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Research Organizations involved in the study

Sporometrics Inc., Canada; National Centre of Agronomic Research (CNRA), Côte d'Ivoire; University Nangui Abrogoua (UNA), Côte d'Ivoire;

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1.0 Executive Summary

Overall significant research findings, key advances, outcomes and innovative outputs are summarized for each corresponding specific objective.

Objective 1) to better understand the bio-ecology of the CILY phytoplasma, and the environmental and socio-economic factors involved in its epidemiology, and the impact of the disease on the coconut industry. The CILY phytoplasma was mapped in over 80 % of the CILY-affected and 30 % of asymptomatic coconut palms with a predicted westward spread within Grand-Lahou. Trunk borings were recommended as the most suitable plant tissue for sampling. The epidemiological origin and spread of the CILY phytoplasma was hypothesized to be from either the Central or West Regions in Ghana affected by the Cape St. Paul Wilt Disease (CSPWD) that destroyed the Ghanaian coconut industry in the last 20 years. The CILY phytoplasma was phylogenetically distinguished from the strains in Ghana and Mozambique, and from the rest of the LY phytoplasmas in Central and North America, the Caribbean, South Asia and the Pacific. A new genus and species within the Typhlocybinae subfamily, Nedotepa curta Dimitriev was found as the potential vector of the CILY phytoplasma, and six different plant species from five botanical families identified as alternative hosts that may play a role in disease spread: Poaceae (Paspalum varginatum, Pennisetum pedicillatum), Verbenaceae (Stachytarpheta indica), Plantaginaceae (Scoparia dulcis), Phyllantacea (Phyllantus muellerianus) and Cyperacea (Diplacrum capitatum). Phytoplasmas of group 16Srl were found in mixed infection with the CILY phytoplasma in 5 % of the palms, which may imply a polyphagous potential insect vector leading to a more complex epidemiology and control of the disease. The assessment of the socioeconomic impact of CILY revealed over 90 % of farmer illiteracy in Grand-Lahou, and very limited access for women to land, training and market, which urged approaches to better educate farmers and empower women. An econometric model was developed to predict the land use change of the coconut cultivated area and to re-allocate the CILY-devastated areas and help reviving the coconut industry in Grand-Lahou.

Objective 2) to develop and adapt control strategies to reduce the impact of the CILY. New diagnostics corresponding to LAMP (loop-mediated isothermal amplification) based on the ribosomal protein gene of the CILY phytoplasma were developed for its early and specific detection in both field and laboratory. These set new paths for further disease epidemiology research, and are important tools for Ivoirian plant health authorities and research organizations to support CILY disease surveillance, quarantine, screening, and management. Nine coconut cultivars and hybrids were recommended for long-term screening and assessment of their resistance based on their genetically closely relatedness to the hybrid resistant to CSPWD in Ghana. Cultivars and hybrids were planted in five pilot farms in Grand-Lahou, and two farms in Ghana for their follow-up within the post-project action plan. New bacterial and fungal endophytes with biocontrol potential for the CILY phytoplasma were identified, which included Bacillus, Enterobacter, Streptomyces, Aspergillus, Candida, Cryptococcus, Purpureocillium, Burkholderia Pseudomonas, Trichoderma, Penicillium, and Fusarium with cultures available for further field-testing; as well as, a new bacterial endosymbiont Pectobacterium within the potential vector Nedotepa curta. These may be used for further long-term disease and vector control programs. For the very first time on phytoplasma research, a new species of parasitoid within the genus Anagrus (Anagrus nedotepae) was identified parasitizing the eggs of the potential vector Nedotepa curta, which opens more feasible and practical ways of vector control, and a new source of income for farmers due to the very fast and easy-to-implement massive rearing of the parasitoid. Farmers were recommended to intercrop coconut with plantain for higher revenue, and to apply poultry manure since results indicated that this latter boosts flower development, which will lead to a higher volume of seednuts for farmers. No seed-borne transmission was found for the CILY phytoplasma; however, instructions for farmers on how to manage seednut exchange have been included in the disease management plan and as a policy brief. The follow-up of the assessment and cost-benefit analyses of both

intercropping and poultry manure trials was also included within the post-project action plan. A new disease management plan was developed and translated into a factsheets, a farmer field mini-guide and policy briefs for farmers, stakeholders and policy makers.

Objective 3) to strengthen local capacity to diagnose, implement and disseminate control strategies, through the involvement of natural and social scientists, authorities, policy makers, stakeholders and farmers, empowering women's role in addressing gender inequity in Grand-Lahou. Nine training-courses on field and lab basic and advanced technology for phytoplasma diagnosis and research, and over ten workshops, including Gender Workshops were held in Côte d'Ivoire, Ghana and Canada. A total of six Ivoirian MSc students and 1 PhD graduated, and 4 MSc are already involved in PhD programs. The Ministry of High Education and Scientific Research of Côte d'Ivoire attended the last workshop 'Lessons Learned from CSPWD'. New approaches were innovated to increase disease awareness in farmers, stakeholders and policy and decision makers, as well as to empower women within the coconut production chain. These included: field schools, plant clinics, Women Groups and Women Coconut Fairs. Ten field schools trained 1,960 farmers (1,568 men and 392 women for around 700 families) and 180 extension agents on proper coconut farming and disease management, which superpassed the expected figures. Nine plant clinics mobilized over 671 farmers, villagers and processors (478 men, 193 women), and addressed plant diseases not only in coconut, but also in over 20 other crops such as cocoa, cassava, citrus, okra, coffee, peanut, yam, beet, plantain, tomato, maize, etc. The Major and Prefect of Grand-Lahou, the Canadian Ambassador in Côte d'Ivoire, Representatives of the Ivoirian High Education and Scientific Research Ministry, and ten village Chiefs attended the plant clinic held at Braffedon in April 2016. A new disease management plan, and a 3year rehabilitation plan were generated and delivered to farmers, stakeholders and policy makers as factsheets, a farmer field mini-guide, and three policy briefs on CILY environmental and mitigation plans, control of movement of coconut germplasm, and dissemination of good cultural practices. Eight Women Coconut Fairs mobilized 550 participants (390 men, 160 women), and allowed women sell out their coconut products, from crafted and food products, cosmetics to home furniture, whose number increased from 14 to 26 (64 %) from 2015 to 2016. Women coconut farmers organized themselves for the very first time in Grand-Lahou in six Women Groups, currently planting cassava yards as a new source of income. A video on project impact in the coconut farming community of Grand-Lahou is available at COWALY. The video is currently under validation for its launching on Canada TV. The in-field detection LAMP technology was transferred to UNA and CNRA partners through a hands-on training-course held at UNA during November 2016. New recent findings include the identification of the CILY phytoplasma infecting cassava orchards nearby the CILY-affected coconut farms, which impacts directly on food security for the smallholder coconut farmers of Grand-Lahou. The project was an example of good partnership between natural and social scientist from Sporometrics, UNA, CNRA and third-party organizations in project implementation and dissemination. It strengthened collaboration among scientists from Canada, Ghana, Italy, France, and Mozambique, and enhanced the international image of Canada supporting research in African developing countries.

A post-project action plan was developed to be implemented till February/2020 to support and assess the continuation of project activities that require long-term monitoring, as well as for the adoption of the gender and communication strategies. Details on project outcomes are provided in the following sections and annexes, and at the COWALY website (http://cowaly.com/progress).

2.0 Research problem

Côte d'Ivoire lethal yellowing (CILY) has impacted farmers' family nutrition and limited women's income in Grand-Lahou. It was anticipated that CILY would decimate the Ivorian coconut grove by 2020 if prompt actions were not taken. Therefore, the general objective of the research was to provide and communicate new needed information to allow authorities, policy makers, stakeholders and farmers to better control CILY. The overall progress of project implementation and outcomes contributed to provide that information, which became innovative knowledge contributions and gender-responsive solutions to engage famers, stakeholders and policy makers in addressing the disease problem.

Conventional strategies for phytoplasma containment are based on vector control combined with good field management, and resistant cultivars, when available. Therefore, promising putative resistant cultivars were urged to be locally identified for long-term monitoring in resistance trials. The promising cultivars identified in the project were planted in pilot coconut farms under different disease pressure levels, whose outcome after monitoring will provide farmers with reliable(s) CILY-tolerant cultivars suitable for a rehabilitation plan. Moreover, data from gender-responsive socio-economic surveys allowed the development of a econometric model to assess the coconut land use change. Such plan is required for the re-orientation of the coconut industry in Côte d'Ivoire assisted by the econometric model to re-allocate the areas already devastated by CILY in Grand-Lahou. Information on the plant-pathogen-vector interaction is critical for any control strategy for phytoplasma diseases. CILY phytoplasma was characterized by using ribosomal and non-ribosomal genes, which revealed its incidence, distribution, possible origin, and distinction among western and eastern variants for further prediction of future outbreaks. Besides, since early disease detection methods needed to be implemented, data was used by project partners to develop a LAMP method for the in-field specific detection of the charecterized CILY phytoplasma to support surveillance, resistance screening and prevent disease spread. Very little information exists in West Africa for LY vector or secondary plant hosts, so the identification of the potential vector and secondary plant hosts of the CILY phytoplasma was used to generate a disease management plan with guidelines for alternative plant hosts' removal and monitoring potential vector populations. Such plan was made available to farmers, stakeholders and policy makers as policy briefs and a farmer field mini-guide.

