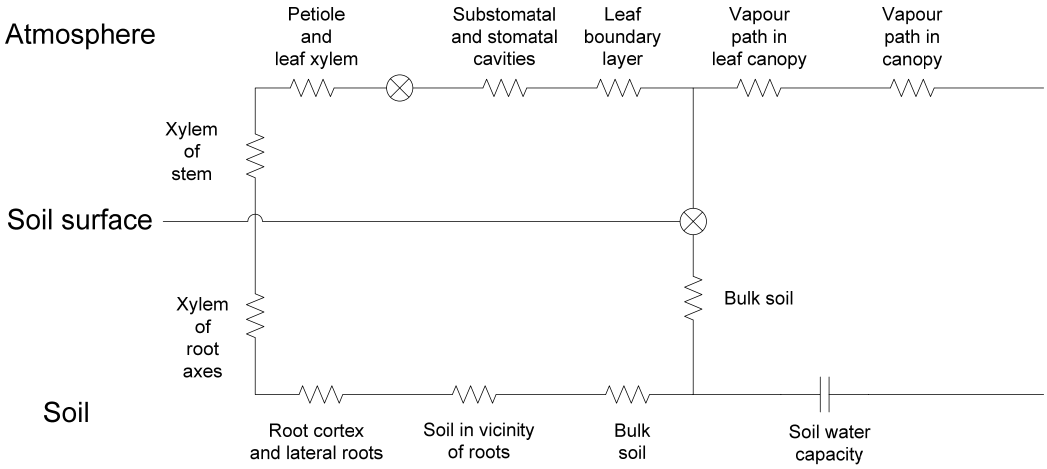


Figure 2 The Ohm's law analogy of the soil-plant-atmosphere continuum showing the pathways of water transfer from bulk soil, either directly or via a plant, to the atmosphere. Resistances (wavy lines) and points of phase change from liquid to gas (cross in a circle) are shown. (from Gregory, 2006)

2. Strategies deployed by plants to access essential soil resources

The strategy of root proliferation as a means to increase competitive advantage is seen in the soil exploration patterns (root foraging strategies) of many species. For example, Mordelet et al. (1996) measured the distribution of roots and N in the patchy savanna of Cote d'Ivoire dominated by the palm tree *Borassus aethiopum* (Mart.). Root mass and total N concentration were significantly greater under clumps of trees (and termite mounds) than outside the same clumps and mounds. Palm trees extended their roots as far as 20 m towards the nutrient-rich patches where they proliferated. This foraging strategy of root proliferation under tree clumps or termite mounds results in both a large area explored and efficient resource exploitation, because high root lengths only occur in nutrient-rich patches.

For a given mineral nutrient, feedback regulating signals on the nutritional status of the shoot to the roots may lead to contrasted responses of the uptake system in different plant species as discussed below for iron. Depending on their response to iron deficiency, plants can be classified into two categories or strategies (Strategy I and Strategy II). In both strategies the responses are confined to the apical zones of growing roots and are fully repressed within about one day after re-supply of iron. Strategy I is typically for dicots and non-graminaceous monocots, and characterized by at least two distinct components of iron deficiency responses: increased reducing capacity and enhanced net excretion of protons. In many instances also the release is enhanced of reducing and/or chelating compounds, mainly phenolics (Olsen et al., 1981; Marschner et al., 1986). These root responses are often related to changes in root morphology and anatomy, particularly in the formation of transfer cell-like structures in rhizodermal cells. In leaves of all plant species the major symptom of iron deficiency is inhibition of chloroplast development. For roots, however, both morphological and physiological changes brought about by the deficiency and responses to this lack of iron depend upon plant species. In both dicots and monocots, with the exception of the grasses (graminaceous species), iron deficiency is associated with inhibition of root elongation, increase in the diameter of apical root zones, and abundant root hair formation (Romheld and Marschner, 1981; Chaney et al., 1992). These morphological changes are often associated with the formation of cells with a distinct wall labyrinth typical of transfer cells. These transfer cells may be induced either in the rhizodermis or in the hypodermis (Landsberg, 1989). The iron deficiency-induced formation of rhizodermal transfer cells (Kramer et al., 1980) is part of a regulatory mechanism for enhancing iron uptake.

Drew et al. (1973), Drew and Saker (1975, 1978) and Drew (1975) demonstrated that barley responded to a localized supply of nitrate, ammonium or phosphate (but not potassium) by increasing the number of primary lateral roots per unit length of axis. Those laterals became longer and, in turn, carried more secondary laterals compared with plants receiving a uniform supply of nutrients.

3. Root system architectures / rooting profiles

3.1 Common types of architectures

Root architecture, the spatial configuration of a root system in the soil, is used to describe distinct aspects of the shape of root systems. Lynch (1995) stated that studies of root architecture do not usually include fine details such as root hairs, but are primarily concerned with the general arrangement of roots within the entire root system of an individual plant. From the architecture, both the topology (a description of how individual roots are connected through branching) and the distribution (the presence of roots in a spatial framework) can be derived, whereas neither topology nor distribution can be used to derive architecture. Root architecture is quite complex and varies between and within plant species. Drawings of excavated root systems of crops and other species show the differences in shape between monocotyledons and dicotyledons and allow some broad generalizations to be made about the depth of rooting and the relative distribution of roots (Kutschera, 1960). Nearly all such drawings show that, with the exception of the tap root which grows almost vertically throughout, most other root axes grow initially at some angle relative to the vertical but gradually become more vertically orientated. Gravitropic responses combined with responses to light, water and soil mechanical impedance, together with the predominance of vertical cracks in deeper soil layers, produce these patterns.

Root architecture's importance lies in the fact that many of the resources that plants need from soil are heterogeneously distributed and/or are subject to local depletion (Robinson, 1994), In such circumstances, the development and growth of root systems may become highly asymmetric, and the spatial arrangement of the root system will substantially determine the ability of a plant to secure those resources (Lynch, 1995), Such ideas have been investigated in a series of experiments and models using common bean (Bonser et al., 1996; Ge et al., 2000). While root trajectories are essentially under genetic control, phosphorus deficiency was found to decrease the gravitropic sensitivity of both the tap root and the basal roots, resulting in a shallower root system. It was hypothesized that the shallower root system was a positive adaptive response to low soil P availability by: first, concentrating roots in the surface soil layers where soil P availability was highest; and second, reducing spatial competition for P among roots of the same plant. This hypothesis was tested by modeling root growth and P acquisition by bean plants with nine contrasting root systems in which basal root angle was varied but not root length or degree of branching. Shallower root systems acquired more P per unit carbon cost than deeper root systems and in soils with higher P availability in the surface layers, shallower root systems acquired more P than deeper root systems because of less inter-root competition as well as increased root exploration of the upper soil (Ge et al., 2000). In practice, the plant may have multiple resource constraints to contend with (e.g. heterogeneously distributed P and soil water) and will try to optimize its investment in roots. Ho et al., (2004) investigated this optimization with respect to beans grown under different combinations of water and P availability. They postulated that an ideally optimized plant would grow roots deeper into the profile until the marginal benefit of extra deeper roots exactly equaled the marginal cost of constructing those roots; through modeling, they found (Ho et al., 2004) that the basal root angle would be shallower for localized shallow P, and deeper for localized deep water compared to the case of uniformly distributed water and P. When P was concentrated in the surface and water was located deep, the optimal basal root angle depended on the relative rates of change with depth in the values ascribed to the available resources. While useful in indicating general principles, it should be remembered that not all of the responses of roots to a heterogeneous environment (e.g. changes in branching frequency and root hair growth) are yet captured in such models; this remains a substantial challenge.

The branching patterns (topology) of individual roots have implications not only for resource capture but also for the construction costs of roots (Fitter et al., 1991). In topological terms, roots can be considered as a mathematical branching tree, with links that are either exterior (ending in meristems) or interior (i.e. internodes). Links have geometrical properties, including length, radius, angle and direction of growth, and are distributed in a defined pattern; as in most branching trees (e,g. the trachea in the lung), the diameter increases with increasing magnitude of the individual link. Fitter et al. (1991) employed a simulation model to demonstrate that a herringbone topology (where branching occurs along a single main axis) with long interior and exterior links is associated with high exploration efficiency, although such a pattern is also characterized by large tissue volumes and hence high construction costs. (Figure 3). Such predictions were only partially supported by the experimental results of Fitter and Strickland (1992) in which *Trifolium repens* L. became more herringbone-like as soil water content increased (contrary to prediction) but *Mercurialis perennis* L. responded as expected to both irrigation and N and P additions. Topological considerations alone, though, are unlikely to be the sole adaptive trait to particular soil environments. For example, Bouma et al. (2001) found that roots of Chenopodiaceae in a salt marsh changed from herringbone-like at low elevation to dichotomous at higher elevations but that the Gramineae showed no such relationship. Moreover, root diameter was not related to link magnitude thereby undermining the basis of the estimates of construction efficiency proposed by Fitter et al. (1991).

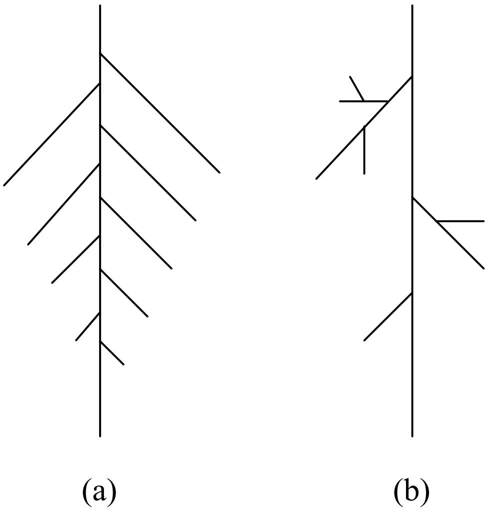


Figure 3 Diagram showing the distinction between (a) herringbone, and (b) dichotomous branching patterns (from Fitter et al., New Phytologist; New Phytologist Trust, 1991.)

Not only does root topology and root system architecture respond to soil heterogeneity, but the form of the root system may, indeed, induce soil heterogeneity. In grassland and savanna systems, caespitose (i.e. tussock or bunch) and rhizomatous perennial grassed represent two distinct forms of grass. In rhizomatous grassed, nutrients can accumulate in the rhizomes but do not accumulate in the soil whereas in the caespitose grasses, both carbon and nitrogen accumulate in soils directly beneath plants resulting in fine-grained soil heterogeneity (Derner amd Briske, 2001). The ‘islands’ of nutrients appear to accumulate beneath caespitose grasses even when they are small, suggesting that they are present throughout much of the plant’s life. Plant-induced increases in nutrient concentrations do not form beneath the rhizatomous species and the large nutrient pool beneath such species in a semi-arid community was largely a consequence of niche separation for microsites characterized by deeper soils with higher amounts of water and nutrients.

In broad-scale agriculture where single crops are grown with inputs of fertilizers, there has been little consideration until recently of root architecture, but with the increasing emphasis on the more efficient use of water and nutrients in production systems, this is starting to change. For example, in soils where P availability is low, selecting genotypes with appropriate architecture may increase soil exploration by roots and raise yields (Lynch and Beebe, 1995). Equally important in other areas is the ability of roots to capture nutrients such as nitrate that might otherwise leach from the soil profile into water coursed. Dunbabin et al. (2003) have shown the role that root architecture may play in this regard and the importance to quickly producing a high density of roots in the topsoil on the sandy soils that they studied. In many part of the world, though, mixed cropping is important either with crops grown together as intercrops or with different crops grown in sequence, as is the growing of trees and crops in agroforestry associations. In such systems, root architecture and distributions are important parameters to consider as they determine both the spatial competition and spatial complementarity of root systems (van Noordwijk et al., 1996)

3.2 Functions of root system architectures

The root system serves several functions simultaneously (Gregory, 2006). It provides a stable platform for the shoot so that the photosynthetic organs can intercept sunlight, and forms a network that can exploit the water and nutrient resources of the soil. The availability and mobility of soil resources varies depending on the particular resource being considered, so that in contrast to the shoot which is essentially harvesting only two resources, light and carbon dioxide, the roots and root system have evolved to cope with a more challenging environment.

3.2.1 Root anchorage

Although anchorage is a major function of the root system, it has not received as much attention as other functions such as water and nutrient absorption. While it has long been assumed that anchorage is merely a byproduct of roots 'main' function as an absorbing organ, it is now realized that the need for anchorage influences the overall root system size and shape (Ennos, 2000).

3.2.2 Water uptake

Water is essential to the life of terrestrial plants and to biota that live in the soil. It carries nutrients in the soil to the roots, is the solvent for, and medium of, most biochemical reactions within plants, and its loss from plants is of the driver of CO_2 exchange with the atmosphere. For most plants, soil is a major source of water, so that the modalities through which soil water is acquired by roots has been, and continues to be, a major topic of soil/plant research (Gregory, 2006).

3.2.1 Nutrient uptake

Unlike water, there is no potential external demand for nutrients that can be readily calculated. Demand for nutrients is driven by the metabolic demands of the plant, and the plant exerts considerable, but not always perfect, control over the quantities of nutrients and other ions that are allowed to enter it. In general, all higher plants have similar requirements for nutrients, although there are some minor variations. An element is essential to a plant if: (1) a deficiency makes it impossible for the plant to complete its life cycle; (2) such deficiency is specific to a particular element and can be prevented or corrected by supplying this element; and (3) the element is directly involved in the physiological or biochemical functions of the plant (Marschner, 1997)

3.3 Interactions

3.3.1 Root systems and competition for resources within the root system

The issue of the size of root system necessary to take up resources in sufficient amounts has been examined in detail in the crop production literature. However, there is no single answer to this question as it is influenced by many factors including the size, architecture and activity of the roots as well as the behavior of the particular resource under consideration in the soil. In general, a large, more intensely branched root system can extract the plant’s requirements from a soil more efficiently than a smaller root system, but the optimal size for a particular resource varies so that there can appear to be an element of redundancy or overprovision in many systems if a mobile resource is used as the basis for comparison (Gregory, 2006). For example, van Noordwijk (1983) calculated that a root length density of 0.1-1.0 cm cm^{-3} throughout the upper 0.2 m soil layer would be sufficient to supply the N requirements of most crop plants, whereas a root length density of 1-10 cm cm^{-3} would be required for less mobile nutrients such as P. For water, the required a root length density is similar to that needed for nitrate, assuming that roots are in intimate contact with the soil but rises to 1-5 cm cm^{-3} if there is an appreciable soil/root contact resistance (Veen et al., 1992). Under usual evaporative demand conditions and assuming that all roots are equally and uniformly active within a soil volume for which spatially uniform supply conditions prevail, root length density values ranging from 0.5 to 10 cm cm^{-3} are sufficient to cover plant needs. These values cover the range of root length density commonly measured for a range of crops in the cultivated layer of many soils.

3.3.2 Effect of soil patchiness

However, this does not hold under conditions of soil patchiness, i.e. spatially heterogeneous distribution of soil resources (Fitter, 1994), which have been reported to trigger “root races” in which vast amounts of assimilates are used to produce profuse roots (Passioura and Wetselaar, 1972), in an effort to secure benefit from resource enriched spots (Hodge et al., 1999). Farley and Fitter (1999) examined proliferation responses of roots of seven plant species chosen because they coexisted at a single site and would therefore encounter a similar suite of patch characteristics. The plants were offered patches of soil or a soil/sand mixture set in a background of sand. The patches varied in size (40, 70 and 160 cm^3), but the probability of encounter was the same for all patches. Only five of the seven species proliferated roots in patches. The two that did not (*Oxalis acetosella* and *Viola riviniana*) had the smallest root systems and thickest roots of the group. There was also evidence that their nutrient uptake depended on mycorrhizal associations to a greater extent than that of the other species. All other species showed a proliferation response, but each did so in a unique fashion. One species (*Glechoma hederacea*) was sensitive to patch size and two other species (*Siliene dioica* and *Veronica montana*) responded to patch quality. Two species changed specific root length (length of root per unit weight of root) on encountering the patches, with finer roots being grown in patches. Four showed a change in branching pattern, becoming less herringbone-shaped in architecture in the patch, as predicted by theoretical models (Fitter et al., 1991).

Such idiosyncrasy of response means that it will be exceptionally difficult to predict the effect of complex variation in patch attributes, such as occurs naturally in soils, on species mixtures. At the same time, this opens up obvious opportunities for species' coexistence and niche differentiation. These would arise from differential responses to a range of spatial and temporal patchiness. Species that respond weakly to nutrient-rich patches by proliferation may do so more strongly by physiological changes (Fitter et al., 2000).

3.3.3 Root plasticity

Roots probably evolved plastic responses to their environment as they differentiated as specialized tissues throughout geological times (Raven and Edwards, 2001), optimized to explore and utilize resources in heterogeneous soils (Leyser and Fitter, 1998). Root plasticity is also a response to intra- and inter-specific competition. Robinson (2001), for example, showed that plastic root responses are triggered by intra-specific competition in a wheat monoculture but do not necessarily lead to greater uptake rates. Nutrient availability is known to influence many facets of root system morphology (Ford and Lorenzo, 2001): root branching, root growth (with growth of main axes generally less affected by nutritional effects than higher order axes), root diameter, root angle (e.g., low P availability decreases the angle of emission of basal roots in bean [*Phaseolus vulgaris* L.], soybean [*Glycine max* (L.) Merr.], and pea [Liao et al., 2001]), root hair length and density, and production of specific root types (cluster roots [Skene, 2000] or drought-induced roots [Vartanian, 1996]). The response of plants to variations in the location of nutrients has been well studied (see review by Robinson, 1994) compared to the influence of temporal variations in nutrient concentrations on root plasticity. Experimental observations of root responses to variations in the spatiotemporal availability of nutrients have generally been made under conditions wherein access to nutrients was artificially reduced. For example, a classic experimental design consists of providing nutrients to a small portion of the root system only, while the rest of it grows in nutrient-poor or sterile soil (Drew and Saker, 1975). Roots respond to such a heterogeneous system in two ways (Robinson, 1996): (i) the nutrient inflow rate increases but then returns to normal within hours, or (ii) roots proliferate toward and within the nutrient rich patch over a period of several days, while root growth in the rest of the root system is inhibited. These trends vary depending on the plant species, with the induced increases in root growth and nutrient uptake varying over one order of magnitude or with a total lack of response in some species (Robinson, 1996). The stimulation in uptake rate seems to be sensitive to the nutrient considered and the duration of the starvation period. Root proliferation appears less dependent on the nutrient considered (except for K in some species). Localized responses are generally assumed to be caused by direct nutritional benefits to the roots directly exposed to nutrient patches, but there is some evidence that they can also involve indirect, sophisticated mechanisms (Pierret et al., 2007a).

The area over which a plant takes up resources such as water and nutrients, or otherwise alters its environment, is considered as its zone of influence. Characterizing this zone is important because its size and shape determine the total resources available to an individual, and the overlapping of zones determines the probability of competition between neighbouring plants (Casper et al., 2003). Bray (1954) was among the first to appreciate that the zones of influence, and hence competition, for neighbouring plants would depend on the mobility of the resource under consideration; zones of influence for mobile resources such as nitrate being much greater than those for immobile nutrients such as phosphate.

Smethurst and Comerford (1993) used analytical solutions to model solute movement and uptake. With their model, they tested the sensitivity of nutrient uptake by two competing root systems where mass flow and diffusion of nutrients were explicit. Species with higher rates of nutrient uptake or root production effectively captured a larger fraction of the nutrient supply, reducing the supply to the competing root system. As such, it should not be assumed that plants that have higher $I_{\max}$ (the maximum inflow rate) necessarily will be better competitors for N. For example, as shown by Smethurst and Comerford (1993), plants that produce and maintain more root length per unit nutrient secured (through pre-emption) a greater fraction of the nutrient supply from competitors.

3.3.4 Root Signaling

It is well established that abscisic acid (ABA) accumulates in root meristems exposed to drought stress (Ribaut and Pilet, 1991) and that its transport to the shoot acts as a non-hydraulic root signal leading to inhibition in shoot and leaf elongation and a decrease in stomatal aperture.

There are numerous and often contradictory reports on the effect of ethylene on root growth; variability of responses appears to depend on applied concentrations. Low concentrations (<1 mg l^{-1}) may enhance root elongation, whereas high concentrations severely inhibit root elongation but simultaneously increase root diameter and root hair formation (Michael, 1990; Jackson, 1991b cited by Marschner, 1997). Light strongly inhibits root elongation, the receptor being the root cap, and ethylene is causally involved in the signal transduction (Eliasson and Bollmark, 1988). The most remarkable effect of elevated ethylene concentrations is the formation of aerenchyma in the root cortex which occurs in response to waterlogging as a mechanism of adaptation of roots to submerged conditions.

4. Concluding remarks and aim of the work

In natural and agro-ecosystems, strategies deployed by plants to access essential soil resources through their root systems vary according to species (genetic determinism), but also according resource availability or spatial distribution (Doussan et al., 2003; Hodge, 2004). In addition, within the same species, resource acquisition strategies will vary (be modulated) depending on the development stage of the plant and/or environmental constraints (soil structure or bulk density, aeration, toxicity, etc...). Such constraints on resource acquisition are likely to induce competition within the root systems of individual plants and between root systems of different neighbouring species: the former being likely to affect root topology while the latter might alter root system architecture as a whole, as well as root position within the soil profile.

For obvious agronomic and environmental reasons, being able to design cropping systems in which plant access to resources is optimized is of foremost importance to all stakeholders involved in crop production, from farmers to policy makers and governments. In this perspective, the ultimate aim of this these is to assess, in the specific context of young rubber tree plantations of NE Thailand, whether it is possible to implement crop or legumes combinations whose strategies for resource acquisition are complementary, in order to maintain or restore an optimal functioning of this agro-ecosystem.

CHAPTER II - INTRODUCTION: General context of the research on below-ground interactions in young rubber tree plantations of northeast Thailand

1. The rubber tree industry

Rubber tree (*Hevea brasiliensis* Mull. Arg.) is the source of natural rubber which is derived from the sap, or latex, produced by this tree. Natural rubber is used for the production of many household and industrial products. Industrial uses of rubber started in Europe during the 1750s with the production of flexible tubes and syringes from rubber solutions (Baulkwill, 1989). At present, car tires and tubes consume more than half of the worldwide rubber production. The remaining part of the production is taken up by the general rubber goods sector, which includes products such as rubber bands, erasers, adhesives, balloons, clothing, gloves and health care products (Porritt, 1926 quoted by Baulkwill, 1989). In 2007, it was estimated that rubber was grown on 8.95 million hectares (FAO quoted by Office of Agricultural Economics, 2009)

Rubber tree is one of the most widely grown tree and most important commercial crops of Thailand’s agricultural sector. In Thailand, as per 2008, rubber was grown over about 2.67 million hectares and yielded an average 1,738 kg per hectare (Office of Agricultural Economics, 2009). The produced quantity and revenue from exported rubber and products in 2008 were 2,832,125 tons and 223,628.2 million bahts (approximately 5 billion euros), respectively (Office of Agricultural Economics, 2009).

In Northeast Thailand, the Royal Thai Government’s policy is to increase rubber tree plantations to increase farmers’ incomes and to improve farming sustainability. The area covered by rubber tree plantations in Northeast Thailand is increasing every year and has almost doubled between 2001 and 2005, from 76,238 to 152,890 hectares (Office of Agricultural Economics, 2006). Rubber trees are grown using a spacing of 7 – 8 meters between rows (Office of Agricultural Economics, 2007). In general, trees reach a tappable girth of 50 cm (equivalent to a diameter of ~ 15 cm at breast height), after an initial growth period of 7 years. During this initial period of tree establishment, the length of which varies depending on clones, climate and soil conditions (Vinod et al., 1996), the soil between rows can be left as fallow. However, although the fallow period plays an important role in maintaining soil fertility and protecting the soil surface from erosion, during that time, the land does not generate immediate income to the farmers. In addition, fallow can also induce increased weed pressure. An alternative to fallow is to grow inter-row crops or inter-crops. Laosuwan et al. (1988) reported that during the immature stage, particularly in the first 2-3 years, a large proportion of the total field area lies underutilized by the new trees. This area is suitable for growing inter-crops which can provide alternative sources of income or food for domestic consumption.

2. Inter-cropping

Inter-cropping consists of growing two or more crops simultaneously in the same field. Inter-cropping represents a particular form of crop intensification. Crop intensification is concerned with both the timing and spacing of crops within a given system. In an inter-cropping system there is potentially competition or facilitation between inter-cropped species during all or part of the cropping cycle (Francis, 1986).

In young rubber tree plantations, farmers commonly use two types of inter-crops. A First type is legumes which are primarily employed to improve soil nitrogen levels; the general expectation when inter-cropping rubber with legumes is that N fixation by the inter-crop will enhance the growth of young rubber tree. Popular legumes for inter-cropping with rubber are *Vigna ungiculata* (cowpea), *Calopogonium caeruleum*, *Calopogonium mucunoides, Centrosema pubescens*, *Pueraria phaseoloides* and *Mucuna cochinchinensis* (Office of Agricultural Economics, 2007; Rubber research Institute of Thailand, 2007; Watson, 1989). In addition, if leguminous shrubs are used, the foliage can provide a high-protein forage or a nutrient-rich mulch (Craswell et al., 1998). The second type of inter-crops includes crops which have a commercial value, such as, for example, cassava, peanuts, corn, chilli, and eggplant (Rubber research Institute of Thailand, 2007). Farmers who grow marketable inter-crops do so to derive income over the 7-year period following planting, during which immature rubber trees cannot be tapped.

Joshi et al. (2006) reported that currently available ‘improved’ rubber cultivation technologies are biased towards large scale monocultures but less suitable for smallholders who have different needs, resources and are more exposed to risks. Mixed/diverse systems can enhance productivity and reduce the risk associated with the volatility of rubber prices. Attractive rubber prices at a given point in time tend to encourage farmers to adopt intensive monocultures, but diversification, such as, typically, rubber agroforests, represents a better alternative than monocultures for rubber smallholders, as diversified systems offer flexibility for periods during which rubber prices drop (Joshi et al., 2006). The paper by Joshi et al. (2006) also explores the different types of interactions between trees and inter-crops when grown together, from competitive or complementary.

2.1 Common rubber tree intercrops

Cassava (*Manihot esculenta* Crantz, of the Euphorbiaceae family) is one of the important root crops of the tropics, grown mainly for its starchy edible roots. The original home of cassava is considered to be North East Brazil. In Asia, its cultivation is limited to a few countries such as, Indonesia, India and Thailand. It is used as a major source of carbohydrate in many African, Asian and American countries. Cassava is a perennial shrub, 1 to 5 m in height, with the stem branching or non-branching. It is harvested after a period of 9-12 months in hot areas and 16-24 months in cooler or dryer areas. The edible roots are adventitious roots, swelled by secondary thickening and deposition of starch and are conventionally referred to as cassava tubers. Usually, 5-10 tubers are produced per plant. These tubers are cylindrical or tapering, 15-100 cm long and 3-15 cm across each and occasionally branched.

Corn or maize (*Zea mays*, *Poaceae or* Gramineae Family) is the most valuable cereal crop of global importance. Corn is used for human consumption and for animal feeding. Besides, it is used in the manufacture of starch, syrup, sugar and industrial spirit. The products of milling include corn grits, meal, flour, germ and germ oil (Palaniappan and Sreenivasan, 1993).

Groundnut or peanut (*Arachis hypogaea* L., *Fabaceae or* Leguminosae Family) is a valuable cash crop and one of the most important food legumes of the tropics (Rao, 1993). Groundnut is harvested over an area of 145 million rais (23.2 million ha), with a production of 34.7 million tons (Table 1). Groundnut is grown as a sole crop and also as an inter-crop with young rubber tree plantations in the Northeast of Thailand.

Table 1 The harvested area, production and yield of groundnut in 2007.

Country/Region	Harvested area (x 1,000 rai)	Productions (x 1,000 tons)	Yield per rai (kg)
World total	145,812	34,722	238
Thailand	201	54	264
Northeastern, Thailand	79	20	258

Source: Food and Agriculture Organization of the United Nations quoted by Office of Agricultural Economics Thailand – 1 rai = 1,600 m^2 (2009)

3. The effect of inter-cropped species on rubber tree growth and yields

3.1 Effects of inter-crops on rubber tree growth

Laosuwan et al. (1988) tested different combinations of inter-cropping treatments between 1981 and 1986. They found differences in girth increments during certain periods of rubber growth. Legume cover and pineapple were more conducive to the growth of rubber than any other crops. Both crops covered the ground from the beginning of rubber planting and provided improved soil moisture conditions. Other crops, including legumes, cereals and banana gave similar growth rate of rubber and none of these crops, as compared with control, adversely affected the growth of rubber.

Wibawa et al. (2006) tested the planting of rubber with *Paraserianthes falcataria* in double row spacing (4 x 3 x 16 m): up to 18 months, growth was comparable to that in a system with normal spacing 6.7 x 3 m. The gap of rubber girth between those two treatments increased afterward and started to be significant after 24 months. The presence of *P. falcataria* at different densities reduced rubber growth significantly since 24 months. At 51 months, rubber girth at inter-cropped plots was 30% and 15% less than that at monoculture with normal and double row spacing, respectively. The monthly girth increments in inter-cropped, mono- and double-spacing plots were 0.6, 0.8 and 0.9 cm respectively. The slowest increment was observed during dry season during which the inter-crop reduced the girth increment by as much as 70% compared to the control and 50% compared to the monoculture with double row spacing.

Rubber tree canopy in double row spacing plots started to shade the soil after 30 months. About 60% of the incident light penetrated in the rubber tree monoculture with double row spacing, about 70% in the monoculture with normal spacing and between 36 and 52% in inter-crop treatments. After 54 months, in all double row plots, light intensity was less than 35%, however in normal density the light intensity was 50%. These data indicated that the intra-plant competition for light may start earlier in plots with inter-crop and in plot without intercrop with double row spacing, compared to normal spacing plots.

In line with the above-mentioned data, the light conditions in between rubber rows and in inter-crop rows, varied depending on treatments: *P. falcataria* with a density of 750 plants/ha reduced light intensity after 18 months, and a lower reduction in light intensity was associated with lower *P. falcataria* density. *P. falcutaria* shaded the soil more than 50% after 18 months.

Wibawa et al. (2006) showed that rubber growth in double row spacing plots was comparable to that in normal spacing (control) plots. These results were better than that mentioned above. This may be related to the wider rubber row spacing and the rubber clone used, RRIC 100, a fast growing clone. This trial also indicated that planting perennial inter-crops after rubber (almost 2 years) is a good strategy to minimize high competition with rubber. *Eucalyptus sp.* planted under rubber did not significantly reduce rubber growth. However, *Acacia mangium* a fast growing timber tree planted at the same time as rubber, competed with rubber and affected its growth very significantly two years after establishment. Rubber reached a tappable size between 56 to 63 months after planting. Up to 62 months after planting, no significant difference was observed between rubber tree girth in double row with or without inter-crop and monoculture with normal.

The findings from this experiment may be very useful as a recommendation basis for planting rubber in double row spacing (4 x 3 x 16 m), i.e. as an alternative to normal, single row spacing (6 x 3 m or 7 x 3 m). The land in the wider space in between double rows (14 m) can be used by farmers to grow food crops over a longer period (more than three years) and for perennial tree crops (timber or fruit trees). The light intensity is expected to remain at levels higher than 70% up to 54 months after planting. The longer the inter-cropping period allowed by this design makes the plantation safer from the pressure of external factors (fire, pests and market fluctuations).

Wibawa et al. (2006) also demonstrated that legume cover crops can assist in indirectly improving the growth of young rubber trees through weed pressure control. For example, they reported that creeping legumes were very efficient at controlling *Imperata* grass. To this end, *Pueraria* was slightly better than *Mucuna* (statistically significant improvement of rubber tree growth). While among the erect legumes, *Flemingia* was good but *Crotalaria* proved disappointing.

Mainstone (1963) quoted by Watson (1989) compared a mixed cover of *Pueraria phaseoloides* and *Centrosema pubescens* with non-leguminous, naturally occurring covers, in the presence of high and low N fertilizer regimes, in immature rubber tree plantations. Rubber tree growth was significantly higher with the mixed legume covers, even compared to the natural cover with high N regime, enabling the first trees to be opened for tapping at 67 months after budding. Others were opened for tapping at 4-monthly intervals over the succeeding 2 years, until the last, in the naturals/low N treatments, were opened at 91 months from budding. At this stage the legume treatment showed a mean advantage in tree girth of 8.2 cm over the naturals.

However, it must be noted that besides positive impacts such as increased soil N levels or protection of the soil surface from erosion, inter-cropping of young rubber tree plantations with legumes such as *Pueraria phaseoloides*, has also been reported to have some undesirable effects on soil chemistry. In particular, since roots of legumes absorb cations in greater amounts than anions, these plants tend to have a marked acidifying effect on soils (Bolan et al., 1991; Tang et al., 1998). It was also found that, in Thailand, the depth of soil affected by acidification can reach one meter under *Stylosanthes hamata* (Noble and Palmer, 1998).

3.2 Effects of inter-crops on rubber tree yields

Laosuwan et al. (1988) reported that the latex yield of rubber inter-cropped with banana (724 ml/10 plants) and pineapple (675 ml/10 plants) was significantly higher than that in monocrop (control: 522 ml/10 plants) and other inter-cropping treatments. Among other inter-crops, the mungbean-peanut treatment was more conducive to increased rubber yields. Upland rice and weeds (control) resulted in the lowest rubber yields.

Due to the earlier opening, over the first 4 years of tapping, the legume treatment plots out-yielded the naturals, by 74 percent in the low N treatments, and by 31 percent in the high N. Not unexpectedly, high N had no effect on yield of the legume plots, but increased that of the naturals by 34 percent. Over the first 10 years of tapping, the legume treatment provided, a mean advantage of 20 percent in cumulative yield, but by the 10th year the mean advantage had fallen to only 5 percent, at an overall yield level of 2,253 kg/ha (Mainstone, 1969 quoted by Watson, 1989).

4. Competition problems potentially associated with inter-cropping

4.1 Resources use by inter-cropped species

During growth and development, plants intercept light, and absorb water and nutrients to produce biomass. Some of this biomass is the harvestable yield. Since crop growth depends on the use of light, water and nutrients, these factors are vital resources for most agricultural activity (Trenbath, 1986).

Although growth factors are distributed heterogeneously in space and time, plant species are able to intercept and absorb them with parts of their shoot and root systems adapted specifically for this (Trenbath, 1986).

Understanding how plants compete with each other to intercept radiation has been a subject of interest to agricultural scientists. Most of the research efforts have been aimed at solving problems related to the optimization of radiation and other resources used by plants. This requires a quantitative understanding of the competition between the plants. Some plants are better competitors than others due to physiological or morphological reasons. These differences provide a framework for selection of crops that could be successfully inter-cropped for optimum radiation interception, hence biomass productivity (Jalloh, 2003). Leaf area index, leaf display, leaf area duration and plant height have been identified as the main plant morphological characters that confer advantages in competition for radiation (Berkowitz, 1988 quoted by Jalloh, 2003). In an comprehensive review on biological efficiencies in multiple-cropping systems by Francis (1989) quoted by Jalloh (2003), it is shown that traditional inter-cropping with food crops (maize/bean, sorghum/pigeon pea, banana/coffee, maize/cassava) involving plants with different sizes and growth cycles gave a better vertical distribution of leaves in the total canopy and, as such, improved radiation interception and increased biomass productivity. Francis (1989) also showed how a tall C_4 species combined with a shorter C_3 crop enhanced total use of radiation in the mixture. Nelliat et al. (1974) quoted by Jalloh (2003) also showed that double hedgerows of cocoa and pineapple between coconut, forming a multi-storeyed system, were very efficient for the spatial use of radiation, each crop being well adapted to its particular radiation micro-environment resulting in higher productivity. In the next paragraph, we will describe below-ground interactions which, like above-ground interactions, can lead to altered plant productivity.

4.2 Below-ground interactions in inter-cropping and agroforestry systems

A major aim of inter-cropping and agroforestry practices is to increase the overall productivity of land and/or its sustainability by optimizing the use of environmental resources (light, water and nutrients) by plants. In theory, means of achieving this aim include: (i) minimizing the amount of below-ground competition by separating the root systems either in space (spatial complementarity) or time (temporal complementarity); (ii) improving access to resources through the facilitating action of one or more of the system's components; and (iii) utilizing resources that would otherwise be lost from the system (through leaching past the root zone). In practice, below-ground interactions are complex and difficult to measure, so that progress in designing improved and sustainable systems of production has been slow, although some general principles are beginning to emerge (Gregory, 2006).

A key issue in crop/crop, tree/crop or tree/pasture systems is to determine the degree of competition or, conversely, complementarity that exists between combined species, in particular with regards to the distribution of roots and activities of the root systems (van Noordwijk et al., 1996; Willey, 1996 quoted by Gregory, 2006). Temporal complementarity is the best documented mechanism for increasing yields in intercrop systems (Willey, 1996 quoted by Gregory, 2006). For example, in the sorghum/pigeon pea system, the two crops have maturity periods of typically 100 and 180-200 days, so that the major demands for resources such as light, water and nutrients differ in time. The result is that the inter-crops make better use of resources over time than the two sole crops. This is easily demonstrated for light where appreciably greater interception of radiation has been measured in many temporal systems (Natarajan and Willey, 1980 quoted by Gregory, 2006).

A similar ‘two-tier’ effect exists when a shallow-rooted species is combined with a deep-rooted species: such a combination is potentially beneficial to the system’s functioning, particularly if the deep-rooted species does not explore the upper profile extensively. Perfect examples of such a combination are rare (Willey, 1996 quoted by Gregory, 2006).

Cannell et al. (1996) quoted by Gregory, 2006 suggested that agroforestry systems may be more productive than sole crop systems if the trees are able to access resources that are under-utilized by crops. Where trees and crops are grown together, it has been suggested that exploiting the different rooting depths of trees and crops might increase resource capture without introducing severe below-ground competition. Ideally, to minimize competition between tree and crop, trees should have a deep root system with little root proliferation near the top of the soil profile, thereby allowing the crop to utilize resources at the top of the profile while the tree accesses resources in deeper layers (Schroth, 1995 quoted by Gregory, 2006).

Haishui and Kejun (1998) reported several successful inter-cropping models for smallholder rubber plantations in China. For example, this paper reports that different food, fodder and vegetable crops could successfully be cultivated in association with rubber, amongst which, the most common were sweet potatoes, maize, sorghum, cassava and peanuts. Some of these crops could even be cropped twice a year. Land preparation included plowing, trench digging, ridging and fertilizer application. The total cost was 750 - 1,000 Chinese Yuan (RMB)/ha and the gross income was 1,200 - 1,500 Chinese Yuan (RMB)/ha, that gave only around 500 Chinese Yuan (RMB)/ha of net profit (roughly 55 euros/ha). Due to its low profitability, this type of inter-cropping is only suitable for rural areas where a large and cheap labour force is available. This experience showed that the most profitable models of food inter-cropping were rubber inter-cropped with maize, sweet potato or peanut.

In terms of soil conservation, agroforestry systems that combine legume shrubs, fruit trees or coffee (*Coffea* spp.) with rubber (*Hevea brasiliensis*) provide useful economic returns, but were not found to provide better soil protection than grass strips or pineapple (*Ananas comosus*) planted along contour lines (Craswell et al., 1998).

Droppelmann et al. (2000) reported biomass yields for sorghum and cowpea as a function of planting distance from rows of pruned and non-pruned rubber trees. Sorghum yields in row position closest to rows of pruned rubber trees (0.5 m) were lower than those further away. However, biomass yields in all row positions of both treatments did not differ statistically from yields in mono-crop stands. Since the canopies of the non-pruned trees were much larger than those of pruned trees throughout the cropping season, the intercrop rows were shaded and were submitted to a different radiation regime. Rainfall during this period (May to July) was relatively high (136 mm of effective rainfall), and rainfall interception was probably higher closer to the tree row (Monteith et al., 1991 quoted by Droppelmann et al., 2000). These two phenomena could have been the reason for the increase in biomass towards the middle of the alley, but the differences were not statistically significant. No differences between row positions were found for the cowpea crops. Biomass productions of the cowpeas were an order of magnitude lower than the corresponding sorghum crops and were associated with a much higher variability.

Droppelmann et al. (2000) found the highest overall productivity when trees were planted at high density, pruned and inter-cropped with annual species (IPH). They also suggested (Droppelmann et al., 2000) that inter-cropping pruned trees with annuals was advantageous in terms of productivity per unit area when compared to mono-specific stands of trees or annuals; they concluded that hedgerow intercropping of *Acacia saligna* with annual crops and tree pruning are effective management tools to increase overall biomass production and productivity per area in a runoff irrigated system of an arid region in northern Kenya.

Stirzaker et al. (2000) considered tree belts between cropped fields in southeastern Australia, a system for which they assessed the above-ground performance of the crop and below-ground leakage of water and nutrients. In dry years, crop growth was clearly suppressed nearby tree lines, while the problem was small or non-existent in wet years, indicating that much of this competition is for water. In this system, it is expected that trees use almost all of the available water directly below their crown; away from the crown, leakage rises until it reaches a level identical to that of a field with no trees (Figure 4).

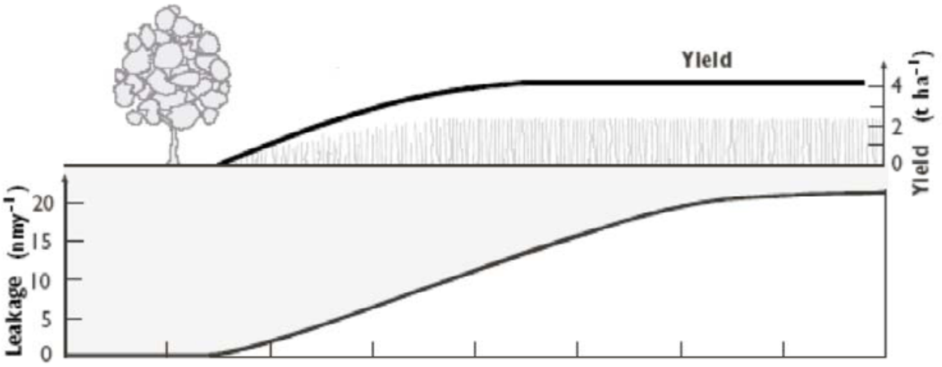


Figure 4 Yield and leakage in a perennial – annual association. Yield and leakage are assumed to be zero at the base of the tree and increase with distance from the belt to levels characteristic of a sole crop (Stirzaker et al., 2000).

Negative effects of tree hedges on yields of annual intercrops were found at the tree/crop interface in most studies in semi-arid regions under rainfed conditions (Govindarajan et al., 1996; Jama et al., 1995; Rao et al., 1991 quoted by Droppelmann et al., 2000). The reported poor inter-crop yields under rainfed conditions were probably due to a lack of water available for the inter-crop as a result of the presence of trees. In this case, or in the case of a shallow soil profile, available water is primarily located in the top layers of the profile, and a high level of competition – with low chances for complementarity – can be expected. Thus, in semi-arid environments with inadequate water supply (i.e. < 1000 mm rainfall per year) the perennial tree component produces too little biomass to benefit annual intercrops (e. g.: N_2-fixing and mulching) and/or poses the risk of becoming too competitive (Rao et al., 1997 quoted by Droppelmann et al., 2000).

5. Context of the research and working hypotheses

Soils in Northeast Thailand are generally of poor agronomic standard and the climate is characterized by a long dry season during which rubber trees are likely to rely on their deep root system to take up water and other essential resources. This area is a marginal area for rubber tree cultivation because the average annual rainfall varies from 1,000 to 1,600 mm, which is often lower than the recommended minimum of 1,400 mm and systematically lower than the optimum of more than 1,800 mm (Jacob, 2009). The soils of NE Thailand also generally exhibit a horizon of high mechanical impedance immediately below the topsoil (typically located between 20 and 40 cm). Depending on soil moisture conditions, this horizon acts as a barrier for the roots of the majority of crops (Hartmann et al., 2001). It also potentially represents a limiting factor for the development of rubber tree cultivation in NE Thailand.

It has been demonstrated that, under certain biophysical conditions, legumes cropped in association with young rubber trees can have beneficial effects on soil protection, soil structure or N availability. Because they fix atmospheric N through symbiosis with rhizobia, many leguminous plant species have the capacity to grow in N poor soils, in all climatic zones. Inter-cropping with legumes is an effective practice for establishing productive crops on N-deficient tropical soils, through atmospheric nitrogen fixation (Giller et al., 1991; Ledgard and Giller, 1995). For this reason, perennial legumes such as *Pueraria phaseoloides* are commonly inter-cropped with young rubber trees (*Havea brasiliensis*). Legumes are used in the restoration of soils impoverished by intensive exploitation, erosion or desertification, in arid or semi-arid regions, for example in the rehabilitation of mining sites. In tropical areas, annual or perennial legumes have also proved to have a strong potential for soil structure improvement, together with a strong potential for nitrogen fixation. It is for example the case for the *Vigna* or *Stylosanthes* genders*,* several species of which are resistant to drought and salinity. A recent experimentation by Lesturgez et al. (2004) proved that *Stylosanthes hamata* can, after only two years, re-colonize and create many macropores in the compact horizons of the soils of NE Thailand. However, one known problem associated with the inter-cropping of rubber trees with legumes in the acidic soils of NE Thailand is the net increase in soil acidity which results from preferential cation uptake by these plants. So there is already some awareness that legumes may pose some problems when used as inter-crops in rubber tree plantations in NE Thailand. It is also known that many perennial legumes, like *P. phaseoloides,* survive with difficulty during the long dry season which characterizes NE Thailand's climate.

In addition, as all inter-crops, whether they be perennial legumes of annual food crops, share the same soil volume and resources with trees, one cannot rule out, *a priori*, the possibility that they could also, at least to some extent, compete with them. Therefore, it is likely that water stressed inter-crops compete with *H. brasilliensis* for water extraction, at key developmental stages for the trees. For example, Little et al (2002) conducted a 7 year experiment which showed that a clonal eucalypt hybrid (*Eucalyptus grandis x E. camaldulensis*) responded more to the reduction of competition from weeds than from any N fixation from cowpea (*Vigna ungiculata)* (whilst, the most profitable option remained inter-cropping with cowpea with pre-germination application of herbicide, due to the savings resulting from fewer weeding operations). If such competition occurs in rubber tree plantations, it is expected to alter both the fine and coarse root development of these trees.

In addition to altering or restricting the development (and resulting root architecture) of young rubber tree roots, competitive effects such as those described above could also have long-term consequences on the functioning of mature trees. Current knowledge suggests that a concomitance of adverse soil conditions (including soil compaction and inter-individual competition for resources), could induce a late and subtle stress favouring a necrotic reaction against tapping stress (Hartmann et al., 2006; Do et al., 2010). This stress results in the so-called *trunk phloem necrosis* (TPN) syndrom or *bark necrosis*, a pathology that affects many rubber tree plantations in northeast Thailand (Nandris and Chrestin, 1991). Under this scenario, inter-individual competition for water would heighten the effects of punctual water stress when a short dry spell occurs during early stages of tapping and be involved in the emergence and extension of the syndrome within a stand (Isarangkool Na Ayutthaya et al., 2007).

6. Main question addressed by this research

Given the context described above, we propose to conduct detailed monitoring of rooting patterns under field and laboratory conditions in presence and absence of several intercrop candidate species, so as to determine the extent and modalities of putative competitive/facilitative effects between theses inter-crops and rubber trees.

7. Expected outputs and outcomes of the research project

7.1 Scientific outputs and outcomes

The main expected scientific output of this research is the production of an experimental dataset about rooting patterns of immature rubber trees as a function of competition/facilitation between rubber trees and some food inter-crops. This experimental dataset encompasses quantified indicators of rubber tree and inter-crop root development such as root length densities, root surface area densities, soil volume occupancy by roots, root diameters, dry root biomass density, specific root length, root growth rates, etc...

One first expected scientific outcome of this work is to produce new knowledge about below-ground interactions between immature rubber trees and associated inter-crops. In particular, the work attempts to document some of the mechanisms that influence the root growth and root system development of rubber trees in presence of food inter-crops, through the quantification of, e.g. changes in root length densities, root system architecture or root growth rates. It has long been recognized that both intra- and inter-specific interactions between plants trigger plastic behaviours through which their growth patterns, including root development, are substantially modified (Weaver and Clements, 1938; Schenk et al., 1999). Recent studies showed that both annual/annual and perennial/annual inter-cropping systems, could induce alterations of the rooting profiles of both the main and inter- crops (Li et al., 2006; Mulia and Dupraz, 2006).

Another expected scientific outcome of the work is to document, at least indirectly, whether inter-cropping immature rubber trees is likely to influence the trees gain access to essential resources, and if yes, how. To this end, a special emphasis is laid on interpreting root measurements in terms of below-ground competition, which has been identified as a potentially important determinant of rubber tree development (e.g. Harja1 et al., 2005), at least under lower-stress conditions, or facilitation which is currently believed to prevail under high-stress conditions (Li et al. 2007; Raynaud et al., 2008).

7.2 Applied outcomes for farmers

A first expected applied outcome of this work is the production of new knowledge on root exploration strategies in young rubber tree plantations that can be used to improve inter-cropping options in the smallholder rubber tree farms of NE Thailand. A central objective of multi-specific agro-ecosystems, such as inter-cropping and agroforestry, is to sustainably maintain or increase land productivity by optimizing the use of environmental resources (light, water and nutrients) and services (e.g. interactions with soil micro- and macro-fauna) needed for plant growth (Gregory, 2006). The diversity of root systems corresponding to a multi-specific vegetation cover offers options to utilize soil and even bedrock resources over an extensive range of lateral and vertical distances (Stone and Kalisz, 1991; Calder et al., 1997; Jackson et al., 2000; Gonkhamdee et al., 2009).

Ultimately, it is expected that this research will help improve inter-cropping options for the rubber tree farmers of NE Thailand. This should translate into recommendations regarding inter-cropping designs, including inter-crop specific composition, that are most suitable to the biophysical and socio-economical context of NE Thailand. In this perspective, cropping system optimization will be driven by efforts to reach an optimal trade-off between crop/inter-crop competition, optimal access to resources by rubber trees and inter-crops, enhanced ecosystem services (e.g. reduced runoff and leaching, improved soil structure and fertility) and economical profitability.

CHAPTER III: METHODOLOGY

1. Introduction

1.1 Tools used to assess rooting patterns

1.1.1 Literature review of methods used to sample and measure roots

One reason for studying plant root is to investigate the influence of environmental factors on the development of plant root systems. Classically, methods used to study root systems and their architecture document aspects related to the shape of the root system, the amount of roots within the root system, and the dynamics of plant root growth. Scientific studies on root systems were started in the eighteenth century (Bohm, 1979); therefore, there exists a vast array of methods to study roots and root growth such as, for example, simple excavations of observation pits, augering of soil core, profile and glass wall methods, the monolith method (used to count root occurrence), or various container methods (Smit et al., 2000). There are also indirect methods that can be used to document root distribution and/or activity, such as the monitoring of soil water content, which provides information about how much and where in the profile is water taken up by roots (e.g. Calder et al., 1997); tracer-based methods, using either radioactive or stable isotopes, that have been used to unravel vertical and horizontal displacement of soil water from moister to drier zones in the soil by plant roots (also know as hydraulic lift or hydraulic redistribution; Richards and Caldwell, 1987), or deep nutrient uptake (Kristensen and Thorup-Kristensen, 2004). In many situations, available time and equipment will limit the type and range of measurements made and will therefore influence the choice of root parameters monitored/estimated.

Atkinson (2000) reported that Harper et al. (1991) divided methods available to study roots into two groups. The first containing whole plant excavations (e.g. Kutschera, 1960), the broad family of profile wall (e.g. Lesturgez et al., 2004), pinboards (e.g. Passioura and Wetselaar, 1972) and soil cores methods (e.g. Heeraman and Juma, 1993), some isotope applications (e.g. Barber and Ozanne, 1970), resin embedding techniques (e.g. Stewart et al., 1999) and Nuclear Magnetic Resonance Imaging (NMRI) (e.g. Menon et al., 2007).

This first methodological group provides information on roots and root distributions at a given point in time, in relation to a given standing crop. The second methodological group includes methods such as rhizotrons (e.g. McDougall, 1916; Thaler and Pagès, 1996), mini-rhizotrons (e.g. Devereux-Joslin and Wolfe, 1999) and in-growth bags (e.g. Matamala et al., 2003), and some isotopic techniques (e.g. Guo et al., 2008), which allows the assessment of changes in rooting patterns with time and/or of root turnover. Some techniques have also been developed to investigate root architecture in 3-D (e.g. Kaestner et al., 2006) and the interplay between roots and soil (e.g. Krebs, 1994; Moran et al., 2000). . Root measurement methods that can be used to relate roots to the physical properties of soils were reviewed by Atkinson and Mackie-Dawson (1991) quoted by Atkinson (2000). They concluded that there is no single method of root measurement applicable for all situations. Prior to deploying a given methodology or set of approaches, it is important to articulate the key questions to be addressed, which will orient the root system parameters (taking into account their functional significance) to be measured. Others factors influencing the choice of methods are likely to be the availability of equipment and facilities, the crop and or soil to be investigated and the type of root system effect of interest. For example, to explore putative links between the occurrence of Trunk Phloem Necrosis (TPN) and root system development in a rubber tree (RRIM 600) plantation of NE Thailand, Pierret et al. (2007b) employed a methodology employed including destructive sampling for physical separation of roots after soil washing, root mapping on soil profile walls, rhizolocation (i.e. the physical detection of coarse roots using a strong metal probe pushed in the soil), architectural characterisation of coarse woody roots, and electrical measurements by capacitance Chloupek (1972; 1977) and earth impedance (Aubrecht et al., 2006; Čermák et al., 2006).

Similarly, within the framework of a study designed to test the feasibility of the integration of three African fruit trees into agroforestry systems based on their rooting patterns, Oppelt (2003) deployed a hybrid methodology to gather knowledge about coarse root architecture as well as information about the fine root distribution and their morphology. This approach, based on detailed description of whole root architectures, included some core sampling prior to the excavation of root systems. In addition, Oppelt et al. (2001, 2000) investigated the root systems using semi-automatic digitizing and computer-based 3-D reconstruction techniques. Topological analysis was carried out to investigate branching patterns as basic determinants of root architecture.

1.1.2 Literature review of methods used to characterize rooting patterns based on simple root measurements.

1.1.2.1 Soil exploration and exploitation by roots

Competitiveness/complementarily between rooting patterns has been assessed using the concepts of soil *exploration* and *exploitation* (Hughes et al., 1995)*,* which are used to quantify the different strategies deployed by roots to acquire resources. The former reflects the spatial distribution of roots within a reference soil volume, while the latter is related to the intensity with which the soil is colonized by roots, hence how soil resources are likely to be mobilized by the plant. For example, two species with highly dichotomous root systems associated with high root length densities in the same soil layers will represent a situation where co-occurrence of high explorative and exploitative potential might lead to competition between species. In contrast, if one of the two species is replaced by a species with a dominant *herringbone* root configuration (least lateral extension, deeper, taproot-dominated root architecture, and limited branching) then, the potential for competition between the two species will be reduced. Of course, these are mere *indicators* of a *potential* for competition and not an *actual* measurement of a competitive process.

The literature record shows that there are many definitions of the two concepts of soil exploration and exploitation. Fitter (1987) and Fitter et al. (1991) compared different patterns of root branching with respect to construction costs and intra root competition for nutrients, using a link-based topological model. Fitter and Stickland (1992) predicted that a herringbone architecture, which is relatively expensive to build but minimizes inter-root competition, is favourable for slow-growing species from habitats where soil resources are scarce. The relatively cheap construction costs of dichotomous root systems were predicted to be favourable for fast-growing species from nutrient-rich habitats. Berntson (1994) presented a model based on size-dependent and size-independent aspects of root system architecture in terms of depletion volume. Van

Noordwijk et al. (1996) defined lengths of the longest (deepest) root as a rough indicator of exploration and the total length or surface area of live roots as a parameter quantifying exploitation. The development and functioning of a given root system have been defined by Harper et al. (1991) as an evolutionary response to the spatio-temporal variability of resource availability and the corresponding constraints to growth. In this perspective, using undisturbed soil sections embedded in synthetic resin, Stewart (1997) developed a '*root exploration index*' which reflects the effectiveness of a given root distribution to explore soil solid space or total soil space, given the distribution of macropore structure. This root exploration index which explicitly takes into account the influence of soil structure on root spatial distributions was found to be an effective tool for the accurate prediction of root water uptake (Moran et al., 1996).

Simple parameters and/or combinations of simple parameters can be used to assess competitiveness/complementary effects between cropped and intercropped species, such as e.g. root length and volume densities, or the cumulative root length percentage in some soil layers. By combining these parameters within some information about root distribution within the soil volume, several authors have defined root exploration indices. For example, Hughes et al. (1995) defined a root exploitation index, E(phi), as the proportion of the soil volume which contains roots at RLD greater than or equal to some specified value and an exploration index, E(0), defined as the proportion of the soil volume which contains roots at any RLD greater than zero. These indices are dependent on sample size, as are all volumetric or soil-coring data. More recently, Oppelt (2003) defined an exploitation index E(Φ) which corresponds to the proportion of soil volume in which Root surface Area Density (RAD) >Φ (as opposed to the exploration index, which is the proportion of soil which is explored by any roots RAD>0).

1.2 Analysis of root growth potential based on apical diameter measurements

Analysis of roots strength as a carbon sink can be assessed by measuring the apical diameter of the roots: it has been shown that, in many species (among which, rubber trees), root apical diameter is related to root potential growth rate and is a good indicator (proxy) of growth potential (or C sink strength) (Pagès et al., 1995; Thaler and Pagès, 1996; Pagès et al., 2010). Pagès et al. (2010) reported that various root morphological markers and their distances relative to the root tip were, with some variability, proportional to elongation rates of corn root. In rubber tree, root development is periodic: when leaves develop, root elongation is depressed and branching increases and conversely (Thaler and Pagès, 1996). Such a periodic development corresponds to both a competition mechanism for C assimilates within the plant and a compensation/plasticity process through which assimilates are allocated within the root system depending on local environmental conditions and the potential for sustained resource supply they represent (correlative-inhibition theory). Only thick roots are able to sustain secondary growth and become perennial. Hence, it can be hypothesized that careful monitoring of root apical diameters might allow detecting early signs of “deficient” root development. It is also known that thicker roots develop in denser soils (Bengough et al., 1997), so if small apices develop in dense soils, it is a sign that despite the fact that the plant should develop thicker roots under such conditions, another factor (competition from the intercropped species?) is altering their response to the environmental stress.

Such an approach could potentially provide indications as to whether environmental conditions (including not only factors such as soil physical constraints or water stress, but also competition pressure) have an effect at the level of actively growing roots. This can be viewed as a complement to the study of root architecture, which documents the cumulative effect, over a given time period, of the biophysical environment on root architecture. Unlike architectural observations, this approach provides an indication as to whether, at similar developmental stages, rhizogenesis differentiates depending on environmental conditions. The question is the same but the tools are different, and one advantage of apical diameter measurements is that they are not as labour intensive as architectural observations.

1.3 Choice of root parameters measured in this work

1.3.1 Field and laboratory monitoring of fine roots

In this work, a deliberate choice was made to study only fine roots. A clear, definition of what fine roots are, including anatomical, dimensional, functional and physiological considerations, is still missing (Zobel, 2003). However, the literature record shows that efforts to improve this situation have recently increased (Zobel et al., 2005a; 2005b; 2006) and it is now clear that, in both annual and perennial plants, roots <1 mm in diameter form a structurally and functionally complex population which is the dominant component of the root system (Pierret et al., 2007a) . While it is they most likely account for most of the root length in many plant species, fine roots are most often underestimated because of their small size and near transparency (Costa et al., 2001). Therefore, there is a need to improve current knowledge and understanding of fine roots to support better prediction and management of biogeochemical cycles at all scales from that of the single plant to the global level (Jackson et al., 1997; McCully, 1999; Norby et al., 2004; Thomas et al., 2004). To do this, it is necessary to measure the quantities, occurrence, sizes and functions of fine roots under a variety of conditions.

Beyond this general rationale, several specific reasons reinforced the choice of placing the focus of this work on fine roots. These reasons are related to the facts that:

1. the annual crops studied develop only fine, short-lived roots,
2. the rubber trees studied in the field were young rubber trees (<4-year old) whose coarse architecture was not fully developed (particularly for the 1-year old trees studied in 2006),
3. although important to understand the long-term functioning or a plantation, coarse root dynamics plays a role at time-scales that are unlikely to play a decisive role in short-term adaptation and responses to highly dynamic populations of intercrop fine roots, and finally,
4. although the literature on rubber tree provides good insight into coarse root architecture (e.g. Carron et al., 2000), root biomass (e.g. Wauters et al., 2008), fine root dynamics and architecture (e.g. Le Roux, 1994), there is a relative paucity of data on fine rubber root length densities (RLD), diameters and spatial distributions as influenced by inter-cropping practices.

2. Experimental Materials and methods

2.1 Greenhouse Experiment

The objective of the greenhouse experiment was to assess the detail of below-grow interactions between young RT and IC species. These experiments carried out under semi-controlled and simplified conditions (non-limiting supply of water and nutrients, homogeneous growth medium) were undertaken to explore the competitive/complementary effects between young rubber trees seedlings and a range of intercrop species. This has been achieved by growing plants in medium-sized containers (rhizoboxes) filled with vermiculite. Root growth was observed and recorded at two-day time intervals by tracing the position of newly appeared roots on a transparent acetate sheet placed over the transparent side of the rhizobox. The duration of the experiments was long enough for lateral root growth to result in co-occurrence of roots of the two species in shared soil volumes.

2.1.1 Greenhouse experiment setup

A greenhouse experiment was conducted at the Agronomy Unit’s field site at Khon Kaen University, Khon Kaen, Thailand (N16° 28' 15.3" E102° 48' 38.6") (Figure 5) form June 2007 to mid of December 2008, until corn ears were ready to be harvested, groundnut was 130 days after planting (harvested time) and cassava and rubber root were contacted. Root growth of rubber trees and corn were monitored using rizhoboxes, i.e. thin containers with a transparent wall through which roots can be observed at regular intervals (see detailed description below), a simple concept that dates back to at least the early 1900s (e.g. McDougall, 1916). A total of three replicate rhizoboxes with rubber tree and intercrop (corn, groundnut and cassava) grown together, on the one hand, and three replicate rhizoboxes with rubber tree alone, on the other hand, were prepared and monitored.

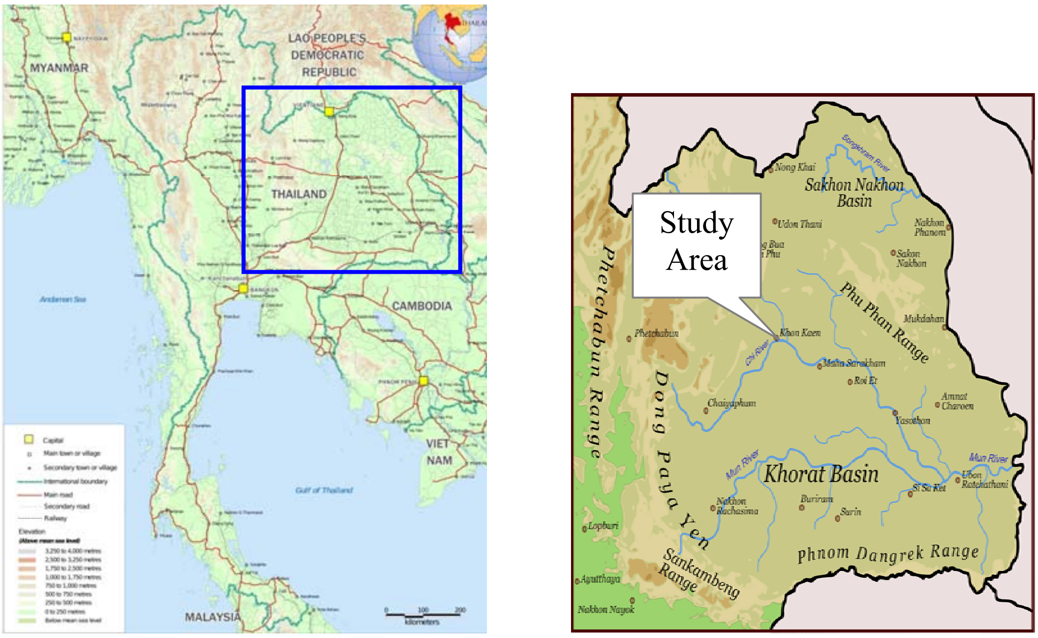


Figure 5 Location of the greenhouse study area.

2.1.2 Treatments

2.1.2.1 *Hevea brasilliensis* alone

2.1.2.2 *Hevea brasilliensis* + *Zea mays*

2.1.2.3 *Hevea brasilliensis* + *Arachis hypogaea* L. var.Tinan 9

2.1.2.4 *Hevea brasilliensis* + *Manihot esculenta* Crantz var.Rayong 5

2.1.3 Plant material

2.1.3.1 Seedlings of *Hevea brasilliensis* var. RRIM 600 (the Rubber Research Institute of Malaysia), approximately 8-12 months old after bud grafting were used. The seedlings were nursed in moist vermiculite (Agra-vermiculite, Dutch Greenery Co., Ltd.) for approximately 1 month, in order to encourage root system development prior to transplantation in rhizoboxes.

2.1.3.2 Seeds of *Zea mays* (hybrid glutinous corn Big White^{TM} 852 trade band) were used. Corn seeds were pre-germinated 3-4 days and subsequently transplanted in the rhizoboxes, 2 cm below the growth medium surface.

2.1.3.3 Seeds of *Arachis hypogaea* L. var.Tinan 9 were used. Groundnut seeds were germinated 3-4 days and subsequently transplanted in the rhizoboxes, 2 cm below the growth medium surface. Groundnut seedling was inoculated with rhizobium var.

TAL1000, THA205 strain CIAT2434 3850 3918 (stylo) when were placed in the root observation boxes.

2.1.3.4 Stem segments of *Manihot esculenta* Crantz var.Rayong 5, collected on 8 months old plants were used. The cassava stems were planted directly in the root observation boxes.

2.1.4 Rhizoboxes and growing conditions

The rhizoboxes (root observation boxes) were PVC boxes 2 cm thick, 100 cm deep and 104 cm wide (internal dimensions). The back of each rhizobox was made of opaque PVC which was 4 mm thick. The sides and bottom were made of square aluminum 2x2 cm. The bottom aluminum were perforated the holes (2 mm in diameter) for drainage at 5 cm intervals through the bottom of the rhizobox. The front plate was made of 4 mm thick transparent acrylic plastic (Pan Asia Industrial Co., Ltd.). As investigated elsewhere, acrylic plastic shows no detrimental effect on growth and survival rate of roots (Withington et al., 2003) A transparent polyester sheet (Polyplex Plc., Ltd., Thailand) was placed over the Plexiglas in order to record root development at regular time intervals (Figure 6). To minimize photo- and thermo-tropic responses from the roots, shutters lined with black plastic and thick aluminium foil were attached to the front of the rhizoboxes at all times, except when roots were traced on acetate sheet. The transparent wall was exposed only for the purpose of root tracing.

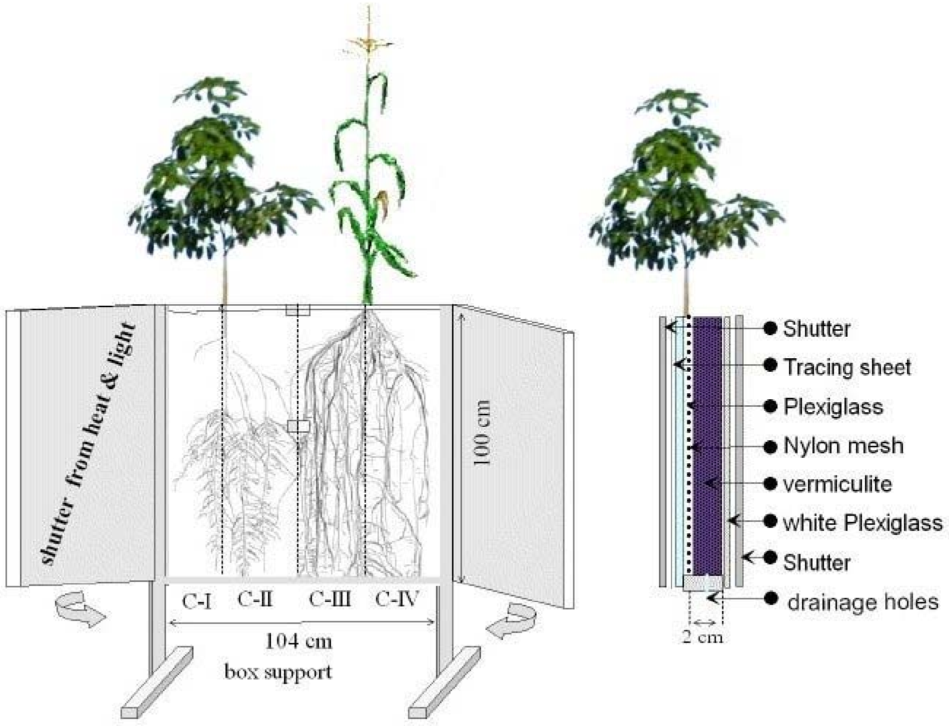


Figure 6 Schematic representation of the rhizobox experimental setup.

The rhizoboxes were packed with moist fine vermiculite (maximum grain diameter of 2 mm, Agra-vermiculite, Dutch Greenery Co., Ltd., Holland) (169.24 % moisture by weight). A nylon mesh (average pore diameter 150 µm) was stretched over the vermiculite, behind the front Plexiglas sheet. Most of the root system could thus be constrained to develop in the virtually two-dimensional space between the transparent front pane and the nylon mesh.

Rubber tree seedlings were implanted between the window and the nylon mesh. Rubber trees and corn plants were implanted in identical positions, 50 cm apart from each other, in all replicate rhizoboxes. The plants were kept under non-limiting supply of water and nutriments via daily watering with Hoagland’s solution (Epstein and Bloom, 2005); the solution was supplied by capillarity using a thick wick in contact with the whole surface of the vermiculite, at the top of the box. Supply was discontinued once some solution started to drain freely from the bottom of the rhizoboxes. The plants were grown in a weed free environment with pests and diseases kept in check. Plants were grown until the root was connected.

The experiment was carried out under an open-sided shelter covered with a translucent plastic roof that transmitted approximately 56% of the incident light. Air temperature, relative humidity and the photo-period were recorded daily over the whole duration of the experiment. The average air temperature, relative humidity, the photoperiod and photo synthetically active radiation (PAR) was 27.4 °C, 84.6%, 7.4 hours and 385 µmol $m^{-2}s^{-1}$.

2.1.5 Measurements of root development

Root emergence and growth were measured every two days by tracing the new growth increments with waterproof colored pens on the transparent plastic sheet placed over the front pane. A new color was used for each observation date. At the end of the experiment, these transparent sheets were scanned using an A4 Epson Perfection V700 Photo, at resolution of 600 dpi: the whole surface area of each 50x100 cm transparent sheet was scanned as 12 separate A4 sections which were subsequently stitched together using the GNU Image Manipulation Program (Gimp 2.6 – 2009, http://www.gimp.org/) to produce a mosaic image of the tracings corresponding to the entire root system. These images were subsequently used to digitize root system architectures using the DART software.

2.1.5.1 Digital representations of root systems using the DART software

DART (Data Analysis of Root Tracings) is freeware based on human vision to identify roots, particularly across time-series. DART produces a description of root system architecture in which each root is represented as a series of ordered links encapsulating specific information about the considered root and is connected to other roots. The list of specific attributes encapsulated in DART root descriptions includes: an identification number, the branching order, the date of emergence, the parent root identification number, the distance to the current's and parent's root base, and the cumulative root length at each observation date. DART is particularly suitable for developmental analyses of complex root system architectures. The population of links constitutes the root system architecture (RSA). DART helps in studying RSA and in producing structured and flexible datasets of individual root growth parameters. It is written in JAVA and relies on manual procedures to minimize the risks of errors and biases in datasets (Le Bot et al., 2009; http://www.avignon.inra.fr/psh/outils/dart_software) (Figure 7).

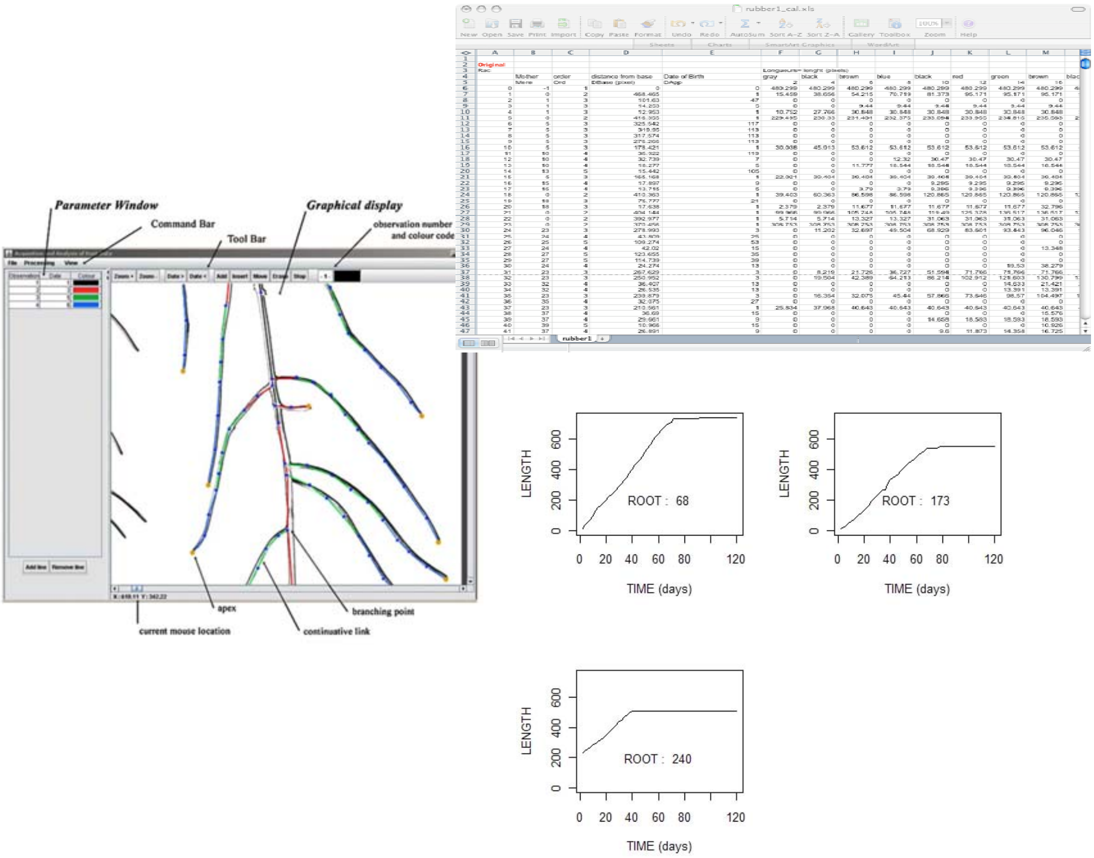


Figure 7 The DART software

2.1.5.2 Analysis of root system dynamics at the scale of the entire root system

Since they include chronological information about the elongation of individual roots, DART output files were particularly useful to compute parameters related to root system dynamics, such as the overall root length, root length density and growth rates, at any given time.

In particular, root system dynamics was investigated via the computation, over three time intervals, namely 0-10, 10-15, 15-20 and 20-30 days, of daily changes in root length density (RLD, in cm.cm^{-2}.day^{-1}) in four 25 cm wide and 100 cm high vertical compartments, consisting of two inner compartments, C-II, on the rubber side and C-III on the intercrop side, enclosed in between the outer rubber (C-I) and intercrop compartments (C-IV) (Figure 6). This was achieved, for every time interval, by dividing the total root length included in a given vertical compartment, by the surface area of the compartment and the time interval's duration.

2.1.5.3 Root system trajectories

DART outputs were also used to assess the displacement/trajectory of entire root systems. To that purpose, root system growth was considered to correspond to a diffusion-type process (De Willigen et al., 2002) that proceeds in the downward direction as a function of individual root branching and elongation. Within this conceptual framework, the elongation of every individual root at a given time step is seen as a vector that describes the local movement of the diffusion front; the overall expansion of the root system was thence estimated by computing the vectorial sum of all the individual vectors describing root elongation. Finally, the trajectory of root growth expansion was represented graphically as the chronological cumulation of the vectorial sums calculated at each time step. Based on this approach, a balanced root system development with gravitropic main axes and as many branch roots of similar length on both sides of these main axes, should result in a vertical, downward trajectory. In contrast, any preferential allocation of assimilates to support the growth of main axes or branches in a particular direction should result in a deviation of the root system trajectory in the same direction.

2.1.5.4 Root growth patterns at the individual root scale: root encounters

Using the DART software, root encounters were visually identified in the digitized root systems of the transparency sheets. Variations in root elongation rates during root encounters were estimated using the link coordinates and temporal information, as recorded in DART output files, of at least three root segments of a given root, corresponding to pre-, syn- and post-contact conditions.

Two types of root encounters can occur, namely “crossings” which correspond to the growth of one root towards another root, then around and beyond it, and “parallel contacts” which correspond to one root growing towards another root and then changing its growth direction so that it subsequently grows parallel and in close contact with the other root. These two types of root contacts can occur within the root system of an individual plant (intra-individual root contact) or involve roots of two neighbour plants (inter-individual root contact). Inter-individual root contacts can be intra- or inter-specific depending on whether they involve plants of the same or of two different species. Given the design of our experiments, all inter-individual contacts were necessarily inter-specific.

2.1.6 Measurement of other plant growth parameters

Shoot development

Measured indicators include tree growth indicators such as tree overall height, measured at regular time intervals. The leaf area and length of petiole of every leaf were measurement before harvest.

2.1.7 Statistical analysis

Data exploration and analysis

Data exploration, statistical analysis, and graphical representations of data collected during the experiments were performed using the R language environment for statistical computing and graphics, version 2.9.1 (R Development Core Team, 2009; see http://www.r-project.org/). The two-tailed unpaired (independent) Welch t-test was used to compare parameters for which replicate and identically distributed samples could be obtained; such variables include overall root length, root system daily expansion rate, stem length, and leaf area. The two-tailed paired (dependent) two-sample Welch t-test was used to compare parameters for which independent samples could not be obtained, such as, typically growth rates of individual roots at different times with respect to a root encounter. The Welch t-test was systematically used as in most cases the assumption of equal variances between samples could not be verified.

Additional statistical analyses were carried out using STATISTIX (Version 8). One factor ANOVA was used to analyze the main effect of the treatments, and standard error of the difference (SED) between treatment means is presented.

2.2 Field Experiment

The aim of this experiment was to compare the effects of different rubber tree and intercrop combinations on rubber tree growth and on rubber tree and intercrop root patterns.

2.2.1 Experimental field sites

The field experiments were conducted at three field plots.

2.2.1.1 2006 rainy season experiment

The first experimental field site was located at a farmer's plantations in None Tun village, in the district of Pha Yune, Khon Kaen province in Northeast Thailand (N 16 20'02" and E 102 44'60" Figure 8) and was monitored from 13 July to the end September 2006. The soil was typical of the region (Loamy, siliceous, subactive isohyperthermic Oxyaquic Arenic Haplustalfs; Maha Sarakham series (Msk), i.e. sandy soil with pH 5.26 (1:1, H_2O ratio), and 40.03 µS/Cm, Electrical conductivity (EC). Total rainfall was 1415.4 mm in the experimental year and the cumulated rainfall over the period of the experiment was 784.9 mm and weekly rainfall pattern during the crop period are presented in Figure 9.

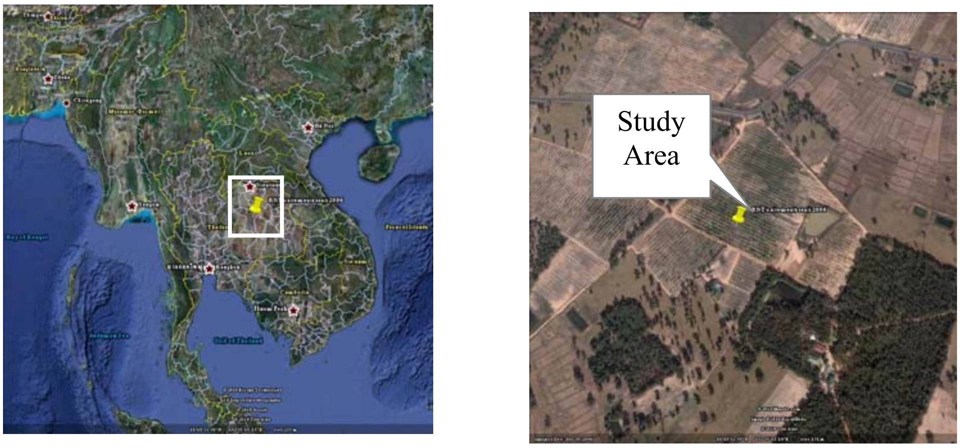


Figure 8 Location of the 2006 field study area.

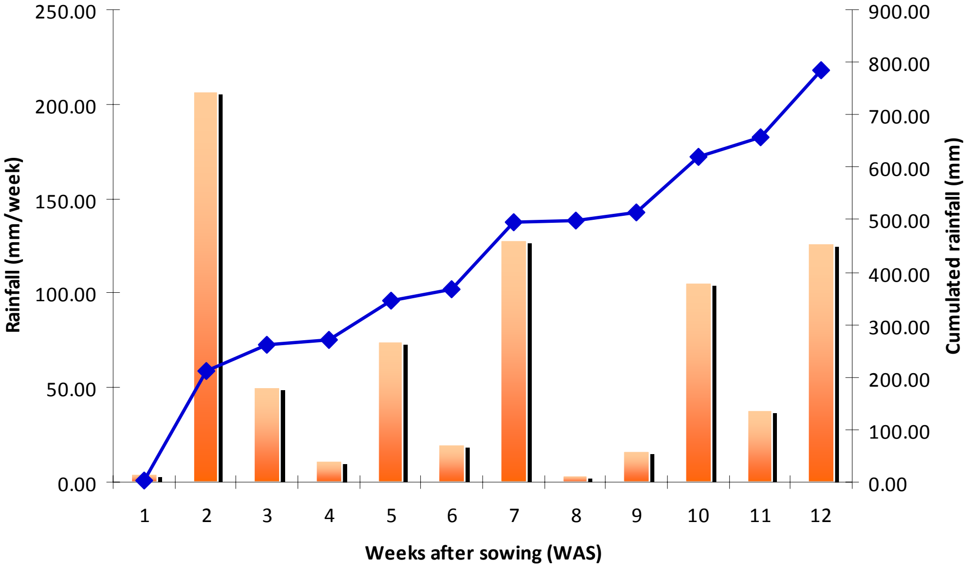


Figure 9 Weekly rainfall after planting until crops harvesting on 2006.

2.2.1.2 2007 rainy season experiment.

The second field site was a plot located at the same farmer's plantations in None Tun village as that used in 2006 (N 16° 19' and E 102° 44', 163 m a.s.l.) the difference being that the experiments were conducted at N 16° 19' not N 16° 20' of the previous year to avoid soil erosion problems in the previous year. The experiment was conducted from 1 August to 13 November 2007. The soil was typical for the region was similar to that of the 2006 plot i.e. sandy soil with pH 6.31 (1:1, H_2O ratio), 0.47 % organic matter, 0.02 % total N, 7.6 ppm extractable P (Bray II), 138.4 ppm extractable K (NH_4OAC), 223.93 ppm extractable Ca (NH_4OAC), 24.2 ppm extractable Mg (NH_4OAC) and 0.02 (1:5, H_2O ratio) Electrical conductivity (EC). Total rainfall was 1,309.80 mm in the experimental year, the cumulated rainfall over the period of the experiment was 765.60 mm and weekly rainfall pattern during the crop period are presented in Figure 10.

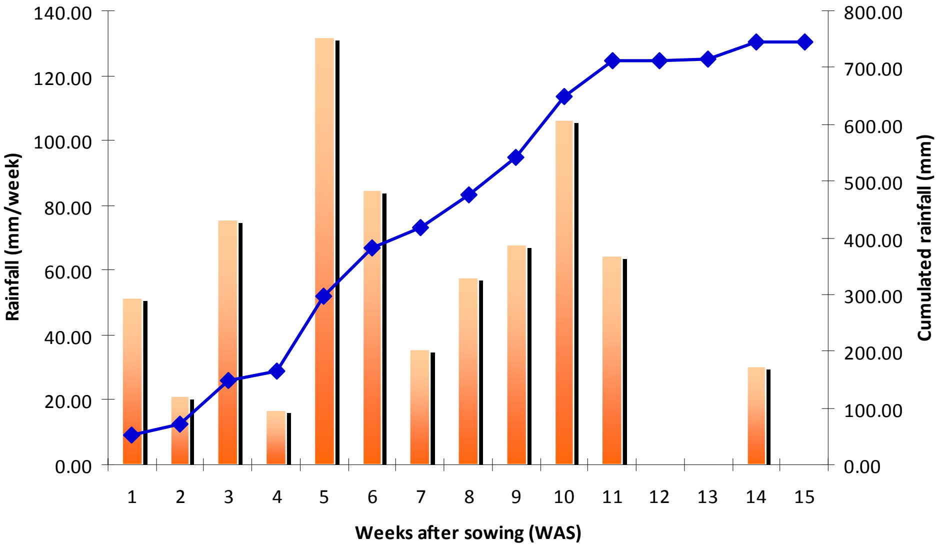


Figure 10 Weekly rainfall after planting until crops harvesting on 2007.

2.2.1.3 2008 rainy season experiment.

The third plot established in 1 July to 28 October 2008, was located in same plot as that used for the 2007 experiment (N 16° 19' and E 102° 44'), the only difference being that the experiments were conducted 20 m above that of the previous year (at 183 m a.s.l.) to avoid water-logging problems observed lower in the landscape the previous year. The soil was typical for the region (Loamy, siliceous, subactive isohyperthermic Oxyaquic Arenic Haplustalfs; Maha Sarakham series (Msk), i.e. sandy soil with pH 5.1 (1:1, H_2O ratio), 0.39 % organic matter, 0.02 % total N, 6.27 ppm extractable P (Bray II), 4300 ppm extractable K (NH_4OAC and 0.01 (1:5, H_2O ratio) Electrical conductivity (EC). Total rainfall was 1,957.60 mm in the experimental year, the cumulated rainfall over the period of the experiment was 864.40 mm and weekly rainfall pattern during the crop period are presented in Figure 11.

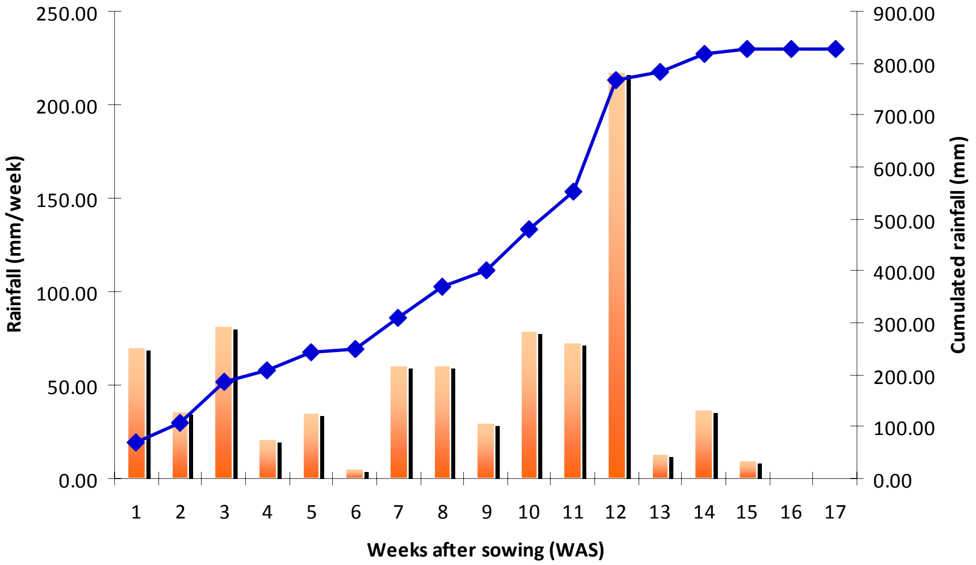


Figure 11 Weekly rainfall after planting until crop harvesting on 2008.

Rainfalls and evapotranspiration (ET_o) were automatically recorded using a Campbell (http://www.campbellsci.com/weather-climate) weather station.

2.2.2 Planting materials

2.2.2.1 Rubber tree

One-year old (2006 rainy season experiment; planting date: August 2005), three-year old (2007 rainy season experiment; planting date: June 2004) and four-year old (2008 rainy season experiment; planting date: June 2004) rubber trees (*Hevea brasilliensis*), clone RRIM 600, were monitored at the Ban None Tun farmer's plantations. Experimental plots were established in zones where rubber trees had relatively uniform heights, stem girth and flush numbers. On average, tree height, stem girth and flush number were 5.22 and 5.90 m, 12.78, 12.95 and 17.04 cm and 9.62, 12.00 and 12.91 flushes in 2006, 2007 and 2008, respectively.

2.2.2.1 Intercrops

In 2006, cowpea (*Vigna unguiculata*) was sown as an intercrop between the one year old rubber tree rows. In 2007 and 2008 one first intercrop was the commercial fresh glutinous corn (*Zea mays*) seeds from EAST-WEST SEED COMPANY, var. name BIG WHITE 852^{TM}, 98 % purity (min), 85 % germ (min). A second intercrop was groundnut (*Arachis hypogaea*) variety Ti Nan 9. Sowing materials of uniform germinating potential were used in each case for the two intercrops: this was achieved by weighing seeds and keeping the heaviest ones (>0.199 and 0.321 g for corn and groundnut, respectively). The groundnut seeds were innoculated with rhizobium var. TAL1000, THA205 strain CIAT2434 3850 3918 (stylo) before sowing.

2.2.3 Cultural Practices

The rubber trees were grown under rain-fed condition. They were grown in a weed free environment with plowing between rubber tree inter row – indicate the frequency or periods of plowing in the inter row. After 2 years, inter-row plowing was stopped. During the two-year first, rubber tree plots were fertilized formular16-8-8 of N, P and K, respectively, at 60 kg ha^{-1} twice per year, at the onset and the end of the rainy season. During the 2008 experiment, fertilizer was applied at 20-20-12, 180 g/plant rates (three year old rubber trees) twice per year at the onset and the end of the rainy season. Pruning of branches was done by the farmer when necessary, i.e. depending on individual tree's development. This practice increases the tapping area later upon maturity recommended by Rubber Research Institute of Thailand.

The crops were grown under rain-fed condition with supply of nutrients based on the fertilizer regime recommended by the DOA (Department of Agriculture). They were grown in a weed free environment with pests and diseases kept in check. The plot size was 3x21 m^2, 4x15 m^2 and 5x12 m^2 on 2006, 2007 and 2008, respectively and there were three replicates.

2.2.4 Field experimental layout

In 2006, inter-crops were planted in rows along the direction of the slope. The distance between rubber trees within the row was 3 m and the distance between tree rows was 7 m. Cowpea was sown at 0.2 x 0.5 m intervals.

In 2007, inter -crops were also planted in rows along the direction of the slope. The distance between rubber trees within the row was 3 m and the distance between tree rows was 7 m. Groundnut and corn were sown at 0.2 x 0.5 m and 0.25 x 0.75 m intervals, respectively (Figure 12).

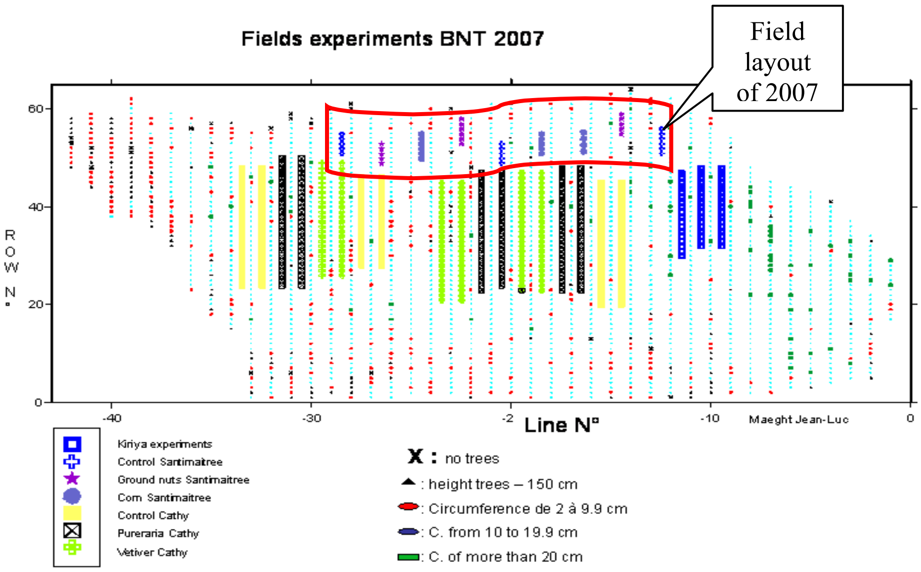


Figure 12 The 2007's field layout

The 2008 experimental plot was located within the same plantation block as that used in 2007 but was located higher in the topography, for the purpose of avoiding water-logging problems observed lower in the landscape in 2007. Crop and inter-crop planting layouts were the same as that used in 2007 (Figure 13).

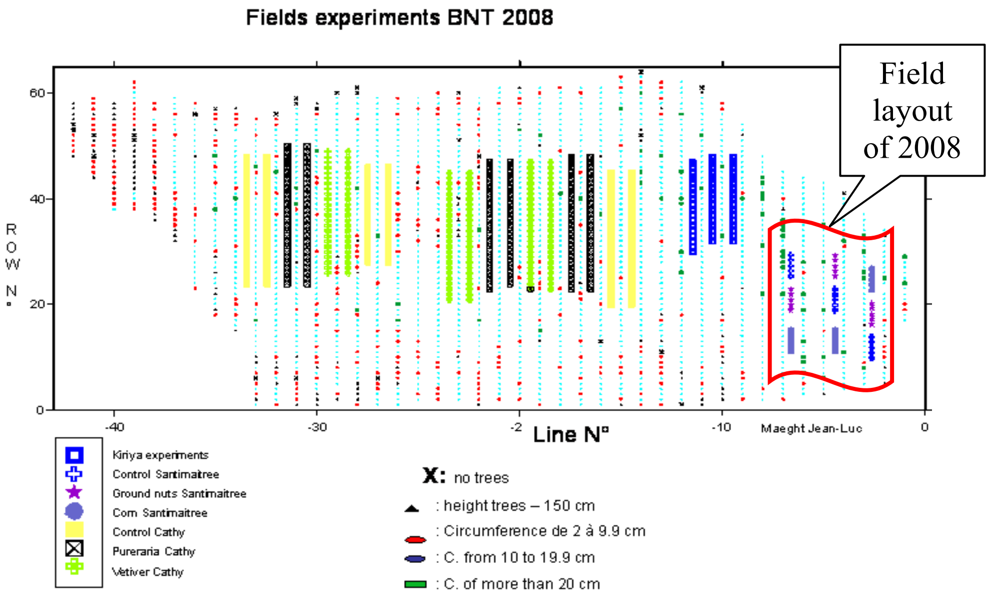


Figure 13 The 2008's field layout

2.2.5 Treatment

2.2.5.1 2006 rainy season experiment.