New trends for phytoplasma disease control rely on endophyte biocontrols and bacterial endosymbionts. Although no information exits on the natural enemies of phytoplasma vectors, the interest increases in finding potential parasitoids to reduce phytoplasma vector populations. Project partners identified bacterial and fungal endophytes, and a new species of parasitoid with potential of becoming a new income source for farmers. Pure cultures and parasitoid rearing are currently available for further field testing of their biocontrol potential. A bacterial endosymbiont was identified within the potential vector, which could be used in a long-term plan for phytoplasma vector endosymbiotic control.

Gender inequalities revealed through gender-responsive socio-economic surveys imposed the need to implement approaches to empower women coconut farmers. Field schools, plant clinics, Women Coconut Fairs, and Women Groups planting cassava yards as alternative cash crop were the most effective tools, especially for women to training, increasing disease awareness, and generating income to support family nutrition. These approaches are to be generalized in other coconut-growing areas of Grand-Lahou and Côte d'Ivoire through a post-project action plan, which was developed to support the project activities that require long-term follow-up, assessment and monitoring, and the continuation of the adoption of the gender strategy.

3.0 Progress towards milestones

PROJECT MANAGEMENT

- 1.1 Research team finalized.
- 1.2 Effective teamwork established between project partners and other collaborators.
- 1.3 Administrative set-up for the project finalized.
- 1.4 Research conditions established and settled.

The project Inception Workshop took place in Nairobi, Kenya in November 2014, attended by 15 participants from Canada, Côte d'Ivoire and Kenya. It included the IDRC Program Officers: Dr. Innocent Butare, Senior Program Specialist, Regional Office for Eastern and Southern Africa, and Dr. Alvaro Paz, Senior Program Officer; Mrs. Anne-Marie Butuba and Mrs. Joyce Wairimu, IDRC Program Assistants - Regional Office for Sub-Saharan Africa; the PIs from Sporometrics, CNRA, and UNA, social scientists and accountants; and representatives of the third-party organizations ANADER and CSIR-OPRI. Signed MOUs and Canadian Food Inspection Agency import permits were presented. The number of researchers involved and roles and responsibilities, project activities by milestones, expected project outputs and outcomes, deliverables, indicators and means of verification were established as part of the M&E plan, and reflected in a gantt chart based on recommendations made by the Program Officers before their implementation. The communication and gender strategies were improved followed Program Officers' recommendations, and responsibilities were assigned to PIs. Project partners identified possible project outcomes to scale-up such as LAMP, field-schools, plant clinics, endophytes, parasitoids, and intercropping. A Steering and a Communication Committee were unanimously approved with responsibilities designated to Pls. The project website COWALY (www.cowaly.com) was successfully launched by Sporometrics. The Ethical Review was issued by the CNER (Comite National d'Ethique et de la Recherche) in Côte d'Ivoire on May 15th 2015. The IDRC Environmental Assessment funds were re-allocated under UNA to support the plant clinics in Grand-Lahou. During the period August 1st 2014 to January 31st 2015, the purchase and delivery of laboratory equipment required by CNRA, UNA and CSIR-OPRI was coordinated by the Canadian PI through biotech companies based in Canada and UK. Greenhouse equipment and installation was conducted by CNRA and UNA through local vendors.

IDENTIFICATION AND CHARACTERIZATION OF THE CILY PHYTOPLASMA(S) AND POTENTIAL VECTORS AND HOST PLANTS

- 2.1 The phytoplasma local strains characterized.
- 2.2 Potential vectors and secondary plant hosts identified.
- 2.3 Germplasm from the International Coconut Genebank for Africa and the Indian Ocean Collection, and from pilot farms ready to be screened for resistance

A multigene approach was used to characterize the CILY phytoplasma affecting coconut palms in Grand-Lahou. Nested PCR and RFLP were conducted on the sequences of three different phytoplasma genes: the 16S rRNA, the secretion-dependent pathway protein (secA), and the ribosomal proteins (rplV-rpsC). The CILY phytoplasma was confirmed as a member of the subgroup 16SrXXII-B ('Candidatus Phytoplasma palmicola'-related strains) detected in 82.9 % of the symptomatic coconut palms, particularly from trunk borings, which was recommended as the best plant part for sampling and disease screening. CILY phytoplasma was also detected in 30 % of the symptomless palms indicating that asymptomatic infection naturally occurs associated with CILY. A single nucleotide polymorphism (SNP) corresponding to the Mboll restriction site on the SecA sequence differentiated the CILY phytoplasma from both the CSPWD (Ghana)

and LY phytoplasma (Mozambique), which was proposed as marker to distinguish among 16SrXXII phytoplasma strains. All the CILY strains shared the rplV-rpsC nucleotide sequence 'ACGTCAAATAAT' that characterizes the Ghanaian CSPWD phytoplasma strains from the Central and Western Regions, where the disease is still active since the 1964 and 1983 outbreaks. This led to hypothesize that the CILY outbreak in Grand-Lahou had its origin from either the Central or Western Region from Ghana. A phytoplasma strain of group 16SrI 'Ca. P. asteris' was detected in 5 % of the symptomatic palms indicating that mixed infection occurs among coconut palms affected by CILY in Grand-Lahou, which may be imposing certain epidemiological constraints for the development of the disease. This result was supported by the first attempts of cultivation of the CILY phytoplasma at the University of Bologna. These results were published in Phytopathogenic Mollicutes 2015 "Analyses based on the 16S rRNA and secA genes identify a new phytoplasma subgroup associated with a lethal yellowing-type disease of coconut in Côte d'Ivoire" (Annex 1), and Annals of Applied Biology 2017 "Detection and differentiation of the coconut lethal yellowing phytoplasma in coconut-growing villages of Grand-Lahou, Côte d'Ivoire" (Annex 2). A newly identified Typhlocybine leafhopper, Nedotepa curta Dmitriev was identified as the potential vector of the CILY phytoplasma. Transmission trials failed to prove N. curta as the vector. Results were submitted to Crop Protection "Identification of a newly described member of the tribe Erythroneurini as a potential vector of the Côte d'Ivoire lethal yellowing phytoplasma in coconut palms sole or in mixed infection with a 'Candidatus Phytoplasma asteris'-related strain" (Annex 3), which is still under review process. Various factors may have influenced the failure of transmission trials such as the non-proper growth of seedlings in the screenhouses due to the excessive high temperatures despite the shedding attempts; an important number of meshed cages corresponding to the field trials were destroyed by the locals in two of the three locations, which made turned down the trials and resume the trials after new meshed cages were constructed; the number of N. curta specimens obtained from the laboratory rearing and released into the meshed fronds (in the field) or seedlings (in screenhouse) was less than 1,000 (as recommended in literature), probably due to the high temperature exposure, and fungal contamination of the rearing plates. New shedding designs and laboratory rearing methods are under study in collaboration with the Ghana entomologists to try new attempts of field trials as part of the post-project plan, when more funding is available. Since the potential vector was confirmed in 73 % of the specimens tested and it is widespread in all the CILY-affected areas, guidelines for their monitoring and management were provided to farmers as a new field mini-guide and included in the new disease management plan. Six plant species from five different botanical families Poaceae (Paspalum vaginatum, Pennisetum pedicillatum), Verbenaceae (Stachytarpheta indica), Plantaginaceae (Scoparia dulcis), Phyllanthaceae (Phyllantus muellerianus) and Cyperacea (Diplacrum capitatum) were identified as alternative plant hosts of the CILY phytoplasma that may play a role in disease spread. These results were published at the Canadian Plant Pathology Journal 2016 "Detection and identification of the coconut lethal yellowing phytoplasma in weeds growing in coconut farms in Côte d'Ivoire" (Annex 4). Guidelines for their removal and treatment of the removed plants were provided to farmers in the "Farmer field mini-guide" (Annex 5, Annex 6), and the new disease management plan (Annex 7, Annex 8), and all the information was put together as policy briefs "Policy Brief for CILY environmental and mitigation plans" (Annex 9, Annex 10); "Policy Brief to control the movement of coconut germplasm" (Annex 11, Annex 12); "Policy Brief to disseminate good cultural practices" (Annex 13, Annex 14). New diagnostics were generated for the CILY phytoplasma. A PCR-RFLP system was developed for the differentiation of the CILY phytoplasmas from the strains in Ghana and Mozambique to support CILY

New diagnostics were generated for the CILY phytoplasma. A PCR-RFLP system was developed for the differentiation of the CILY phytoplasmas from the strains in Ghana and Mozambique to support CILY surveillance and published in Annals of Applied Biology (Annex 2). A LAMP (loop-mediated amplification) PCR based on the ribosomal protein gene was developed and optimized for the specific in-field detection of the CILY phytoplasma. Data analyses from ring tests in Ghana and Côte d'Ivoire have been just completed, so the full article is ready to be submitted to *African Journal of Biotechnology*.