1. *Hevea brasilliensis* alone
2. *Hevea brasilliensis* + *Vigna unguiculata*
3. *Hevea brasilliensis* + *Vigna unguiculata* + PK: 37.5 kg P (TSP) ha^{-1} + 18.75 kg K (KCl) ha^{-1}
4. *Hevea brasilliensis* + *Vigna unguiculata* + NPK: 18.75 kg N (urea) ha^{-1} + 37.5 kg P (TSP) ha^{-1} + 18.75 kg K (KCl) ha^{-1}
5. *Hevea brasilliensis* + *Vigna unguiculata* + NK: 18.75 kg N (urea) ha^{-1} + 18.75 kg K (KCl) ha^{-1}
6. *Hevea brasilliensis* + *Vigna unguiculata* + NP: cowpea with 18.75 kg N (urea) ha^{-1} + 37.5 kg P (TSP) ha^{-1}

2.2.5.2 2007 and 2008 rainy season experiment.

1. *Hevea brasilliensis* alone
2. *Hevea brasilliensis* + *Zea mays*
3. *Hevea brasilliensis* + *Arachis hypogaea* L. var.Tinan 9

2.2.6 Sampling methodology

2.2.6.1 Soil cores

Standard soil sample steel rings (dia. 53 mm, height 50 mm, 100 cm^3 internal volume, Eijkelkamp – www.eijkelkamp.com) were used to collect roots at three depth increments, namely 25, 45 and 65 cm. Two series of soil cores were taken at an equal distance of 1 m from both the row of rubber trees and the outside edge of the intercrop plots (Figure 14). To restrict the number of holes to be augered in the soil, two samples taken just above and below these three soil depth increments were considered as replicates (i.e. 20-25 with 25-30, 40-45 with 45-50 and 60-65 with 65-70 cm). To account for the high spatial variability of rooting patterns (De Silva et al., 1999) such core samples were taken at the three replicate plots, nearly as possible apart from each other, for each treatment. This sampling scheme resulted in a final number of six replicates per depth increment per treatment. These fine root samples were used to measure and compare root length/area densities for the different treatments, using image processing. The total number of samples taken was therefore: 6 cores x 2 augered holes x 3 treatment x 3 replications making up a total of 108 samples.

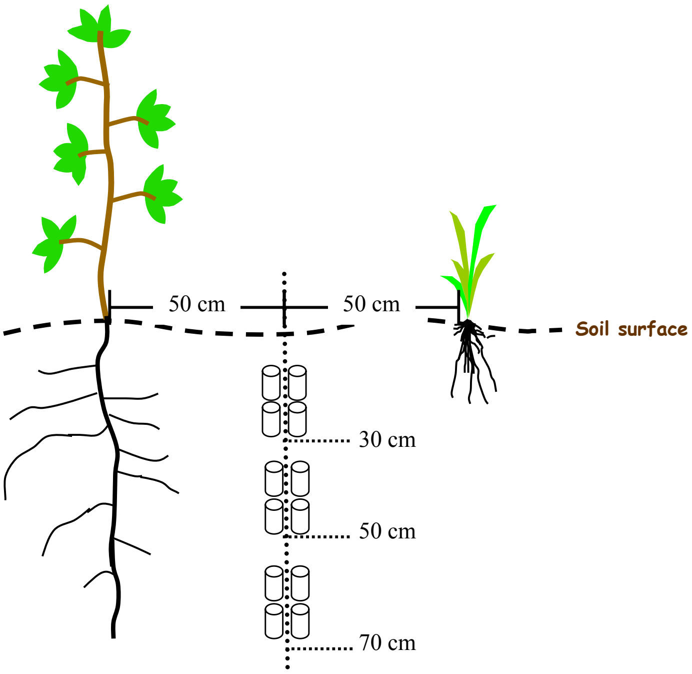


Figure 14 The layout of soil core sampling

2.2.6.2 Root trap

This method is inspired from the ingrowth core method (see e.g., Steingrobe et al., 2000). As soil virtually free of roots is placed in the traps at the time of installation, this approach presents the advantage of allowing sampling of roots that grew during a known period of time. Root traps were made of PVC tubes, installed in the field prior to planting the intercrop. These tubes, 70 mm in diameter and 150 mm long (internal volume = 577 cm^3), were filled with soil from the soil depth at which they were installed and left in situ for the whole duration of the intercropping cycle. Two series of root traps were installed at a 45 degrees angle at three depth increments of 30, 50 and 70 cm respectively, one series oriented towards the rubber trees and the other towards the inter-crop/inter-row (Figure 15). The lower end of root traps was wrapped with fine plastic mesh in order to prevent the soil from falling out from tubes at the time of sampling. A total of 6 cores x 6 treatment x 5 replications i.e. 180 root traps were installed in 2006 while a total of 6 cores x 3 treatment x 3 replications i.e. 54 root traps were installed in 2007. Root traps were not used in 2008 because it was found in 2007 that their installation was problematic in the location where the experiment was conducted, with water saturated soil slumping excessively during excavation.

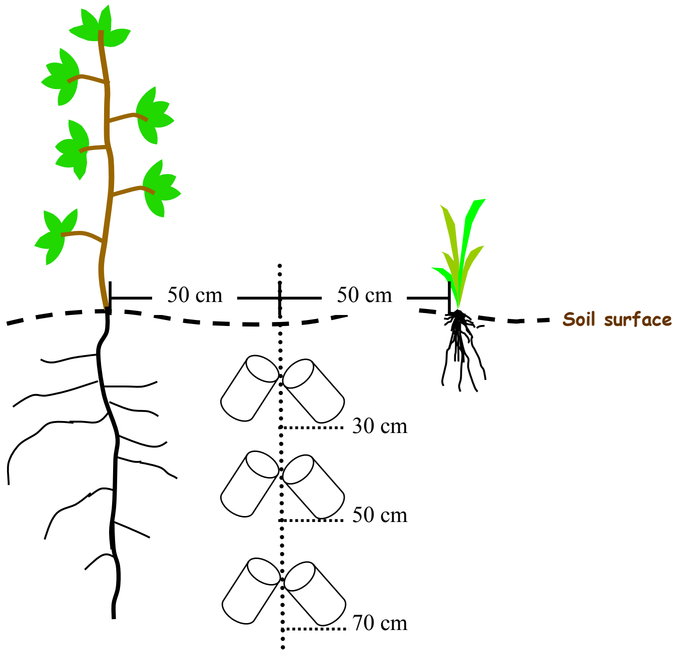


Figure 15 The layout of root trap sampling

2.2.7 Preparation and imaging of root samples

2.2.7.1 Storing soil-root samples before washing

It was not possible to wash out roots from all soil samples immediately after the samples were taken from the field site. Samples were therefore stored for a maximum of about 2-3 days at a temperature of 5 to 7 °C before which prevented root tissues to start decaying (Bohm, 1979).

2.2.7.2 Hand washing of root

The technique of separating roots from soil is a washing process with water aided by hand manipulation. The soil-root sample is suspended in water and filtered through fine-mesh sieves (40 to 70 μm meshes) which retain the roots. Root samples were separated from debris or from all other organic matter using tweezers.

2.2.7.3 Storing root samples after washing

Cleaned roots had to be stored before scanning and measurement. To that purpose, they were placed in opaque plastic bottles and preserved with dilute acetic acid (typical commercial vinegar with 5% acetic acid content) and water, 2:3 ratio, respectively, at temperatures of about 10 °C until being measured in the laboratory. The parameters used to characterize root growth are: root length (and implicitly, root length density as the volume of the soil samples taken was always known), root surface area and volume, root diameter, dry root biomass and specific root length.

2.2.7.4 Production of root images

Each washed root sample was scanned using an Epson Perfection V700 Photo scanner, in light transmission and reflection modes. Root samples were placed in a glass tray the same size as the scanner's transparency window (dimensions ~210×300 mm) and about 20 mm deep. The root sample was transferred into the tray half-filled with water. Special attention was taken to separate every root from each other as much as possible, since overlapping roots block the scanner's incident light as a single large object from which accurate length recovery is not possible. Washed samples used to estimate root length density were processed and analyzed according to the protocol described in Pierret et al., (2007c) (see "Processing of root sample images" section).

2.2.7.5 Separation of roots from different species and processing of root washing samples

After separation from the soil by hand washing in water, root samples obtained from both root traps and soil cores were scanned (see description in this chapter, sections 2.2.7.1 to 2.2.7.3) and the images thus obtained were measured using a specifically designed image analysis procedure (see description in this chapter, sections: 2.2.7.5 to 2.2.8). The separation of rubber roots from that of the intercrops was achieved either by hand, at the time of image scanning, or on the scanned images. Although not planned initially, the latter procedure was found to be more convenient as roots of rubber and intercrop were often tightly entangled with each other and physically separating them, apart from time consuming, resulted in a lot of breakage which further complicated the process of scanning. When done on the scanned images, the separation of two types of roots was achieved using visual criteria such as shape, diameter, branching pattern and color. Both light transmission and reflection images were used for this operation. Examples of the appearance of the different types of roots studied in this work are given in Figure 16 and 17. It has to be noted that such a separation procedure does not guarantee an absolute selection of mixed root samples: in almost all samples, a certain proportion of short and/or very fine (small diameter) roots remains impossible to classify with certainty. It is however the only basic and inexpensive method that can be used for such a purpose. Other, more accurate methods exist for such an analysis, such as near-infrared reflectance spectroscopy (NIRS), the use of plant wax markers or DNA techniques (Jackson et al., 1999; Roumet et al., 2006). However all these techniques rely on expensive and sophisticated equipment that was not available within the framework of this study.

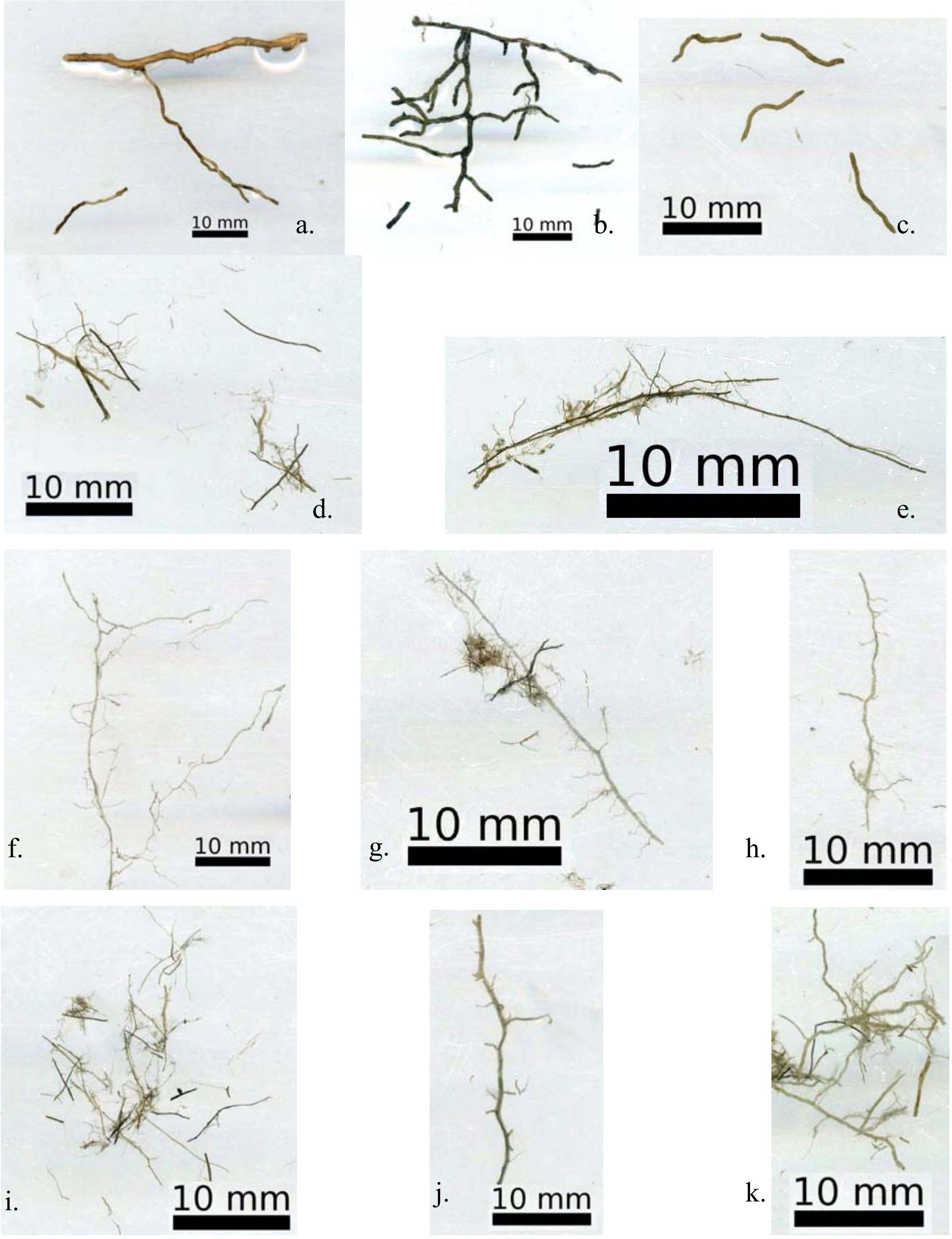


Figure 16 Examples of the appearance of the different types of roots studied. Rubber tree roots (a, b and c), weed roots (d and e), groundnut roots (f, g and h) and corn roots (i, j and k).

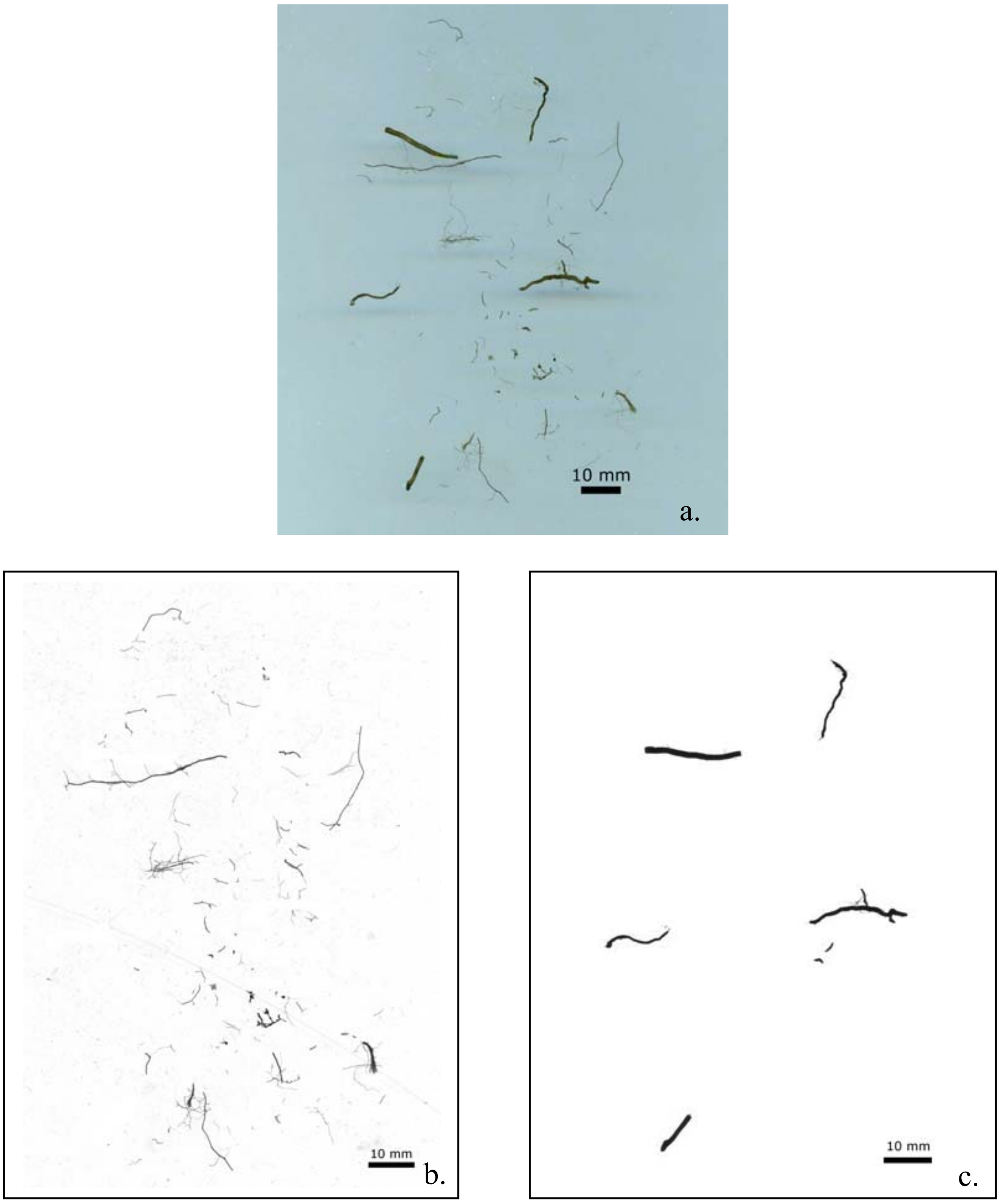


Figure 17 The separation of rubber roots from that of the intercrops was achieved on the scanned images. Light reflection image that show both the roots of rubber tree and intercrop before separation (a). (b) and (c) light transmission image of intercrop (groundnut) and rubber tree roots, after separation , respectively.

2.2.8 Image analysis routines developed and used to measure root systems

To perform the length and radius measurements based on scanned images of washed root samples, an *ImageJ* macro was written. *ImageJ* is a public domain Java image processing program created by the National Institutes of Health and inspired by NIH Image for the Macintosh. It is a powerful image analysis package that runs, either as an online applet or as a downloadable application, on any computer with a Java 1.4 or later virtual machine. Downloadable distributions are available for Windows, Mac OS, Mac OS X and Linux from http://rsbweb.nih.gov/ij/.

A macro is a simple program that automates a series of *ImageJ* commands. A macro is saved as a text file and executed by selecting a menu command, by pressing a key or by clicking on an icon in the *ImageJ* toolbar.

Description of the ImageJ macro used to measure samples obtained from soil washing

The purpose of the first part of the macro is to remove pixels at the image periphery when these pixels are known to include values which could mistakenly be considered as "objects". The size of the image margin to be excluded from analysis must be adjusted by the user. Once, this operation completed, the image is subsequently thresholded, i.e. transformed into a binary image in which objects and background are coded with a unique value. The threshold applied is defined automatically by the function "*FindThres*" which analyses the local slope of the image histogram. First the algorithm looks for a positive change in slope which it keeps as a first value *ThInf*. Then a slope more than 1000 at a "distance" of more than 100 grey levels from *ThInf* is kept as a second value *ThSup*. Finally the threshold value is defined as

$$\text{Thres} = ((95 * \text{ThSup}) + (5 * \text{ThInf})) / 100 \qquad \text{Eq (2)}$$

Note that this approach only works for a simple image with dark objects on a light background, so that two separate and well-defined peaks exist in the histogram (Figure 18).

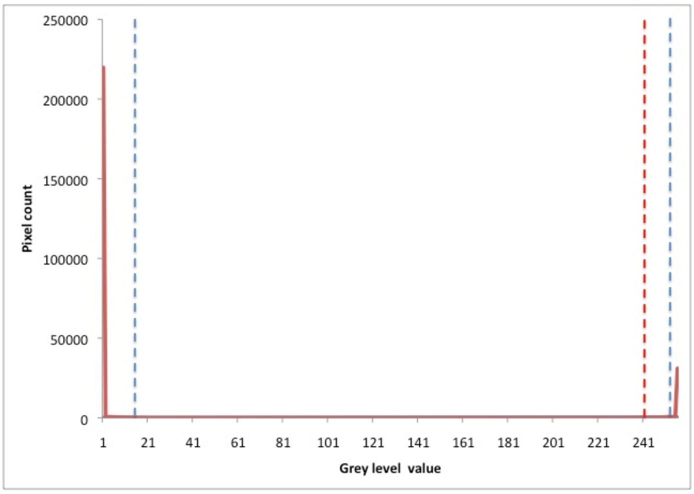


Figure 18 Illustration of the automatic image thresholding approach: the solid line is a typical image grey level histogram; the vertical dotted line to the left is ThInf (grey level16), the one to the extreme right is ThSup (grey level 254) and the dotted line at grey level 242 represent the threshold value Thres.

Next, the macro calls an optional routine "*CleanParticles*" that is used to remove objects that are less than a given size threhold and more than a shape threshold. These two thresholds are user-defined (they can be entered at the time of lauching the macro, using the dialogue windows that automatically open). The shape parameter is the so-called "circularity" index which is built in *ImageJ*. This particle cleaning routine is most useful to remove small debris and grain sands that often get mixed with roots from soil washing samples. This optional routine is run by default but can be unselected by the user when it is deemed unnecessary to conduct this rather computer-intensive operation. This thresholded image is then saved (it will be useful to check these images once the processing completed to assess the validity of the number produced by the macro).

In a second part, the thresholded image is re-opened and skeletonized, i.e. that objects are thinned iteratively until they are represented by only a line one pixel wide (Figure 19). The number of pixels making up this skeleton image is then computed. Multiplied by the image resolution, it provided a first estimate of root length in the image.

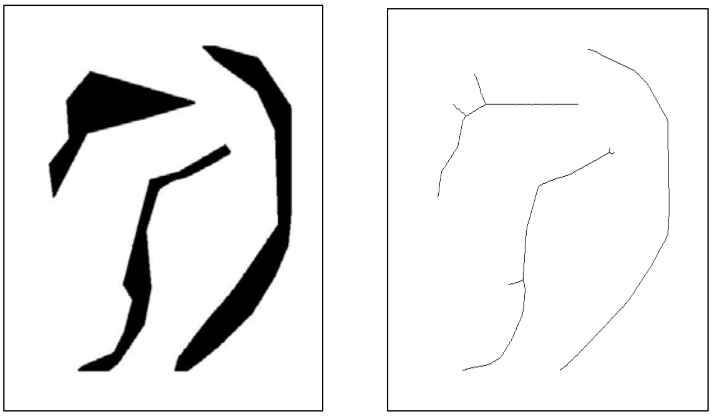


Figure 19 An example image with objects in black (left) and an image of the objects skeletons (right).

The projected surface area is measured by counting the number of object (in our case, roots) pixels in the thresholded image and by multiplying this number by the surface area of an individual pixel (depends on image resolution).

A third part of the macro produces the root radius histograms. With these histograms, root length is expressed as a function of root diameter. To this end, the thresholded image is used to derive the so-called Euclidean Distance Map (EDM) in which pixels corresponding to objects are labeled according to their distance to the edge of the object in which they are included (Figure 20). By combining the EDM and the skeleton images, it is therefore possible to produce a skeleton labeled with discrete root radius values. A table is then created in which, for each radius values, the corresponding number of pixels is reported. By multiplying both root radius and pixels numbers by a factor corresponding to the image spatial resolution, this results in a root radius histogram with values scaled to actual sizes.

Figure 20 The Euclidean Distance Map (EDM) of the image in Figure 16. The darker the grey of shade, the longer the distance.

A fourth part is used to implement an optional length correction; a property of the skeleton-based technique is that the assessed length of objects is underestimated in a proportion directly related to their thickness (or radius). This is because more pixels must be removed from around a thick object than from a thinner one to produce a skeleton (Figure 21). Consequently, using the EDM labeled skeleton produced previously, this part of the macro estimates, object per object, the most likely number of pixels to be added to the figure of the overall root length.

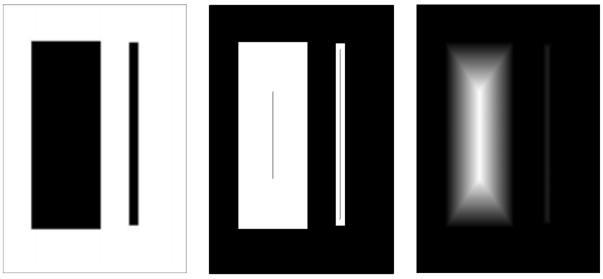


Figure 21 A theoretical image with two objects of identical maximum length but of different thicknesses (left), the skeletons (with outlines of the original objects) corresponding to theses two objects – note that the thick object has a shorter skeleton than the thin one (center) and the Euclidean Distance transform of the image (right).

Finally, the fourth part of the macro is dedicated to calculations of values to be written in the main output file, namely:

The uncorrected root length RL:

$$RL = SkelSz*(25.4/ImRes) \qquad \text{Eq (3)}$$

Where $SkelSz$ is the number of pixels that make up the skeleton and $ImRes$, the image spatial resolution in dots per inch (dpi);

The corrected root length RLc:

$$RLc = RL + (XtraPix * (25.4/ImRes)) \qquad \text{Eq (4)}$$

Where $XtraPix$ is the number of extra pixels found through the EDM-based length correction computation;

The average root diameter $MeanDia$:

$$MeanDia = ActArea/RLc \qquad \text{Eq (5)}$$

Where $ActArea$ is the surface area of objects within the image;

The root volume equivalent $EqVol$:

$$EqVol = (MeanDia/2)^2 * RLc * \pi$$

The root surface area equivalent $EqSurf$:

$$EqSurf = MeanDia * RLc * \pi \qquad \text{Eq (6)}$$

Note: to be able to run the macro, the user must previously install the Morphological Operators for *ImageJ* (download as a single zip file from: http://www.dentistry.bham.ac.uk/landinig/

2.2.9 Root biomass measurements

Following scanning, each root sample was oven dried at 80°C for 48 h, after which individual sample weight was measured using a Adventurer^{TM} OHAUS precision balance (precision 0.0001 g). Given the total root length RL of a sample and the corresponding dry root biomass (DRB), it is possible to derive its specific root length SRL (in m of fresh root length per g of dry root biomass) by computing the ratio:

$$\mathrm{SRL} = \mathrm{RL}/\mathrm{DRB} \qquad \text{Eq (7)}$$

2.2.10 Running the Macro

To start the Root Processing *ImageJ* macro, it is necessary to first start the *ImageJ* program. The macro itself can thence be launched from the Plugins menu, in *ImageJ* main window's menu bar. This is done by selecting Plugins>Macros>Run... from the *ImageJ* toolbar (Figure 22)

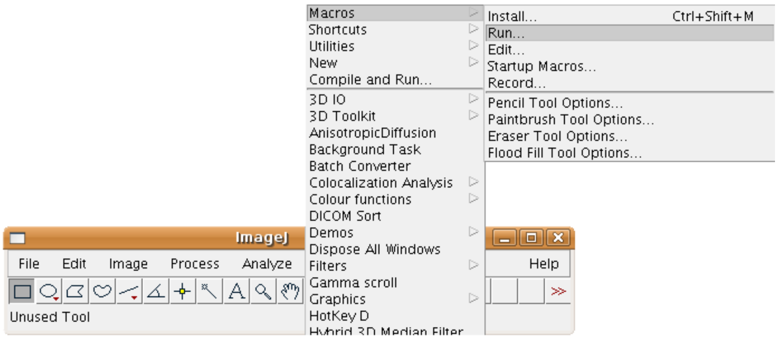
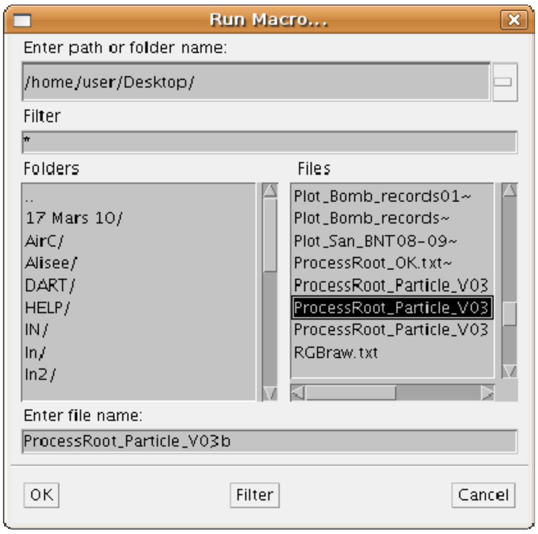


Figure 22 Launching the root processing macro from the *ImageJ* toolbar

This will the opening of a “Run Macro...” window (Figure 23), from which the user needs to select the text file in which the macro has been previously saved. Navigation to this file is possible by selecting appropriate folders in the left hand side part of the “Run Macro...” window. Once in the right directory, select the macro by clicking on its name.



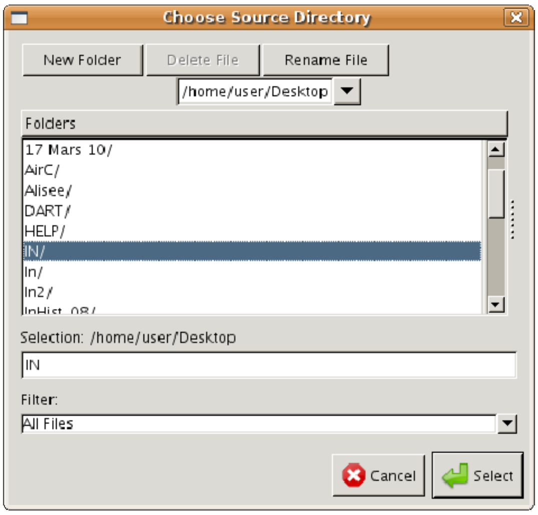
Enter path or folder name: /home/user/Desktop/

Filter: *

Enter file name: ProcessRoot_Particle_V03b

Figure 23 Selecting the macro from the “Run Macro...” window

As soon as the macro is started, the user is prompted to select a source directory, by selecting it in the “Choose Source Directory” window (Figure 24). The source directory is a directory in which all the files images that need to be processed must be stored prior to launching the macro.



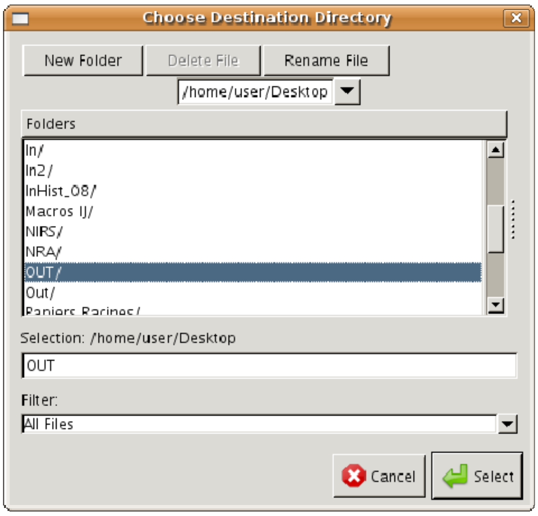
Selection: /home/user/Desktop

IN

Filter: All Files

Figure 24 Selecting the source directory

Once the source directory selected, the user is prompted to select a destination directory, by selecting it in the “Choose Destination Directory” window (Figure 25). The destination directory is an empty directory which must be created by the user prior to launching the macro and in which all the macro's output files will be recorded.



Selection: /home/user/Desktop

OUT

Filter: All Files

Figure 25 Selecting the destination directory

Finally, it is necessary to set a number of parameters, prior to running the macro. To this end, the user is prompted with and "Input Data" window (Figure 26). In the first box, at the top of this dialog window, the user must enter the spatial resolution of the images to be processed, in "dots per inch" (dpi). Note that all the images contained in the source directory must have the same spatial resolution. If not, the output measurements produced by the macro will only be valid for those images which have a resolution equal to that entered in the box. The default value for this parameter is 600 dpi.

The next box (Width of excluded border) requires input for the width of the image margin that may need to be excluded from the analysis (typically because it contains non root-related information such as, e.g. the edge of the tray used during the root scanning process).

Below is a "Perform particle cleaning" tick box. This box should be ticked to exclude undesirable objects from the analysis, such as small debris or sand grains. The size of the smallest particle included in the analysis can be adjusted here, as well as a shape parameter (a value of 1 and 0 corresponding to a circle and line, respectively). By default, objects down to 1 mm^2 and up to 0.75 in circularity are included in the analysis.

Finally, a “perform root length correction” tick box can be selected if loss of length related to the skeleton approached used by the macro for estimating root length needs to be accounted for. This applies chiefly when thick roots, with diameters of several tens of pixels are included in the images to be analyzed. Note that this correction is rather computing intensive and will substantially slow down the overall processing.

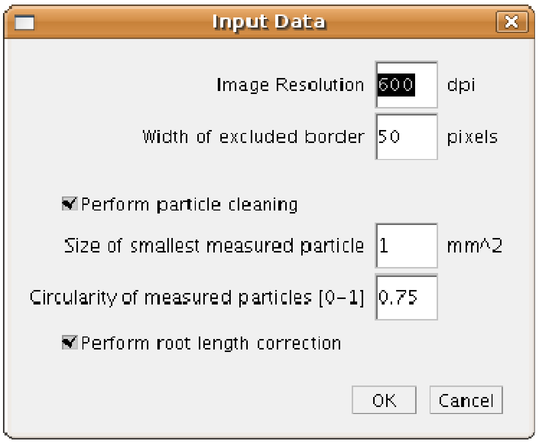


Image Resolution 600 dpi

Width of excluded border 50 pixels

☑ Perform particle cleaning

Size of smallest measured particle 1 mm^2

Circularity of measured particles [0-1] 0.75

☑ Perform root length correction

Figure 26 The input data dialog box, used to set input data and processing options.

CHAPTER IV: RESULTS

1. Results of the rhizobox experiments

1.1 Introduction

In this section, we present the results of the rhizobox experiments. Data related to both root system and shoot development of rubber tree and three intercrop species candidates, namely, corn, cassava and groundnut, are presented. The level of detail presented for these three treatments, respectively rubber tree × corn, rubber tree × cassava, rubber tree × groundnut is very unequal due to practical/experimental constraints. One first reason for this is that, in the case of the rubber tree × groundnut treatment, the root development of groundnut plants was greatly hampered, probably due to a fungal attack on roots. Using an additional set of three replicate rhizoboxes, it was finally possible to grow disease-free plants. However, even then, two out of three groundnut plants did not develop any contact with their neighbour rubber tree and the last one developed only 4-5 roots contact with rubber roots. This adverse experimental situation precluded the study of interactions of the two plants at the individual root level; it also importantly reduced the interest of studying the interactions at the whole root system level as there was little evidence for direct interaction between the two plants. A second problem that occurred with the rubber tree × cassava treatment was that cassava developed profuse amounts of roots that resulted in extremely complex architectures that are prohibitively long to digitize for analysis with the DART software. In addition, the fact that cassava grew such extensive and profuse root systems also raised the issue of the space limitation imposed by the rhizobox experimental setup on the development of such plants. Indeed, it was observed that cassava roots reached the limits of the growth container very rapidly and that they grew with such vigor that a large proportion of them ended up growing behind the nylon mesh, directly into the vermiculite infill behind. It therefore can be said that these experimental circumstances were sub-optimal for the analysis of interactions between rubber tree and cassava at the whole root system level. However, as cassava roots grew extensively in all directions, including that of the rubber trees and developed a number of root contact with rubber roots, we analysed changes in growth rates associated with these contacts and present the results of this analysis in this chapter. Finally, the rubber tree × corn treatment was the only one for which serious experimental difficulties did not occur. This explains why most of the results presented below focus on this treatment.

1.2 Overall root system development (rubber tree -corn association)

1.2.1 Overall root system length

The overall length of young rubber tree and corn root systems was on average 18.96 (±2.8) and 57.57 (±3.8) m, respectively (figures in brackets are 95% confidence intervals, n=3), corresponding to a growth period of over 9 weeks (from 5 March to 20 May 2008) (Figure 27). Young rubber tree root growth was rather regular throughout the experiment, as indicated by the constant slope of the cumulative root length as a function of time (Figure 27), at least until about 55 DAS; past this period, the slope is less, indicating a reduction in the rate of rubber tree root system expansion. In contrast, corn root system development displays three distinct phases: a first period from the onset to about 14 days during reach corn root system expansion was comparable to that of rubber tree; a second phase, from about 14 to 45 days during which corn root systems rapidly expanded, as indicated by a slope more than four times that of rubber tree in (Figure 27); and finally a third phase, past 45 DAS during which the expansion of corn root systems stopped as indicated by the plateau in the cumulative root length curve (Figure 27).

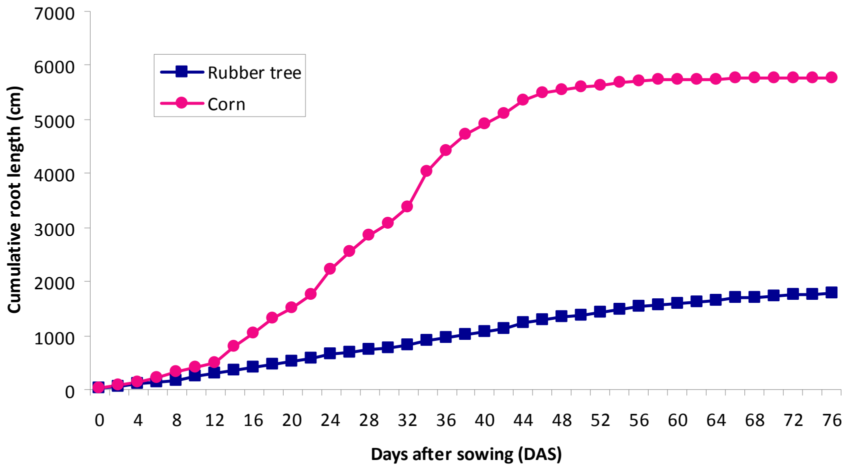


Figure 27 Root growth dynamics at the root system level.

Total root length of corn which planted with young rubber tree was range 50.01 – 61.91 m. Total root length of rubber tree which planted with corn and rubber tree alone ranged from 14.39 to 24.06 m and 3.71 to 13.44 m, respectively. Rubber tree root system length, whether grown alone or in association with corn, was significantly lower than that of corn ($p<0.01$; $n=3$; Welsh two-sample t-test); the root system length of rubber trees grown alone was not significantly different from that of rubber trees grown in association with corn (Figure 28).

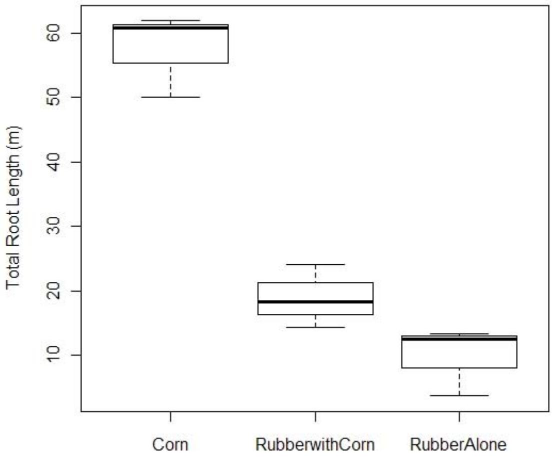


Figure 28 Total root length of corn grew with young rubber tree, rubber tree grew with corn and rubber tree alone.

1.2.2 Root system expansion rate

On average, the expansion rate of young rubber tree root systems grown with corn ranged from 14.27 to 21.07 cm per day during the 2 - 8 DAS period. It ranged from 16.07 to 46.35 cm per day during the 10 - 54 DAS period and then declined, ranging from 4.15 to 19.51 cm per day over the 56 to 76 DAS period (Figure 29). The average root system expansion rate of corn ranged from 12.49 to 47.86 cm per day during the 2 - 12 DAS period. It increased during the 14 - 44 DAS period, during which it fluctuated from 93.17 to 331.79 cm per day and then decreased over the 46 to 76 DAS period, with a range from 0.57 to 58.99 cm per day (Figure 29).

All three corn plants exhibited a series of four 10-day growth cycles, from c. the 10th DAS until c. the 60th DAS (Figure 29) according to which the daily root system expansion varied. The precise timing and the amplitude of these cycles varied depending on the replicate considered and were not related to variations in environmental conditions (air temperature, PAR, or photo-period).

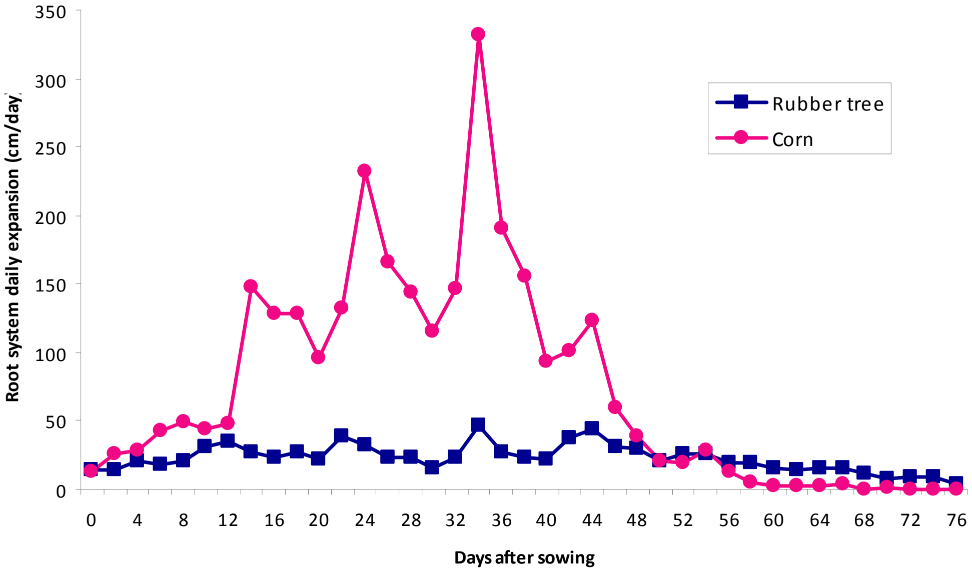


Figure 29 Average (n=3) root system daily expansion rate (in cm/day) of corn and rubber tree plants grown in association in the rhizoboxes.

1.2.3 Root branching, number of growing roots and growth rates of growing roots

In corn, root branching followed three main phases (Figure 30): from 0 to 17 DAS during which root numbers increased little, indicating limited branching; from 17 to 50 DAS, during which branching produced about 20 more roots every day; and beyond 50 DAS, corresponding to the end of the branching period, with no or little further increase in root numbers. From these observations we can therefore define the 17-50 DAS period as the period of active corn root branching.

In rubber, root branching followed the first two phases described for corn but the reduction in root branching from 50 DAS onward did not apply (Figure 30): in one case only (Box 6) the branching rate clearly dropped after 61 DAS, but in the two other cases, there was only a slight and progressive reduction of branching rate after 50 DAS compared to that of the 17 to 50 DAS period. In addition, one plant (Box 4) had a much higher branching rate than the two others.

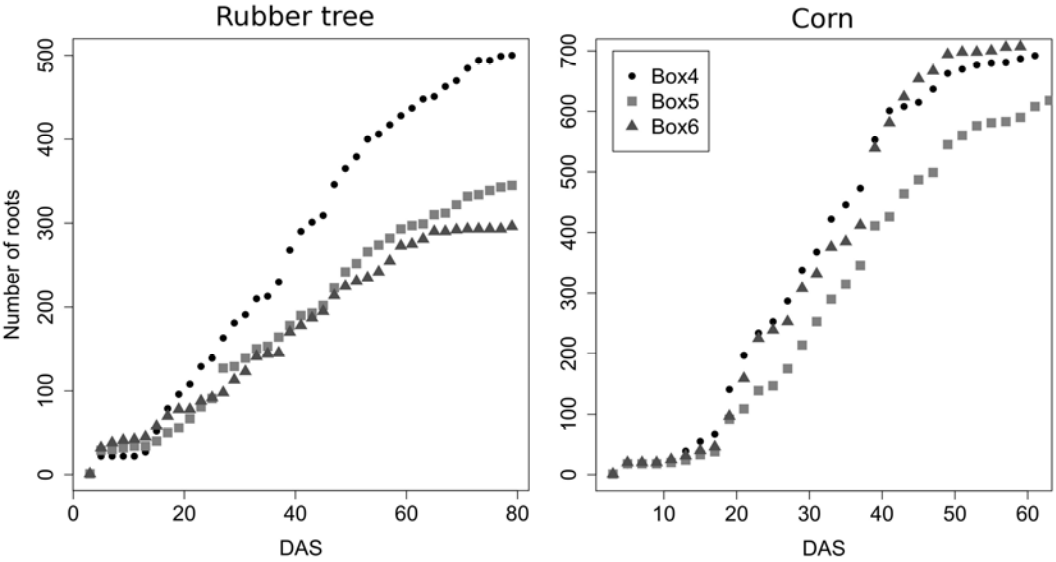
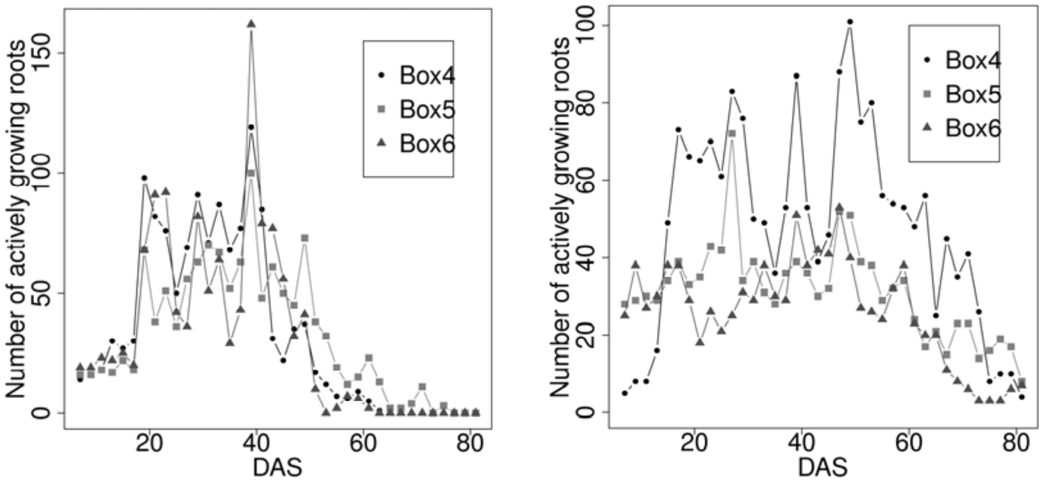


Figure 30 Cumulative numbers of roots in rubber trees grown in association with corn and corn grown with rubber trees. The slope of the curves is indicative of the root branching rate.

The number of actively growing roots was computed for both corn and rubber trees grown in association. The patterns corresponding to corn show 3 successive phases (Figure 31), roughly 0-18, 20-55 and >55 DAS which correspond more or less to the three phases of root system expansion described earlier. There is, during the 20-55 DAS period - which corresponds the phase during which the numbers of growing roots are the highest - a large variability at a short time scale (typically 5 days). Over these short periods of time, the number of actively growing roots can double or conversely be halved (with even more extreme variations around 40 DAS).

In rubber trees grown with corn, the number of actively growing roots does not appear to show any clear phases such as that identified in corn. There is also a greater inter-individual variability. Similarly to what was observed in corn, it can be seen that the number of actively growing roots can vary hugely over short periods of time (typically 5 days). This is particularly true for the individual in Box 4 (Figure 31).



Rubber tree Corn

Figure 31 Number of actively growing roots in rubber trees grown in association with corn (left) and corn grown with rubber trees (right).

The distributions of growth rates over each of the two-day observation periods were computed and plotted for both the corn and rubber trees grown in association. Overall, they demonstrate that, despite some variability, growth rates of individual roots did not vary much with time in both corn and rubber. Remarkably, in corn, the cycles in root system expansion reported earlier do not seem to correspond to similar cycles in root growth rates (Figure 32).

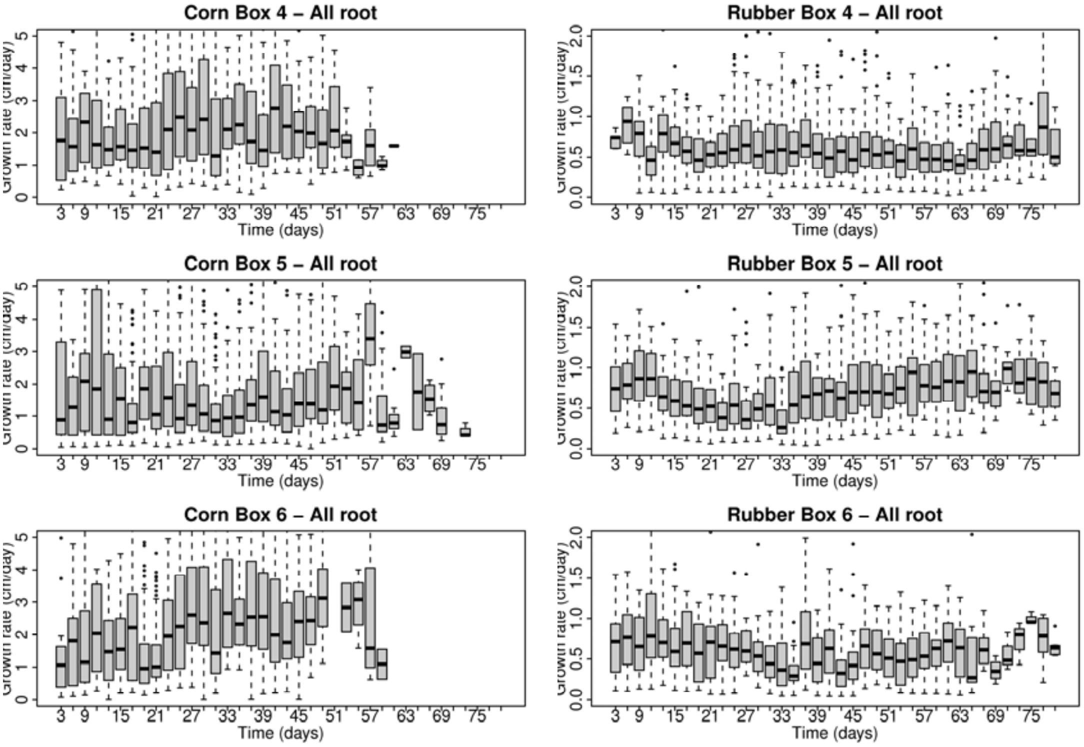


Figure 32 Distributions of growth rates in corn grown in association with rubber trees (left) and rubber trees grown with corn (right).

1.2.4 Spatial distribution of RLD variations

Regarding the spatial distribution of roots of the two species grown in association, the result show that all three corn increased root length density (RLD) faster in compartment C-III (which corresponded to the half of the corn root system growing on the side of the rubber tree root system) (Figure 33), followed by compartments C-IV and C-II (which corresponded to the half of the corn root system growing on the side opposite to the rubber tree root system and to an intrusion of corn into the rubber tree side of the rhizobox, respectively). RLD variation in corn reached a maximum, between 10 and 15 days after the onset in two replicates and between 15 and 20 days in one replicate. In two out of the three replicates corn plants developed measurable amounts of roots in compartment C-I (i.e. an intrusion of corn into the most distant edge of the rhizobox, on the rubber tree side) (Figure 33).

On the other hand, two out of three rubber trees increased RLD faster in compartment C-I, followed by compartment C-II (which corresponded to the halves of the rubber tree root system growing on the side opposite to the corn root system and on the side adjacent to the corn root system, respectively). For rubber trees, RLD variation was the highest between 20 and 30 days after the onset, i.e. after corn had reached its first relative maximum growth rate over the 0-30 DAS period. Noticeably, the replicate rhizobox in which the rubber tree increased RLD the fastest in compartment C-II, was also the one where the corn increased RLD at the lowest rate in this same compartment (Figure 33). In the two replicates (boxes 5 and 6) for which corn grew most roots towards to rubber root zone (C-I and C-II), the RLD of rubber tree was lower in compartment C-II than C-I, while the opposite configuration prevailed in box 4, in which the corn root system intruded much less into the rubber tree side (Figure 33). Remarkably, in none of the three replicates did rubber trees grow roots into the corn root zone (C-III and C-IV).

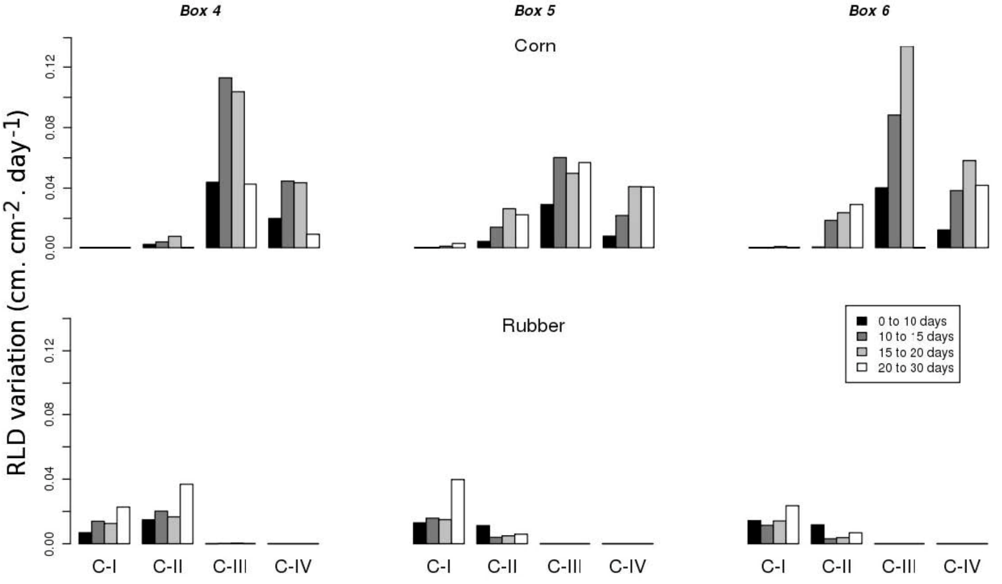


Figure 33 Variations in Root Length Density (RLD) for corn (top row) and rubber trees (bottom row) over three successive periods, namely 0-10, 10-15, 15-20 and 20-30 DAS, in the four adjacent compartments (25-cm wide and 100-cm high). C-I is vertical-half volume of rubber root in marginal side, C-II is vertical-half volume of rubber root in middle side, C-III is vertical-half volume of corn root in middle side, and C-IV is vertical-half volume of corn root in marginal side.

1.2.5 Root system trajectories

Although the root system trajectories of the three corn plants differed with regards to the values of the vertical and horizontal components and the precise timing of change in growth pattern, all had common characteristics (Figure 34), namely: an initial phase of about 12-15 days during which there consistently was a preferential growth in the direction of the rubber tree, a second phase, from c. day 15 to c. day 40, during which the opposite trend prevailed, and a final phase during which root system development was generally more balanced (Figure 34). However, the corn plant in box 4 was the closest to a balanced root system trajectory overall, while that in box 5 was the most laterally spread out, resulting in a shorter cumulative vertical component, and that in box 6 strongly shifted to the direction opposite to the rubber tree from c. day 35 to c. day 45.

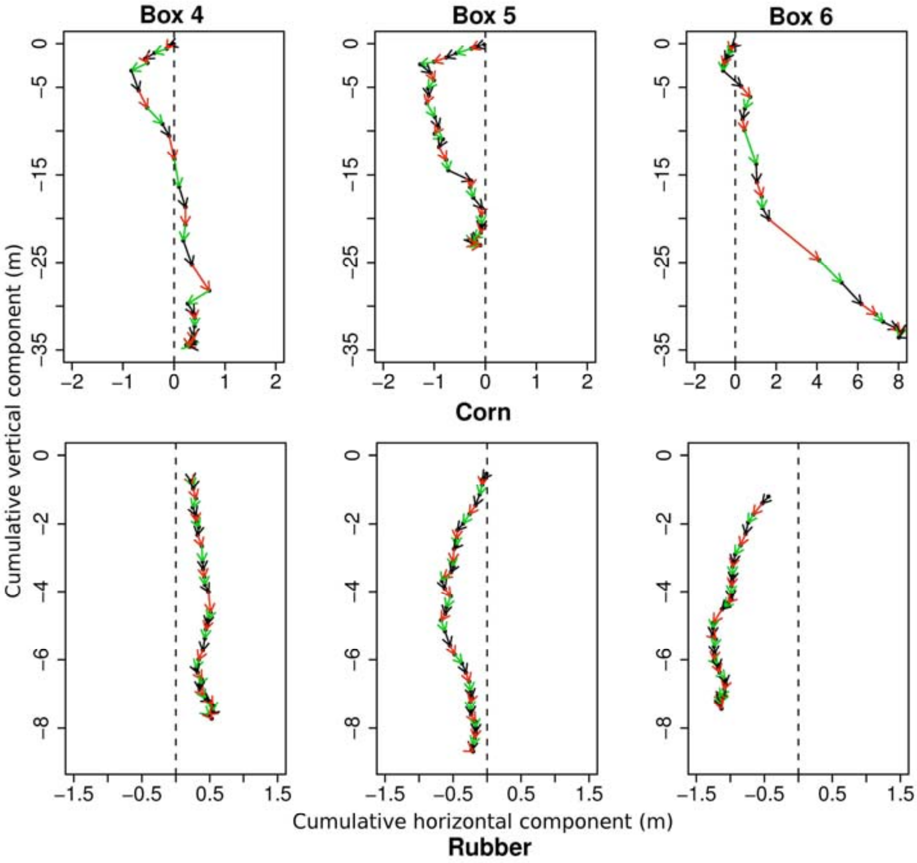


Figure 34 Root system trajectories of the three corn plants (top row) and associated rubber trees (bottom row).

Trajectories of rubber trees associated with corn were less variable, with more gentle lateral shifts than that observed for corn, and smaller horizontal and vertical cumulative components than corn.

Remarkably, even though there was no simple relationship between the trajectories of corn and associated rubber, the end of the initial phase of corn root system expansion towards the rubber tree coincided, in all three replicates, with the time at which corn roots first encountered rubber tree roots (Figure 35).

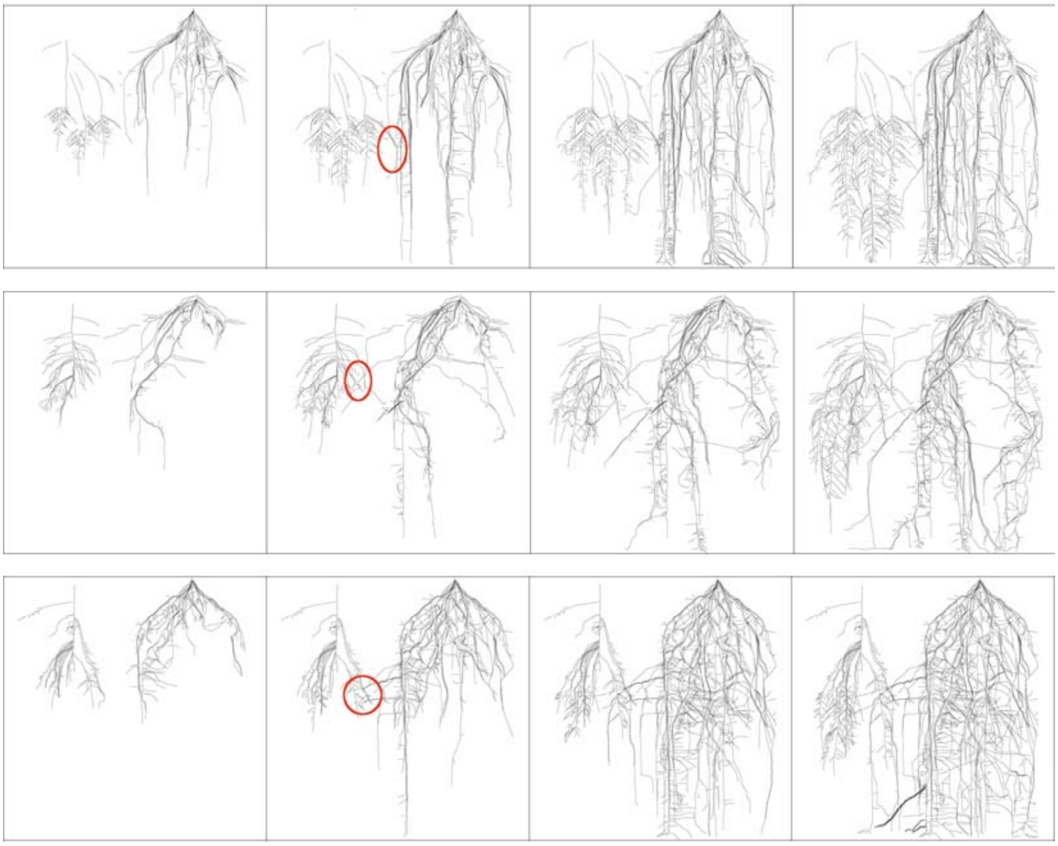


Figure 35 Root system architecture of the corn and rubber tree plants in the three replicate rhizoboxes over three successive periods, namely 0-10, 10-15, 15-20 and 20-30 DAS (from left to right). In all cases, corn extended long lateral branches that encountered the adjacent rubber tree root system during the second period (10-15 DAS, as indicated by circles overlaid on the corresponding images) which is also the point in time at which corn root system trajectories shifted direction (Figure 34).

1.3 Root growth rate analysis at the individual root scale

1.3.1 Corn and young rubber tree

Overall, out of the three replicate corn × rubber experiments, we could identify 90 inter-specific contact points out of which 12% were parallel contacts. In all cases, rubber tree roots were contacted by corn roots, and a single corn root could encounter up to 17 rubber tree roots. Because of the low occurrence of parallel contacts, and because they are likely to be induced by experimental conditions (the space left for growth in the third dimension was extremely limited) we did not analyze them as a specific case. An illustration of the spatial arrangement of encounters of corn and rubber tree roots is given in Figure 36.

Figure 36 Photograph of encounters of corn and rubber tree roots. The width of the imaged field is approximately 30 cm. Inset: general view of the corresponding rhizobox with both the below- and above-ground parts of the plants visible

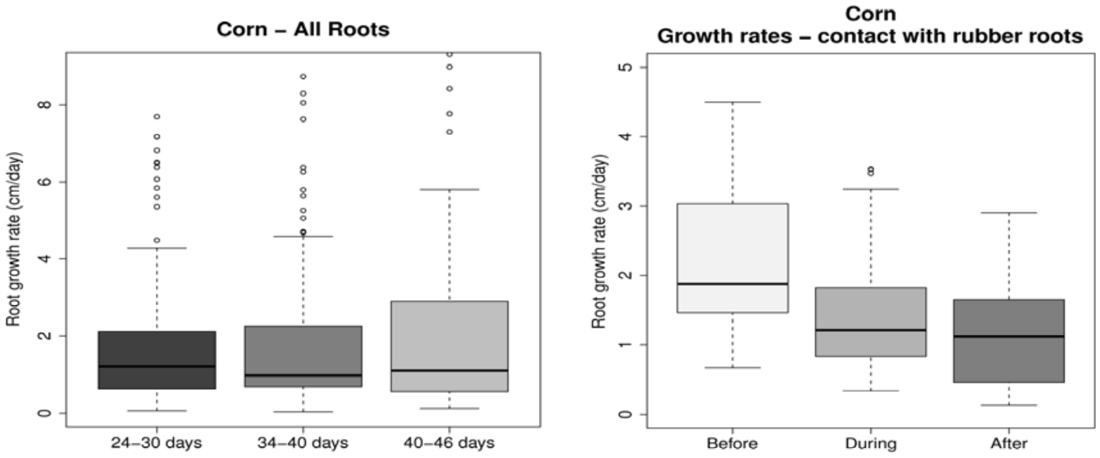
1.3.1.1 Inter-specific root contacts

Inter-specific root contacts occurred as early as 10 and as late as 74 days after the onset, respectively. Corn and rubber tree roots involved in root contacts were 2nd and 3rd order laterals that had an elongation period of over 19 days on average (minimum: 12, maximum 32) and 1st to 3rd order laterals with an average elongation period of 25 days (minimum: 10, maximum 42), respectively.

To avoid computing statistics that include the potential confounding effect of changes in elongation rates with time, we compared the pre-, syn- and post-contact elongation rates of roots involved in encounters, with that of all other roots over time intervals that encompassed the average time (in days after sowing, das) of pre-, syn-, post-contact, i.e. roots of similar age classes. We selected 3 time periods of similar duration (6 days) that corresponded to pre-, syn-, post-contact, and that were centered on the average age of root segments belonging to roots that were involved in encounters, i.e. 27, 37 and 43 DAS for the pre-, syn- and post-contact periods. The thus defined intervals were the 24-29, 34-39 and 40-45 DAS. An illustration of the distributions of growth rates thus computed and their variations with time is given in Figure 37a which shows that, in the case of corn, when considering all growing root over the pre-, syn-, post-contact periods, there were no significant variations in root growth rates.

The results of pre- syn- and post-contact growth rates show that individual corn roots involved in root encounter grew at slower rates as soon as contact with corn occurred ($p<0.001$, Welsh two sample t-test; $n=33$. Figure 37b) and this effect persisted in the post-contact period.

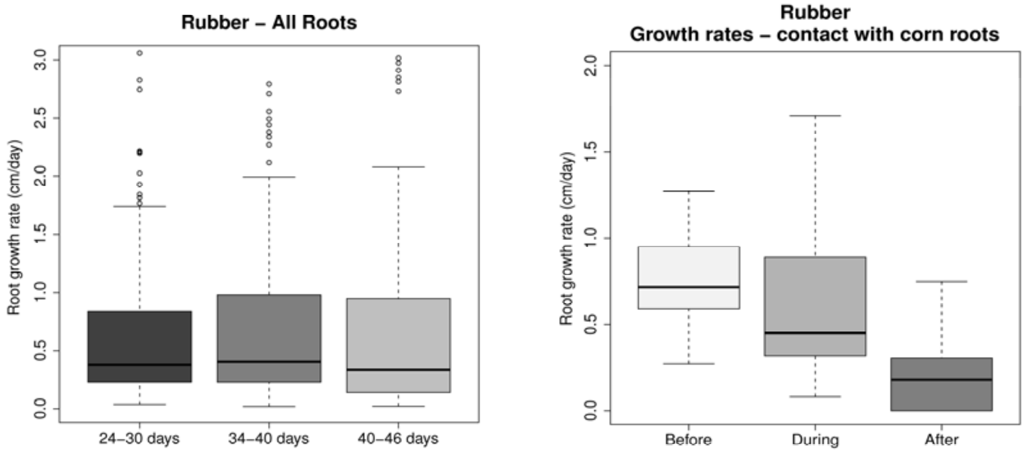
The pre-, syn- and post-contact growth rates were, 2.21, 1.56 and 1.11 cm/day, respectively. The comparison between Figure 34a and 34b also shows that the median pre-contact root growth of corn roots involved in encounters was higher than that of other roots of the same age range. Conversely, the median post-contact root growth of corn roots involved in encounters was lower than that of other roots of the same age range.



a. b.

Figure 37 (a) Corn root growth rates for all growing roots over the pre-, syn-, post-contact periods (n=105). (b) Corn root growth rates of roots involved in inter-specific root contacts (n=33).

A similar analysis as that just presented for corn was conducted for rubber roots involved in root encounters. Growth rates of all roots over the same 24-29, 34-39 and 40-45 DAS intervals were computed and compared to the pre-, syn- and post-contact growth rates of rubber roots contacted by corn roots When considering all roots over the pre-, syn- and post-contact time intervals, no significant variation in growth rate could be detected (Figure 38a). Conversely, root growth rates of rubber roots contacted by corn roots dropped significantly from 0.73 before contact, to 0.62 and 0.21 cm/day during and after contact, respectively ($p<0.001$ in both cases; Welsh two sample t-test, n=32).(Figure 38b).



a. b.

Figure 38 (a) Rubber tree root growth rates for all growing roots over the pre-, syn-, post-contact periods (n=117). (b) Rubber tree root growth rates of roots involved in inter-specific root contacts (n=32).

A summary of this analysis of the effects of inter-specific root encounters at the individual root level is provided in (Table 2). Overall, these results indicate that inter-specific root encounters induced a significant reduction in both rubber tree and corn root elongation rates. The time-related decline in elongation of corn axile roots reported by Pagès and Pellerin (1994) does not appear to be a likely confounding factor that would explain the change in elongation observed for corn roots that encountered rubber tree roots.

Table 2 Statistical summary of inter-specific root encounters

I. Inter-specific root encounters			
	(a) Before	*(b) During*	*(c) After*
Average observation time (DAS)	27	37	43
Corn Roots			
Mean growth rate (cm day^{-1})	2.21	1.56	1.11
p-value (n=33)	*(a)-(b):* 0.014	*(a)-(c):* <0.001	*(b)-(c):* 0.049
Rubber Roots			
Mean growth rate (cm day^{-1})	0.73	0.62	0.21
p-value (n=32)	*(a)-(b):* 0.256	*(a)-(c):* <0.001	*(b)-(c):* <0.001

II. Comparison with all other Roots			
	(a) Before	*(b) During*	*(c) After*
Sampling date range (DAS)	[24-30[	[34-40[	[40-46[
Corn Roots			
Mean growth rate (cm day^{-1})	1.86	1.90	2.21
p-value (n=105)	*(a)-(b):* 0.479	*(a)-(c):* 0.314	*(b)-(c):* 0.357
Rubber Roots			
Mean growth rate (cm day^{-1})	0.68	0.86	0.83
p-value (n=117)	*(a)-(b):* 0.243	*(a)-(c):* 0.337	*(b)-(c):* 0.453

1.3.1.2 Intra-specific root contacts

There was a significant ($p<0.001$) decrease in rubber tree root elongation rate following encounters with another rubber root (Table 3-III). In contrast, intra-specific encounters did not alter the elongation rates of corn roots. Intra-specific contacts occurred about 10 days earlier on average in corn than rubber (Table 3-III).

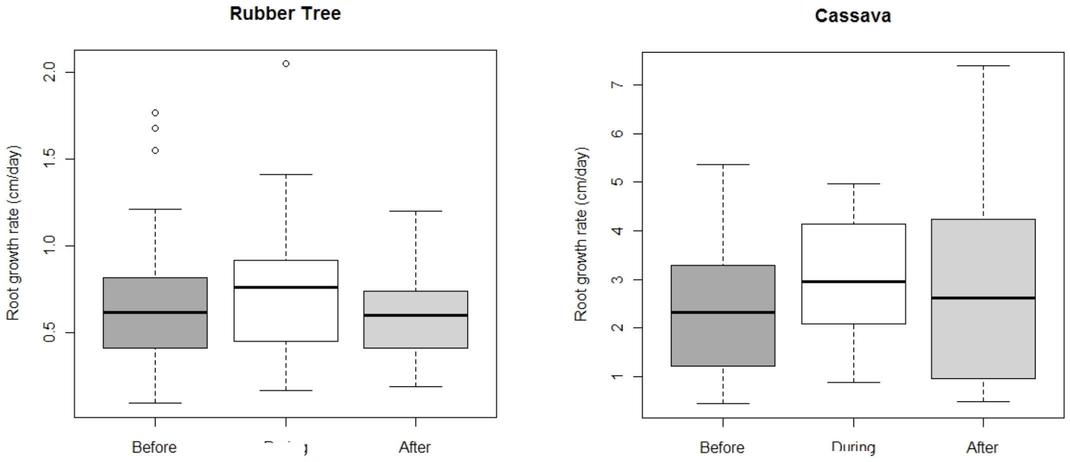
Table 3 Statistical summary of intra-specific root encounters

III. Intra-specific root encounters			
	(a) Before	*(b) During*	*(c) After*
Corn Roots			
Average observation time (DAS)	22	24	27
Mean growth rate (cm day^{-1})	2.11	2.54	2.21
p-value (n=54)	*(a)-(b):* 0.171	*(a)-(c):* 0.733	*(b)-(c):* 0.245
Rubber Roots			
Average observation time (DAS)	26	35	35
Mean growth rate (cm day^{-1})	0.75	0.71	0.46
p-value (n=68)	*(a)-(b):* 0.564	*(a)-(c):* <0.001	*(b)-(c):* <0.001

IV. Comparison with all other Roots		
Corn Roots		
	(a) Before	*(b) During and After*
Sampling date range (DAS)	[20-24[	[24-30[
Mean growth rate (cm day^{-1})	1.73	1.86
p-value (n=86)	*(a)-(b):* 0.449	-
Rubber Roots		
	(a) Before	*(b) During and After*
Sampling date range (DAS)	[24-28[	[34-38[
Mean growth rate (cm day^{-1})	1.00	0.87
p-value (n=102)	*(a)-(b):* 0.417	-

1.3.2 Cassava and young rubber tree

Results related to the effect of encounters between cassava and rubber tree roots (crossing) are reported in Table 4. These results indicate that, quite in contrast with the case of the rubber tree x corn treatment, there never was any effect of root encounters on the growth rates of both rubber tree and cassava roots (Figure 39).



a. b.

Figure 39 (a) Rubber tree root growth rates of roots involved in inter-specific root contacts (n=32). (b) Cassava root growth rates of roots involved in inter-specific root contacts (n=12).

Table 4 Statistical summary of inter-specific root encounters of cassava and young rubber tree

Inter-specific root encounters			
	(a) Before	*(b) During*	*(c) After*
Cassava Roots			
Average observation time (DAS)	88	94	97
Mean growth rate (cm day^{-1})	2.37	3.01	2.79
p-value (n=12)	*(a)-(b):* 0.273	*(a)-(c):* 0.576	*(b)-(c):* 0.771
Rubber Roots			
Average observation time (DAS)	74	81	87
Mean growth rate (cm day^{-1})	0.72	0.75	0.62
p-value (n=32)	*(a)-(b):* 0.829	*(a)-(c):* 0.338	*(b)-(c):* 0.203

1.3.3 Groundnut and young rubber tree

The monitoring of root growth at two-day intervals during the experimental period revealed that, in this association, physical contact between groundnut and rubber tree occurred, to a limited extent, only once out of 6 attempted replicates, hence precluding any analysis of the effect of direct root contacts on root growth rates.

1.4 Analysis of the above-ground development of rubber trees and intercrops

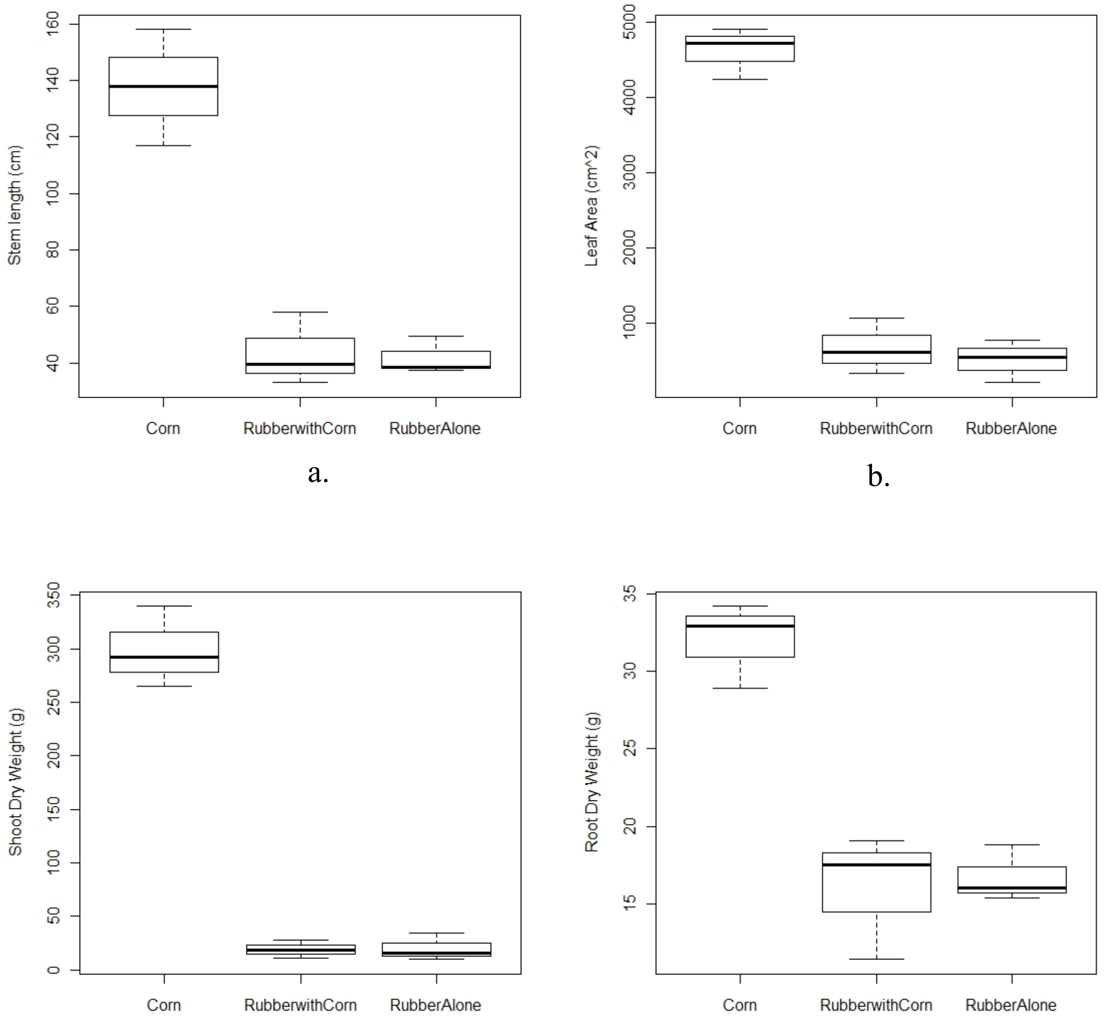
1.4.1 Corn and young rubber tree

The stem length of corn planted in association with young rubber trees ranged from 117.00 to 158.00 cm. The stem length of rubber trees planted with corn and grown alone ranged from 33.00 to 58.00 and from 37.00 to 39.00 cm, respectively. There was no significant difference in the above-ground development of rubber tree in both situations (Figure 40a).

The leaf area of corn planted with young rubber tree ranged from 4,240.47 to 4,904.29 cm^2. The leaf area of rubber trees grown in association with corn rubber tree and alone ranged from 326.10 to 1,063.42 cm^2 and 198.68 to 770.47 cm^2, respectively. Here again, there no significant difference between the leaf area of rubber trees grown alone that of corn grown in association with corn (Figure 40b).

The dry shoot biomass of corn ranged from 264.85 to 339.80 g/plant. The dry shoot biomass of rubber trees planted with corn and rubber tree grown alone ranged from 10.9 to 27.8 and 9.6 to 34.82 g/plant, respectively. There was no significant different between both rubber tree situations regarding this parameter (Figure 40c).

Corn dry root biomass ranged from 28.90 to 34.20 g/plant. Dry root biomass of rubber trees planted with corn and rubber trees grown alone ranged from 11.40 to 19.10 and 15.40 to 18.80 g/plant, respectively, with no significant different between the rubber tree situations (Figure 40d).



a. b.

c. d.

Figure 40 Descriptive parameters of the above-ground development of corn associated with young rubber tree, young rubber tree associated with corn and young rubber tree control on rhizobox experiment: stem length (a), leaf area (b), dry shoot biomass (c) and dry root biomass (d).

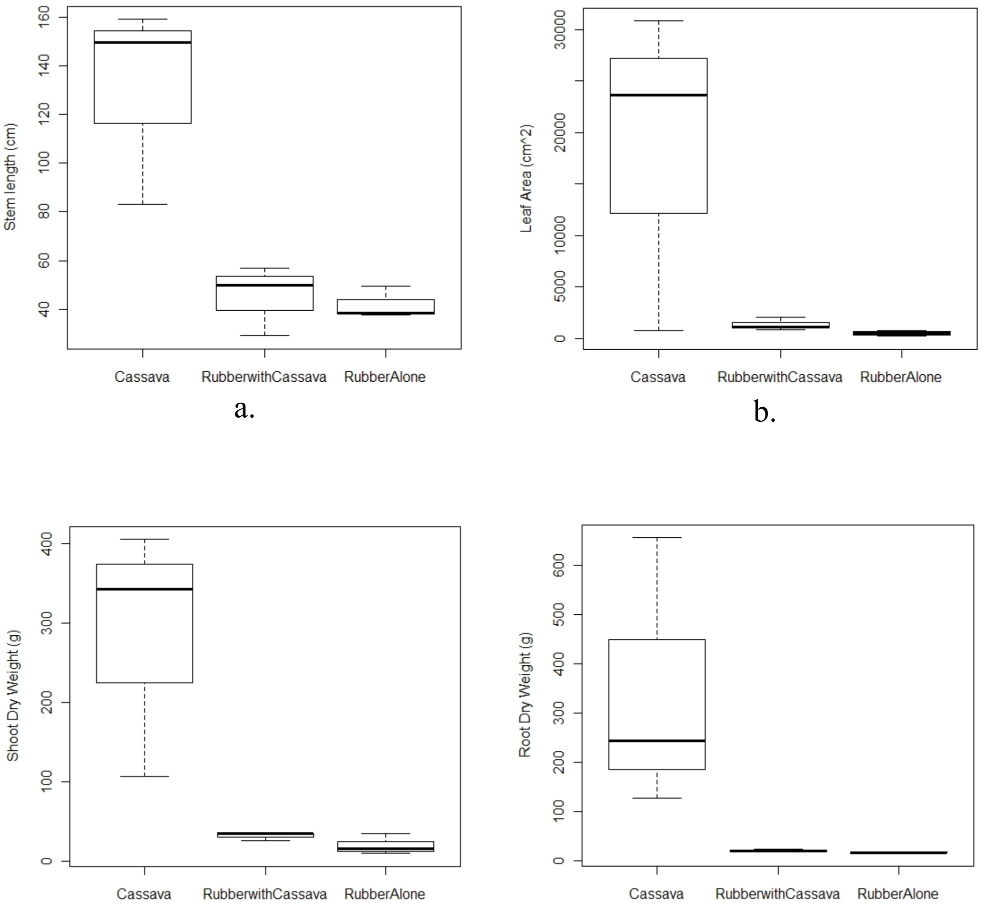
1.4.2 Cassava and young rubber tree

The stem length of cassava grown with young rubber tree ranged from 83.00 to 159.00 cm. The stem length of rubber trees grown with cassava and grown alone ranged from 29.00 to 56.80 cm and 37.50 to 49.50 cm, respectively, indicating no significant difference between the rubber trees grown in the two situations (Figure 41a).

The leaf area of cassava grown with young rubber trees ranged from 788.06 to 30,840.40 cm^2. The leaf area of rubber trees grown with cassava and grown alone ranged from 858.80 to 2048.05 cm^2 and 198.68 to 770.46 cm^2, respectively, showing no significant difference between the rubber trees grown in the two situations (Figure 41b).

Dry Shoot biomass of cassava ranged from 107.36 – 405.50 g/plant while that of rubber trees planted with cassava and grown alone ranged from 26.30 to 34.99 and 9.60 to 34.82 g/plant, respectively. There was no significant difference between rubber trees in the two situations (Figure 41c).

Dry root biomass of cassava planted with young rubber trees ranged from 128.00 to 656.20 g/plant, while that of rubber trees planted with cassava and grown alone ranged from 18.29 to 23.52 and 15.40 to 18.80 g/plant, respectively, indicating no significant difference between rubber trees grown in the two situations (Figure 41d).



a. b.

c. d.

Figure 41 Descriptive parameters of the above-ground development of cassava associated with young rubber tree, young rubber tree associated with cassava and young rubber tree control on rhizobox experiment: stem length (a), leaf area (b), dry shoot biomass (c) and dry root biomass (d).

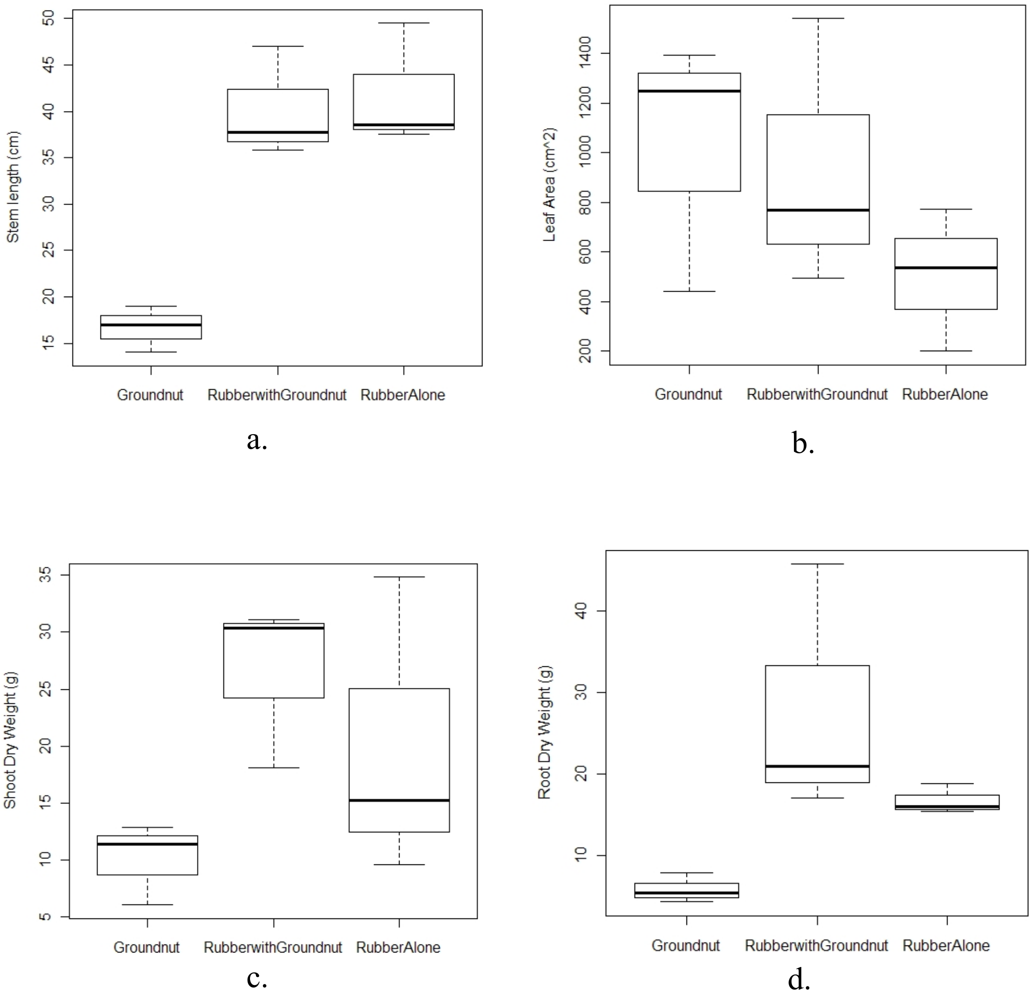
1.4.3 Groundnut and young rubber tree

The stem length of groundnut grown with young rubber trees ranged from 14.00 to 19.00 cm. The stem length of rubber trees grown with groundnut and grown alone ranged from 35.80 to 47.00 cm and 37.50 - 49.50 cm, respectively, indicating trend to increase of range but no significant difference between the rubber trees grown in the two situations (Figure 42a).

The leaf area of groundnut grown with young rubber trees ranged from 439.18 to 1394.89 cm^2. The leaf area of rubber trees grown with groundnut and grown alone were ranged from 495.13 to 1540.40 cm^2 and 198.68 to 770.46 cm^2, respectively, showing to decrease of range but no significant difference between the rubber trees grown in the two situations (Figure 42b).

Dry shoot biomass of groundnut ranged from 6.04 – 12.87 g/plant while that of rubber trees planted with groundnut and grown alone ranged from 18.11 to 31.12 and 9.60 to 34.82 g/plant, respectively. There was trend to decline of range but no significant difference between the rubber trees in the two situations (Figure 42c).

Dry Root biomass of groundnut planted with young rubber trees ranged from 4.30 to 7.90 g/plant, while that of rubber trees planted with groundnut and grown alone ranged 17.00 to 45.7 and 15.40 to 18.80 g/plant, respectively, indicating trend to decrease of range but no significant difference between rubber trees grown in the two situations (Figure 42d).



a. b.

c. d.

Figure 42 Descriptive parameters of the above-ground development of groundnut associated with young rubber tree, young rubber tree associated with groundnut and young rubber tree control on rhizobox experiment: stem length (a), leaf area (b), dry shoot biomass (c) and dry root biomass (d).

1.5 Comparative assessment of the overall development of young rubber tree grown in association with cassava, corn and groundnut

Overall, there was no significant difference between the stem lengths of rubber trees grown in association with different inter-crops and alone (Figure 43).The stem length of rubber trees grown with cassava, corn, groundnut and grown alone ranged from 29.00 to 56.80 cm, 32.90 to 58.00 cm, 35.80 to 47.00 cm and 37.50 to 49.50 cm, respectively. Rubber trees grown with cassava were higher than rubber trees grown with corn, groundnut and grown alone, while the stem length of rubber tree planted in association with groundnut and grown alone were very similar.

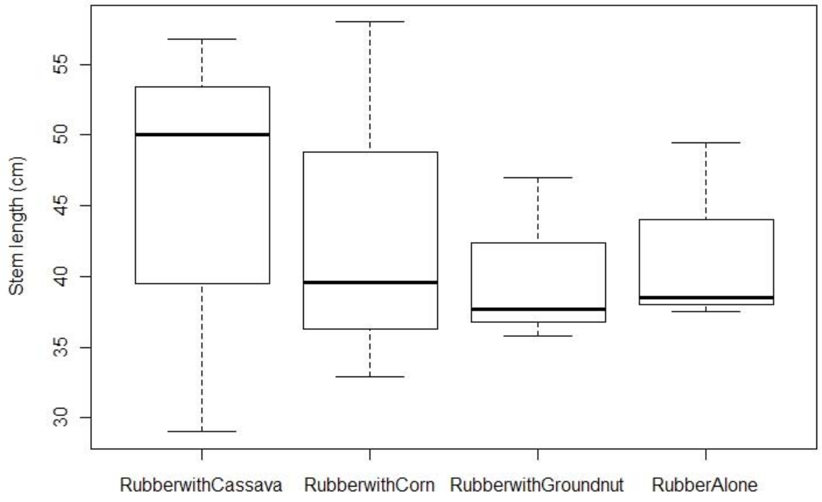


Figure 43 Stem length of young rubber tree grown with cassava, corn, groundnut and rubber tree control.

The leaf area of rubber trees grown with cassava, corn, groundnut and grown alone ranged from 858.80 to 2,048.05 cm^2, 326.09 to 1,063.41 cm^2, 495.13 to 1,540.40 cm^2 and 198.68 to 770.46 cm^2, respectively. Rubber trees grown with cassava had a larger leaf area than rubber trees grown with corn, groundnut and grown alone. The leaf area of rubber trees grown alone was the lowest and was significantly different than that of rubber trees grown with cassava (Figure 44).

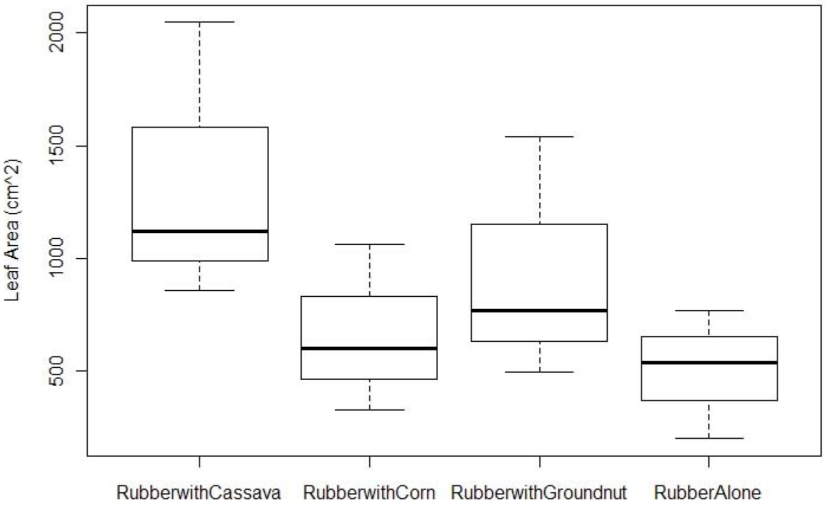


Figure 44 The leaf area of young rubber tree grown with cassava, corn, groundnut and rubber tree control.

Dry shoot biomass of rubber trees grown with cassava, corn, groundnut and grown alone ranged from 26.31 to 34.99 g/plant, 10.90 to 27.80 g/plant, 18.11 to 31.12 g/plant and 9.61 to 34.82 g/plant, respectively. Rubber trees grown with cassava were had a higher dry shoot biomass than rubber trees grown with corn, groundnut and rubber tree control, but there was no significant difference between the rubber dry shoot biomass of the trees in the four conditions (Figure 45).

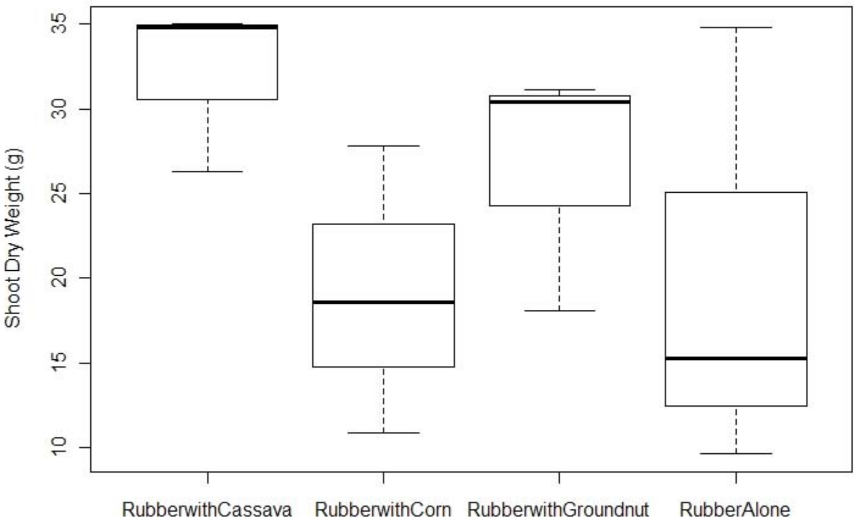


Figure 45 Dry shoot biomass of young rubber trees grown with cassava, corn, groundnut and rubber tree control.