Training was an important component of the research project to raise disease awareness, build knowledge capacity and transfer technology. CNRA and UNA staff were fully trained through two training-courses on field and laboratory methods for detection, identification and characterization of the CILY phytoplasma from coconut palms, non-coconut plants and Hemipteran insects in 2015 and 2016 at UNA for a total of 30 attendees from UNA and CNRA. Project partners received two hands-on training-courses on LAMP-PCR (for the CSPWD phytoplasma and specific for the CILY phytoplasma) for a total of 28 attendees in 2015 and 2016. Two hands-on training-courses were held in 2016 for project partners, one at UNA and one at CNRA for RNA extraction, and data analysis and interpretation. A training-course Plant Clinics and Going Public' was held in 2015 at UNA for 15 attendees from UNA, CNRA, and ANADER with plant clinic experts, Dr. Eric Boa (UK) and Dr. Jeffrey Bentley (Bolivia), which inaugurated the first project plant clinic in Grand-Lahou (Annex 15). Two versions of the hands-on training-courses 'Advanced Technology Applied to Phytoplasma Research' with Canadian and Italian instructors were held in 2015 and 2016 at the University of Toronto for a total of 28 participants including project partners from Côte d'Ivoire and Ghana, and attendees from Mozambique and Saudi Arabia. The training-course was acknowledged at the 'Arts and Science News' from the Faculty of Arts and Science of the University of Toronto "African-Canadian collaboration bears fruits" (Annex 16). A Workshop 'Grand-Lahou: la maladie du jaunissement du cocotier gagne du terrain' was organized in October 2014 at CNRA for the official start of the project, and attended by UNA and ANADER, and the Major of Grand-Lahou (Annex 17). A training-course was provided in 2016 to extension agents and project partners at UNA on the main coconut diseases and control methods. Two workshops "Lessons Learned from the Cape St. Paul Wilt Disease in Ghana" were organized, one in Takoradi, Ghana in 2015 with attendance of 22 natural and social scientist; and the other one at UNA in 2016 with the attendance of the Ministry of High Education and Scientific Research of Côte d'Ivoire (Annex 18). Details on the screen for resistance to CILY are summarized under milestone 4.3.

EMPOWER WOMEN IN CILY MANAGEMENT

2.4 Gender strategy implemented.

As part of the gender strategy, five workshops on gender were held as part of the field schools at Brafedon, Likpilassié, Doudougbazou, Gredjiberi and Palmindustrie V2. Three mini-workshops on environment management were held at Doudougbazou, Lahou Kpanda and Likpilassié. A special session on health and nutrition was included for the field schools at Doudougbazou, Gredjiberi and Likpilassié led by an expert nutritionist from UNA. Two versions of the Gender Workshop "Towards Creating Gender-Responsive Agricultural Research and Development Programs" were held at the University of Toronto in 2015 and 2016, attended by a total of 40 project partners, four invited speakers (Ontario Universities and NSERC-Canada), four international attendees (Mozambique, Italy, Colombia, Saudi Arabia), and 160 students, academics, and faculty members of the University of Toronto, including one Econometrics specialist of the Department of Economics. Attendees discussed gender issues associated with the scientific research and approaches to empowering women within the food production chain and strategies to promote equal access to resources and opportunities. The 2015 Gender Workshop was acknowledged at the 'Arts and Science News' (Annex 16) from the Faculty of Arts and Science of the University of Toronto. Special activities were organized for the social scientists from Côte d'Ivoire and Canada, which included meetings with Econometrics and Gender specialists from Ontario Universities, and farmers from the Toronto's largest urban farm, and a visit to the Toronto Lending Tool Library to learn how to implement the lending tool library in Grand-Lahou to support women through field schools and plant clinics.

UNA and ANADER conducted gender-responsive surveys to identify the gender issues, and the main constraints for women associated with the coconut production in Grand-Lahou. Gender issues were related

to the poor knowledge of the coconut plant and farming, as well as on the coconut lethal yellowing disease; the difficult access to quality seed; the scarcity of arable lands; the lack of labor; the lack of organization of stakeholders (male and female producers, processors); the absence of markets for products (coconut oil, attiéké); the poor knowledge on coconut product diversity. The main constraints to women's participation within the coconut production chain include the no access to land and control over land: women are denied access and control over farmland, they should work in their husband's field, or the field inherited from their late husbands, or relatives; no access to labor: women's farms are weedy and produce little due to the lack of farm workforce; no access to training/extension; no access to agricultural innovations (seed, processing equipment, new varieties, loans), for instance, women use ratoons as seeds; no means of transportation from the farm to the village due to poor road conditions, especially during the rainy season; lack of organization of women producers and processors that impact on the selling/trading capacity for women and aggravate poverty; no access to market: the geographical isolation increases the challenges of agricultural marketing, and the low selling prices affect women especially; lack of proper tools for copra clearing and shelling: women clear and shell by hand; low production of non-coconut crops; no access to loans for product selling/marketing. One important step towards addressing these limitations was the creation of Women Groups in five of the CILY-affected villages to promote processing and commercialization of agricultural and coconut products, the planting of cassava yards as an alternative food and cash crop, and the implementation of field schools, plant clinics, Women Coconut Fairs, gender workshops and capacity building. The gender issues identified, as well as, the highlights from field schools and plant clinics, including the Women Coconut Fairs and Women Groups have been published at the International Journal of Agricultural Extension and Rural Development 2017 "Field schools and plant clinics: effective agricultural extension approaches to fight the coconut lethal yellowing disease and improve livelihoods of smallholder farmers in Grand-Lahou, Côte d'Ivoire (Annex 19).

ASSESSING THE IMPACT OF THE DISEASE ON THE COCONUT INDUSTRY

3.1 Socio economic and environmental impacts of the disease on coconut farm families livelihoods assessed.

Gender-responsive surveys were conducted to assess the socio-economic impact of CILY in the livelihoods of smallholder coconut farmers in Grand-Lahou. The survey data evidenced 90% of illiteracy for coconut farmers (89.14 % for those with farms affected by CILY showing much less experience on coconut farming); the inequality of gender roles in Grand-Lahou, emphasizing the limited access to resources for women farmers; the surveys evidenced smallholder farmers with CILY affected farms incurred in higher food and health expenses compared to those with non CILY-affected farms. Informing farmers that CILY is associated with an increase of food- and non-food related expenses may encourage their engagement at mitigating the impact of the disease and improving disease management. Recommendations were made to address gender inequities and to promote awareness for smallholder farmers in preventing disease spread; and approaches for better education access, which included the implementation of field schools with emphasis on training for women farmers, plant clinics, Women Coconut fairs and the support to Women Groups. The results on surveys related to the socio-economic impact of CILY were published at the African Journal of Agricultural Economics and Rural Development 2016, Vol 4(9), p. 463-79, "Socio-economic impact of the coconut lethal yellowing disease on Ivorian smallholder coconut farm families" (Annex 20). Moreover, an econometric model of land use change in Grand-Lahou was produced based on an extensive panel data set developed using satellite images and land use maps from 1990 to 2012 collected from the CCT (Centre of Cartography and Tele-detection) from five Administrative Divisions. The analysis derived from the econometric model will be used to predict the future land use change in Grand-Lahou and its spatial distribution until 2042. CILYfree buffer zones with nourishing soils less prone to erosion and with agronomic enhancing capacity will be

identified to relocate coconut farms devastated by CILY and revive the coconut industry in Grand-Lahou. Results on the econometric model are under review, Land Use Policy (Annex 21).

A study on the CILY effect of socio-economic and human environments was conducted by UNA and ANADER in the Avikam Island. Over 90% of the coconut plantations were destroyed, so an estimate of 10% of young people left the village or moved to Abidjan since their main activities were related to the coconut selling. Due to the collapse and closing down of the local coconut processing company SICOR, an estimate of 30% of men and women were unemployed. This affected families, especially women, since their husbands abandon them to find new jobs in Abidjan or other urban cities. Women organized themselves so far into six Women Associations or Women Groups with a total of 173 members. The Groups aim to produce cassava seed yards from improved cassava varieties, which should provide them with an alternative cash/food crop as insurance against the losses due to CILY. However, this has generated gender issues as many men do not accept the change, so more field schools and workshop have been included within the post-project plan to enhance CILY disease awareness, and to sensitize the community on gender.