Dry root biomass of rubber trees grown with cassava, corn, groundnut and grown alone ranged from 18.29 to 23.52 g/plant, 11.40 to 19.10 g/plant, 17.00 to 45.70 g/plant and 15.40 to 18.80 g/plant, respectively. Rubber trees grown with groundnut had a higher dry root biomass than that in other treatments. However, there was no significant between the dry root biomasses of rubber trees in the four treatments (Figure 46).

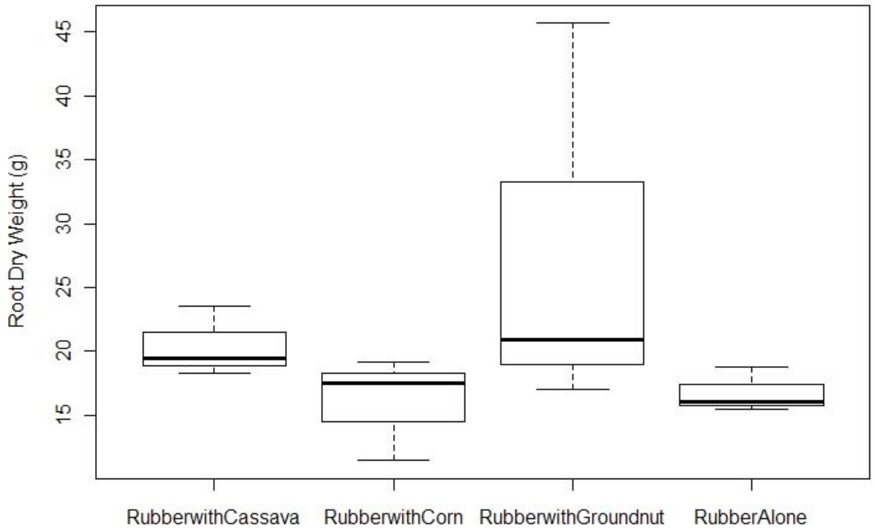


Figure 46 Dry root biomass of young rubber trees grown with cassava, corn, groundnut and rubber tree control.

1.6 Discussion and conclusions

This experiment investigated how the root development of young rubber trees grown in association with another plant, in rhizoboxes, is affected, at both the whole root system level and the individual root level. In addition, this work also provided information about the effect of the plant association on the overall development of rubber trees, based on the measurement of four parameters, namely, stem length, root and shoot dry biomass and leaf area.

Specifically, in the case of the rubber tree – corn association, our results suggest that plants were able to 'sense' and adjust their root system development according to that of their neighbour. Such a scenario is supported by the facts that 1. the growth trajectories of corn root system were initially oriented towards the rubber trees, 2. corn plants grew unusually long laterals when some of their main axile roots were close to the rubber tree root systems, 3. both individual corn and rubber tree roots grew at lower elongation rates following encounters with each other, 4. the overall root length expansion of rubber trees in presence of corn was significantly higher than when they were grown alone and 5. was also significantly higher while the overall growth rate of corn was the highest (i.e. until ear formation), and 6. rubber and corn root expansion rates varied concomitantly.

In the case of cassava, the analysis of root encounters did not reveal any interaction such as that observed for corn and rubber: neither did rubber tree nor cassava roots undergo any change in growth rates during or following contacts.

Finally, in the case of the rubber tree × groundnut association, a very limited number of root encounters were not sufficient to conduct an analysis.

Interestingly, measurements conducted on above-ground parts of all the plants did not reveal any effect, and noticeably, no negative effect, of growing rubber trees in association with plants like corn, cassava or groundnut.

2. Results of the field experiments

2.1 2006 Field experiment

2.1.1 Root trapping effect

In this section, we present the results of root measurements made on rubber tree and cowpea (*Vigna unguiculata)* roots that grew during the 2006 rainy season. First, we pooled together all of the treatments (i.e. control without legume, with legume without fertilizer and the four treatments with legume and different fertilizer combinations) to assess whether the root trap device used for this experiment allowed the detection of some competitive behaviour from one or both of the system's two components.

Root length measurements (Figure 47) indicate that, for cowpea, there was on average a sharp decrease in root length with depth in both the traps oriented toward the legume and rubber sides. However, there was no difference between the legume root lengths measured in the two series of root traps, indicating that legume roots colonized the soil enclosed in differently oriented traps equally at all depths.

In contrast, for rubber tree, although there was no difference between average root lengths measured in the two root trap series installed at 50 and 70 cm, the average root length found in the root trap installed at 30 cm and oriented towards the inter-row was less than that found in the root trap installed at the same soil depth but oriented towards the rubber tree row (Figure 47). However this different is not quite significant (p=0.105, two-tailed unpaired t-test, n=24). This result indicates that, at 30 cm, rubber probably was not at a competitive advantage and was not able to explore the soil volume as thoroughly than 1. in deeper soil horizons and 2. than the neighbour cowpea plants.

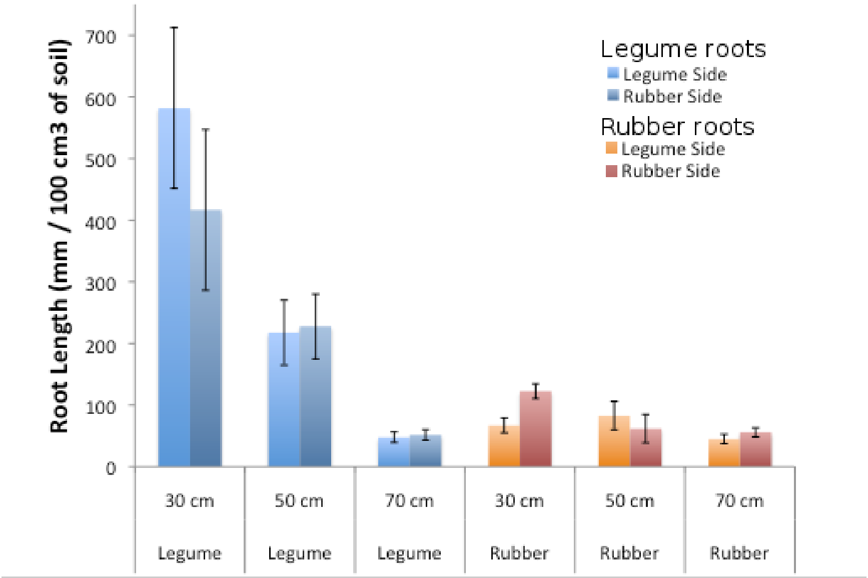


Figure 47 2006 Field experiment - Average root length for cowpea and rubber tree, at three depth increments, as measured in root traps oriented towards the inter-row (Legume side) or the rubber tree row (Rubber side) (error bars are 95% confidence intervals)

Additionally, specific root length (SRL) measurements (Figure 48) show that, the SRL of cowpea was on average higher than that of rubber tree, and that it did not vary substantially with depth or depending on root trap series. Similarly, in rubber tree, SRL was rather invariable with depth and in both root trap series (Figure 48).

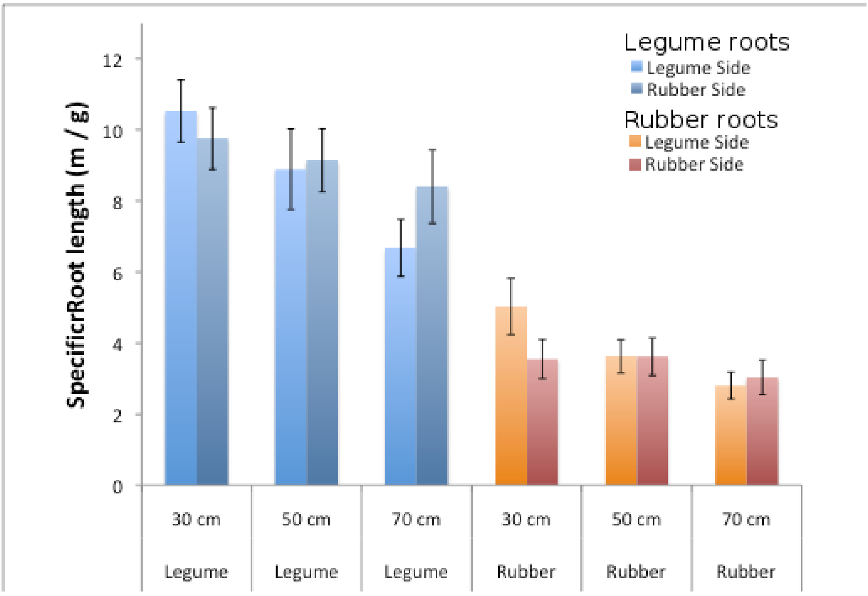


Figure 48 2006 Field experiment - Average specific root length for cowpea and rubber tree, at three depth increments, as measured in root traps oriented towards the inter-row (Legume side) or the rubber tree row (Rubber side) (error bars are 95% confidence intervals)

Altogether, these result indicates that there was no detectable trapping effect at the three soil depths investigates and that, therefore, results derived from the two root trap series could be treated as replicate measurements of rubber tree and cowpea roots characteristics at all depths investigated.

2.1.2 Effects of fertilization and the presence of cowpea

In what follows, we present root measurements made on samples collected in 2006 considering the possible effects of several combinations of fertilization and presence/ absence of cowpea (*Vigna unguiculata)* on the rooting characteristics of both rubber tree and cowpea. For every treatment, samples from the two root trap series taken at a given soil depth, were pooled together, based on the finding presented in the previous paragraph, that there was no detectable root trapping effect.

The measured cowpea root lengths were very homogeneous in all treatments. The relative root length contribution of cowpea can be estimated from the comparison of treatments T2 to T6 with T1, which corresponds to weed roots in the rubber tree alone treatment (Figure 49). In all rubber tree + cowpea treatments, root lengths at 50 and 70 cm were similar. In contrast, at 30 cm, there was a signifcantly higher root length in the cowpea +PK treatment,(two-tailed unpaired t-test, $p<0.05$; $n=8$) than at the same soil depth in the cowpea+NPK and cowpea+NP treatments.

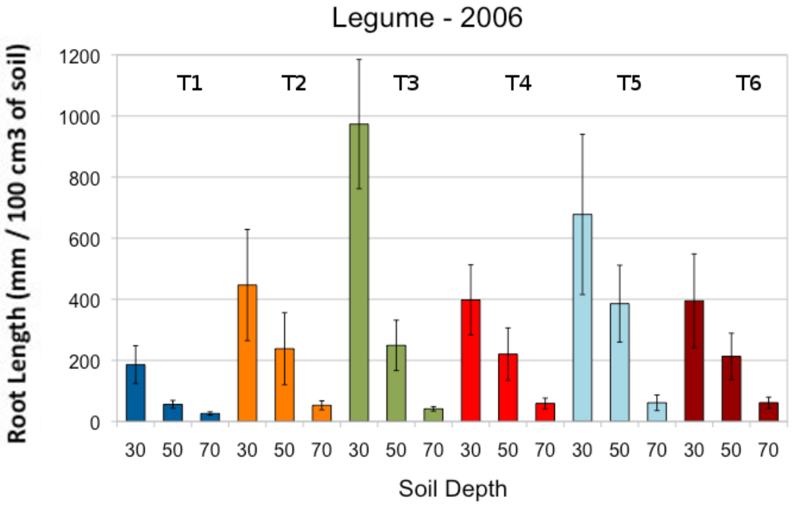


Figure 49 2006 Field experiment - Average root length for cowpea, at three depth increments and different combinations of fertilization (error bars are 95% confidence intervals) (treatments are: T1 - *Hevea brasilliensis* alone; T2 - *Hevea brasilliensis* + *Vigna unguiculata* without fertilizer; T3 - *Hevea brasilliensis* + *Vigna unguiculata* + PK: 37.5 kg P (TSP) ha^{-1} + 18.75 kg K (KCl) ha^{-1}; T4 - *Hevea brasilliensis* + *Vigna unguiculata* + NPK: 18.75 kg N (urea) ha^{-1} + 37.5 kg P (TSP) ha^{-1} + 18.75 kg K (KCl) ha^{-1}; T5 - *Hevea brasilliensis* + *Vigna unguiculata* + NK: 18.75 kg N (urea) ha^{-1} + 18.75 kg K (KCl) ha^{-1}; T6 - *Hevea brasilliensis* + *Vigna unguiculata* + NP: cowpea with 18.75 kg N (urea) ha^{-1} + 37.5 kg P (TSP) ha^{-1}).

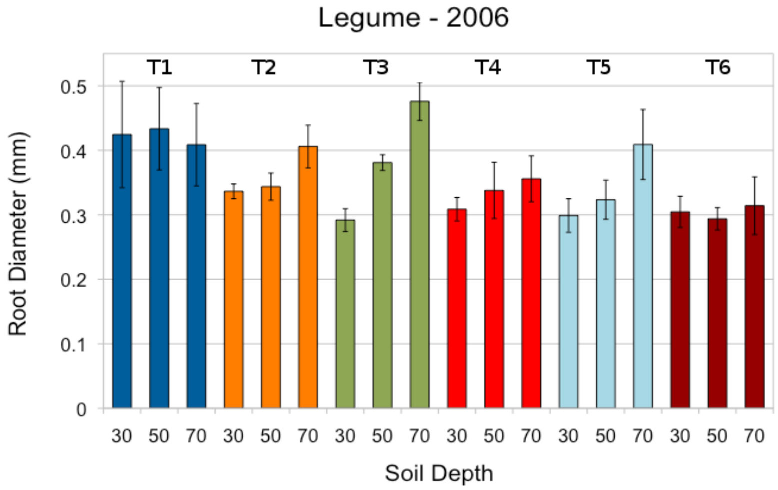


Figure 50 2006 Field experiment - Average root diameter for cowpea, at three depth increments and different combinations of fertilization (error bars are 95% confidence intervals). See Figure 49 for meaning of treatments.

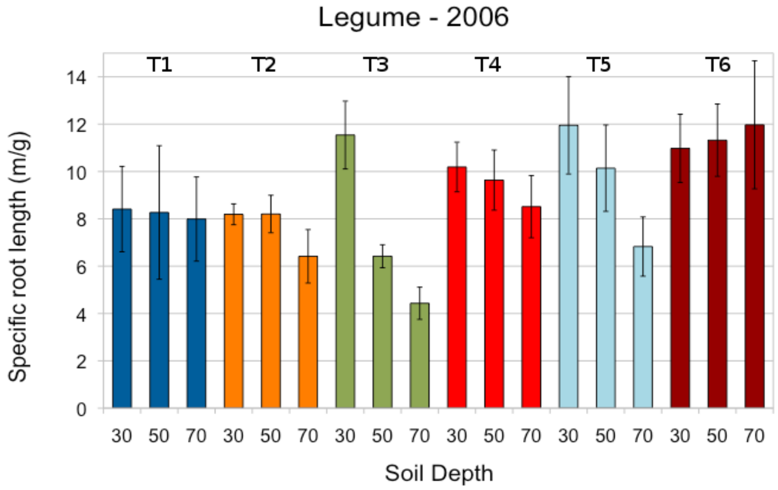


Figure 51 2006 Field experiment - Average specific root length for cowpea, at three depth increments and different combinations of fertilization (error bars are 95% confidence intervals). See Figure 49 for meaning of treatments.

On average, the root diameters of cowpea (Figure 50) were similar between treatments and at all depths and also not dissimilar from that of weeds in the control treatment. Only the T3 treatment showed a clear increase of root diameter with depth ($p<0.01$, two-tailed unpaired t-test; $n=8$). Cowpea SRL (Figure 51) were also of the same order of magnitude across treatments and relative stable with soil depth, except in the case of T3 (Legume +PK) for with SRL decreased significantly from 30 to 50 and from 50 to 70 cm ($p<0.05$ and $p<0.001$, respectively; two-tailed unpaired t-test; n=8).

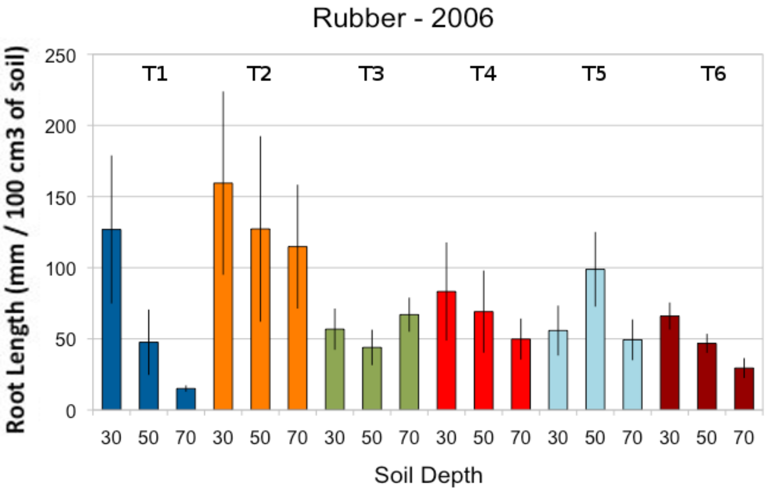


Figure 52 2006 Field experiment - Average root length for rubber trees, at three depth increments and different combinations of fertilization (error bars are 95% confidence intervals). See Figure 49 for meaning of treatments.

The measured rubber tree root lengths, although variable to some degree, were most significantly different across treatments. One effect of the cowpea association (with and without fertilization) was that it signicantly increased the root length of rubber at the 70 cm depth increment (Figure 52, $p<0.05$, two-tailed unpaired t-test; n=8).

Average rubber tree root diameters and specific root length (Figures 53 and 54) did not show any significant difference across treatments. SRL tended to decrease with depth in treatments T2 to T5 but differences between values were never significant.

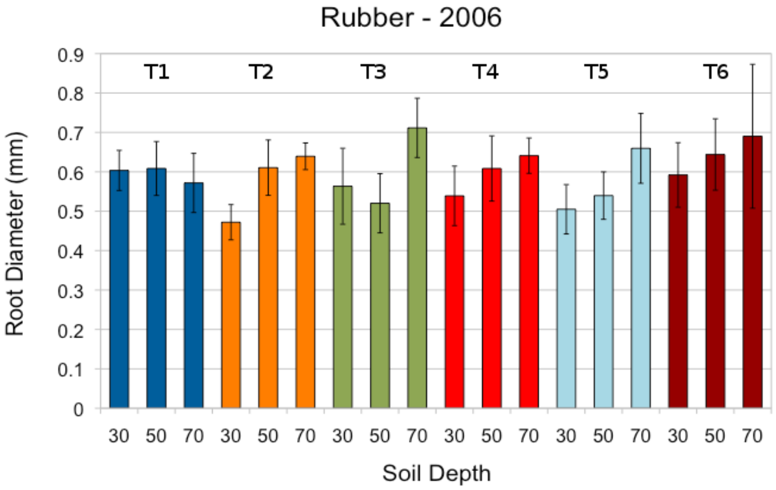


Figure 53 2006 Field experiment - Average root diameter for rubber tree, at three depth increments and different combinations of fertilization (error bars are 95% confidence intervals). See Figure 49 for meaning of treatments.

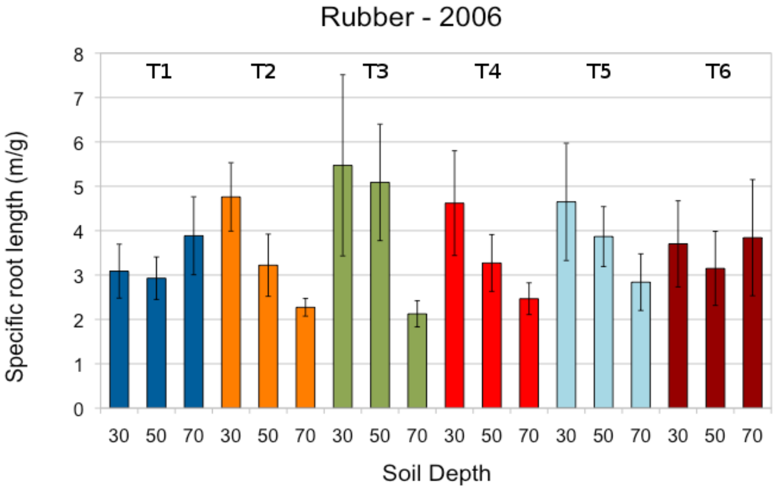


Figure 54 2006 Field experiment - Average specific root length for rubber tree, at three depth increments and different combinations of fertilization (error bars are 95% confidence intervals). See Figure 49 for meaning of treatments.

2.2 2007 Field experiment

2.2.1 Analysis of unsorted root length densities

In this section we present the results of the root measurements conducted on unsorted samples, i.e. including both rubber tree and intercrop roots. This first set of results allows assessing how the measured soil profile is colonized by plant roots under different inter-cropping treatments. It must be mentioned that the weather conditions during the 2007 experiment had an adverse effect on the emergence of both corn and groundnut inter-crops: heavy rainfall that occurred during the first week after sowing induced slumping of the freshly tilled sandy soil surface, which upon subsequent drying, formed a sealed surface that prevented a large proportion of seedlings from emerging (Figure 55). Moreover, later during the growing cycle, namely on the 3rd, 5th, 6th and 10th week after sowing, abundant rainfall (Figure 10) induced water logging conditions sustained over periods of several days. These water logging conditions that prevailed in shallow subsoil layers (at soil depths of approximately 30 to 50 cm), further impeded the development of inter crops.

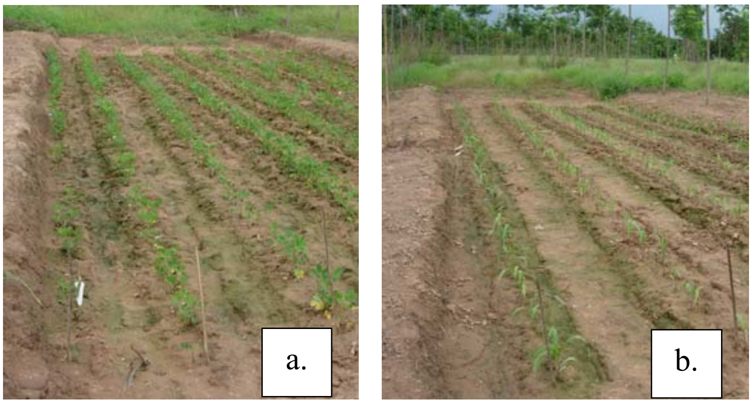


Figure 55 Soil crusting and impaired plant emergence: groundnut (a), and corn (b) in August 2007.

When comparing the two rooting profiles corresponding to the auger samples collected on the side of the rubber row line and on that of the inter-row, it clearly appears that, despite the proximity between the two series of samples, there were important differences in RLD at some depths, particularly at the 20-25, 25-30, 40-45 and 45-50 depths increments in the control and groundnut treatments. This result demonstrates that the variability of RLD at the 5-10 cm scale is already high (Figure 56).

Overall, it also appears that there was a much higher variability in RLD values at all soil depths in the rubber tree x corn treatment. When considering the average profiles (Figure 57) in both cases (inter-row and row sides) an increase in RLD can be observed at depth (60-65 and 65-70 depth increments) in both the corn and groundnut treatments compared to the control, but considering the variability of the measurements, it is not possible to confirm that this is anything else than a trend.

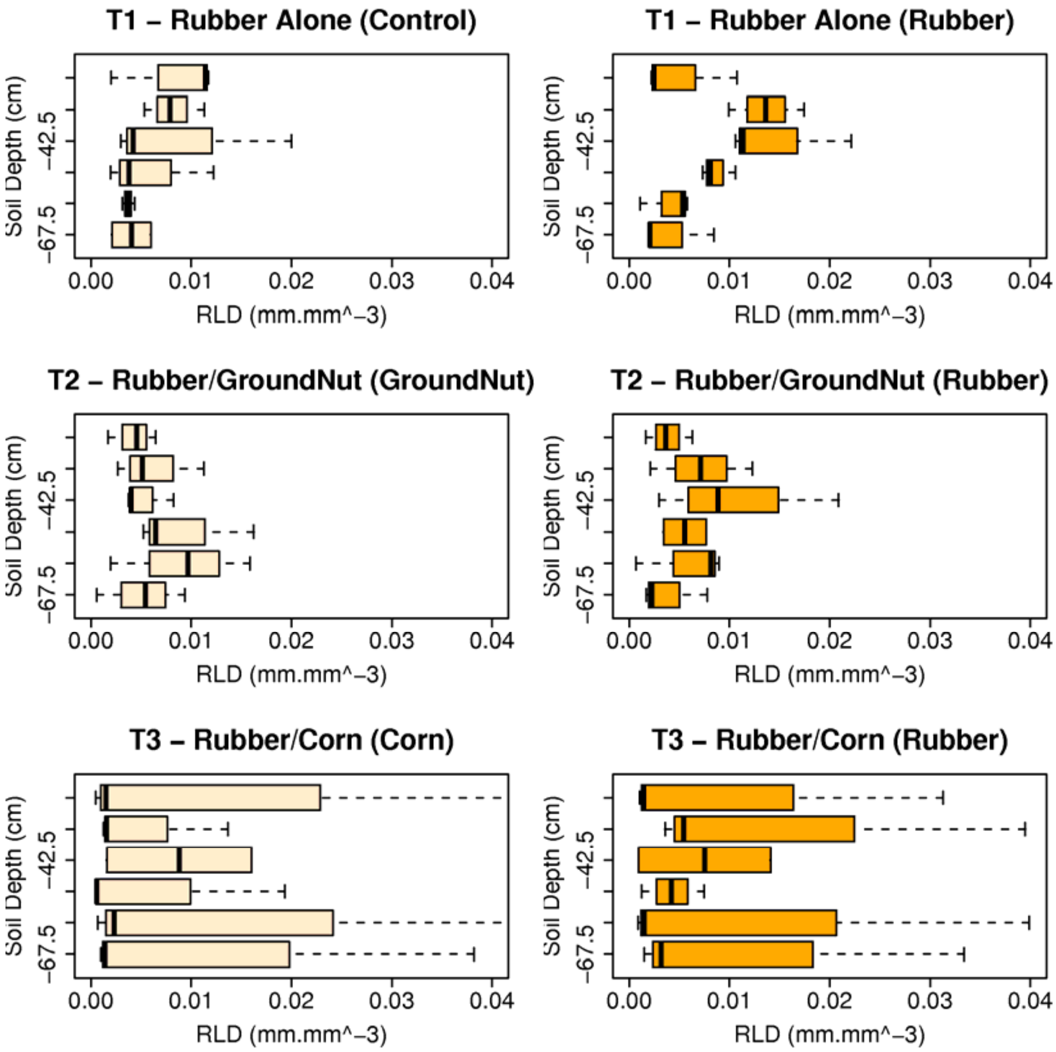


Figure 56 Root length density profiles of unsorted rubber tree and inter-crop roots at the end of the 2007 rainy season (November 2007). Inter-crop side (left) and rubber tree side (right) (measurements made on roots washed free of soil, using augered soil samples)

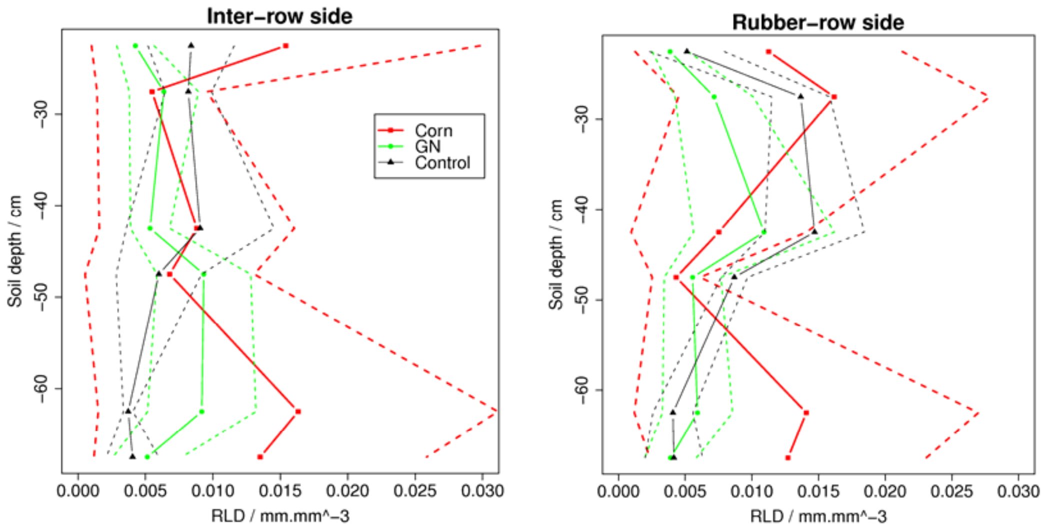


Figure 57 Average inter-row and row side rooting profiles, at the end of the 2007 rainy season (November 2007). Inter-crop side (left) and rubber tree side (right) (measurements made on roots washed free of soil, using augered soil samples).

The root trap experiment did not indicate any clear trend: the only observation that can be made is that, compared to the control, root growth in both corn and groundnut treatments seemed to be somewhat stimulated (as indicated by higher RLD) and more variable at all depths from 30 to 70 cm (Figure 58), and to a greater extent in the corn than the groundnut treatment. Results of the rubber alone treatment show that RLD was higher near the soil surface (depth increment 30 cm) and decreased moderately with depth (Figure 58). This RLD pattern was not found in the corn and groundnut treatments.

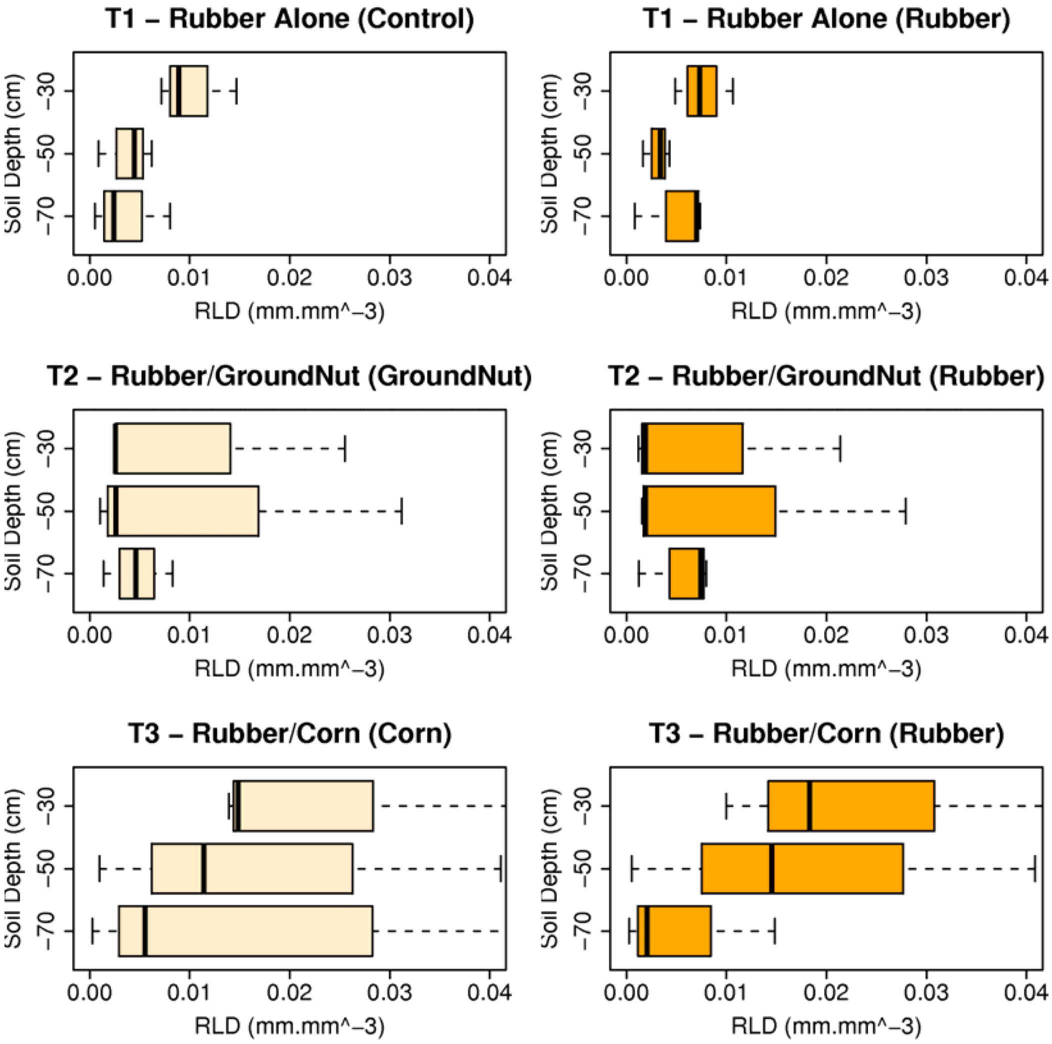


Figure 58 Root length density of rubber tree and inter-crops at the end of the 2007 rainy season (November 2007). Measurements made on roots washed free of soil, using soil samples collected by means of root traps. Intercrop side (left) and rubber tree side (right).

2.2.2 Analysis of sorted rubber tree and inter-crop roots

In the following section, we present the results of the root measurements conducted on sorted samples, i.e. after the production of two separate images, one corresponding to rubber tree roots only and the second to inter-crop roots only. In order to be able to carry out some basic statistical analysis of the data, augered samples were pooled as follows: successive depth increments 20-25 and 25-30, 40-45 and 45-50 and 60-65 and 65-70 cm were pooled together to form samples for the three 25, 45 and 65 cm depth increments.

Once this done, the two thus re-sampled profiles were pooled together to create on single dataset. Root trap samples from soil depths of 30, 50 and 70 cm were pooled together with composite augered samples corresponding to the three 25, 45 and 65 cm depth increments, respectively.

2.2.2.1 Control treatment

In this treatment, RLD was of the same order of magnitude at both 25 and 45 cm (0.297 and 0.304 cm cm^{-3}, respectively) while it was significantly lower (75 cm cm^{-3}) at 65 cm, ($p<0.01$, two-tailed unpaired (independent) Welch t-test n=12). For rubber, RLD varied from 0.250 to 0.403 and 0.236 cm cm^{-3}at 25, 45 and 65 cm, respectively (no significant difference with soil depth). Average RLD of weed and rubber – 0.225 and 0.307 cm cm^{-3}, respectively - were not significantly different (Figure 59).

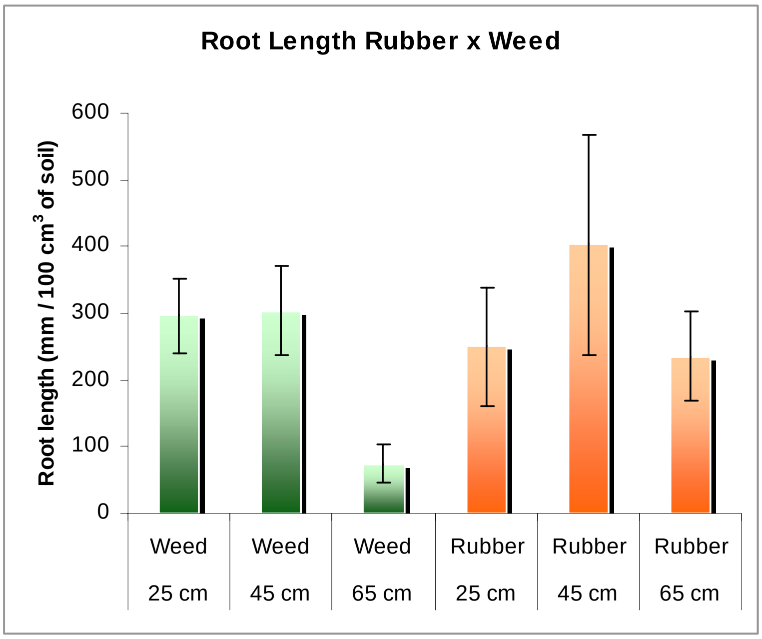


Figure 59 Root length of rubber tree and weed roots at different depth at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

Average root diameters of rubber tree increased with depth from 0.309 to 0.401 and 0.585 mm at soil depths of 25, 45 and 65 cm respectively (the value at 25 cm was significantly lower ($p<0.05$; n=12 and $p<0.001$; n=12) than that at 45 and 65 cm, respectively and the value at 45 cm was significantly different ($p<0.01$; lower) from that at 65 cm). For weeds, root diameter varied with depth from 0.181 to 0.220 and 0.166 mm at 25, 45 and 65 cm respectively (the value at 25 cm being significantly different ($p<0.05$; n=12) from that at 45 cm). Average root diameters of weed and rubber – 0.19 and 0.43 mm, respectively - were highly significantly different ($p<0.001$; n=36) , which means that rubber tree were on average more than 2 times thicker than weed roots (Figure 60).

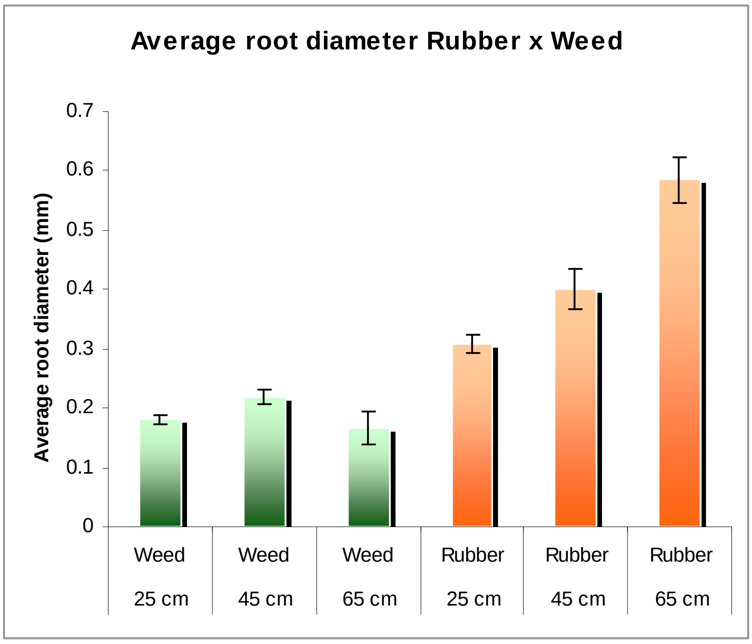


Figure 60 Average root diameter of rubber tree and weed roots at different soil depths, at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

Root diameter and length had a direct incidence on root surface area (Figure 61) and soil volume occupancy by roots (Figure 62) which were at least double in rubber tree than weed at all soil depths. For weed roots, both parameters, varied moderately between 25 and 45 cm and were significantly reduced at 65 cm. For rubber trees, both parameters increased with depth and were significantly higher at 45 and 65 cm than at 25 cm (except for root surface area at 65 cm).

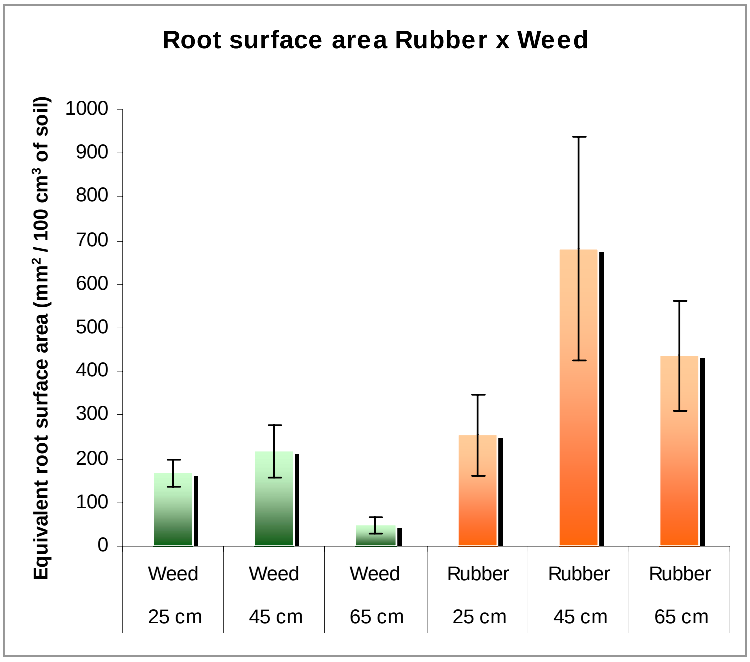


Figure 61 Root surface area of rubber tree and weed roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

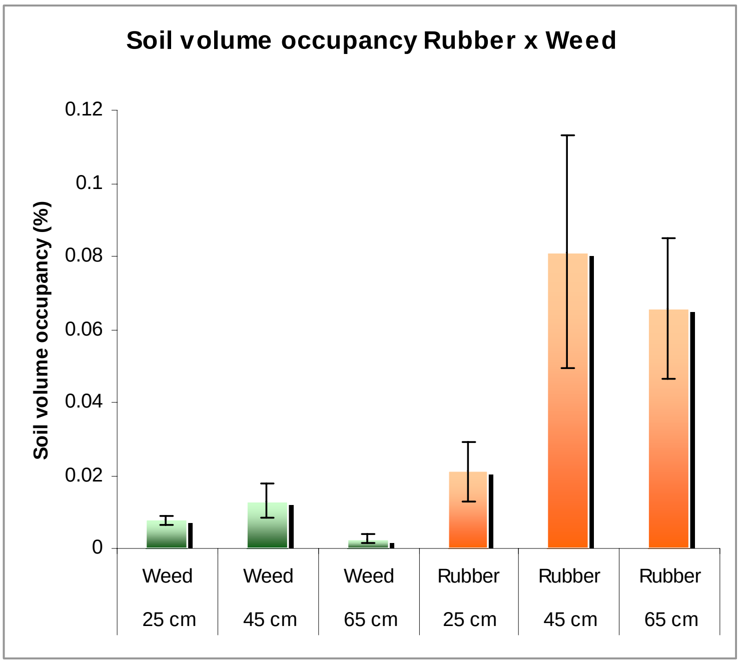


Figure 62 Soil volume occupancy of rubber tree and weed roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

Estimated specific root length showed that weed and rubber tree differed highly significantly with regards to this parameter ($p<0.001$), which averaged at about 24.70 and 6.80 m/g in weed and rubber tree, respectively. The average specific root length of weeds varied between soil depth from 29.84 to 20.17 and 23.75 m/g, at 25, 45 and 65 cm respectively (the value at 25 cm being significantly higher ($p<0.05$; $n=12$) from that at 45 cm). In rubber, there was a monotonous decrease in SRL with depth from 10.33 to 6.77 and 3.30 m/g, at 25, 45 and 65 cm, respectively (the value at 25 cm being significantly different ($p<0.05$; $n=12$) and ($p<0.001$; $n=12$) from at that at 45 and 65cm, respectively, and the value at 45 cm being significantly lower ($p<0.01$; $n=12$) than that at 65 cm) (Figure 63).

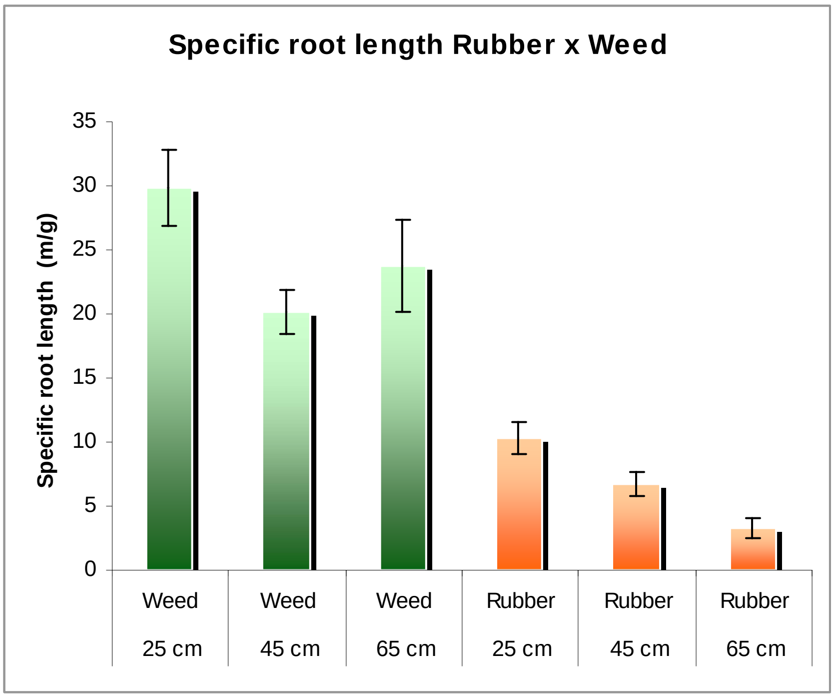


Figure 63 Specific root length of rubber tree and weed roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

2.2.2.2 Groundnut x Rubber treatment

In this treatment, the average RLD of groundnut and rubber – 0.378 and 0.107 cm cm^{-3}, respectively - were significantly different (two-sample t-test $p<0.001$, $n=12$), which indicates that , in this treatment, the RLD corresponding to groundnut roots was on average at least 3 times that of the RLD contributed to by rubber tree roots. Compared to the control treatment, the RLD corresponding to rubber roots was also much lower - 0.107 and 0.307 cm cm^{-3} in the groundnut and control treatment, respectively). The average RLD of groundnut did not significantly vary with soil depth, and varied from 0.318, 0.475 and 0.342 cm cm^{-3} at 25, 45 and 65 cm, respectively. The RLD corresponding to rubber trees tended to increase with soil depth, ranging from 0.072 to 0.120 and 0.127 cm cm^{-3} at 25, 45 and 65 cm, respectively. These variations were not statistically significant (Figure 64).

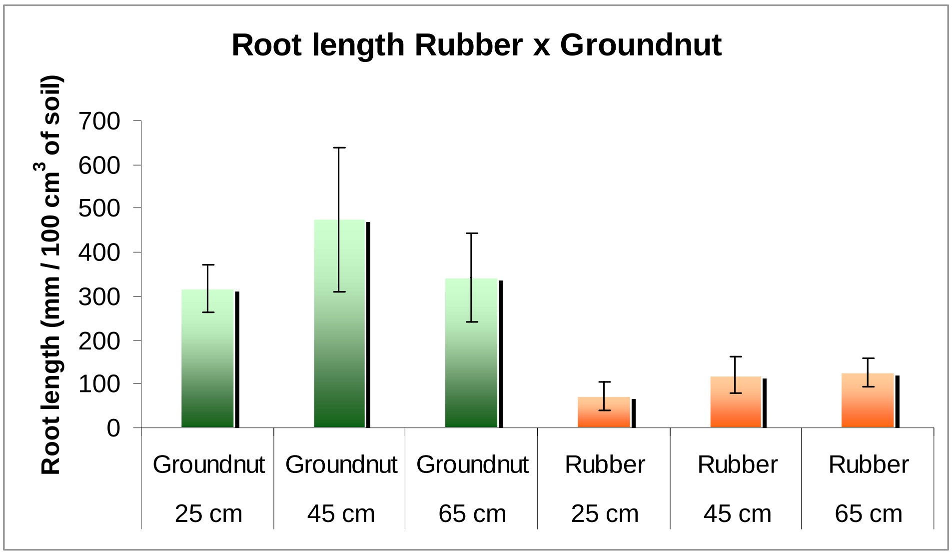


Figure 64 Root length of both rubber tree and groundnut roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

Groundnut roots were of very similar diameters at all depths increments, at an average of about 0.22 mm, while the diameter of rubber tree roots varied non-significantly from 0.43 to 0.64 and 0.51 mm at 25, 45 and 65 cm, respectively (Figure 65). However, average root diameters of groundnut and rubber tree – 0.225 and 0.541 mm, respectively - were highly significantly different ($p<0.001$; n=36), indicating the fact that groundnut roots were on average at least twice thinner than rubber tree roots, hence validating root diameter as a valid criterion for separating rubber tree roots from that of other species.