DISSEMINATE RESULTS TO THE SCIENTIFIC COMMUNITY

3.2 Scientific presentations at international seminars.

Presentations in international events included the 3rd Meeting of the International Phytoplasma Working Group (IPWG), celebrated in Mauritius in January 2015 with attendance of 60 participants from 25 countries (Annex 22). As part of the IPWG Meeting, a round table "Fighting emerging palm diseases in Africa and the Americas" was conducted by Dr. Yaima Arocha Rosete (Canada) and representatives from Saudi Arabia, Mozambique, France, and Dr. Ndede Yankey (Ghana); results were published in the 2015 Proceedings of the Meeting in the international phytoplasma journal Phytopathogenic Mollicutes, Vol 5 (1-Supplement): S57-58 (Annex 1). Project partners presented a poster at the 21st IOM (International Organization of Mycoplasmology) Meeting in Australia on July 2016 with 170 delegates from 28 countries (Annex 23). Our research was also presented at the 47th Conference of the Asian and Pacific Coconut Community (APCC) in Indonesia September 2016 as oral presentation by Dr. Konan Konan; and at the XVIIe édition des Journées Scientifiques Internationales de Lomé (JSIL 2016) in Togo as oral presentation by Dr. Eric Kwadjo (Annex 24).

EMPOWER FARMERS AND STAKEHOLDERS IN CILY DIAGNOSIS

3.3 Key stakeholders trained. Six plant clinics and Women Coconut Fairs organized.

Ten field schools were organized by UNA and were established at eight localities of Grand-Lahou. Training modules were designed based on data from gender-responsive socio-economic surveys focused on Production, Plant Protection, and Marketing. Field schools covered 1,086.5 ha and trained 1,960 farmers (1,568 men, 392 women), 180 extension agents and circa 700 families on coconut biology and farming, disease resistance assessment, quality seed, cultural practices, and plant and environment protection. Special training modules were designed for Women Groups (total of 173 women farmers) on cassava farming and processing of coconut into cakes, candies and biscuits aim at encouraging potential small business opportunities. Results on field schools were also published (Annex 19).

Nine plant clinics mobilized 671 farmers, villagers, and processors (478 men, 193 women); and addressed plant disease queries not only in coconut, but also in twenty other crops such as cacao, cassava, citrus, okra, coffee, peanut, yam, beet, plantain, tomato, maize, etc. Plant clinics showed to be a suitable platform to engage stakeholders and policy makers as for the plant clinic held at Braffedon in April 2016, which was attended by the Major and Prefect of Grand-Lahou, the Canadian Ambassador in Côte d'Ivoire,

Representatives of the Ivoirian High Education and Scientific Research Ministry, and ten village Chiefs. Appreciation words from the Canadian Ambassador in Abidjan on the plant clinic and the project impact (Annex 25) are available. During this plant clinic, farmers, stakeholders and policy makers were taught through factsheets and physical plant samples on those species that should be removed from the coconut plantations: Paspalum vaginatum, Pennisetum pedicillatum, Stachytarpheta indica, Scoparia dulcis, Phyllantus muellerianus, and Diplacrum capitatum since they harbour the CILY phytoplasma. Besides, a display of tube traps and tools used to capture insects in the field, as well as stereo microscopes to learn the different stages of N. curta development were made available to educate famers on how to identify them in the field and report to UNA and CNRA. Eight Women Coconut Fairs mobilized 550 participants (390 men and 160 women), and allowed new opportunities of marketing and small businesses for them. Women sell out their coconut products, from crafted and food products, cosmetics to home furniture, whose number increased from 14 to 26 (64 %) from 2015 to 2016. From the interaction with representatives from copra and husk small business that attended the Fairs, women coconut farmers in Grand-Lahou became interested in processing of coconut oil and soap, as well as seed processing into copra and desiccated coconut They also learned that coconut husk was a potential source for small business as they were able to see huskbased items like cleaning brushes, bags, floor mats. ANADER implemented the 'Centre de Formation en Machinerie Agricole' (CEFMAG) for agricultural equipment rental, and is mediating the approaches to make the service available for women in Grand-Lahou to rent equipment for land preparation. Women coconut farmers organized themselves for the very first time in Grand-Lahou in six Women Groups, which are currently planting cassava yards as a new source of income. Results on plant clinics were also published (Annex 19).

DEVELOP CONTROL STRATEGIES

- 3.4 Methods of control strategy tested.
- 3.5 Bacterial endosymbionts, and bacterial and fungal endophytes identified for the development of long-term biological vector control strategies.

Bacterial and fungal endophytes with biocontrol potential for the CILY phytoplasmas were identified, which include well known species as biocontrols of other bacterial, fungal and phytoplasma diseases, for instance in grapevine. These were *Bacillus* (*B. flexus*); *Burkholderia* (*B. tuberum*), and *Pseudomonas* (*P. nitroreducens*, *P. pseudoalcaligenes*), *Trichoderma*, *Penicillium*, and *Fusarium*. The endophytes were recovered by a high-throughput culturing method 'dilution-to-extinction' from trunk, leaves and rhizosphere of CILY-affected coconut palms, and were confirmed by Illumina-based microbiome profiles. Results on the new bacterial and fungal endophytes identified were accepted in the African Journal of Biotechnology (Annex 27). Pure cultures are available for preparing spore suspensions to test in coconut plantations established under different disease pressure to assess their biocontrol potential. PCR and microbial community studies on the potential vector (*N. curta*) populations identified Bacterial species *Cardinium*, Acetic Acid Bacteria (AAB), and a new species within the genus *Pectobacterium* as endosymbionts with potential to be used for a future program of vector endosymbiotic control. Results on the bacterial endosymbiont are under reviewing for submitting to PLOS One journal.

Poultry manure assessment trials were established at the Genebank at the Marc Delorme Station for two years. The goal was to assess the effect of poultry manure in increasing resilience on coconut palms affected by CILY. The trials were based on previous reports on positive effect of the poultry manure in the improvement of the agronomic performance of coconut plantations in India. In Côte d'Ivoire, CILY-affected coconut palms do not bear more than 15 nuts/tree/year compared to a non CILY-affected palms, which yield 104 nuts/tree/year, 240 g of copra/nuts and 3.5 t of copra/ha/year. Statistics after two year

observations and data collection showed that coconut palms treated with poultry manure had an increase in the number of flowers from 8 to 10 per palm and per inflorescence. These results suggest that the application of poultry manure provides coconut palms affected by CILY with certain level of resilience against the disease, so it is anticipated that the palms could reach half of the standard yield measured for non CILY-affected palms if poultry manure application continues consistently. This represents a considerable gain for coconut farmers, producers and villagers. The follow-up of the poultry manure assessment trials for an extended period of 2 more years was specified within the post-project action plan.

DEVELOP NEW DIAGNOSTIC METHODS

4.1 Seed-borne transmission assessed.

Embryo cultures were established from coconut seednuts collected from four CILY-affected villages, PCR tested for phytoplasma, and in vitro cultured. Fifteen embryos survived after 4 months of successful and not-contaminated in vitro culture, so, there are currently 15 seedlings being monitored for CILY symptoms and for phytoplasma. No phytoplasma has been detected so far in any of the seedlings and plantlets obtained through embryo in vitro culture. So, there is as yet no evidence that the CILY phytoplasma is seedborne transmitted through the seedling to cause disease in progeny palms in Côte d'Ivoire. These results confirm previous results in Ghana and Mexico, where the LY phytoplasma was detected in embryos but not in the progeny palms. Nevertheless, other phytoplasmas have been found in new progenies as for the confirmed cases of tomato, lime and alfalfa in Europe and Middle East, so the possibility of seed-borne transmission cannot be ruled out. Therefore, monitoring of the germinated seedlings will continue for 2 years at CNRA, as part of the post-project plan. If the CILY phytoplasma is found in progenies of any breeding program, it may indicate possible seed-borne transmission, which would prevent planting seedlings in disease-free regions since it would introduce the phytoplasma into these areas. This would force the production of pure seeds from varieties located in CILY-affected areas, which would negatively impact the phytoplasma-free movement of germplasm. For that reason, guidelines for the control of germplasm movement were provided to farmers, stakeholders and policy makers under the new disease management plan (Annexes 6 and 7), and as a policy brief (Annexes 11 and 12). Moreover, 1960 farmers (1568 male and 392 female) were trained on how to establish their own coconut nurseries through the ten field schools established in Grand-Lahou. Farmers are aware that such nurseries are the safest source of seedlings for farmers, and the most practical approach to control seed exchange among farmers, to reduce import of planting material through the border, and to prevent disease spread.

ASSESS THE IMPACT OF THE DISEASE ON THE ENVIRONMENT COCONUT PRODUCTION AREA

4.1 Environmental impact of disease and project implementation assessed.

Information on soil observations during the IDRC-DFATD project, and the records of environmental variables such as rainfall and temperature in Grand-Lahou obtained from SODEXAM (*Société d'Exploitation et de Devéloppement aéroportuaire, Aéronautique et Météorologique*) were considered to develop environmental and mitigation plans for the CILY-affected coconut farms to be disseminated within the coconut farming community. Our surveys performed in the Grand-Lahou area confirmed that farmers do not use chemicals in the coconut plantations, so no remediation or chemical disposal sub-plan was included in the environmental plan. The highest temperature was 27.6 °C, the hottest month was April (mean 27.45 °C), and the coolest month was August (mean 24.35 °C); annual temperature means were 26°C and 26.3°C. Temperature ranges (22-26°C) are reported to enhance the phytoplasma multiplication rate within the plant and insect hosts. Although no data on the biology of *N. curta* are available, it is possible that the current warm temperature may favour the insect vector migration, and consequently the spread of CILY in the coconut farmlands of

Grand-Lahou. During 2015, January (1.2 mm) and September (7.7 mm) received the lowest mm of rain fall, and June received the most rain (787.6 mm). Rainfall means were 175.87 mm in 2014 and 117.4 mm in 2015, which were considerable below the ideal rainfall requirement (1,200 – 3,000 mm/year). Due to the severe droughts during 2014 and 2015, and early 2016, CILY became more widespread in Grand-Lahou. In fact, it was noticeable the faster transit of coconut palms from disease stage 3 to terminal stage (telephone pole), particularly in the westernmost areas. Since prolonged dry spell requires irrigation, this was an aspect considered within the environmental plan.

Observations of soil conditions in the villages of Badadon, Braffedon, Adjadon, Palmindustrie V1, V2 and V3, and Yaokro indicated that severely CILY-affected farms show higher levels of erosion. This was associated with the removal and burning of palms as soils get more exposed to waterlogging, fragmentation and drought. During periods of heavy rain, a higher frequency of waterlogging around CILY-affected palms was observed, which influenced a fast transition of palms to the terminal disease stage. A new technique 'slow down' that does not include burning learned from the Ghana Workshop as well as guidelines for the management of CILY phytoplasma secondary plant hosts were included increases the incidence of CILY and its environmental impact in the farms of Grand-Lahou.included as in the environmental and mitigation plans aim at mitigating the environmental impact of burning palm trees or CILY incidence to the natural ecosystem of the coconut groves of Grand-Lahou. There were observations on plastic and metal waste increase in the last 5 years in Grand-Lahou, particularly in coconut farms affected by CILY. Although no data is available on how the plastic or metal waste could be related to soil erosion, or how it affects the ecosystem in Grand-Lahou, it is known that plastic pollution has a global negative impact for natural ecosystems, so training on plastic waste management was included as part of the environmental and mitigation plan. Environmental management and mitigation plans (Annexes 28 and 29) and a policy brief "Environmental and Mitigation Plans for CILY in Grand-Lahou" (Annexes 10 and 11) have been developed for farmers, stakeholders, authorities, policy and decision makers in Grand-Lahou.

DEVELOP CONTROL STRATEGIES

4.3 Various methods of control put in place.

Guidelines were developed for farmers and stakeholders to implement new field practices to properly manage the secondary plant hosts for the CILY phytoplasma, and the potential vector *N. curta* populations, and the establishment of on-farm nurseries as the safe source of seedlings for farmers. Guidelines were first disseminated at the plant clinic plant in Braffedon that was attended by the Canadian Ambassador, the Grand-Lahou Major and the Representative of the Ministry of High Education and Scientific Research. A new disease management plan containing all these aspects (Annexes 7 and 8) was developed, disseminated, implemented and monitored through the field schools established in Grand-Lahou, as well as community workshops. Currently, farmers know to recognize, remove and dispose the weeds that host the CILY phytoplasma, differentiate the stages of *N. curta* and its peak season, and report to UNA and CNRA, and establish their own on-farm coconut nurseries.

Nine promising cultivars/hybrids: NVS, NJM, NVM, NBO, NJG, NVT NRM, NVE and NRC, genetically closer to the Ghanaian resistant hybrid GVSXGVT were confirmed as the candidates to be included in the post-project long-term resistance trials (minimum 3 years) in pilot farms in Côte d'Ivoire (Grand-Lahou) and Ghana to assess for resistance or tolerance to CILY. Pilot farms in Grand-Lahou include two in Adjadon, one in Noumouzou, one in Badadon, and the control at the Marc Delorme Station, CNRA (plot N°152), which have been already planted with such varieties, and are ready to perform the resistance assessment trials. Varieties NVS, NJM, NVM, NBO, NJG, NVT NRM, NVE, NRC and GVSXGVT have been already selected for the long-term resistance trials, and included in the post-project action plan. Those with better performance will

be selected for the rehabilitation plan to be implemented in Grand-Lahou after 3 years of assessment of resistance trials. A rehabilitation plan "Coconut rehab plan" (Annexes 30, 31) with the corresponding follow-up plan was generated and discussed among project members, and executives of CNRA, UNA, ANADER, and CSIR-OPRI (Ghana), and informed to stakeholders through a field school.

Intercropping coconut-plantain-maize trials were established at the Marc Delorme Station. An extensive drought period during the rainy season caused a delay on the planting of corn and the the growth rate of the plantain plants and its harvest. Nevertheless, the profitability index was estimated based on the cumulative balance over the four years of the net cash-flow of coconut-to-food crops compared to the pure coconut crop. It showed that the association for 4 years of the coconut crop was more profitable with the plantain (833,950 F CFA/ha) than with the maize (123,000 F CFA/ha). The advantages of intercropping plantain or corn with the coconut crop are that the income generated by the associated crops allowed covering the operating expenses until the coconut came into production. The follow-up of intercropping trials has been also included in the post-project action plan.

EMPOWER FARMERS AND STAKEHOLDERS IN CILY MANAGEMENT

4.4 Capacity of scientists, authorities, policy makers, stakeholders, and farmers increased.

As part of the IDRC-DFATD project, two MSc students at UNA, Kodjo Thierry (male) and Ouattara Winnie (female) graduated. Kodjo's MSc thesis focused on the study of *N. curta*, the potential vector of the CILY phytoplasma. Winnie's MSc thesis was related to the alternative plant hosts of the CILY phytoplasma. These two students are in the process of registering as PhD students at UNA. Another female MSc student, Dadié Reine Prisca, graduated at the University 'Felix Houphouët Boigny' (UFHB), "Population et environnement en Côte d'Ivoire: regard sociologique sur le jaunissement mortel du cocotier dans le département de Grand-Lahou". MSc students at CNRA included Daramcoum N. Marie-Pierre from UFHB on the agromorphological characterization of coconut hybrids for the selection of cultivars tolerant to CILY. Marie-Pierre is now a registered PhD to study the selection of tolerant cultivars and the transmission mechanisms of the CILY phytoplasma for February 2018. Student, Zoro Abel Arnaud successfully finalized his MSc on the molecular diagnosis of the CILY phytoplasma, and MSc Tagro Nathan Raymond on the molecular characterization of the CILY phytoplasma. PhD student, Beugré N'jiha Isabelle is scheduled to finalize by April 2018 on the inventory of the entomofauna and the biology of the potential vector of the CILY phytoplasma. In summary, six graduate students associated with UNA and CNRA successfully completed their MSc, and two PhD students are scheduled to finalize in 2018.

Nine plant clinics and eight Women Coconut Fairs were organized as part of the IDRC-DFATD project. Plant clinics mobilized 671 farmers, villagers, and processors (478 men, 193 women), while Fairs mobilized 550 participants (390 men and 160 women). Ten field schools were organized by UNA and established at eight localities of Grand-Lahou, which trained 1,960 farmers (1,568 men, 392 women), 180 extension agents and circa 700 families on coconut biology and farming, disease resistance assessment, quality seed, cultural practices, and plant and environment protection. Special training modules were designed for six Women Groups created for the very first time in Grand-Lahou (total of 173 women farmers). Training was focused on cassava farming and processing of coconut into cakes, candies and biscuits aim at encouraging potential small business opportunities. For more details on field schools, plant clinics, Women Coconut Fairs and Women Groups see milestone 3.3 and publication (Annex 19).