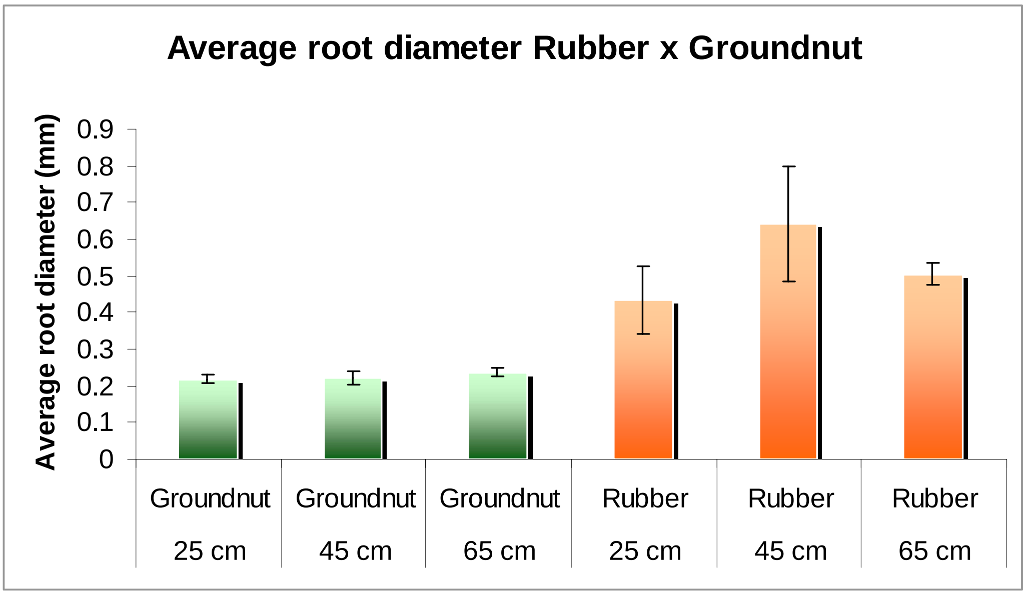


Figure 65 Average root diameter of both rubber tree and groundnut roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

The root surface areas were of the same order of magnitude in groundnut and rubber tree at all depths increments, except for rubber tree at 25 cm which was significantly lower (at 25 cm being significantly lower from that at 65 cm $p<0.05$ t-test; n=12) than all other rubber and groundnut values, down to 66 mm^2 / 100 cm^3 of soil (Figure 66). Soil volume occupancy values were also of the same order of magnitude in groundnut and rubber tree at all depths increments except for rubber tree at 45 cm, which increased to 0.06 % of soil volume occupancy (this increase was not statistically significant) (Figure 67).

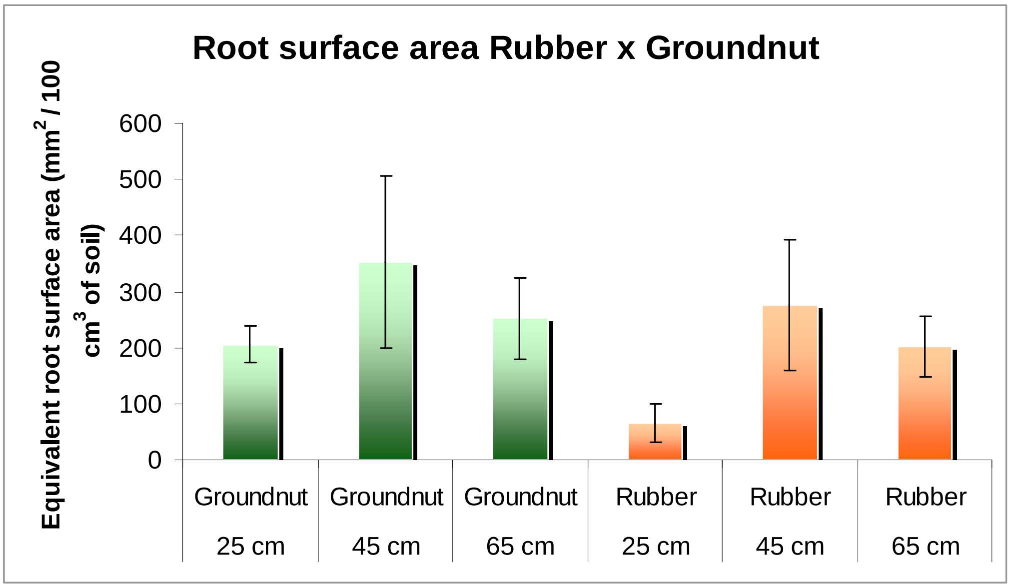


Figure 66 Root surface area of both rubber tree and groundnut roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

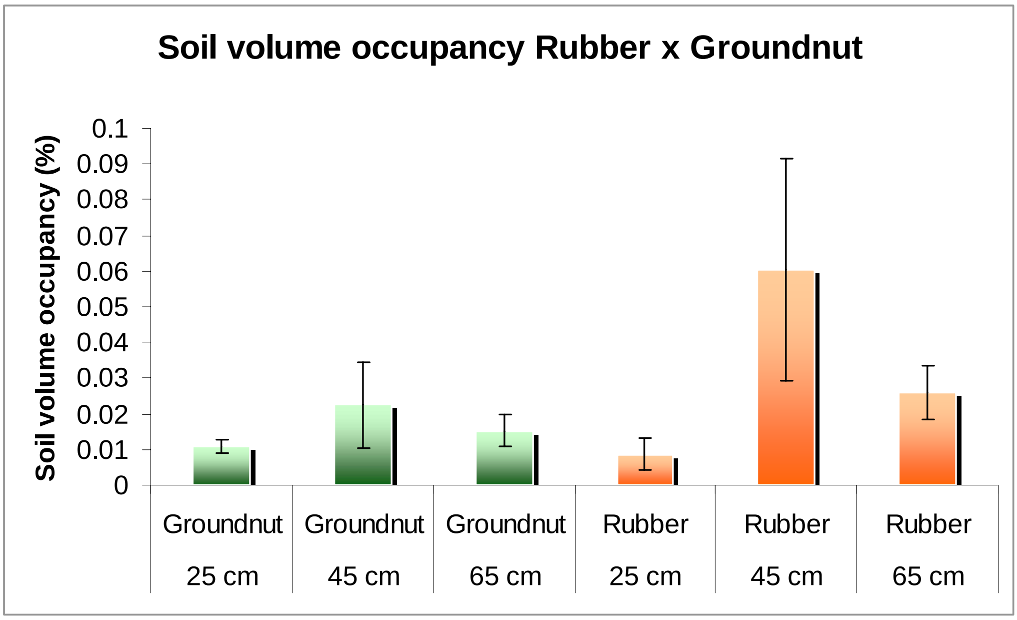


Figure 67 Soil volume occupancy of both rubber tree and groundnut roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

Estimated specific root lengths (SRL) of groundnut were of the same order of magnitude at all depths increments while SRL of rubber tree decreased with depth but not in a significant manner. The average specific root length - at about 20 and 5 m/g in groundnut and rubber, respectively,- were highly significantly different ($p<0.001$; n=36), which means that groundnut roots had on average a SRL 4 times than that of rubber tree roots (Figure 68).

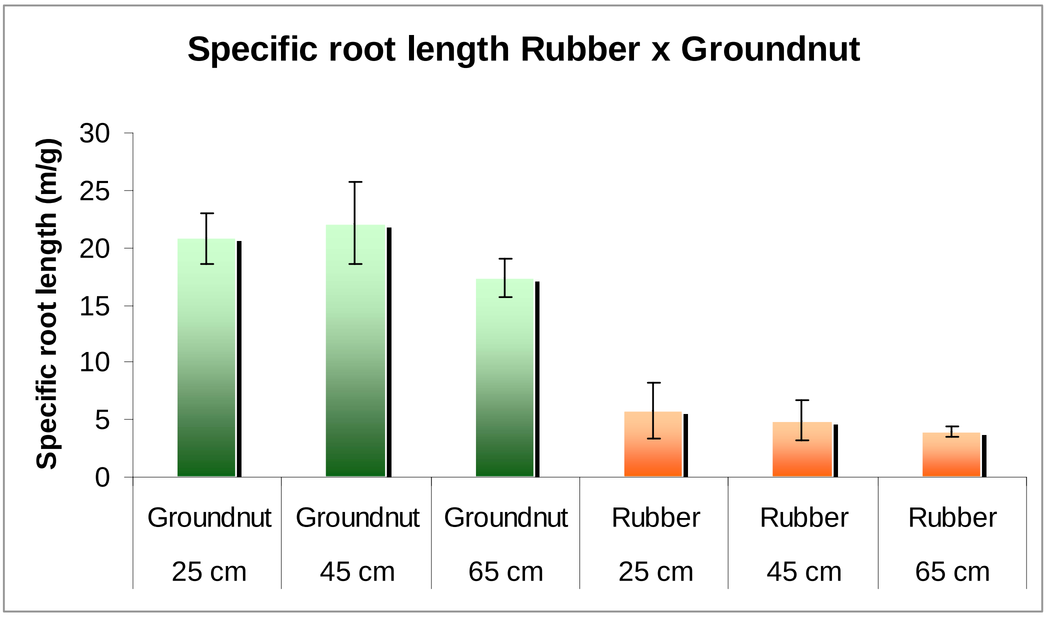


Figure 68 Specific root length of both rubber tree and groundnut roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

2.2.2.3 Corn x Rubber treatment

In this treatment, for both corn and rubber, higher and comparable RLD values were found at soil depths of 25 and 65 cm. At 45 cm, the corn RLD dropped significantly, down to 0.278 cm cm^{-3} while that of rubber was reduced to 0.062 cm cm^{-3}. Average RLD of corn and rubber – 0.609 and 0.133 cm cm^{-3}, respectively - were significantly different ($p<0.01$; n=36), which shows that corn developed on average at least 4 times more RLD than rubber trees in this treatment (Figure 69).

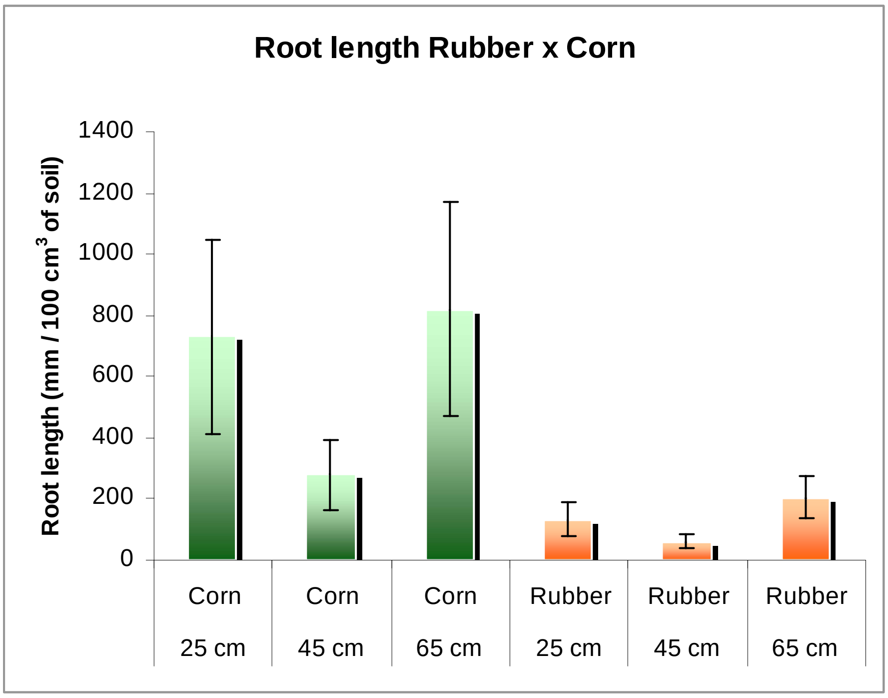


Figure 69 Root length of both rubber tree and corn roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

Average root diameters of corn increased from 0.171 to 0.191 and 0.222 mm at soil depths of 25, 45 and 65 cm respectively (the value at 65 cm was significantly higher ($p<0.05$; $n=12$) than that at 25 cm). In rubber, root diameters ranged from 0.39 to 0.50 and 0.53 mm at 25, 45 and 65 cm respectively (the value at 25 cm being significantly lower ($p<0.05$; $n=12$) from that at 65 cm). Average root diameters of corn and rubber – 0.19 and 0.48 mm, respectively - were highly significantly different ($p<0.001$; $n=36$) (Figure 70).

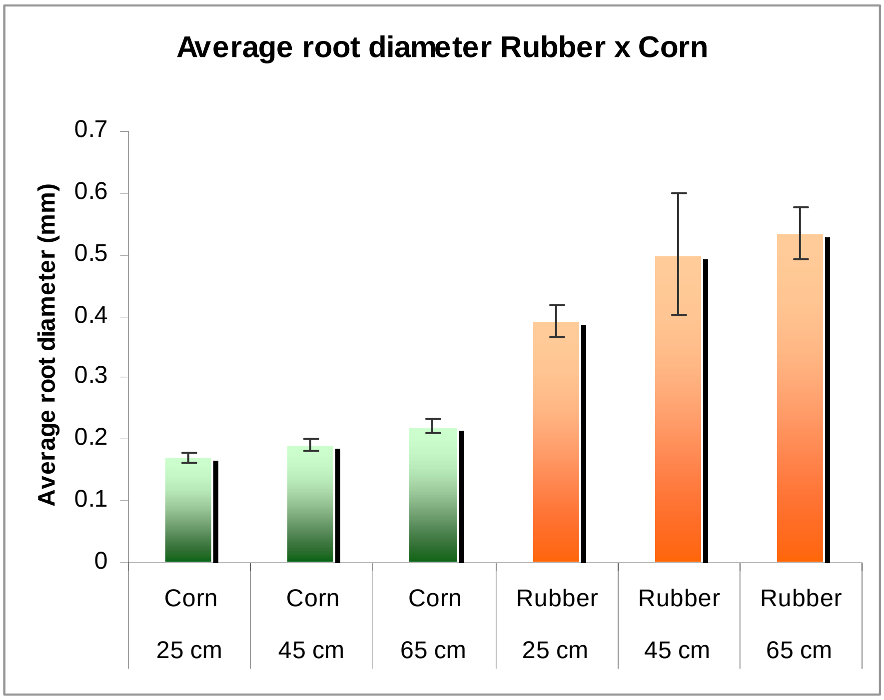


Figure 70 Average root diameter of both rubber tree and corn roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

These results about root diameter and length had a direct incidence on root surface area (Figure 71) and soil volume occupancy (Figure 72): as corn developed on average 4 times more roots length than rubber but with roots that were on average 2.5 times thinner than rubber tree roots, corn tended to develop higher surface areas than rubber tree on average, but had a soil volume occupancy similar to that of rubber (and sometime lower, such as for example at 65 cm where rubber tree roots were the thickest and rubber RLD the highest.

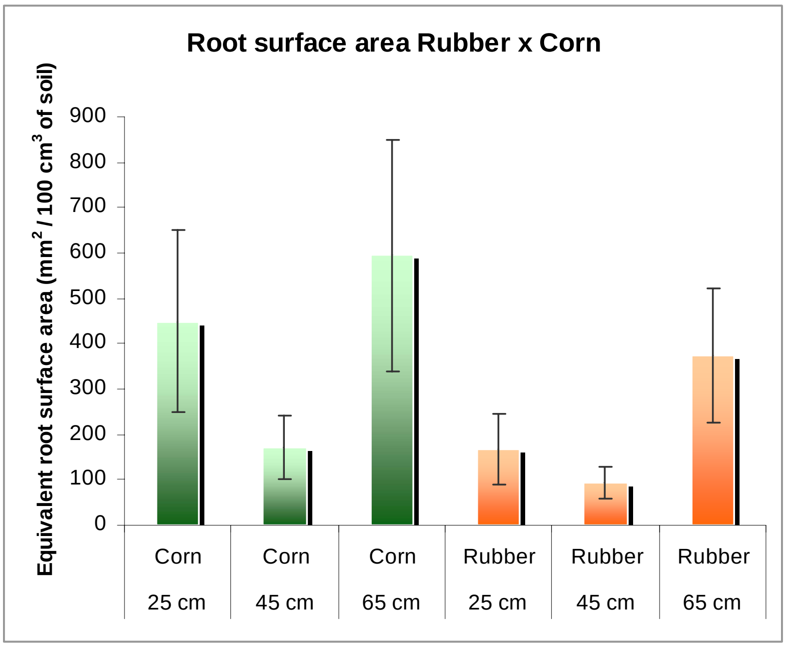


Figure 71 Root surface area of both rubber tree and corn roots at different soil depths at the end of the 2007 experiment. Error bars are 95% confidence intervals

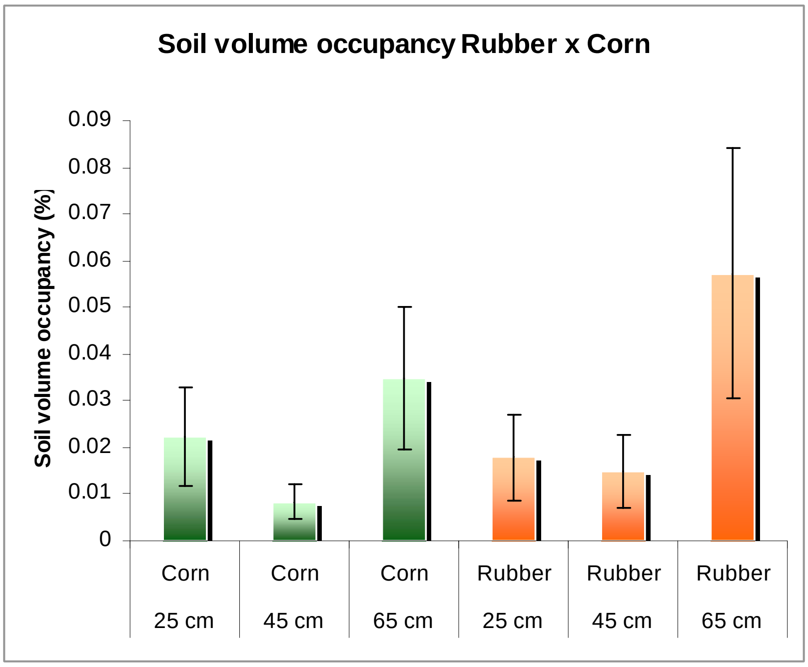


Figure 72 Soil volume occupancy of both rubber tree and corn roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

Figures about estimated specific root length showed that corn and rubber differed highly significantly with regards to this parameter ($p<0.001$), which averaged at about 26.11 and 5.93 m/g in corn and rubber, respectively. The average specific root length of corn decreased from 33.47 to 25.6 and 19.27 m/g, at 25, 45 and 65 cm, respectively (the value at 25 cm being significantly different ($p<0.01$; n=12) from that at 65 cm. In rubber, specific root length varied with soil depth from 6.42 to 7.34 and 4.03 m/g, at 25, 45 and 65 cm respectively, (the value at 25 cm being high significantly different ($p<0.05$; n=12) from at 65 cm) (Figure 73). These SRL values are of the same order of magnitude as that reported by Kooistra et al. (1992) for corn who reported a range of 3.26-23.8 m/g. Similarly for rubber tree, the values found here fall within the range reported by Pierret et al. (2007b) for the same RRIM 600 rubber tree clone, in a 13 year plantation in NE Thailand.

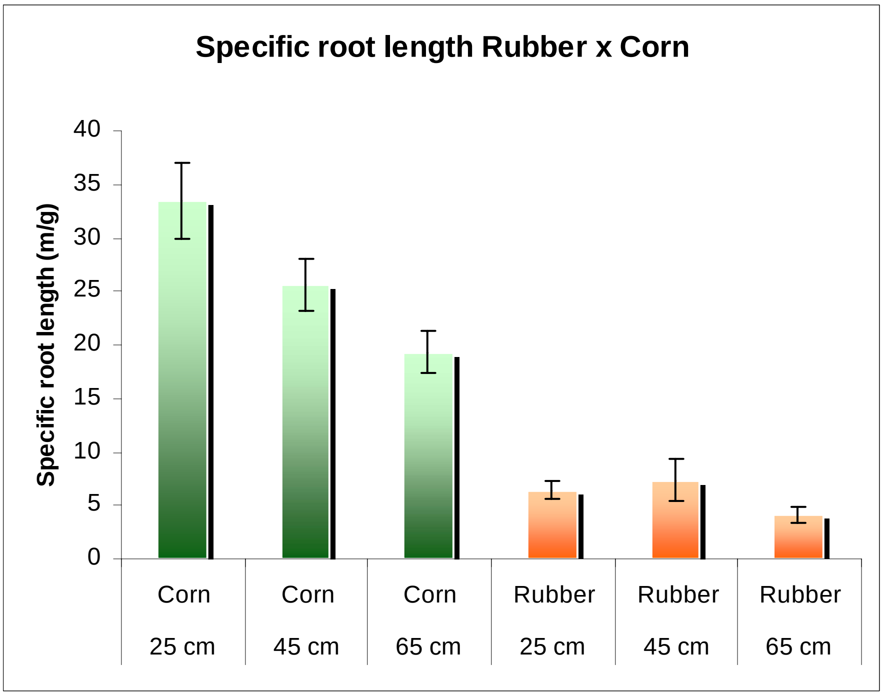
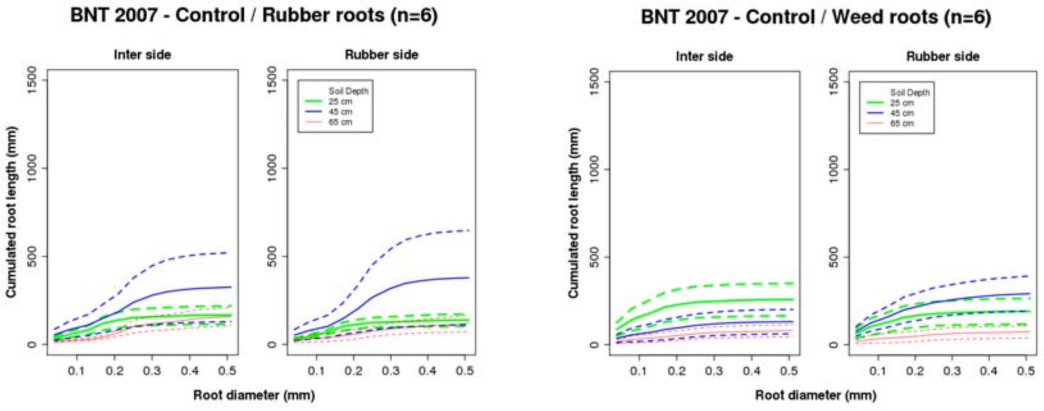


Figure 73 Specific root length of both rubber tree and corn roots at different soil depths at the end of the 2007 experiment (November 2007). Error bars are 95% confidence intervals

2.2.3 Root diameter distributions of sorted rubber tree and inter-crop roots

2.2.3.1 Control treatment

The rubber and weed root diameter distributions, along the two augered profiles are shown in Figures 74a and 74b, respectively. For rubber, distributions at 25 and 65 cm were very similar while that at 45 cm was more variable. In all cases, roots < 0.5 mm in diameter encompassed most of the total root length as indicated by the plateauing of all curves at this or a lower value. Root diameter distributions of weeds were similar at all depths and plateaued at even lower root diameter values (c. 0.3 mm) which is consistent with the fact that weed roots are on average thinner than rubber tree roots.

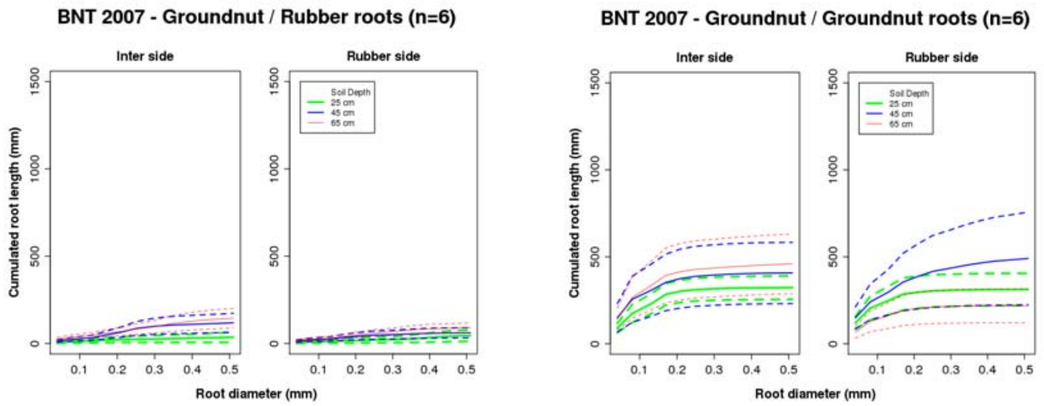


a. b.

Figure 74 Field experiment 2007 - Root diameter distributions of rubber (a) and weeds (b) along two augered profiles, at three soil depth increments (solid lines are average cumulative root length values corresponding to a given root diameter; dotted lines represent 95% confidence intervals).

2.2.3.2 Groundnut treatment

The rubber and groundnut root diameter distributions, along the two augered profiles are shown in Figures 75a and 75b, respectively. For rubber, distributions at all depths were similar and plateaued at root diameter values c. 0.5 mm. Groundnut root diameter distributions were characterized by a variability intermediate to that observed in the weed and corn treatments (Figure 75b). Although groundnut root diameter distributions tended to plateau at root diameter values of c. 0.2 mm, the distribution corresponding to groundnut roots at 45 cm on the rubber row side did not.

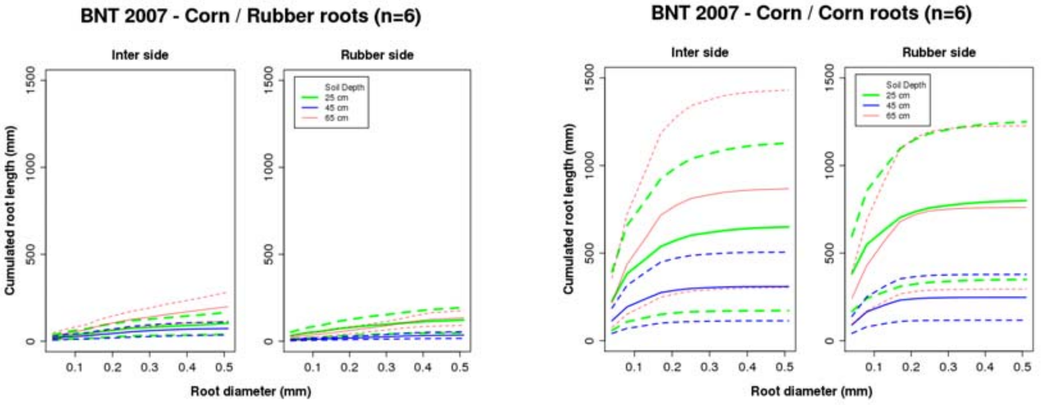


a. b.

Figure 75 Field experiment 2007 - Root diameter distributions of rubber and groundnut along two augered profiles, at three soil depth increments (solid lines are average cumulative root length values corresponding to a given root diameter; dotted lines represent 95% confidence intervals).

2.2.3.3 Corn treatment

The rubber and corn root diameter distributions, along the two augered profiles are shown in Figures 76a and 76b, respectively. For rubber, distributions at all depths were similar and plateaued for root diameter values <0.5 mm except in the case of the distribution corresponding to soil depth 65 cm on the inter-row side. Corn root diameter distributions were characterized by an important variability as shown by the large 95% confidence intervals (Figure 76b). All corn root diameter distributions plateaued between 0.2 and 0.3 mm confirming the small average diameter of corn roots compared to that of rubber tree roots.



a. b.

Figure 76 Field experiment 2007 - Root diameter distributions of rubber and corn along two augered profiles, at three soil depth increments (solid lines are average cumulative root length values corresponding to a given root diameter; dotted lines represent 95% confidence intervals).

2.2.4 Analysis of the above-ground development of rubber trees and intercrops

This analysis was based on measurements made on 32 individual rubber trees in each treatment. The number of rubber tree flushes in all treatments ranged from 0 to 5 flushes (Figure 77a). The average number of flushes in control rubber trees was higher than in other treatments, at 2.37 flushes. The average number of flushes in rubber trees associated with groundnut and corn were moderate and lower at 2.23 and 2.20 flushed, respectively. There was no significant difference in the number of flushes between the three field treatments (Table 5).

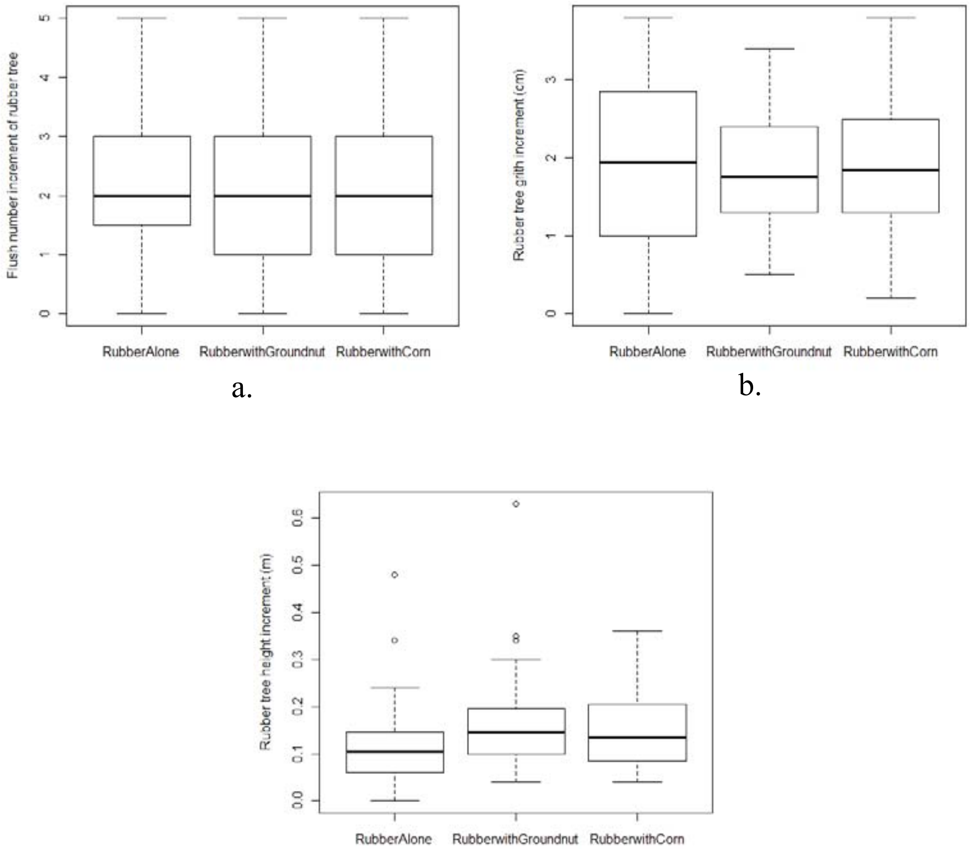
Rubber tree girth increment of all treatments ranged from 0.0 to 3.8 cm (Figure 77b). The average rubber tree girth increment for trees planted without intercrop, 1.99 cm, was higher than that of intercropped treatment. The average girth increment for rubber trees associated with groundnut and corn were 1.87 and 1.86 cm, respectively. The differences in tree girth increments between treatments were not significant (Table 5).

The rubber tree height increment for all treatments ranged from 0.00 to 0.48 m (Figure 77c). The average height increment of trees associated with groundnut was 0.17

m, i.e. higher than in other treatments. The average height increments of rubber trees associated with corn and without intercrop were as 0.15 and 0.13 cm, respectively. There was no significant difference in tree height increment between the three treatments (Table 5).

Table 5 Rubber tree growth (flush number, girth, and height) over the duration of the 2007 experiment (112 DAS, from 1 August to 13 November 2007)

Treatment	**Flush number increment (flush)**	**Girth increment (cm)**	**Height increment (m)**
Rubber alone	2.37	1.99	0.13
Rubber + Groundnut	2.23	1.87	0.17
Rubber + Corn	2.20	1.86	0.15
P-value (n=32)	0.8491	0.7924	0.2013
F-test	ns	ns	ns
LSD	0.05	0.05	0.05
CV	55.66	42.91	67.24



a. b.

c.

Figure 77 Growth increment (flush number (a), girth (b), and height(c)) of rubber trees over a 112-day observation period (from 1 August to 13 November 2007; n=32).

Shoot, root and yield biomass of groundnut and corn are reported in Figure 78. This figure shows that the average shoot and root biomass of groundnut were 13.92 and 6.13 g per plant, respectively. The average yield biomass was 4.06 g (dry weight per plant). For corn, stem and root dry biomass were 32.68 and 15.67 g, respectively, and the average yield was 23.49 g (dry weight per plant). The Root:Shoot ratio of groundnut without yield, corn without yield, groundnut including yield and corn including yield were 0.47, 0.49, 0.73 and 0.29, respectively (Figure 79).

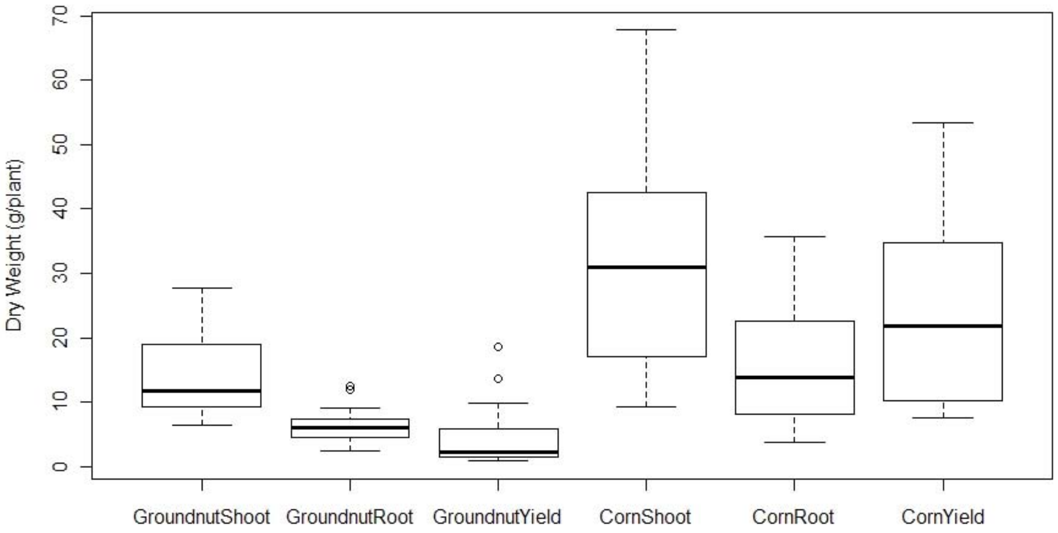


Figure 78 Shoot, root and yield biomass of intercrop at the end of the 2007 experiment (n=30).

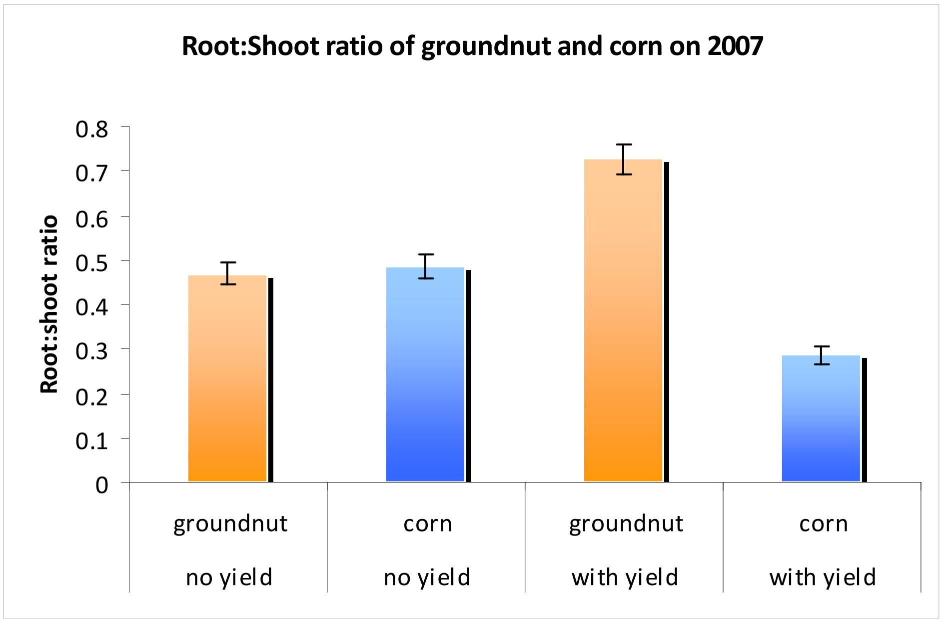


Figure 79 Root:Shoot ratio of intercrop at the end of the 2007 experiment (n=30).

2.2.5 Intercrop yield

Groundnut yielded 0.53 ton ha^{-1} of good pod (dry weight), that is the equivalent of

32.11 g per 100 seeds (Table 6). Corn yielded 33,580 ears ha^{-1}, or the equivalent of 2.36 ton ha^{-1} ear (fresh weight), and 18,518 commercial ears ha^{-1} (Table 7).

Table 6 Groundnut yield component in different parts of the cultivated slope area in 2007.

Treatment	**Pod dry weight (ton ha^{-1})**	**100 seed dry weight (g)**
Groundnut up land	0.48	34.74
Groundnut moderate land	0.76	34.08
Groundnut low land	0.34	27.52
Mean	0.53	32.11

Table 7 Corn yield component in different parts of the cultivated slope in 2007.

Treatment	**Ear numbers (ear ha^{-1})**	**Ear fresh weight (ton ha^{-1})**	**Commercial ear numbers (ear ha^{-1})**
Corn up land	20,740.74	0.79	1,481.48
Corn moderate land	58,518.52	5.01	43,703.70
Corn low land	21,481.48	1.27	10,370.37
Mean	33,580.25	2.36	18,518.52

2.3 2008 Field experiment

2.3.1 Analysis of unsorted root length densities

In this section we present the results of the root measurements conducted on unsorted samples, i.e. including both rubber tree and intercrop roots. As in the case of the 2007 experiment, this first set of results allows to assess how the measured soil profile is colonized by plant roots under different inter-cropping treatments. As opposed to 2007, special care was taken to make sure that the emergence of both corn and groundnut inter-crops would not be hampered by heavy rainfall. To this end, a fine mesh (mesh size approx. 1 mm) was installed horizontally, a few centimeters above the soil surface in order to reduce the kinetic energy of incident rainfall drops. The mesh was removed once plants had established themselves strongly enough.

Similarly to what we already observed in 2007, despite the proximity between the two series of samples that make up the two rooting profiles, there were important differences in RLD at some depths, particularly at the, 40-45 and 45-50 depths increments, in the control and groundnut treatments, and at 20-25 cm in the corn treatment. This result confirms our previous conclusion that the variability of RLD at the 5-10 cm scale is quite high (Figure 80). In line with what occurred in 2007, RLD variability in the rubber tree x corn treatment was higher than that in the control and groundnut treatments, although to a lesser extent, except for depths 40-45 and 45-50 cm on the inter-crop side.

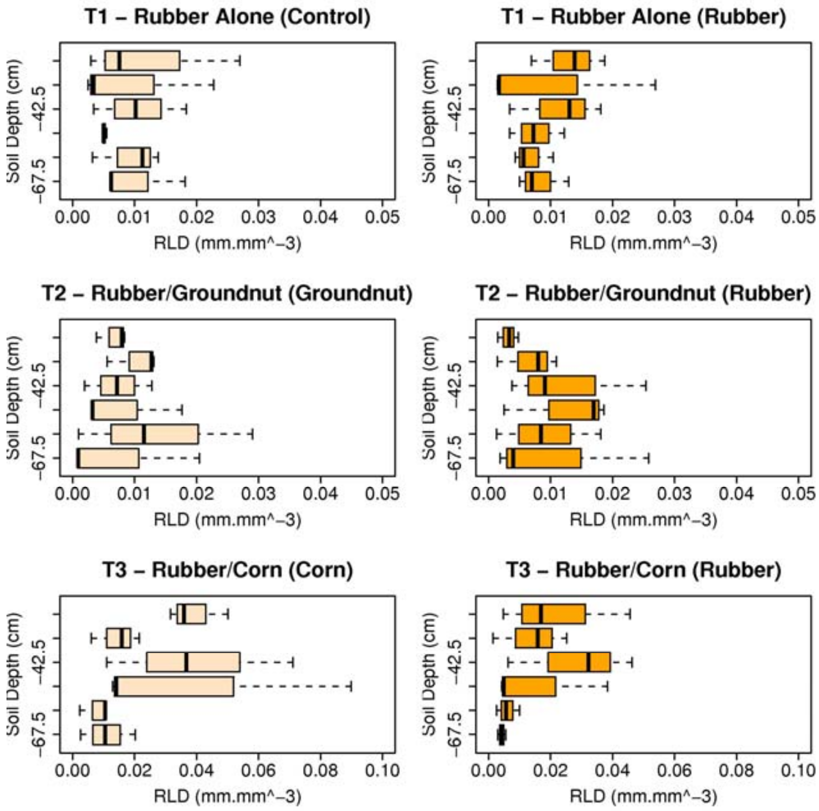


Figure 80 Root length density of unsorted rubber tree and inter-crop roots at the end of the 2008 rainy season (November 2008). Inter-crop side (left) and rubber tree side (right) (measurements made on roots washed free of soil, using augered soil samples).

When considering the average RLD profiles (Figure 81), there appears to be a significant increase in RLD associated with the corn inter-cropping, at the 40-45 and 45-50 cm depths increments, in the inter-row profile. There was also some reduction in rubber tree root growth when associated with groundnut at 20-25 cm (Figure 81).

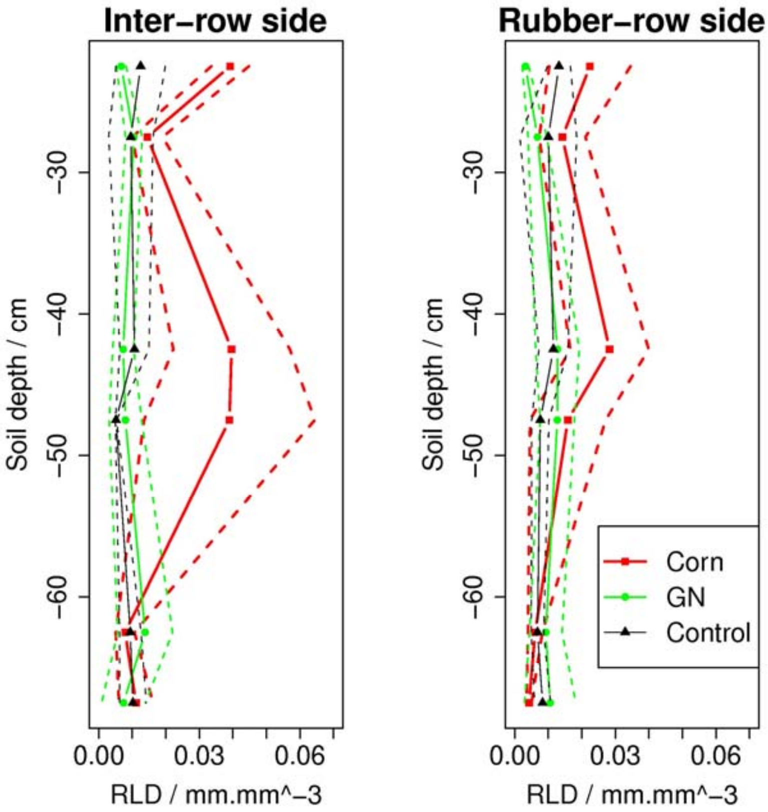


Figure 81 Average inter-row and row side rooting profiles, at the end of the 2008 rainy season (November 2008). Inter-crop side (left) and rubber tree side (right) (measurements made on roots washed free of soil, using augered soil samples).

2.3.2 Analysis of sorted rubber tree and inter-crop roots

In the following section, we present the results of the root measurements conducted on sorted samples, i.e. after the production of two separate images, one corresponding to rubber tree roots only and the second to inter-crop roots only. In order to be able to carry out some basic statistical analysis of the data, augered samples were pooled as follows: successive depth increments 20-25 and 25-30, 40-45 and 45-50 and 60-65 and 65-70 cm were pooled together to form samples for the three 25, 45 and 65 cm depth increments. Once this done, the two thus re-sampled profiles were pooled together to create on single dataset.

2.3.2.1 Control treatment

In this treatment, the weed root length density declined monotonously with soil depth, from 0.498 to 0.286 and 0.116 cm cm^{-3} at 25, 45 and 65 cm, respectively, (the value at 25 cm being highly significantly different (two-sample t-test $p<0.05$; (n=12) from that at 65 cm). For rubber, on the contrary, there was a non-significant trend towards RLD increases with depth, from 0.293 to 0.307 and 0.498 cm cm^{-3} at 25, 45 and 65 cm, respectively. Average root length densities of weed and rubber – 0.300 and 0.366 cm cm^{-3}, respectively - were not significantly different (Figure 82).

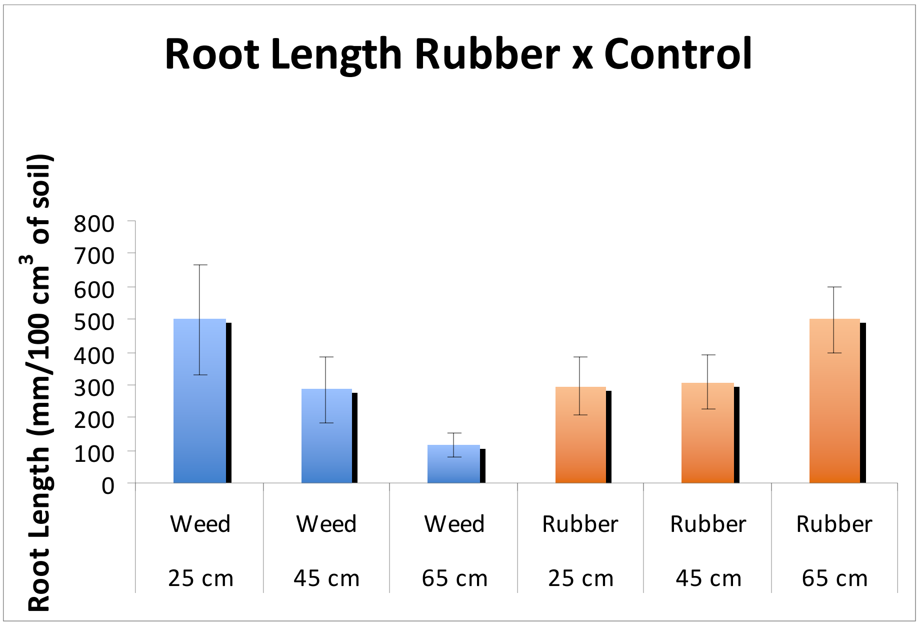


Figure 82 Root length of both rubber tree and weed roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

Average root diameters of weed decreased with soil depth from 0.200 to 0.187 and 0.151 mm at soil depths of 25, 45 and 65 cm, respectively, and the value at 65 cm was significantly different ($p<0.05$; n=12) from that at 25 cm. With rubber, root diameters were very similar at all depths increments. Average root diameters of weed and rubber – 0.18 and 0.31 mm, respectively - were highly significantly different ($p<0.001$; n=36) (Figure 83).