DISSEMINATE RESULTS TO THE COCONUT-PRODUCING COMMMUNITY

4.5 Impact of the project documented.

COWALY website (http://cowaly.com) currently shows all the international events and published scientific papers. Those include: online publication of the Gender Workshop at the 'Arts and Science News' from the Faculty of Arts and Science of the University of Toronto (Annex 16); the Technical Manuals from the training-courses held at the University of Toronto, UNA, and CNRA (http://cowaly.com/progress); the reports from the Gender Workshops held at the University of Toronto, and the Lessons Learned Workshop held in Ghana (http://cowaly.com/progress); and a video on Plant Clinics (Annex 32), and the Project Video (Annex 33). Scientific papers include those published in the Canadian Journal of Plant Pathology (Annex 4), Annals of Applied Biology (Annex 2), International Journal of Agricultural Extension and Rural development (Annex 19), African Journal of Agricultural Economics (Annex 20), New Disease Reports in Plant Pathology (Annex 46), Zootaxa (Annex 47). Workshops and media releases are also presented at COWALY such as "Valorisation des résultats de la recherche: un fonds de 500 millions de FCFA mis en place" (Annex 18, UNA Workshop), "Grand-Lahou: la maladie du jaunissement du cocotier gagne du terrain" (CNRA Workshop) (Annex 17); "La culture de coco menacée par la maladie du jaunissement à Grand-Lahou" (Annex 34); "A fair the coconut tree took place the locality Badadon on in of (Grand-Lahou)" http://news.abidjan.net/h/527940.html (Annex 35); "Coconut Concerns", Clear Life Issue 2, Spring 2016 (Annex 36); and the IDRC CILY short story plant clinics & field schools (Annex 37), this latter one is one of the best project story telling on plant clinics, field Schools, Women's Coconut Fairs, and Women Groups.

4.6 Information disseminated to stakeholders.

One poster and six factsheets were generated an some examples are shown in Annexes. Three were produced on the alternative hosts for the CILY phytoplasma (Annex 40), one on the identification and field recognition of the insect vector (Annex 38), one on the field identification of CILY symptoms at each disease stage (Annex 39), and one on the new disease management plan were generated to disseminate the implementation of the new disease management package. One poster was presented at the IOM Meeting in Australia, July 2016 (Annex 22).

EMPOWER WOMEN LIVING IN THE AFFECTED COCONUT ZONE

5.1 The part of income generated by women increased.

Quantities of coconut products sold by women increased.

Eight Women Coconut Fairs were organized in six villages of Grand-Lahou during 2015 and 2016 with a total of 550 participants (390 men and 160 women). See publication (Annex 19).

Table 1. Coconut products displayed and sold by women farmers in the Coconut Fairs.

Villages	Coconut Products	Participants des villages			Date
Villages		Men	Women	Total	Date
Badadon	Flower pots, tables, lamp base, cooking utensils, crafted jewelry (earrings, bracelets), sweetened grilled coconut, cake, toffee, coconut oil soap, crafted items (ice container, jewelry box), trays, toothpicks holders, coconut-based home furniture.	92	35	127	04/03/2015

TOTAL		390	160	550	
Yaou	oil soap, sweetened grilled coconut, fresh nuts, coconut butter, toffee	26	12	38	19/11/2016
Braffedon	Broom; coconut cake, coconut oil, sweetened grilled coconut, coconut oil soap, fresh nuts. Broom; coconut cake, coconut oil, coconut	30	8	38	18/08/2016
Tiapoum	Coconut wood rafters, coconut wood planks; firewood; coconut oil-based mosquito repellent; coconut fish food, charcoal, broom, coconut oil, copra, coconut water; seednuts, coconut milk, sweetened grilled coconut, grated fresh coconut, toffee, coconut cake, coconut oil soap, crafted jewelry.	59	7	66	21/09/2016
Palmindustrie V3	Coconut oil, broom, sweetened grilled coconut, coconut cake, toffee.	48	10	58	18/08/2016
Liboli	Flower pots, crafted items (cups, jewelry box), chairs, coconut oil, coconut broom, sweetened grilled coconut, coconut cake, toffee.		13	43	20/05/2016
Doudougbazou	Coconut oil, broom, coconut cake, coconut oil base soap, sweetened grilled coconut, toffee, biscuits.	52	18	70	28/01/2016
Palmindustrie V1	Mats made of coconut leaves, coconut oil, broom, coconut milk-based pudding, fried rice cooked with coconut oil, coconut cake, sweetened grilled coconut, coconut oil soap.	53	57	110	12/11/2015

Women farmers displayed and sold hand-made coconut products, which ranged from crafted products, cosmetics to home furniture. The range of coconut products displayed and sold by women increased from 14 to 26 (64 %) from 2015 to 2016. Coconut products included crafted items, coconut charcoal, coconut-based fish food, copra, coconut milk, and coconut firewood, coconut leaf planks, coconut-based mosquito repellent, etc. Approximately 20 % of women sold hand-made coconut cakes at the Fairs, and one of them started-up a small coconut business between the village area and Abidjan.

Increased number of women involved in income generating activities

Six Women Groups were created in 2015 in six villages of Grand-Lahou. With a total of 173 registered members in 2016, the Groups included Binkadi in Palmindustrie V1 (30 members), Solidarity in Palmindustrie V3 (30 members), Club Victorie in Palmindustrie V2 (30 members), Ebo Ebo in Yaokro (30 members), Union in Doudougbazou (25 members), and Effozoui Assia in Badadon (28 members). These Women Groups are currently planting 2 ha of cassava orchards in areas of coconut land where no coconut plantations exist due to the impact of CILY. See publication (Annex 19).

Currently, over 80 non-registered have been trained in the field schools on land preparation and cassava planting, and are all involved in the harvest and selling of cassava. It is noteworthy that at the beginning of the project there were no Women Groups and towards the end of the project the number has increased to circa 300 women (registered and non-registered) coconut farmers that grow cassava as an alternative income generating activity. These cassava yards are an alternative food and cash crop for women to cover the losses of coconut palms due to CILY, and help family income and nutrition. They have become a new

initiative to support crop diversification and to empower women farmers to become independent and enable them to improve their family's nutrition and income. Considering women that sell coconut products, the number has notably increased from 90 during the 1st year to 160 in the second and last year of the project. These figures indicate that both activities implemented as part of the IDRC-DFTAD project have greatly contributed to the increase of the number of coconut women farmers involved in new income generating activities such as the cassava yards and the buying/selling of coconut products.

EMPOWER FARMERS AND STAKEHOLDERS IN CILY MANAGEMENT

5.2 Technology scaled up.

<u>640 coconut farm families (40/village) trained on management of resistant cultivars, including proper access to resistant cultivars.</u>

All ten field schools established in Grand-Lahou completed all seven training modules previously designed (Table 2).

Table 2: Training modules designed for field schools in Grand-Lahou.

Themes	Training Modules			
Production	Module 1	Coconut crop and farming: coconut biology, coconut quality nursery and seed, coconut planting techniques		
	Module 2	Coconut seed quality		
	Module 3	Coconut cultural practices: establishment of a nursery; planting techniques		
	Module 4	Plant and Environment Protection: fight against bushfire; disease and pest control (CILY); alternative hosts for the CILY phytoplasma		
	Module 5	Establishing plantations		
Marketing	Module 6	Organization of coconut producers		
	Module 7	Marketing techniques: market research, sell/buy, price setting		

Ten field schools were established in eight villages of Grand-Lahou for a total area of 1086.5 ha, which included Braffedon, Badadon, Yaokro, Lahou Kpanda, Likpilassié, Doudougbazou, Gredjigberi and Palmindustrie V1. Each field school was structured with one class leader, one reviewer and one secretary that received special training, and are now able to teach other farmers. All field schools were established on men-owned plantations. Women-owned plantations were generally remotely distant from the villages, most of them in small areas with a very difficult access. Nevertheless, 244 men and 36 (12.8 %) women farmers attended the field schools for a total of 280 participants per module. Details can be found in the publication (Annex 19).

A total of 1,960 farmers (1,568 men, 392 women), and 180 extension agents were trained by UNA and ANADER, who will be able to train other farmers. These figures superpass the expected ones, which include circa 700 families trained on farming and disease resistance assessment. The training module on disease management included training on planting the nine putative resistant cultivars (supplied by CNRA) in pilot farms to monitor and assess the response of the new emerging palms against CILY. The module also included training on how to establish the coconut nurseries so farmers were able to get their own planting material for their farms aim at minimizing disease spread due to uncontrolled seed exchange. Pilot farms in Grand-Lahou where the nine putative resistant varieties were planted and are ready to perform the

resistance assessment trials include two in Adjadon, one in Noumouzou, one in Badadon, and the control at the Marc Delorme Station, CNRA (plot N°152). Pilot farms from each of the villages (8) where field schools were established are ready to set up the long-term resistance trials upon been provided with the putative varieties from CNRA. As part of the post-project plan, five new field schools are to be established in five CILY-free villages of Grand-Lahou as an approach to educate to prevent disease spread. It is noteworthy, the interest from the villagers, especially youngsters in working for the coconut sector, and extend the field schools to other coconut-growing areas to raise disease awareness.

DISSEMINATE RESULTS TO AUTHORITIES, POLICY MAKERS AND EXTENSION AGENTS

5.3 Policy makers and other stakeholders have access to information and use it.

An information package directed to farmers, stakeholders and policy and decision makers was developed and presented as 'Research Outputs' as part of the 4th Technical Report. These included three policy briefs: Policy Brief for CILY environmental and mitigation plans (Annexes 9 and 10), Policy Brief to control the movement of coconut germplasm (Annexes 11 and 12), Policy Brief to disseminate good cultural practices (Annexes 13 and 14), a new disease management plan, and the protocol for the establishment of on-farm nurseries (Annex 41), as well as the environmental and mitigation plans (Annexes 28 and 29). The package has been now translated into French to access local policy and decision makers, which include the Ministry of Agriculture, the Ministry of High Education and Scientific Research, and the Major of Grand-Lahou and representatives. A document with guidelines for the use and tracking of the information generated has been developed "Guidelines for the use and tracking of information" (Annexes 42 and 43).

The policy briefs, new disease management plan, protocol for the establishment of on-farm nurseries, as well as the environmental and mitigation plans were discussed among project members during strategic meetings with ANADER. The new disease management plans, protocol for the establishment of on-farm nurseries, as well as the environmental and mitigation plans were discussed with farmers and extension agents through the field schools, as well as through meetings with ANADER.

Printed copies and cover letters corresponding to the three policy briefs were sent to the Major of Grand-Lahou and representatives, and the Ministry of High Education and Scientific Research. The latter will take actions to send the information to the Ministry of Agriculture. The documents have been made available in both English and French at the COWALY website (http://www.cowaly.com).

A farmer field mini-guide "Farmer field mini-guide" (Annexes 5 and 6) was developed in both French and English, which compile a summary of the new disease management plan as a suitable in-field use tool for farmers and stakeholders. The field mini-guides were first handed to farmers in two reference farms, Badadon and Braffedon during the Evaluation Visit of Dr. Innocent Butare to Grand-Lahou in November 2016. The mini-guides were improved from inputs from farmers, re-printed and handed to the field school teachers during the Final project Meeting on December 14th 2016 to disseminate to the rest of the Grand-Lahou villages.

DEVELOP CONTROL STRATEGIES.

5.4 Coconut industry rehabilitated.

No data are available at this phase of the project to support a significantly decrease of the incidence of CILY, neither an increase of the coconut oil production in only two years and half of project implementation.

The project has developed outcomes towards reaching out these purposes, and results will be visible upon accrued figures for the next three years as described in the post-project action plan. Actions have been

taken to initiate the implementation and monitoring of a new disease management plan, and environmental and mitigation plans, as well as, protocols for the establishment of on-farm nurseries and the application of new field practices that go along with a well-structured gender strategy. Besides, approaches such field schools, plant clinics, Women Groups and Women Coconut Fairs have become already common practices adopted by the coconut farming community of Grand-Lahou with the support of ANADER and the extension agents. The adoption, improvement, assessment, and monitoring of the implementation of such outcomes by farmers, stakeholders and decision makers will contribute to provide the expected figures. For instance, farmers started to implement the new disease management plan since its first launch at the plant clinic in Braffedon in April 2016, and are currently using the farmer field mini-guide developed.

Recent observations from extension agents and field schools teachers estimate that the following estimates of coconut farmers in Grand-Lahou villages attending the field schools are currently:

- -removing the alternative hosts of the CILY phytoplasma and making compost out of them (~ 60 %),
- -immediately informing CNRA when palm trees start showing disease symptoms (~ 70 %),
- -felling diseased palms at disease stage 3, which was almost impossible during the first year of the project implementation (~ 50 %),
- -preparing pieces of land to make their own seed supply orchards (~ 60 %),
- -intercropping with plantain or maize (~ 60 %).

A rehabilitation plan "Coconut rehab plan" (Annexes 30 and 31) with the corresponding follow-up action plan was generated. All recommendations given by CSIR-OPRI (Ghana), CNRA third-party organization were included since they were directly involved in the replanting program in Ghana to fight CSPWD. The plan was discussed among project members, and executives of CNRA, UNA, ANADER, and CSIR-OPRI (Ghana), and informed to farmers and stakeholders in field schools. The rehabilitation and post-project action plans are available at the COWALY website in English and French (http://cowaly.com/available-documents).

5.5 Post-project action plan developed and communicated.

A post-project action plan "Post Project Action Plan" (Annexes 44 and 45) responding to each specific objective was generated and discussed among project members, and executives of CNRA, UNA, ANADER, and CSIR-OPRI (Ghana), and informed to farmers and stakeholders in field schools. It is available at COWALY. Post-project strategy to scale-up, use of resistant cultivars and continue gender strategy is shared with stakeholders and policy makers and is available at COWALY website (http://cowaly.com/available-documents).

4.0 Synthesis of research results to date

4.1 **Objective 1**. To better understand the bio-ecology of the causal agent of the lethal yellowing disease in Côte d'Ivoire, biophysical, environmental and socio-economic factors involved in its epidemiology, and the impact of the disease on the coconut industry.

Mapping of the CILY phytoplasma incidence and phytoplasma characterization

The CILY phytoplasma was detected in in over 82.9 % of the CILY-affected coconut palms with a predicted westward spread within Grand-Lahou. Trunk borings were recommended as the most suitable plant tissue for sampling. The nucleotide sequence 'ACGTCAAATAAT' in the ribosomal protein sequence gene reported

as characteristic for the CSPWD phytoplasma strains of the Central and West Regions in Ghana was found in the CILY phytoplasma isolated from all the symptomatic palms. This supports the hypothesis that the CILY phytoplasma affecting Grand-Lahou may originate and spread from either Central or West Regions in Ghana towards the south littoral of Grand-Lahou. The CILY phytoplasma was confirmed as a member of group 16SrXXII-B 'Candidatus Pytoplasma palmicola'-related strains along with the Ghanaian strain that causes CSPWD. CILY phytoplasma was shown to be phylogenetically closely related to the group 16SrXXII-A 'Ca. P. palmicola' that embraces the lethal yellowing isolate in Mozambique. It is also far related to the lethal yellowing groups that affect the regions of the Central and North America and the Caribbean, as well as South Asia and the Pacific. The CILY phytoplasma was distinguished from the strains from Ghana and Mozambique based on its secretion protein and ribosomal protein gene sequences.

Phytoplasmas of group 16SrI were found in mixed infection with the CILY phytoplasma in 5 % of the palms surveyed, which makes epidemiology and control of the disease more complex. These findings urge to develop more effective disease management strategies, particularly for those villages where mixed infection was identified.

Part of the results of this section was included in the 3rd and 4th Technical Report. Results have been accepted as a publication in the high impact factor journal Annals of Applied Biology "Detection and differentiation of the coconut lethal yellowing phytoplasma in coconut-growing villages of Grand-Lahou, Côte d'Ivoire" (Annex 2).

Potential vector and alternative hosts of the CILY phytoplasma

A new genus and species within the Typhlocybinae subfamily, Nedotepa curta Dimitriev was confirmed as the potential vector of the CILY phytoplasma (Annex 3, publication under review in Crop protection, "Identification of a newly described member of the tribe Erythroneurini as a potential vector of the Côte d'Ivoire lethal yellowing phytoplasma in coconut palms sole or in mixed infection with a 'Candidatus Phytoplasma asteris'-related strain"). Transmission cage trials are currently on-going and will be included in the extension phase of the project to prove Nedotepa curta's vector capacity and to study aspects of its biology. This is the first report of a xylem feeder identified as potential vector of a phytoplasma disease in coconut. Phytoplasma vectors of the Typhlocybinae include Empoasca papayae, proven as vector of the phytoplasma associated with Bunchy Top Symptom of papaya; E. decedens in Italy for the European stone fruit yellows or in Lebanon for almond witches' broom; E. decipiens in Saudi Arabia for the lime decline disease and alfalfa witches' broom; and the Ranunculus virescence in Italy; and almond witches' broom in Lebanon; E. kraemeri for phytoplasmas affecting citrus species (C. sinensis and C. limon), coffee (Coffea arabica), periwinkle (Catharanthus roseus), and tabebuia (Tabebuia heterophylla) in Puerto Rico, and E. fabae and Erythroneura ziczac found as carriers of 'Ca. P. asteris' in Canada. Phytoplasmas of group 16Srl were also found in being carried by few specimens of the potential vector Nedotepa curta. This may imply that the potential vector is polyphagous and maybe able to transmit both the CILY and 16Srl phytoplasmas. These findings urge to develop more effective disease management strategies, particularly for those villages where mixed infection was identified. More sounded surveys should be performed in an extension study phase in order to determine the epidemiological factors in both palms and insect populations related to CILY sole and mixed infection.