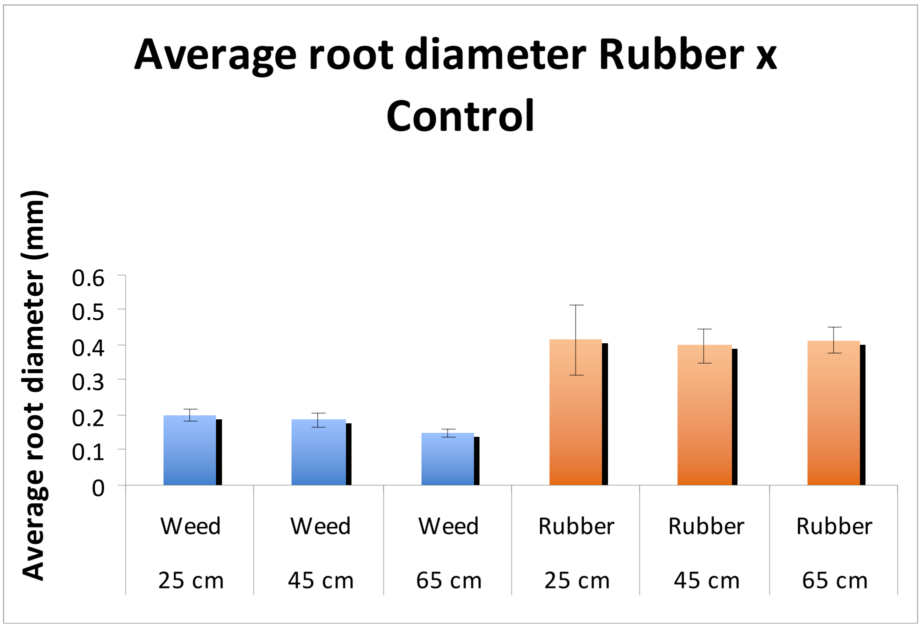


Figure 83 Average root diameter of both rubber tree and weed roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

While root surface area and soil volume occupancy of weed declined with soil depth that of rubber trees increased. The average surface area of weed and rubber tree roots, at 156.35 and 473.68 $mm^2/100\ cm^3$ of soil, respectively, were highly significantly different ($p<0.001$; $n=12$) (Figure 84) while the average soil volume occupancy of weed and rubber tree roots, at 0.55 % and 6.71 %, respectively, were highly significantly different ($p<0.001$; $n=36$) (Figure 85)

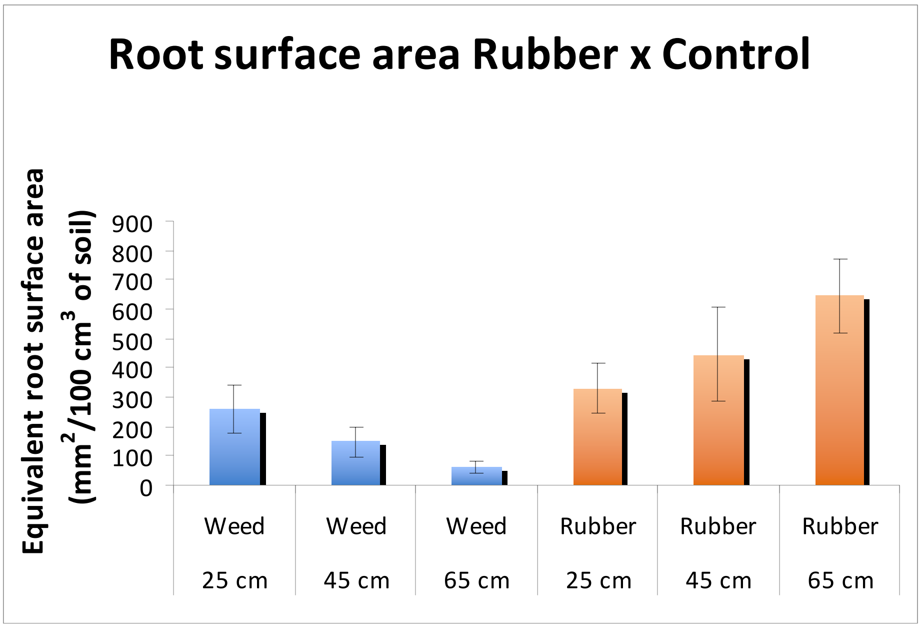


Figure 84 Root surface area of both rubber tree and weed roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

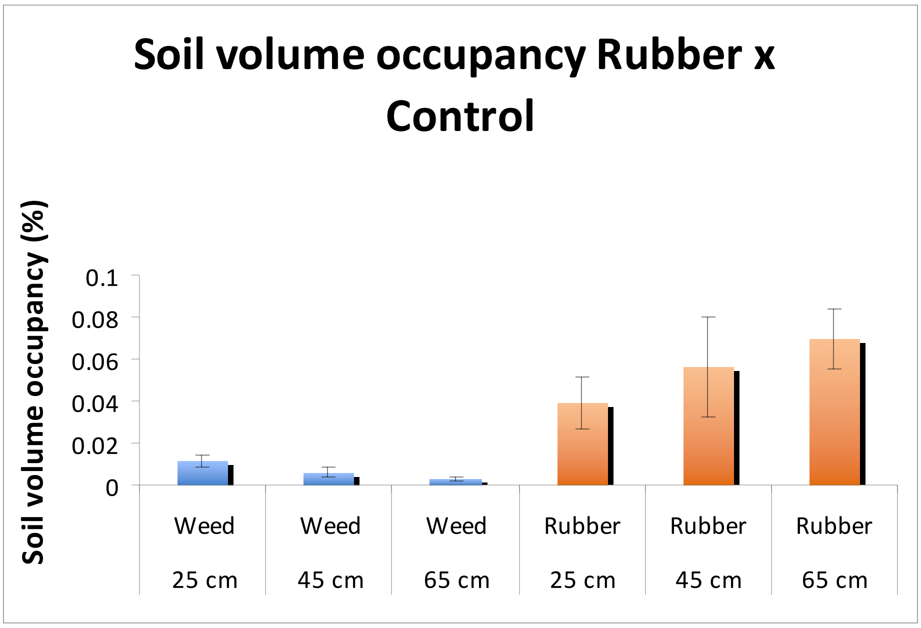


Figure 85 Soil volume occupancy of both rubber tree and weed roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

The estimated weed specific root length increased with soil depth while that of rubber tree slightly decreased. Average estimated SRL were 38 and 10 m/g in weed and rubber tree, respectively. The specific root length of weed was therefore significantly higher than that of rubber tree ($p<0.001$; $n=36$) (Figure 86).

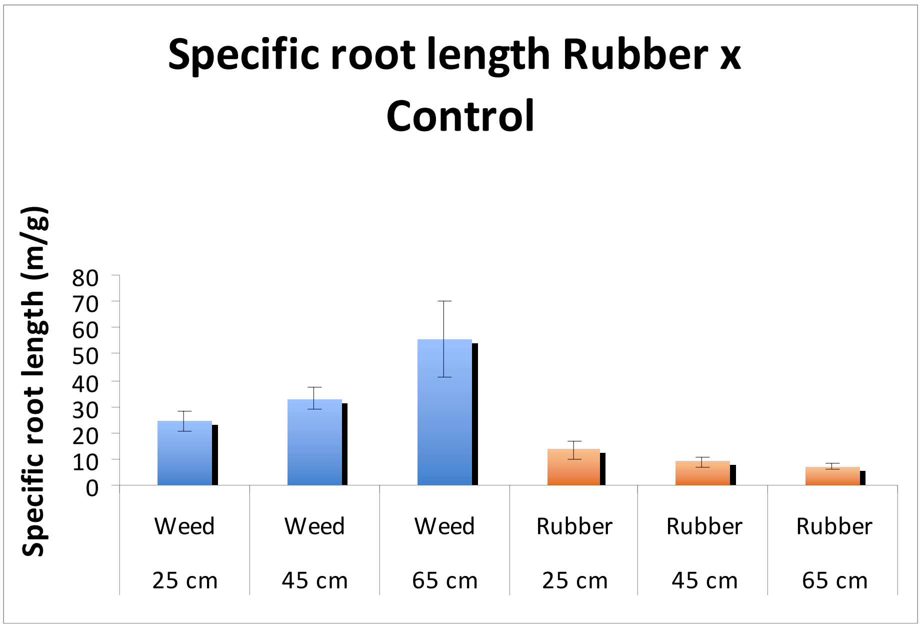


Figure 86 Specific root length of both rubber tree and weed roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

2.3.2.2 Groundnut x Rubber treatment

In this treatment, rubber tree roots and groundnut developed similar root length densities at soil depths of 25 and 45 cm, with RLD values of about 0.25 and more than 0.4 cm cm^{-3} at 25 and 45 cm, respectively, in both plant species. At 65 cm, the groundnut RLD dropped back down to 0.243 cm cm^{-3} while that of rubber increased to > 0.5 cm cm^{-3}. Average root length density of groundnut and rubber – 0.314 and 0.415 cm cm^{-3}, respectively – were not significantly different (Figure 87).

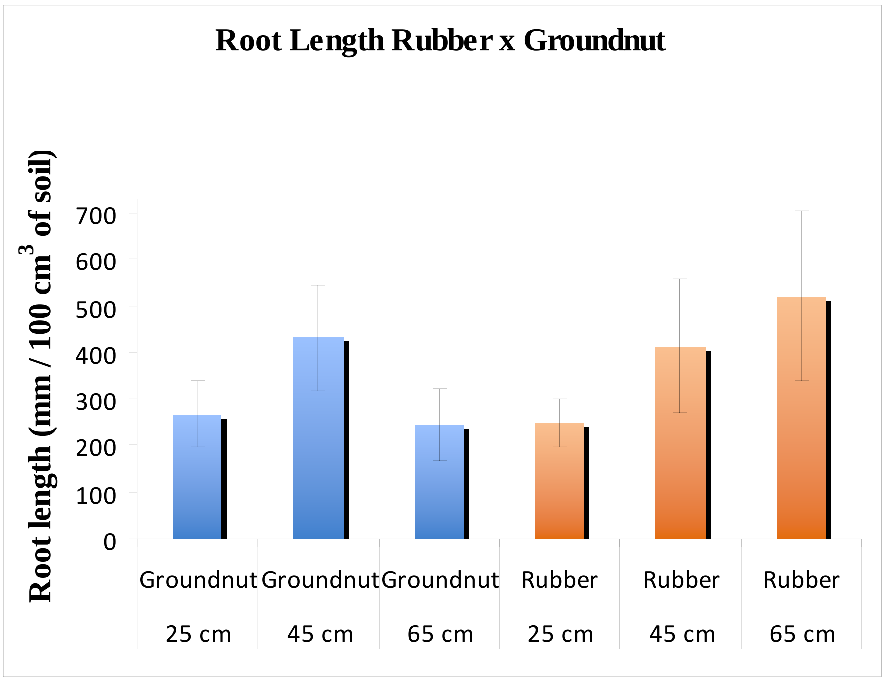


Figure 87 Root length of rubber tree and groundnut roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

Average root diameters of groundnut increased, although not significantly, from 0.14 to 0.16 and 0.176 mm at soil depths of 25, 45 and 65 cm, respectively. The average root diameter of rubber, on the other hand, almost doubled from 0.25 mm at 25 cm to 0.515 mm at 65 cm (two-sample t-test $p<0.01$; $n=12$). The average rubber tree root diameter also increased significantly ($p<0.05$; $n=12$) from 0.25 mm at 25 cm to 0.39 mm at 45 cm. Overall, the average root diameters of groundnut and rubber tree, 0.16 and 0.38 mm, respectively, differed with a high degree of significance ($p<0.001$; $n=36$) (Figure 88), indicating that, root diameter is a simple valid criterion for separating rubber tree roots from that of other species.

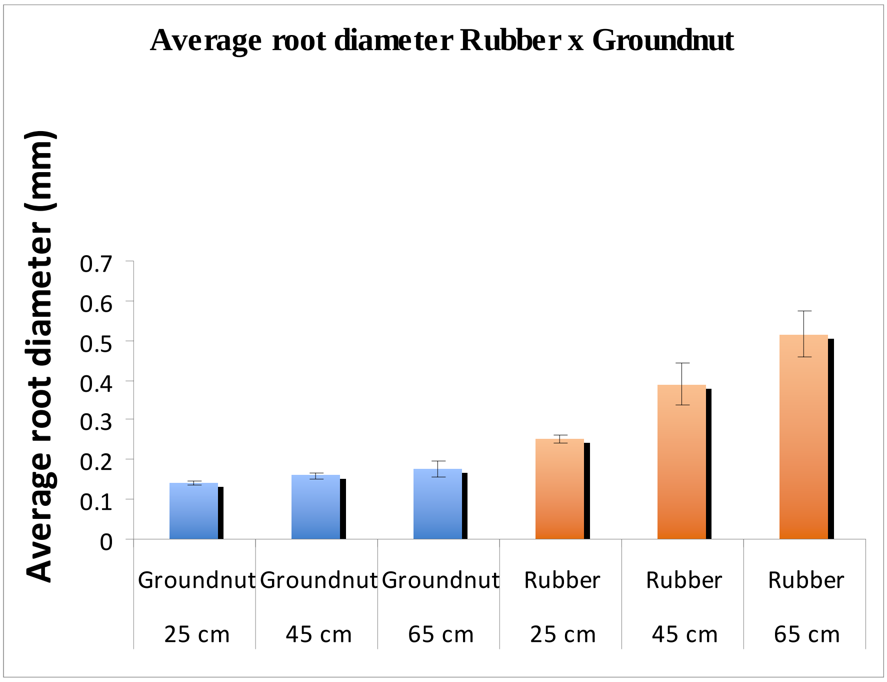


Figure 88 Average root diameter of both rubber tree and groundnut roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

While root surface area (Figure 89) and soil volume occupancy (Figure 90) were of the same order of magnitude in groundnut at all soil depth increments, in rubber tree, these parameters increased significantly with soil depth. The average root surface area and soil volume occupancy of rubber tree were more than 3.5 and 9.6 times that of groundnut, respectively, which indicates that rubber tree developed much larger exchange surfaces and occupied a much larger fraction of the soil volume than groundnut.

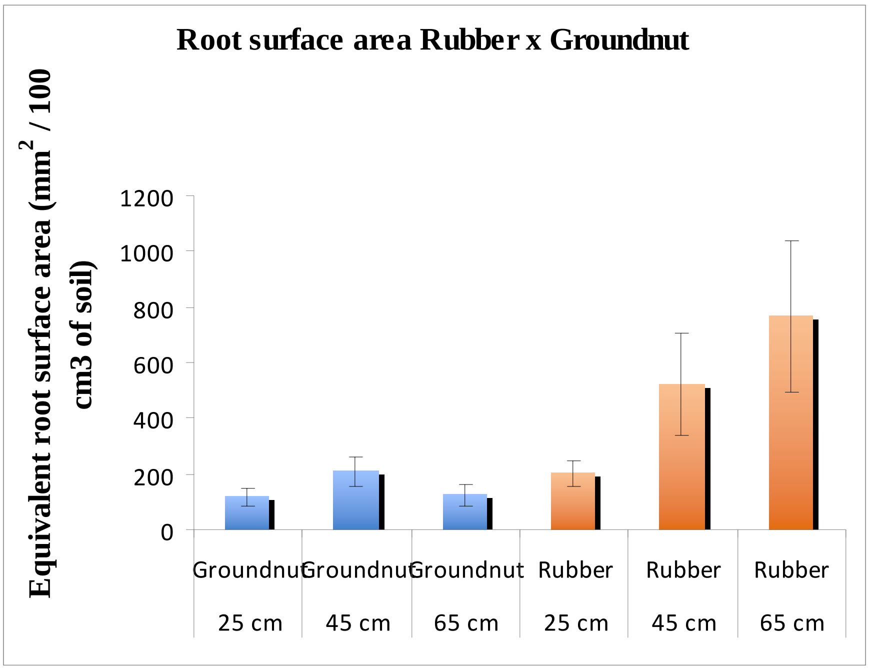


Figure 89 Root surface area of rubber tree and groundnut roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

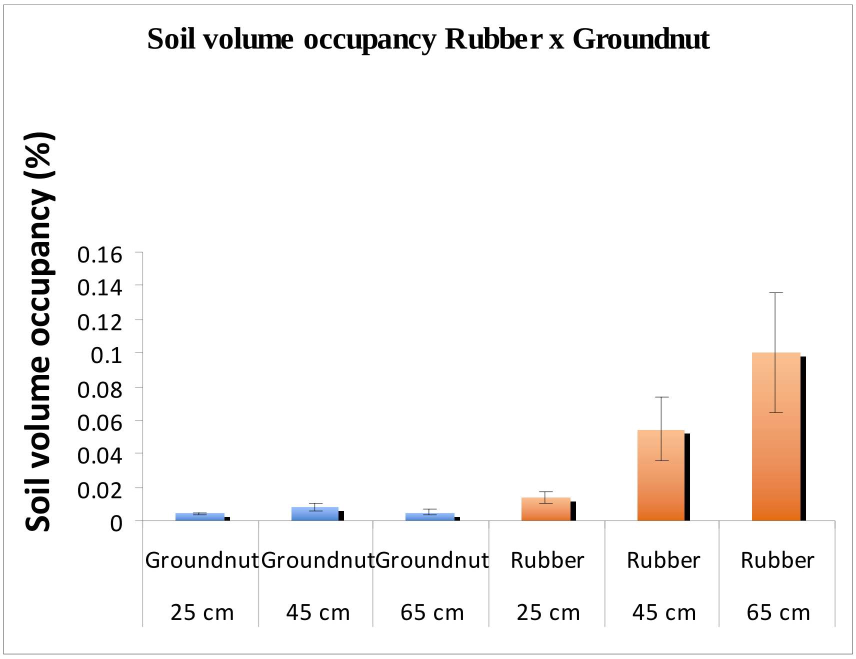


Figure 90 Soil volume occupancy of both rubber tree and groundnut roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

Average estimated specific root length decreased with depth in both groundnut and rubber tree and at about 40 and 10 m/g in groundnut and rubber, respectively. The specific root length of groundnut was therefore significantly higher than that of corn ($p<0.001$; n=36) (Figure 91).

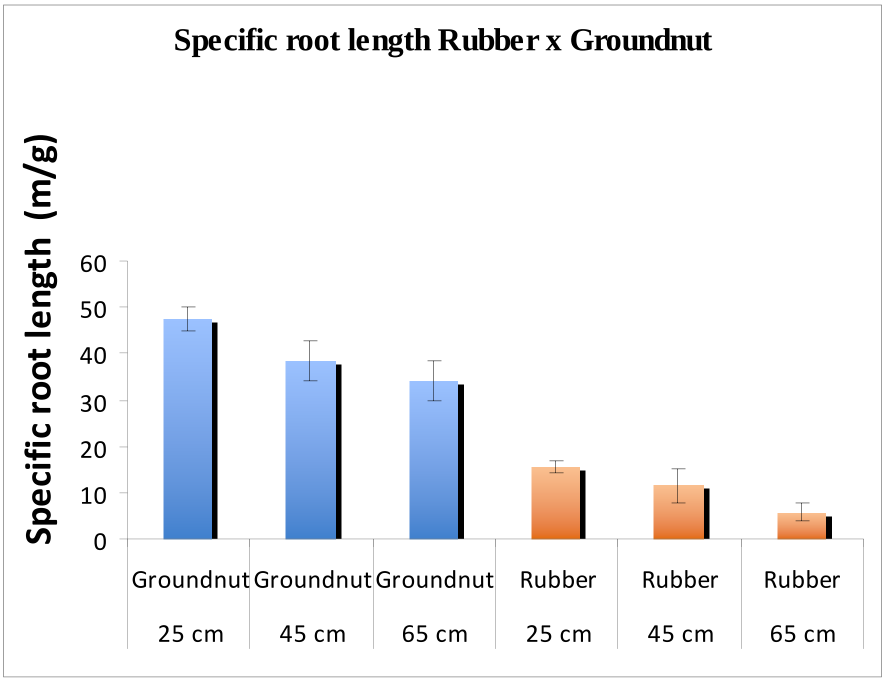


Figure 91 Specific root length of both rubber tree and corn roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

2.3.2.3 Corn x Rubber treatment

In this treatment, in contrast with the two previous cases, there were relatively high and comparable lengths of corn and rubber roots at both 25 and 45 cm (RLD ranging from a minimum of 0.903 and a maximum of 1.625 cm cm^{-3}). The RLD of corn at 25 and 45 cm were significantly higher ($p<0.01$; n=12) than at 65 cm. At 65 cm, the corn RLD dropped significantly, down to 0.142 cm cm^{3} while that of rubber was reduced to only 0.436 cm cm^{-3}, a value significantly higher than that of corn (two-sample t-test $p<0.01$; n=12), but lower than at depths 25 and 45 cm ($p< 0.05$; n=12). Average root length density of weed and rubber – 0.915 and 0.793 cm cm^{-3}, respectively – were not significantly different (Figure 92).

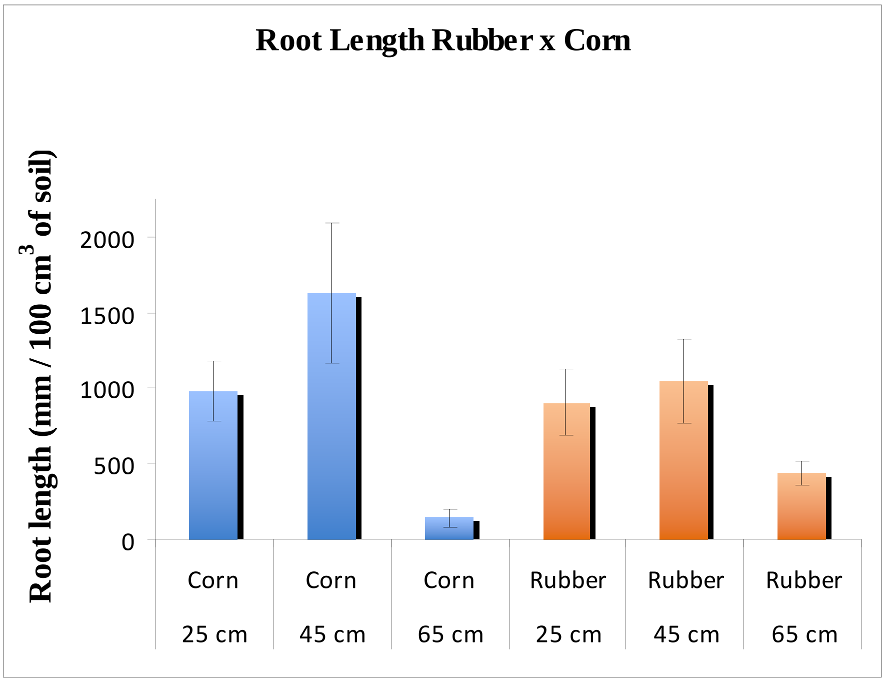


Figure 92 Root length of rubber tree and corn roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

Average root diameters of orn decreased significantly (two-sample t-test $p<0.01$) from 0.173 to 0.142 mm at soil depths of 25 and 65 cm, respectively, while with rubber, there were much greater variations in root diameter between soil depths from 0.37 to 0.31 and 0.44 mm at 25, 45 and 65 cm respectively (the value at 45 cm being significantly different ($p<0.05$; n=12) from that at 65 cm). Average root diameters of corn and rubber – 0.16 and 0.37 mm, respectively - were highly significantly different ($p<0.001$), confirming the fact that rubber roots were on average at least twice thicker as corn roots, a criterion that was used for separating the two species and that also was validated as a criterion to separate groundnut and weed roots from that of rubber (Figure 93).

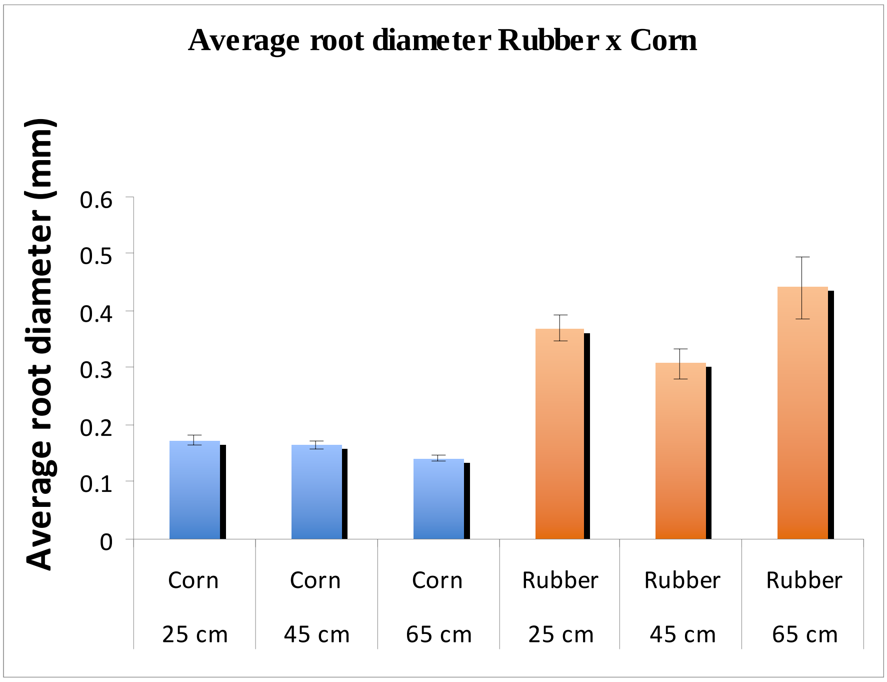


Figure 93 Average root diameter of rubber tree and corn roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

These results about root diameter and length had a direct incidence on root surface area (Figure 94) and soil volume occupancy (Figure 95) which were higher in rubber than corn at all soil depths, and which, at 65 cm, were one order of magnitude higher in rubber than in corn.

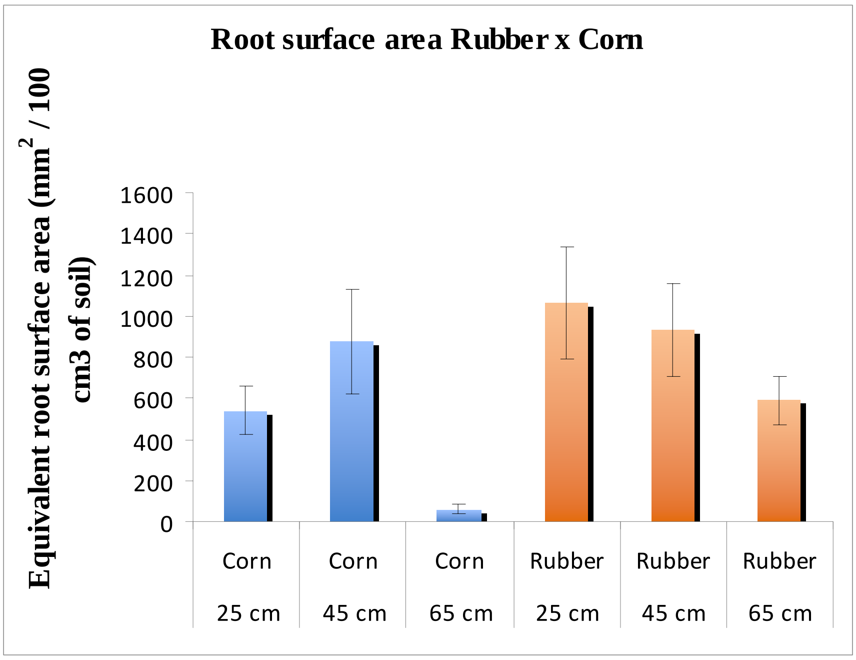


Figure 94 Root surface area of both rubber tree and corn roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals.

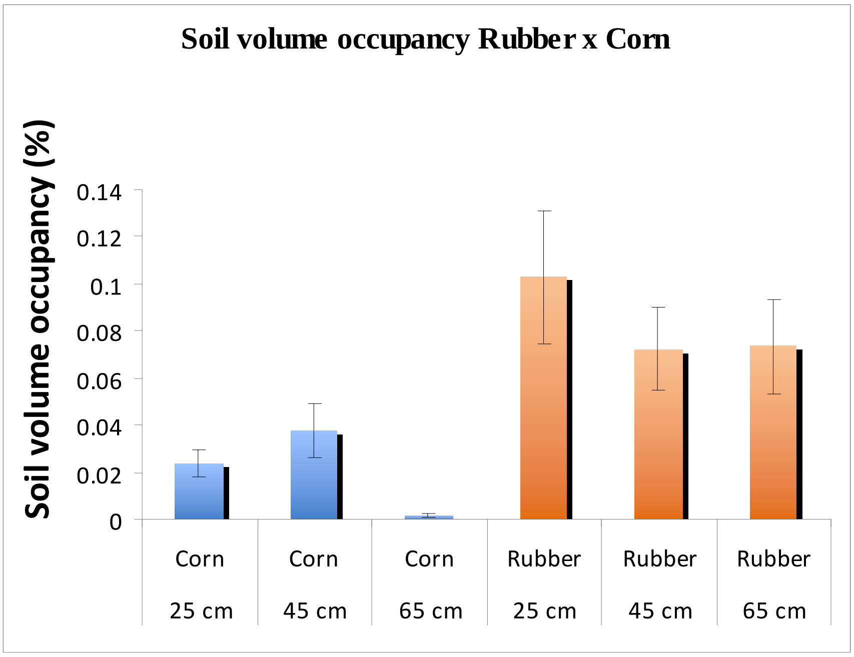


Figure 95 Soil volume occupancy of both rubber tree and corn roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

Figures about estimated specific root length (Figure 96) showed that corn and rubber differed significantly with regards to this parameter, which averaged at about 30 and 9 m/g in corn and rubber, respectively. These SRL values are of the same order of magnitude as that reported by Kooistra et al. (1992) for corn who reported a range of 3.26-23.8 m/g. Similarly for rubber tree, the values found here fall within the range reported by Pierret et al. (2007b) for the same RRIM 600 rubber tree clone, in a 13 year plantation in NE Thailand.

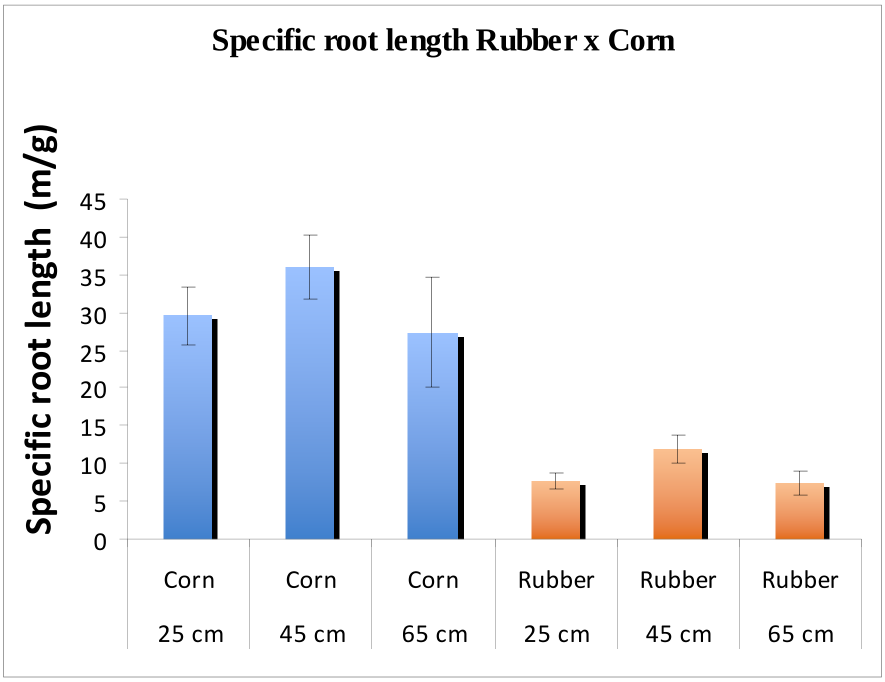


Figure 96 Specific root length of both rubber tree and corn roots at three soil depth increments, at the end of the 2008 experiment (November 2008). Error bars are 95% confidence intervals

2.3.3 Analysis of average root diameter distributions

In the diagram below are some data about average root diameter measurements. It all cases, there is a tendency towards increased root diameters with depth, which might be related to an increased occurrence of rubber tree roots (Figure 97). This is particularly clear in the groundnut and corn treatments.

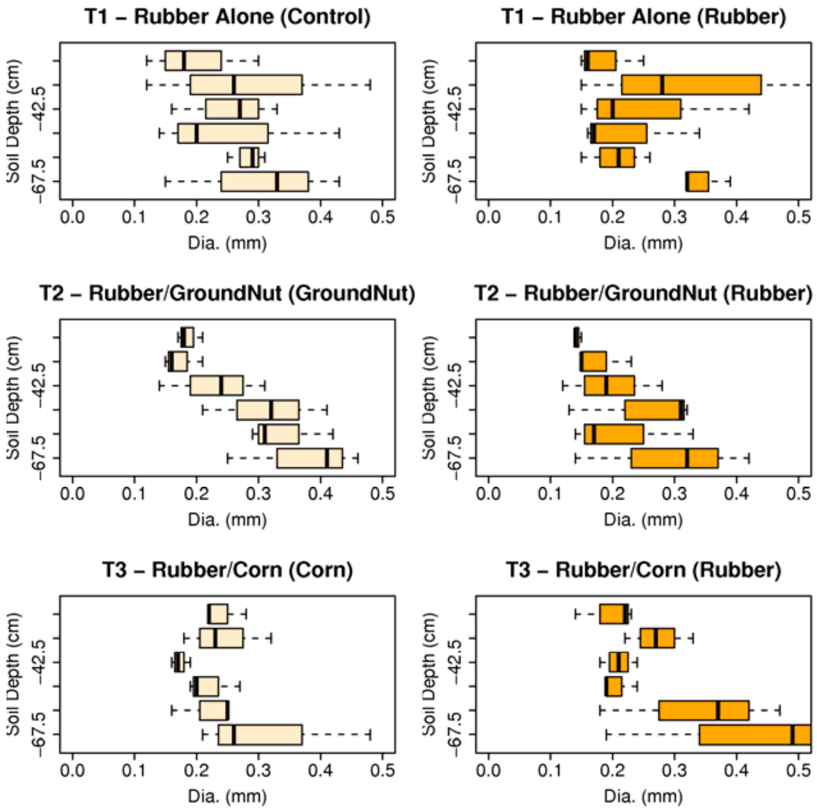
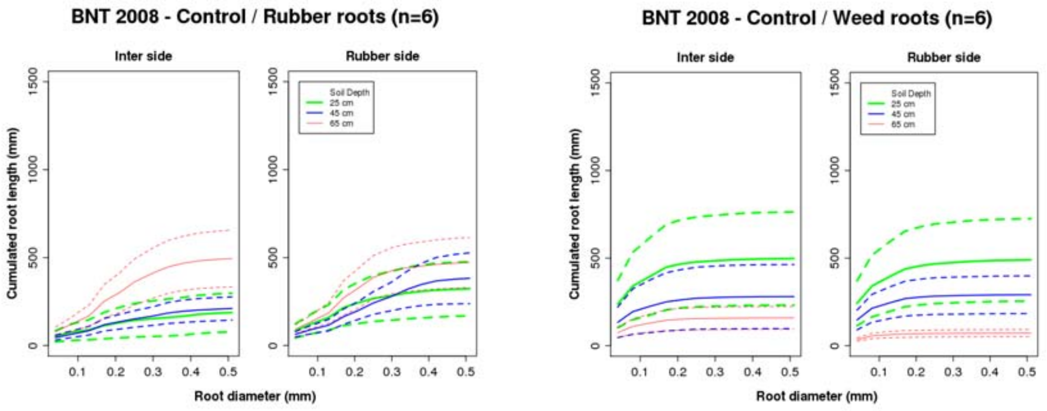


Figure 97 Diameter of unsorted rubber tree and inter-crop roots at the end of the 2008 rainy season (November 2008). Inter-crop side (left) and rubber tree side (right) (measurements made on roots washed free of soil, using augered soil samples).

2.3.4 Analysis root diameter distributions of sorted rubber tree and inter-crop roots

2.3.4.1 Control treatment

The rubber and weed root diameter distributions, along the two augered profiles are shown in Figures 98a and 98b, respectively. For rubber, distributions tended to be similar, expect for the 65 cm distribution on the inter-row side which cumulated more root length from root diameter ranging from 0.3 to 0.5 mm. The distributions corresponding to the 25 and 65 cm soil depth increments also accounted for much more root length than in 2007 (Figure 74a). All distributions plateaued at root diameter values < 0.5 mm. For weeds, root diameter distributions plateaued at values < 0.25 mm (Figure 98b). Weed root diameter distributions of weeds were similar at all depths, except for the one corresponding to the 65 cm soil depth on the rubber tree row side, which cumulated significantly less root length than the other distributions. Overall, there was also more weed root length in 2008 than in 2007, particularly at the two first depth increments (25 and 45 cm).

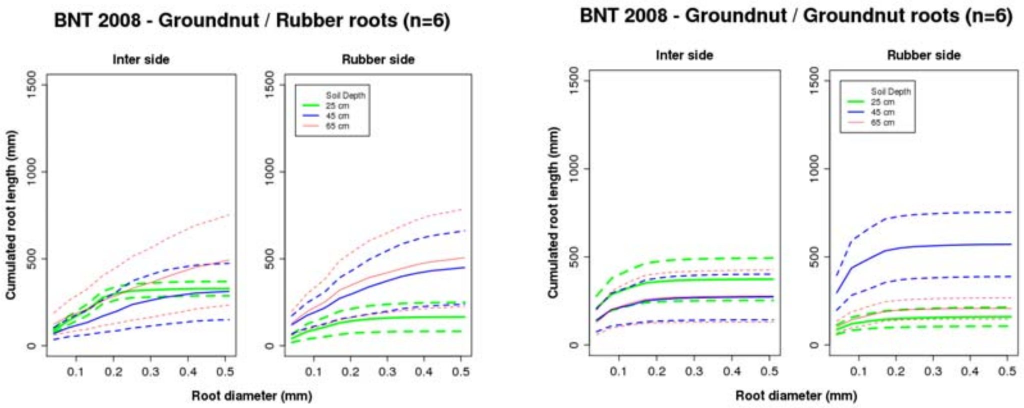


a. b.

Figure 98 Field experiment 2008 - Root diameter distributions of rubber (a) and weeds (b) along two augered profiles, at three soil depth increments (solid lines are average cumulative root length values corresponding to a given root diameter; dotted lines represent 95% confidence intervals).

2.3.4.2 Groundnut treatment

The rubber and groundnut root diameter distributions, along the two augered profiles are shown in Figures 99a and 99b, respectively. For rubber, distributions at depths 45 and 65 cm were similar and did not plateau at root diameter values < 0.5 mm, indicating the presence of thicker roots in these samples. However, at 25 cm, the rubber root diameter distributions plateaued at diameters between 0.2 and 0.3 mm. Groundnut root diameter distributions were not dissimilar to that of weed roots (Figure 98b) and they all plateaued at root diameter values < 0.2 mm. Remarkably, on the rubber row side, the groundnut root diameter distribution corresponding to the 45 cm soil depth cumulated more root length than at the two other soil depth increments (Figure 99b).

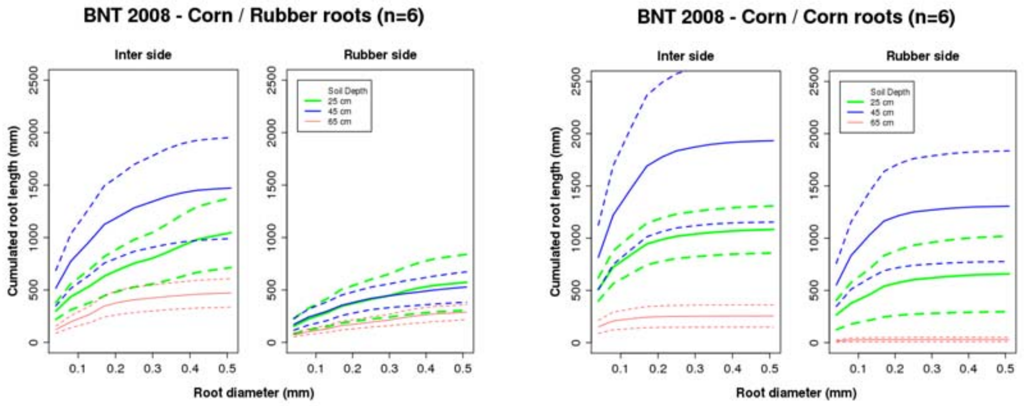


a. b.

Figure 99 Field experiment 2008 - Root diameter distributions of rubber and groundnut along two augered profiles, at three soil depth increments (solid lines are average cumulative root length values corresponding to a given root diameter; dotted lines represent 95% confidence intervals).

2.3.4.3 Corn treatment

The rubber and corn root diameter distributions, along the two augered profiles are shown in Figures 100a and 100b, respectively. For rubber, distributions tended to cumulate more root length on the inter-row side particularly at 25 and 45 cm. In addition, many distributions did not plateau at root diameters < 0.5 mm indicating the presence of thicker roots in these samples. Overall, there was more root length in this treatment than in the same treatment in 2007 (Figure 75a). As in 2007, corn root diameter distributions were characterized by an important variability as shown by the large 95% confidence intervals, except for the 65 cm depth increment (Figure 100b). All corn root diameter distributions plateaued at root diameter values of about 0.2 mm confirming the small average diameter of corn roots compared to that of rubber tree roots. The main difference with the corn root diameter distributions observed in 2007 is that, the most profuse corn root development in 2008 appeared to be limited to soil depths of < 65 cm.



a. b.

Figure 100 Field experiment 2008 - Root diameter distributions of rubber and corn along two augered profiles, at three soil depth increments (solid lines are average cumulative root length values corresponding to a given root diameter; dotted lines represent 95% confidence intervals).

2.3.5 Above-ground development of rubber trees and inter-crops

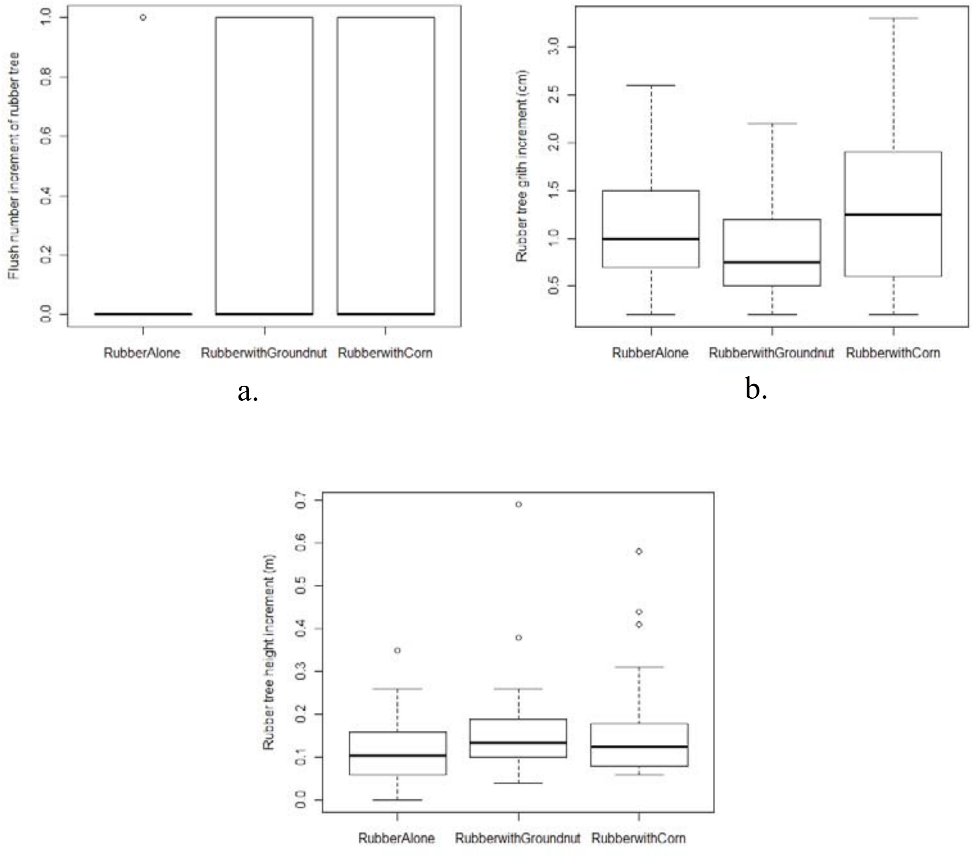
This analysis was based on measurements made on 30 individual rubber trees in each treatment. The number of rubber tree flushes in all treatments ranged from 0 to 1 flush (Figure 101a). The average number of flushes in the corn treatment was significantly higher, at 0.33 flushes per tree, than in the control treatment, at 0.07 flushes, but not different than in the groundnut treatment, at 0.27 flushes per plant (Table 8).

Rubber tree girth increment, in all treatments, ranged from 0.2 to 3.3 cm (Figure 101b). The average rubber tree girth increment associated with the corn treatment was the highest, with an average value of 1.35 cm, compared to 1.16 and 0.91 cm in the control and groundnut treatments, respectively, but these differences were not statistically significant (Table 8).

Rubber tree height increment ranged from 0.00 to 0.69 m across treatments (Figure 101c). The average rubber tree height increments associated with the groundnut and corn treatments were equal and higher, although not significantly, than in that associated with the control treatment (Table 8).

Table 8 Rubber tree increment growth (flush number, girth, and height) over the duration of the 2008 experiment (30 June to 6 November 2008, 126 DAS).

Treatment	Flush number increment (flush)	Girth increment (cm)	Height increment (m)
Rubber alone	0.07 B	1.16	0.12
Rubber + Groundnut	0.27 AB	0.91	0.16
Rubber + Corn	0.33 A	1.35	0.16
P-value (n=30)	0.0353	0.0606	0.1906
F-test	*	ns	ns
LSD	0.05	0.05	0.05
CV	183.35	62.29	76.77



a. b.

c.

Figure 101 Rubber tree increment growth (flush number (a), girth (b), and height(c)) over the duration of the 2008 experiment, i.e. 126 days, from 30 June to 6 November 2008 (n=30).

Shoot, root and yield biomasses of groundnut and corn are reported in Figure 102. This figure shows that, for groundnut, the shoot included, on average, the highest proportion of the plant biomass (22.73 g/plant or >52 % of the plant's dry biomass) followed by grain yield (13.39 g or >30 % of the plant's dry biomass) and root biomass (7.25 g or 17 % of the plant's dry biomass). In corn, grain yield made up, on average, the highest proportion of the plant biomass (39.82 g or 45% of the plant's dry biomass), while the shoot and root included 37.29 and 11.77 g (or 42 and 13% of the plant's dry biomass), respectively. The Root:Shoot ratio of groundnut and corn without grain yield, and groundnut and corn including grain yield were 0.32, 0.31, 0.91 and 0.15, respectively (Figure 103).

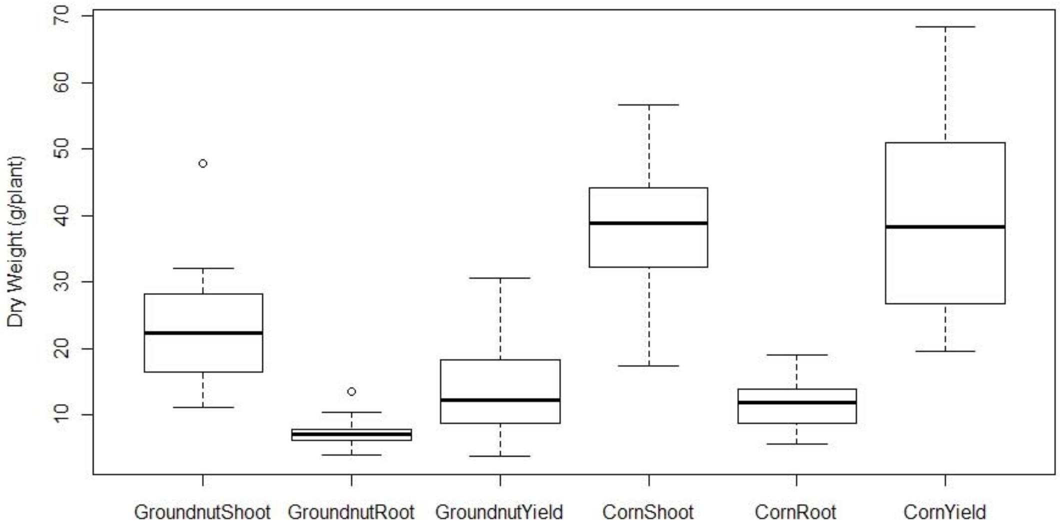


Figure 102 Shoot, root and yield biomass of intercrop by the end of the 2008 field experiment.

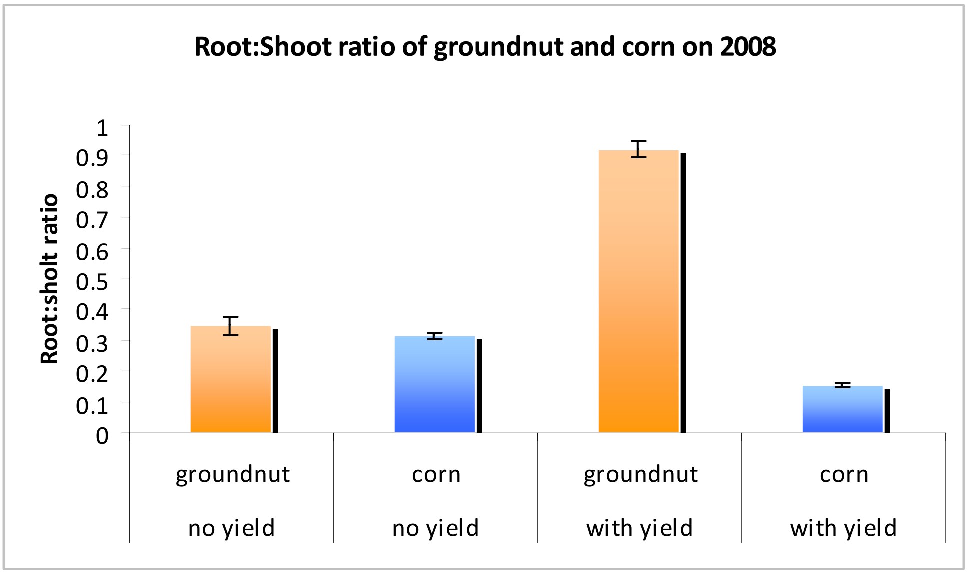


Figure 103 Root:Shoot ratio of intercrop by the end of the 2008 experiment.

2.3.6 Inter-crop yields and profitability of rubber-tree inter-cropping

Groundnut yielded 0.73 ton ha^{-1} good pod dry weight and 26.93 g per 100 seeds. Corn yielded 16,666.67 ears ha^{-1}, 1.24 ton ha^{-1} ear fresh weights and 11,111 commercial ears ha^{-1}. Buranatham et al. (2003) reported much higher yields for corn (livestock variety) and groundnut inter-cropped with rubber trees in plantations of NE, Thailand (5.6 and 2.5 ton ha^{-1}, for corn and groundnut, respectively). However, in southern Thailand, the corn yield associated with young rubber tree was 2.6 ton ha^{-1} (Sangpradap et al., 1993). The yield of inter-crops associated with rubber trees appears to vary widely depending on local biophysical conditions. Overall, the corn and groundnut yields obtained during this experiment appear to be at the lower end of yield ranges commonly reported for these species in agroforestry systems (e.g. Hauser, 2006), and groundnut was probably better suited to the soil and rainfall conditions of the site where the experiment was conducted (i.e. unreliable and most likely limiting rainfall towards the end of the rainy season; low N and P availability).

Buranatham et al. (2003) interviewed farmers about cost, income and net profit related to corn and groundnut inter-cropping over the 2000-2003 period. The investment costs for corn and groundnut were 10,500 and 3,469 Baht ha^{-1}, respectively (approximately 233 and 77 Euros ha^{-1}), not including labour cost. Income earned from corn and groundnut were 37,350 and 37,500 Baht ha^{-1}, respectively (approximately 830 and 833 Euros ha^{-1}). The net profit for corn and groundnut were 26,850 and 34,031 Baht ha^{-1}, respectively (approximately 597 and 756 Euros ha^{-1}). Karanad et al. (1993) indicated that the net profits achieved with corn and groundnut associated with young rubber tree were higher than with other inter-crops such as banana, pineapple, and ruzi grass.

In this experiment, the costs associated with growing corn and groundnut inter-crops amounted to 60,388 and 69,000 Baht ha^{-1} (approximately 1,342 and 1,533 Euros ha^{-1}), respectively, inclusive of labour (at a rate of 160 Baht day^{-1} person^{-1}). Income from corn and groundnut were 74,073 and 19,710 Baht ha^{-1} (approximately 1,646 and 438 Euros ha^{-1}), respectively, leading to a net profit of 13,685 Baht for corn and a net deficit of 49,290 Baht ha^{-1} for groundnut (approximately 304 and -1,095 Euros ha^{-1}), respectively. Clearly, labour cost has a decisive impact on the profitability of inter-cropping practices and our experiments show that it only makes economical sense to grow inter-crops in NE Thailand for farmers who do not have to cover extra labour costs.

2.4 Discussion of the 2007-2008 field experiments

2.4.1 Development of the below-ground compartment of rubber trees and inter-crops

This study provided field observations made in a young rubber tree plantation of northeastern Thailand of below-ground interactions between some inter-crops (i.e. groundnut and corn) and rubber tree. The main root parameters investigated were: root length density, root diameter, root surface area, soil volume occupancy and specific root length.

The results of the 2007 experiment indicate that the average root length density of both rubber alone and rubber tree associated with groundnut declined with depth. In contrast, the average root length density of rubber inter-cropped with corn did not decrease with depth and was similar at 65 than at 25 cm. Anderson (1987) reported that corn root characteristics such as, diameter and specific root length (or root mass per length (in mg m^{-1}) vary as a function of lateral position and depth. Typically, the corn root system starts with a radicle or primary root that emerges from the scutellar node located within the seed embryo. Roots that subsequently emerge from this same node form the seminal root system. The adventitious, or crown, or nodal root system later develops sequentially from individual nodes above the mesocotyl,. As all other *Poaceae* species, corn develops a fibrous root system which includes large amounts of roots that are considered to occur preferentially near the soil surface and it is classically reported that maximum corn root density at maturity occurs in the 15 cm of soil (Mengel and Barber, 1974). Our results obviously show that such a root distribution is not always verified in the field.

The root length density of groundnut, as measured in this work, was very similar at all the three depth increments investigated. In contrast, Hammond et al. (1987) quoted by Rao (1993) indicated that on a well-drained deep sandy soil, the root length density of cv. Florunner was of the order of 1.5 cm cm^{-3} in the 0-30 cm zone (i.e. about five times more than what we have observed) and dropped to 0.1-0.4 cm cm^{-3} at greater depths (while we observed values > 0.4 cm cm^{-3} at 45 cm in 2007 and 2008). Moreover, Zade et al. (1981) observed a progressive decrease in groundnut root weight, root volume and root length with increasing soil depth, with 43% of the total root length measured in the top 0-15 cm layer while the next 15 cm layer accounted for only 32%. Once again, as in the case of corn, our results show discrepancies with previously published reports, part of which may be explained by the fact that we did not investigate the shallowest soil horizons, and also by the fact that strong varietal differences in rooting patterns are known and documented (e.g. Sivasankar et al., 1981).

The root distributions of rubber trees grown in association with inter-crops, as observed in this work, were quite variable and often, root length density was seen to increase with depth. In contrast, Soong et al. (1972) quoted by Krishna (1993) reported that the mean root length density of rubber tree fine roots declined with soil depth in a range of soils. This different trend might be partly related to the fact that we worked with a different clonal variety and to the particular pedo-climatic conditions that prevail in NE Thailand.

2.4.2 Development of the above-ground compartment of rubber trees and inter-crops

2.4.2.1 Effects of inter-crops on rubber tree growth

When considering the effect of inter-crops (groundnut and corn, in this work) on the above-ground development of rubber trees, it was found that none of the above-ground parameters which were measured during the 2007 and 2008 experiments, namely flush number, girth and height increments, differed significantly between treatments with and without inter-crops. One exception though, was the number of flushes in 2008, which increase more in the rubber – corn treatment than in the control and rubber – groundnut treatments. These results are in line with previous reports, such as for example, that of Laosuwan et al. (1988) who found differences in girth increments during certain periods of rubber growth and that legume cover and pineapple were conducive to the growth of rubber trees. Similarly, Mainstone (1963) quoted by Watson (1989) indicated that rubber tree growth was significantly improved by mixed legume covers, even compared to a natural cover with a high nitrogen fertilization regime. Pushparajah and Tan (1970) stated that short term inter-crops such as groundnut or corn may only have a limited negative impact on rubber tree growth and that this can be easily corrected using modest amounts of fertilizers. In contrast, Wibawa et al. (2006) found that at 51 months, the girth of rubber trees inter-cropped with timber trees was 30% less than in a control monoculture treatment (without intercrop).

2.4.2.2 Intercrop yield and water use

Both the groundnut and corn yields obtained in 2007 and 2008 at Ban Non Tum were rather low. Here we investigated the possible links between water availability and these low yields.

Actual evapo-transpiration (ET), represents the sum of water evaporated from the soil and of that transpired by plants. The difference between evapo-transpiration and evaporation is therefore equivalent to soil water use by plants, i.e. the water extracted by the root system from the soil volume under its influence (or rooting zone). To assess soil water use by plants, we can use a general water balance model:

$$P = ET + Q + \Delta S \qquad \text{Eq(8)}$$

Where P = precipitation, ET = evapo-transpiration (where E=evaporation and T=transpiration), Q = runoff and ΔS = change in soil water storage. The depth of water available for transpiration, i.e. for use by plants can be derived from this equation by writing:

$$T = P - (Q + \Delta S + E) \qquad \text{Eq(9)}$$

To assess T numerically, the following assumptions were made: $\Delta S = 0$, which is consistent with the fact that, water levels in piezometers installed in the field did not vary substantially over the course of the experimental period; $Q = 0.3 \times P$ and $E = 2$ mm /day, which are values in line with the results of unpublished results made in Ban Non Tun by an LDD/IRD team (personal communication from J.-L. Maeght, IRD).

ETo, i.e. the reference crop evapo-transpiration (mm/day) for both corn and groundnut, was estimated using the Blaney-Criddle method, which is expressed as:

$$ETo = p\ ((0.46*T\ mean) +8) \qquad Eq(10)$$

Where ETo = reference crop evapo-transpiration (mm/day) as an average for a period of 1 month, p = mean daily percentage of annual daytime hours and T mean = mean daily temperature (°C) (Brouwer and Heibloem,1986).

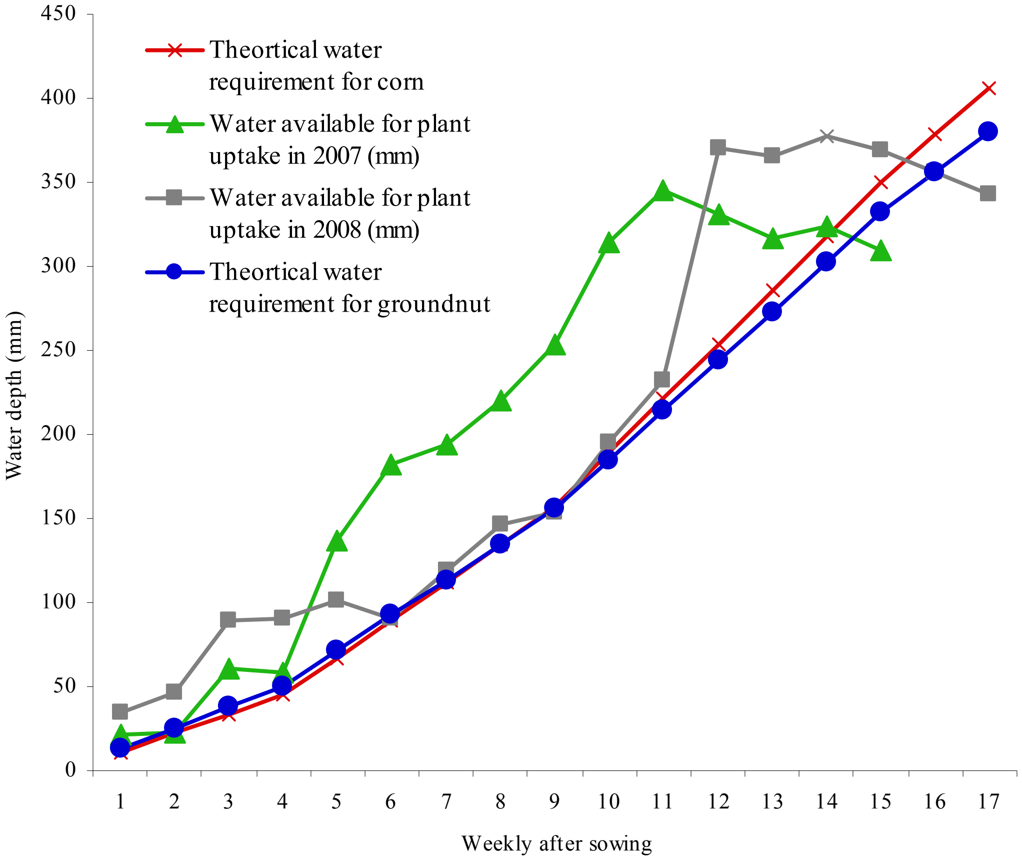


Figure 104 Available vs. required water for plant uptake at Ban Non Tun during the 2007 and 2008 experiments.

For groundnut, water availability is critical between the pegging and pod fill stages (during 11-12 WAS) while for corn, it is critical between the silking and milk stages (during 9-10 WAS) (Hollis, 2002; Wiatrak, 2010; Farahani and Smith, 2010). Figure 1 illustrates the fact that, in 2007 both groundnut and corn potentially suffered water deficit around the 9th WAS and that in both 2007 and 2008, there was a situation of water deficit from the 15th WAS. The first, short water deficit period is rather unlikely to have negatively impacted the yields of corn and groundnut. The second, and more durable water deficit period may have influenced the harvest quality of groundnut which normally ripens from 15 to 19 WAS (Brouwer and Heibloem,1986). During critical peak water use, yield loss due to water stress is substantial and estimated at 6-8% per day of stress (Farahani and Smith, 2010; Haise and Hagan, 1967; Taylor, 1965).

2.5 Results of the 2007 and 2008 field experiments on cassava inter-cropping

In this section we present the results of an experiment that was conducted to assess the development of roots in a rubber tree – cassava treatment in Kra Nuan District, Khon Kaen Province, Thailand, in 2007 and 2008. This treatment was not conducted at the main site in Ban Non Tun, were other treatments were investigated, due to the fact that the owner of the Ban Non Tun plantation did not agree to have cassava grown on his property. This experimental constraint precluded a direct comparison with the results obtained in Ban Non Tun with other inter-crops. However, the results obtained are reported as an indicative example of the type of rooting patterns that can be observed in a rubber tree – cassava inter-cropping system.

2.5.1 Experimental locations and age of plant materials

The 2007 experiment was conducted at Ban Khamhai, Kra Nuan District (N 16° 19’52.7”, E 102° 44’35.7”). At sampling time, i.e. on 25 March 2008, rubber trees and cassava were 34 and 8 months old, respectively. The 2008 experiment was conducted at Ban Srisomboon, Kra Nuan District. At sampling time, i.e. on 23 April 2009, rubber trees and cassava were 35 and 7 months old, respectively. In both cases, the distance between the rubber tree line and the cassava inter-crop was 2.30 m and samples were collected half-way in between (i.e. at 1.15 m from both cassava and rubber trees).

2.5.2 Results

The characteristics of the rooting patterns observed in the rubber tree – cassava treatments in 2007 and 2008 at Kra Nuan are reported in Table 9. The results correspond to unsorted samples, i.e. samples in which roots of both rubber tree, cassava and to some extent, weeds, are included. When considering each year separately, there was no significant variations in RLD, root surface area and soil volume with soil depth. The average root diameter decreased significantly ($p<0.05$, two-sided two samples t-test, $n=12$) from 25 to 45 cm in 2007 and increased significantly from 25 to 65 cm in 2008, but overall there was no differences between the two years. In contrast, RLD was significantly higher at soil depths of 25 and 45 cm in 2008 than in 2007 ($p<0.05$, two-sided two samples t-test, $n=36$).

Table 9 Results of the root measurements made on unsorted (rubber tree + cassava) root samples. For each of the four parameters presented, numbers in brackets are 95% confidence intervals.

	Root parameters	
	2007	**2008**
Soil Depth (cm)	**Root Length Density (cm / cm^3)**	
25 cm	0.119 (0.026)	0.429 (0.128)
45 cm	0.183 (0.038)	0.416 (0.051)
65 cm	0.207 (0.057)	0.340 (0.087)

Soil Depth (cm)	**Mean root diameter (mm)**	
25 cm	0.42 (0.02)	0.24 (0.02)
45 cm	0.36 (0.03)	0.27 (0.02)
65 cm	0.45 (0.03)	0.32 (0.04)

Soil Depth (cm)	**Root Surface Area (mm^2) / 100 cm^3**	
25 cm	150.7 (31.51)	330.45 (106.76)
45 cm	220.55 (48.47)	361.39 (60.08)
65 cm	315.62 (108.25)	261.02 (59.14)

Soil Depth (cm)	**Root Volume (mm^3) / 100 cm^3**	
25 cm	15.61 (3.46)	22.03 (8.05)
45 cm	22.48 (6.01)	26.47 (5.81)
65 cm	39.95 (16.51)	18.03 (4.01)

When considering the development of the above-ground parts of the rubber trees and cassava plants in 2007 and 2008 (Table 10), it appears that in 2007, although virtually the same age than in 2008, the rubber trees were taller, had a larger girth and were bearing more flushes than in 2008. As for cassava plants, although not taller nor bearing more branches in 2008 than in 2007, they had, on average a larger circumference in 2008.

It is noteworthy that, in conjunction with this increased RLD in 2008, cassava stems were thicker and rubber trees were shorter, thinner and developed fewer flushes. Further, it can be seen that roots at 25 and 45 cm in 2008 were also thinner than in 2007, which is consistent with the presence of more cassava roots, in relative terms, in 2008 than 2007 (rubber tree roots consistently being the thickest roots we have observed in all the treatments we have investigated, with an average root diameter of about 0.5 mm). Although these results are not sufficient to conclude firmly about the influence of cassava on rubber tree growth, they suggest that increased cassava vigor (as indicated by higher RLD values and cassava stem thickness in 2008 than in 2007) could be detrimental to the development of associated rubber trees.

Table 10 Results of the shoot measurements made on 30 rubber tree and cassava individuals. For each of the three parameters presented, numbers in brackets are 95% confidence intervals.

	2007	**2008**
Rubber tree		
Height (cm)	623 (16)	443 (2)
Circumference (cm)	13.83 (0.73)	9.93 (0.04)
Number of flushes	15.33 (0.67)	10.1 (0.04)
Cassava		
Height (cm)	168 (5)	150 (1)
Circumference (cm)	4.83 (0.17)	6.05 (0.03)
Number of branches	2.67 (0.33)	2.53 (0.02)

CHAPTER V - GENERAL DISCUSSION AND CONCLUSIONS

1. Rhizobox experiment - discussion

1.1 Methodological advances in the study of root growth dynamics

This experiment was carried out to investigate the effects of candidate annual inter-crops, primarily corn, but also groundnut and cassava, on rubber root growth. Experiments conducted since the mid-1970s have consistently indicated that the inter-cropping of immature rubber can be both economically and agronomically advantageous (Laosuwan, 1996). While it represents a source of income to small farmers during the immature stage of a plantation, it was found to have no detrimental, and under some circumstances, beneficial impacts on the development of rubber trees (Laosuwan et al., 1988).

While there exists a vast body of literature on belowground interactions between plants, most of the existing research on this topic is based either on static observations at a given point in time (Schenk et al., 1999), or of the growth dynamics of individual roots that encounter each other (Mahall and Callaway, 1991). In contrast, in this work, we examined the dynamics of belowground interactions in an inter-specific system, at both the whole root system level and that of the individual root, based on detailed digital descriptions of root systems generated using the DART software (Le Bot et al., 2009).

Digitizing complex root architectures such as that studied in this work was labour intensive: root systems included, on average, more than 4000 individual links (in excess of 8000 in the case of one corn root system), which required an average 40 hours of work per root system. However, this was counterbalanced by the fact that once digitized, a vast range of root growth indicators could be computed. Here, we even showed that novel approaches such as the study root system growth trajectories, which were found to provide new insights into belowground plant interactions, could be developed based on DART records.

A major difference between our experimental setup and that used in previous studies about the influences of separate root systems on one another is that no particular measure was taken to constrain the roots of one plant to encounter that of its neighbour. However, it must be noted that, by design, the rhizoboxes induced a virtually 2-dimensional root system development, which may be seen as factor maximizing the likelihood of inter-individual root contacts. Nevertheless, the spacing between plants (50 cm), even though relatively small, was of the same order of magnitude as common inter-row spacings implemented in the field.

1.2 Growth of the below-ground component

In the case of the corn-rubber tree association, the growth and architectural characteristics of both plants, particularly the overall root lengths, proportions of different branching orders, and root elongation rates were consistent with previously reported values; this indicates that, despite a limited number of replicates, our experimental conditions did not introduce any bias likely to invalidate the results presented. For example, consistently with the findings of Pagès and Pellerin (1994), the length distribution of corn lateral roots was of the same order of magnitude and highly asymmetrical, for all phytomers (mean: 32 mm; median: 10 mm). Similarly, growth rates of both corn and rubber tree roots were comparable to that previously reported (Le Roux, 1994; Pagès and Pellerin, 1994).

The results of the rhizobox experiments also suggest that, in the case of the corn-rubber association, plants were able to sense and adjust their root system development according to that of their neighbour. Such a scenario is supported by the facts that: 1. the growth trajectories of corn root systems were initially oriented towards the rubber trees, 2. corn plants grew unusually long laterals when some of their main axile roots were close to rubber tree roots, 3. both individual corn and rubber tree roots grew at lower elongation rates following encounters with each other, 4. the overall root length expansion of rubber trees was significantly higher in the presence of a corn neighbour and while the overall growth rate of corn was the highest (i.e until ear formation), and 5. rubber and corn root expansion rates varied concomitantly.

However, such sensing of each other plant's roots could not be confirmed in the case of the cassava–rubber association, for which no effect of root encounters on the growth rates of either rubber tree or cassava roots could be detected, even though cassava developed profuse root systems that extensively overlapped that of neighbour rubber trees. This absence of responsiveness might be related to the fact that both cassava and rubber tree belong to the *Euphorbiaceae* family; assuming a chemical mediation of the root growth regulation between individual plants, cassava and rubber could therefore have root exudate 'fingerprints' similar enough for the two plant species not to sense the presence of each other. This would not be the case in the corn-rubber association, hence the contrasted root growth dynamics in these two treatments. Likewise, no sensing of each other plant's roots could be observed in the case of the groundnut – rubber association, but this time, this was probably mainly due to the lack of proximity and absence of physical contact between two plants, which only encountered each other in <20 % of the attempted replicates.

In the case of the corn–rubber association, since care was taken to supply water and nutrients in unlimited amounts, the likelihood that the rooting patterns observed are related to competition for resources appears very remote. In this context it seems legitimate to consider the putative existence of a communication mechanism that enabled corn, and to some extent rubber, to detect and adjust their root system development according to the presence of roots of another plant species within the soil volume they could potentially explore. In the case of rubber trees, changes in root system trajectories were of much lower magnitude than in corn, and it therefore remains unclear whether the two studied species are able to deploy the same strategies to adapt their root system development to that of their neighbour. It is also possible that, given the differences in average root system expansion between the two plants (corn producing at least 5 times more root length daily than the rubber tree seedlings), rubber trees were not in a position to deploy a pre-emptive behaviour that could have been of any use against that of corn. While self-inhibition, i.e. reduced resource allocation to less promising parts of the root system (Falik et al., 2003; 2005) appears to have possibly been at play in rubber trees, which displayed reduced root elongation in both inter- and intra-specific root encounters with corn, this mechanism cannot be invoked for corn which did not react to intra-specific encounters.

Recent evidence suggests that roots are able to detect and avoid the presence of neighbouring roots (Krannitz & Caldwell 1995), and to segregate spatially in 'territories' (Schenk et al., 1999). Root segregation appears to be particularly frequent in resource-limited environments (Schenk et al., 1999); at the whole root system level, root segregation can provide competitive advantages for water and nutrient uptake (Casper and Jackson, 1997) as well as spatial benefits such as limited overlap between individual root systems (Brisson and Reynolds, 1994). Segregated root placement could, at least partly, result from an avoidance mechanism of soil volumes under the influence of other plants, such as soil containing exudates of other roots (Krannitz and Caldwell, 1995). Falik et al. (2003) reported that self/non-self discrimination was partly related to allogenic recognition and to physiological coordination among root parts of the same plant. In their experiments with *Pisum sativum*, they found that root contacts never occurred, a fact they interpreted as a result of self/non-self discrimination. However, such results do not preclude the possibility that, in other species, self/non-self discrimination combined with inter-specific inhibitory effects of root exsudates could promote root growth towards a neighbour and even root encounters. However, some authors have reported that chemically based allo-recognition is unlikely due to rapid decomposition of organic compounds used as "identifying molecules" (Falik et al., 2003). Alternative mechanisms have been suggested, such as a combination of hormonal and electrical oscillations (Souda et al., 1990) that might be perceived by neighbouring roots without direct contact.

Nevertheless, in our experiments, the clear effect of corn and rubber on each other's root growth rates, compared to the absence of effect of cassava and rubber seems to advocate in favour of chemically mediated processes: in this context, the working hypothesis would be that two members of the same family (*Euphorbiaceae*) secrete root exudates that do not act as root growth inhibitors on other members of this family, while corn (a member of the *Poaceae* family) would be sensitive to rubber exudates and vice-versa. The groundnut (a member of the *Fabaceae* family) – rubber tree treatment was expected to provide further evidence of this putative chemical mediation, but as previously explained, due to the poor development of the groundnut plants, this could not be achieved during this study.

1.3 Growth of the aboveground component

In addition to the work on root development, we also examined the influence of the tested plant associations, i.e. corn, cassava and groundnut with rubber tree, on the growth of the above-ground part of the plants.

Overall, both corn and cassava produced much more stem length, leaf area, shoot dry weight (and also root dry biomass), than young rubber trees grown alone or in association with these two plant species. Due to the specific characteristics of these plant species, by the end of the experimental period (11 weeks), both cassava and corn plants were quite higher than the young (8-12 months old) rubber trees. This has significant implications for competition for light between rubber tree and these two species.

In contrast, groundnut developed on average, less stem length, shoot dry weight and root dry weight than young rubber trees associated or not with groundnut. Only the leaf area of groundnut plants was higher than that of young rubber trees.

With regards to the development of young rubber trees, the results of this study show that all general development parameters were slightly higher (although not a statistically significant level in most cases) for trees grown in associated with an intercrop (either corn, cassava and groundnut) than control rubber trees (grown alone).

One reason why no significant effects of inter-cropping on rubber tree development could be detected during this experiment might be related to the fact that the experimental period corresponded to only one inter-cropping cycle. In real plantations, successive inter-cropping cycles might have cumulative effects on the development of rubber trees, and such effects might be statistically significant.

2. Field experiments - discussion

Despite the fact that the rhizobox experiments provided evidence, at least in the case of the corn – rubber tree combination, of processes that alter root growth at both the individual root scale and that of the entire root system, field experiments yielded, not unexpectedly, a more complex picture of below-ground interactions between rubber tree and inter-cropped plants. The high variability of rooting patterns observed in the field was most likely related to the fact that biophysical factors, particularly soil heterogeneity and climate, triggered plastic root growth responses in both rubber tree and inter-crops (Hodge, 2004; Pierret et al., 2007a). Overall though, in both the 2007 and 2008, it could be verified that rubber tree roots were consistently thicker than roots of inter-crops (or that of weeds in control treatments). Rubber tree also developed roots of low SRL (within a range of 5 to 10 m/g) that resulted in high (relatively to inter-crops) soil volume occupancies, particularly at soil depths more than 50 cm, while inter-crop and weed roots were on average at least twice thinner than rubber tree roots and were characterized by much higher SRL than rubber (ranging from 20 to 40 m/g) and comparatively low soil volume occupancies. These characteristics correspond to a contrasted soil exploration strategy between the two plants, with rubber trees investing in costly - but most likely long-lived – roots of low SRL (Bouma et al., 2001; Fitter et al., 1991), while annual crops and weeds would favour the allocation of assimilates to cheaper roots, grown more quickly (as confirmed by the higher root growth rates measured during the rhizobox experiments).

Although the root length density of rubber tree increased markedly from 2007 to 2008 in the groundnut and corn treatments, RLD did not prove a conclusive indicator of competition/complementarity of rubber tree and inter-crops. It was however observed that, in all treatments during the 2008 experiment, the RLD of inter-crops dropped below 50 cm, indicating that most of the below-ground interactions between rubber tree and inter-crops likely occurred in shallow soil horizons, at least in the context studied. Consistently with what was observed during the rhizobox experiments, there was, in 2008 a marked increase in the RLD of rubber tree grow in association with corn, compared to that in other treatments.

Finally, except in the case of cassava (but the corresponding observations were made at another field site), inter-cropping rubber tree with corn and groundnut did not have any significant impact on the development of the rubber trees, as indicated by their girth, height and number of flushes. This result obtained under field conditions is consistent with the results of the rhizobox experiments which also showed no suppressive effect of inter-cropping on the above-ground development of rubber trees.

3. Perspectives for future research

Some of the findings of these experiments appear worth being investigated further, from a more applied/agronomic perspective. For example, it is noteworthy that, over the short observation period of our experiment, neither the above- and below-ground parts of rubber trees appeared to be negatively affected by the presence of nearby inter-crop. If this were confirmed under a wide range of field conditions and over longer periods of time, this would be of influential importance for farming practices. Timing issues need to be documented in detail. For example, Collet et al. (2006) reported that the size of oak root system was considerably reduced by grass competition, even though branching density was not affected. It therefore seems important to clarify the roles of seasonal inter-crops from that of perennial covers such as grass.

Another finding presented in this work that may prove worth investigating further is whether and how some of the “territorial” rooting behaviours observed in rhizoboxes occur under field conditions, and assuming that they do, how they could be used to shape the root system architecture of rubber trees. Ecological research on underground interactions between plants has recently indicated that competition for bio-available nutrients is driven by diverse mechanisms and strongly depends on soil, nutrient, and plant properties (e.g. Raynaud et al., 2008): facilitation would dominate under high-stress conditions, while competition would dominate under lower-stress conditions. Accordingly, on heavily weathered, phosphorus deficient soils, the acidification of the rhizosphere by faba bean inter-cropped with corn was found to mobilize phosphorus in soil volumes and amounts sufficient to benefit the growth and yield of corn (Li et al., 2007). Similarly, using appropriate inter-cropping techniques to stimulate rubber tree root growth towards deeper and moister soil layers could prove beneficial for the long-term productivity of a plantation. This could be of particular interest in locations, such as NE Thailand, where a seasonal drought prevails.

A final area of needed research is to understand how root growth coordination, such as that discussed in this work, translates in terms of crop yields. It has been reported that spatial and temporal shifts in rooting patterns induced by intercrops can be detrimental to the yields of one of the species, due to competition between root systems for one or more resources (Celette et al., 2005; Collet et al., 2006; Li et al., 2006). However, and more unexpectedly, other reports show that combining plant species can lead to yield increases, putatively due to enhanced soil exploration (Li et al., 2006; Mulia and Dupraz, 2006; Malezieux et al., 2009). While detailed analyses of intra- and inter-specific root interactions such as that presented in this work are not sufficient to fully unravel how species grown in combination can functionally complement each other, they represent an essential step towards the design of sustainable agro-ecosystems, which are much needed to meet the worldwide growing demand for food and raw materials.

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APPENDICES

Appendix 1 Composition of a Modified Hoagland Nutrient Solution for Growing Plants

I. Macronutrients

Compound	Molecular weight (g mol^{-1})	Concentration of stock solution (mM)	Concentration of stock solution (g L$^{-1)}$	Volume of stock solution per liter of final solution (ml)	Element	Final concentration of element (µM)	Final concentration of element (ppm)
KNO_3	101.1	1000	101.1	6	N	16000	224
					K	6000	235
$Ca(NO_3)_2$ $4H_2O$	236.16	1000	236.16	4	Ca	4000	160
$NH_4H_2PO_4$	115.08	1000	115.08	2	P	2000	62
$MgSO_4$ $7H_2O$	246.47	500	123.24	2	S	1000	32
					Mg	1000	24

II. Micronutrients

Compound	Molecular weight	Concentration of stock solution (mM)	Concentration of stock solution (g L$^{-1)}$	Volume of stock solution per liter of final solution (ml)	Element	Final concentration of element (µM)	Final concentration of element (ppm)
KCl	74.55	25	1.864	2	Cl	50	1.77
H_3BO_3	61.83	12.5	0.773		B	25	0.27
$MnSO_4$ H_2O	169.01	1	0.169		Mn	2	0.11
$ZnSO_4$ $7H_2O$	287.54	1	0.288		Zn	2	0.13
$CuSO_4$ $5H_2O$	249.68	0.25	0.062		Cu	0.5	0.03
H_2MoO_4 (85% MoO_3)	161.97	0.25	0.04		Mo	0.5	0.05
NaFeDTPA (10% Fe)	558.5	53.7	30	0.3-1	Fe	16.1-53.7	1.00-3.00

Appendix 2 Flush number of rubber trees - 2007 rainy season.

Treatment	**Flush number of rubber trees - 2007**								
	0 DAS	**14 DAS**	**28 DAS**	**42 DAS**	**56 DAS**	**70 DAS**	**84 DAS**	**98 DAS**	**112 DAS**
Rubber alone	11.84	11.84	12.61	13.49	13.74	14.20	14.20	14.20	14.20
Rubber + Groundnut	11.86	11.86	12.48	13.30	13.67	14.02	14.09	14.09	14.09
Rubber + Corn	12.30	12.30	13.20	13.73	14.05	14.40	14.46	14.46	14.49
P-value	0.5753	0.5753	0.2518	0.6299	0.667	0.7038	0.7026	0.7026	0.6578
F-test	ns	ns	ns	ns	ns	ns	ns	ns	ns
LSD	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CV	16.37	16.37	14.32	13.48	12.9	12.58	12.79	12.79	12.79

Appendix 3 Girth of rubber trees - 2007 rainy season.

Treatment	Girth number of rubber trees - 2007								
	0 DAS	**14 DAS**	**28 DAS**	**42 DAS**	**56 DAS**	**70 DAS**	**84 DAS**	**98 DAS**	**112 DAS**
Rubber alone	13.27	13.27	13.66	14.05	14.34	14.61	14.98	15.02	15.26
Rubber + Groundnut	12.76	12.76	13.17	13.54	13.82	14.16	14.39	14.51	14.63
Rubber + Corn	12.83	12.83	13.27	13.64	13.90	14.18	14.47	14.55	14.69
P-value	0.6546	0.6546	0.7037	0.6984	0.6934	0.7486	0.6505	0.7171	0.6239
F-test	ns	ns	ns	ns	ns	ns	ns	ns	ns
LSD	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CV	18.51	18.51	18.48	18.4	18.54	18.65	18.82	19.01	19.08

Appendix 4 Height of rubber trees - 2007 rainy season.

Treatment	Height number of rubber trees - 2007								
	0 DAS	**14 DAS**	**28 DAS**	**42 DAS**	**56 DAS**	**70 DAS**	**84 DAS**	**98 DAS**	**112 DAS**
Rubber alone	5.35	5.35	5.35	5.42	5.43	5.45	5.46	5.47	5.48
Rubber + Groundnut	5.13	5.13	5.16	5.20	5.24	5.26	5.28	5.29	5.30
Rubber + Corn	5.19	5.19	5.22	5.26	5.28	5.30	5.31	5.32	5.34
P-value	0.5137	0.5137	0.6357	0.5121	0.5883	0.5937	0.6296	0.6413	0.6316
F-test	ns	ns	ns	ns	ns	ns	ns	ns	ns
LSD	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CV	15.42	15.42	15.44	14.94	14.84	14.83	14.85	14.82	14.76

Appendix 5 Height of intercrops - 2007 rainy season.

Treatment	Height of intercrop - 2007 (cm)						
	28 DAS	**42 DAS**	**56 DAS**	**70 DAS**	**84 DAS**	**98 DAS**	**112 DAS**
Groundnut	20.33	26.44	30.87	33.20	35.33	34.78	34.78
Corn	47.92	63.43	103.83	109.10	110.87	harvested	harvested

Remark: DAS stands for Days After Sowing of intercrop; groundnut and corn

Appendix 6 Flush number of rubber trees - 2008 rainy season.

Treatment	Flush number of rubber trees - 2008									
	0 DAS	**14 DAS**	**28 DAS**	**42 DAS**	**56 DAS**	**70 DAS**	**84 DAS**	**98 DAS**	**112 DAS**	**126 DAS**
Rubber alone	13.43	13.43	13.43	13.43	13.47	13.50	13.50	13.50	13.50	13.50
Rubber + Groundnut	12.70	12.70	12.70	12.73	12.77	12.97	12.97	12.97	12.97	12.97
Rubber + Corn	12.60	12.60	12.63	12.63	12.63	12.93	12.93	12.93	12.93	12.93
F-test	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LSD	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CV	15.01	15.01	15.15	15.12	14.98	14.87	14.87	14.87	14.87	14.87

Appendix 7 Girth of rubber trees - 2008 rainy season.

Treatment	Girth of rubber trees - 2008 (cm)									
	0 DAS	**14 DAS**	**28 DAS**	**42 DAS**	**56 DAS**	**70 DAS**	**84 DAS**	**98 DAS**	**112 DAS**	**126 DAS**
Rubber alone	16.09	16.17	16.28	16.34	16.43	16.54	16.73	16.89	17.01	17.17
Rubber + Groundnut	17.48	17.56	17.61	17.67	17.74	17.81	18.00	18.12	18.24	18.33
Rubber + Corn	17.56	17.66	17.75	17.85	17.98	18.13	18.33	18.48	18.66	18.81
F-test	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LSD	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CV	21.41	21.27	21.43	21.44	21.33	21.39	21.5	21.57	21.57	21.75

Appendix 8 Height of rubber trees - 2008 rainy season.

Treatment	Height of rubber trees - 2008 (m)									
	0 DAS	14 DAS	28 DAS	42 DAS	56 DAS	70 DAS	84 DAS	98 DAS	112 DAS	126 DAS
Rubber alone	5.79	5.80	5.81	5.81	5.83	5.84	5.86	5.87	5.88	5.88
Rubber + Groundnut	5.93	6.05	5.96	5.98	5.99	6.00	6.02	6.03	6.05	6.06
Rubber + Corn	5.94	6.06	6.07	6.08	6.09	6.13	6.15	6.16	6.17	6.12
F-test	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LSD	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CV	14.16	14.53	13.04	13.01	13.05	12.99	13.05	12.98	12.97	13.46

Appendix 9 Height of intercrops - 2008 rainy season.

Treatment	Height of intercrop - 2008 (cm)						
	28 DAS	42 DAS	56 DAS	70 DAS	84 DAS	98 DAS	112 DAS
Groundnut	22.03	37.47	49.83	59.48	60.77	61.53	62.03
Corn	39	86.5	146.7	149.35	151.13	152.03	harvested

Remark: DAS stands for Days After Sowing of intercrop; groundnut and corn

Abstract

For obvious agronomic and environmental reasons, being able to design and implement agro-ecosystems in which crops have optimal access to resources is of pivotal importance to all stakeholders involved in agricultural production. Intensification techniques such as agro-forestry or the introduction of inter-crops aim to increase land productivity while conserving geochemical cycles, to ensure the sustainability of agro-ecosystems through an optimized use of environmental resources (light, water and nutrients). In theory, there are many ways of achieving such a goal, but in practice, below-ground interactions between plants are complex and difficult to measure, so that progress with the development of sustainable agro-ecosystems has been slow and remains modest. In this context, the objective of this work was to assess the effects of inter-crops on the growth of young rubber trees, based on a detailed analysis of below-ground interactions between the associated plants. The dynamics of below-ground interactions has been studied in rhizoboxes, at both the scale of individual roots and that of the whole root system, using detailed numerical descriptions of root architecture. Such an approach resulted in the design of an innovative method for the analysis of the entire root system dynamics, namely, the analysis of growth trajectories. In the case of the maize-rubber tree association, the experiments in rhizoboxes showed that the below-ground interactions between these two plants can induce changes in root growth, at both the individual root and the whole root system levels. However, such a coordination of rooting patterns could not be confirmed in the case of the cassava-rubber tree and groundnut-rubber tree associations. Not unexpectedly, field experiments provided a rather complex picture of the underground interactions between rubber trees and inter-crops. However, initial results obtained using 'root traps' in a cowpea-rubber tree treatment indicated that these two plants were unlikely to have a marked underground competitive behavior relative to each other. Results of field experiments also indicated that, in general, rubber trees seem to 'invest' in 'expensive roots' of low specific root length, presumably to confer some degree of durability to these organs, while inter-crops favoured the allocation of assimilates to 'cheaper' roots, i.e. roots of much higher specific length, probably in response to a 'fast growth imperative' (an hypothesis supported by the root elongation rate values measured during the rhizobox experiments). Finally, to the possible exception of cassava, inter-crops were found to have no significant impact on the development of young rubber trees, as evidenced by measured changes in tree circumference, height and leaf development. This result is consistent with results from the rhizobox experiments which also showed no inhibitory effect of inter-crops on the above-ground development of rubber trees. Although the work presented in this report does not allow, in itself, to conclude firmly on the issue of the functional complementarity between plants grown in association in inter-cropping systems, they provide preliminary answers to this complex issue together with methods to obtain such answers. Overall, this work therefore represents a contribution to the design of sustainable agro-ecosystems which are becoming increasingly needed in the context of a growing global demand for food and raw materials. In addition, some of the results generated by this work open up prospects for future research for the development of sustainable agro-ecosystems.

Key words: root, root system growth and dynamics, rhizobox, rubber tree (*Hevea brasiliensis* Muel. Arg), inter-crop, below-ground interactions

Résumé

Pour d'évidentes raisons agronomiques et environnementales, être en mesure de concevoir et mettre en place des systèmes de culture dans lesquels les plantes accèdent aux ressources de manière optimale revêt une importance cruciale pour tous les intervenants impliqués dans la production agricole. Les techniques d'intensification telles que la mise en place de cultures d'inter-rang et l'agro-foresterie visent à accroître la productivité globale des terres tout en assurant la durabilité des agro-écosystèmes, via une optimisation de l'utilisation des ressources environnementales (lumière, eau et nutriments) par les plantes et une préservation des cycles géochimiques. En théorie, les moyens d'atteindre ces objectifs sont nombreux mais en pratique, les interactions souterraines sont complexes et difficiles à mesurer, de sorte que les progrès réalisés dans la conception d'agro-écosystèmes améliorés et durables demeurent modestes. Dans ce contexte, l'objectif de ce travail a été d'évaluer, au travers de mesures racinaires detaillées en rhizotron et au champ, les effets des cultures d'inter-rang sur la croissance des jeunes hévéas. La dynamique des interactions souterraines a été étudiée, tant au niveau de la racine individuelle qu'à celui du système racinaire entier, sur la base, notamment, de descriptions numériques détaillées. Une telle approche a permis de proposer, en outre, une voie novatrice pour l'analyse de la dynamique racinaire à l'echelle du systeme racinaire entier, à savoir l'analyse des trajectoires de croissance. Dans le cas de l'association maïs-hévéa, les expérimentations en rhizotron ont permis de mettre en evidence que les interactions souterraines entre ces deux plantes peuvent induire des modifications de la croissance de leurs racines, à la fois à l'échelle de la racine individuelle et à celle du système racinaire entier. Toutefois, une telle coordination des dynamiques racinaires des plantes associées n'a pas pu être confirmée dans le cas des traitements manioc-hévéa et arachide–hévéa. Les expérimentations au terrain ont fourni, de manière assez prévisible, une image complexe des interactions souterraines entre hévéa et cultures d'inter-rang. Toutefois, un premier résultat obtenu par le biais de la mise en place de 'pièges à racines' dans un traitement niébé-hévéa, a été de montrer que ces deux plantes n'avaient pas un comportement compétitif marqué l'une vis-à-vis de l'autre. Il est également apparu que les hévéas paraissent 'investir' dans des racines 'coûteuses', car de faible longueur spécifique, probablement pour assurer une certaine durabilité de ces organes, tandis que les cultures d'inter-rang favorisent l'allocation des assimilâts vers des racines de longueur spécifique élevée, de construction moins 'coûteuses', probablement en réponse a un impératif de croissance plus rapide (suggéré par les taux d'élongation racinaire mesurés au cours des expérimentations en rhizotron). Enfin, excepté le cas du manioc, l'introduction de cultures d'inter-rang telles que le maïs et l'arachide n'a pas eu d'impact significatif sur le développement des jeunes hévéas, comme en attestent l'évolution de leur circonférence, hauteur et développement foliaire. Ce résultat de terrain est compatible avec les résultats des expérimentations en rhizotron qui n'ont démontré aucun effet inhibiteur des cultures d'inter-rang sur le développement de la partie aérienne des hévéas. Bien que les travaux présentés dans ce rapport, ne permettent pas, à eux seuls de conclure de manière définitive sur la façon dont les espèces cultivées en association peuvent se compléter mutuellement sur le plan fonctionnel, ils apportent des éléments de réponse préliminaires à cette question complexe ainsi que des méthodes permettant de les obtenir. Au total, ce travail représente donc une contribution à la conception des agro-écosystèmes durables qui deviennent de plus en plus indispensables dans le contexte d'une demande mondiale croissante en produits alimentaires et matières premières. En outre, certains des résultats obtenus dans le cadre de cette thèse ouvrent des perspectives pour des recherches plus approfondies, avec une finalité agronomique appliquée.

Mots clé: racines, croissance et dynamique du système racinaire, rhizotron, hévéa (*Hevea brasiliensis* Muel. Arg), cultures d'inter-rang, interactions souterraines