Six different plant species from five botanical families were identified as alternative hosts. These may play a role in disease spread: Poaceae (*Paspalum varginatum*, *Pennisetum pedicillatum*), Verbenaceae (*Stachytarpheta indica*), Plantaginaceae (*Scoparia dulcis*), Phyllantacea (*Phyllantus muellerianus*) and Cyperacea (*Diplacrum capitatum*). This is the first record of alternative plant hosts identified for phytoplasmas of the subgroup 16SrXXII-B since in Ghana, no alternative hosts have been proven so far,

despite the same plant hosts are shown to grow within Ghanaian coconut farms. In Mozambique where lethal yellowing is associated with subgroup 16SrXXII-A, secondary hosts identified include the African fan palm *Borasus aethiopum*, and oil palm *Elaeis guineensis*. So, these findings set the basis to expand epidemiology studies in Côte d'Ivoire and Ghana, and elucidate aspects related to disease spread. It is well known that the removal of alternative hosts combined with the use of resistant plant material has proven to be highly effective for the management of coconut lethal yellowing diseases, for instance in Jamaica and Mexico. Since the six plant species identified in Grand-Lahou are secondary hosts of the CILY phytoplasma, they pose a threat for its spread, therefore, they should be removed from all coconut farms as a strategy to effectively manage CILY.

These findings were already included in the 3rd and 4th Technical Reports. Results were put together under a new disease management plan, which was disseminated to farmers, stakeholders and policy makers through factsheets, a farmer's field mini-guide and policy briefs. Results on the alternative hosts have been published in the Canadian Journal of Plant Pathology "Detection and identification of the coconut lethal yellowing phytoplasma in weeds growing in coconut farms in Grand-Lahou, Côte d'Ivoire (Annex 4), and those on the potential vector are currently under review for journal Crop Protection "Identification of a newly described member of the tribe Erythroneurini as a potential vector of the Côte d'Ivoire lethal yellowing phytoplsma in coconut palms sole or in mixed infection with a 'Candidatus Phytoplasma asteris'-related strain" (Annex 3).

Detection of the CILY phytoplasma in cassava orchards of Grand-Lahou.

During the last three months of the project, symptoms of yellowing, mosaic, curling, and size reduction of the leaves were recently observed by farmers in different cassava orchards located nearby the coconutgrowing villages affected by CILY. Such orchards were confirmed in the past as infected by the cassava mosaic virus. Farmers rapidly contacted UNA requesting a field visit. UNA scientists collected cassava leaves from all areas where symptoms were reported. Leaf samples from *Pueraria* sp., commonly growing on the cassava orchards were also collected. Insect specimens were captured with a sweep net and a hand-made aspirator. Total DNA was extracted from all samples collected and subjected to PCR and sequencing with primers generic for phytoplasmas and specific for the CILY phytoplasma (group 16SrXXII-B, *'Candidatus* Phytoplasma palmicola'-related strains). Thirteen out of 21 cassava plants were found positive for the CILY phytoplasma, while one *Pueraria* sp. sample and one insect sample were positive for a phytoplasma strain of group 16SrI *'Candidatus* Phytoplasma asteris'.

PCR and sequencing confirmed the identification of the CILY phytoplasma for all the cassava plants. This finding indicates cassava as a potential alternative host for the CILY phytoplasma. Moreover, it is noteworthy the fact that group 16SrI was also found sole and in mixed infection in 5 % of the coconut palms affected by CILY in Grand-Lahou. For the potential vector, *Nedotepa curta*, two specimens were also found positive for the 16SrI phytoplasma. It is well known the aggressiveness of the 16SrXXII phytoplasma strains since those have wiped out the coconut industries in Côte d'Ivoire and Ghana. The group 16SrI is the phytoplasma group with the most known complex epidemiology and the widest range of plant hosts and Hemiptera phytoplasma vectors. The first report of cassava as a plant host for the CILY phytoplasmas was published as a New Disease Report in Plant Pathology "First report of phytoplasmas affecting cassava orchards in Côte d'Ivoire" (Annex 46). The writing of a full paper on the detection of the CILY phytoplasma in cassava and its mixed infection with cassava mosaic virus strains is under completion to submit to Plant Disease journal "Detection of the Côte d'Ivoire lethal yellowing phytoplasma in mixed infection with cassava mosaic virus strains in cassava orchards of Grand-Lahou.. The presence of both 16SrXXII-B and 16SrI phytoplasmas in the cassava orchards nearby CILY-affected coconut farms, and used for the Women Groups as an alternative source of family food and income, pose a serious threat to the food security of the smallholder coconut

farmers in Grand-Lahou. As part of the post-project activities, Dr. Kra from UNA, responsible for the field schools and plant clinics during the project performed a 45 day-training visit from Feb 2nd to March 18th 2017 at Sporometrics, sponsored by UNA. The visit pursued to complete the studies of CILY phytoplasma in cassava, and to receive training on phytoplasma lab diagnosis and environmental assessment to support further services at UNA for the community.

Socio-economic impact of CILY

The assessment of the socio-economic impact of CILY in the livelihoods of the coconut farmers in Grand-Lahou revealed over 90 % of illiteracy among farmers. There was also an increase in food and health related expenses for those families having farms affected by CILY. Surveys evidenced high inequality levels on gender roles, particularly for women with limited access to land, training and market. Recommendations were provided on developing approaches to better educate farmers and promote disease awareness, and empower women. These results were published in the African Journal of Agricultural Economics and Rural Development Vol. 4 (9), pp. 463-479, "Socio-economic impact of the coconut lethal yellowing disease on Ivorian smallholder coconut farm families" (Annex 20). An econometric model was developed to predict the land use change of the coconut cultivated area towards re-allocating the CILY-devastated areas and help reviving the coconut industry in Grand-Lahou. A full paper is under review in the journal Land Use Policy, (Annex 21).

Research partnerships, governance, research ethics and use of results

Since the beginning of the project, attempts have been made in order to isolate the CILY phytoplasma (16SrXXII-B) in artificial media in collaboration with the Department of Phytoplasmology of the University of Bologna, Italy. The partnership was strengthened through their participation in ring tests for the confirmation of lab results obtained at Sporometrics, UNA and CNRA. Dr. Nicoletta Contaldo was invited as one of the trainers for the Phytoplasma Advanced Research Training held in Canada for the Ivoirian project partners. She also presented at the Gender Workshop held at the University of Toronto. The CILY phytoplasma was for the very first time isolated and recovered from solid artificial media. Besides, the phytoplasma group 16SrI was also recovered from both solid and liquid artificial media supporting the field results about mixed infection. Although, studies should be completed, these preliminary results set the basis for the artificial culture of the CILY phytoplasma. This will speed-up full genome sequencing to unveil genes involved in disease pathogenicity, as well as, the performance of Koch postulates to confirm the CILY phytoplasma as the causal agent of CILY. The writing of a full paper on these results is under completion for its submission to PLOS One "Côte d'Ivoire coconut palm lethal yellowing disease -associated phytoplasmas: molecular and biochemical characterization of strains isolated in cultures". Collaboration with the Department of Economics of the University of Toronto was strengthened from the partnership with Prof. Ismael Mourifié, Econometrics Specialist, who contributed to the socioeconomic impact of CILY in Grad-Lahou, and the development of the econometric model.

Results obtained under Objective 1 are being used as part of the new disease management plan by disseminating factsheets, the farm field mini-guide and policy briefs to farmers, stakeholders and policy makers such the Major of Grand-Lahou, and the Ministries of Agriculture, and High Education and Scientific Research.

4.2 Objective 2. To develop and adapt control strategies to reduce the impact of the lethal yellowing disease on the coconut industry.

New diagnostics developed and validated

A new LAMP (loop-mediated isothermal amplification) PCR-based technology for in-field detection of the CILY phytoplasma was developed by CSIR-OPRI (Ghana) and Sporometrics. Primers were designed and chemically synthesized based on the ribosomal protein gene sequence of the CILY phytoplasma. Strains. The LAM-PCR assay was optimized and validated at CSIR-OPRI and UNA, and transferred to UNA and CNRA partners through a hands-on training-course held at UNA during November 2016. Project partners at UNA and CNRA are now fully equipped and trained for the early screen of CILY in the field since the LAMP-PCR allows the on-site and accurate detection of the CILY phytoplasma directly from coconut palms surveyed in CILY-affected farms. The method has been included as part of the post-project action plan to assess for the CILY phytoplasma in resistance trials. The writing of the full manuscript on the LAMP-PCR for the CILY phytoplasma is under completion for its submission to the African Journal of Biotechnology "Detection of the Côte d'Ivoire lethal yellowing phytoplasma using a ribosomal protein gene based assay and an internal control based on a WRKY transcription factor gene of coconut".

Moreover, the CILY phytoplasma was also differentiated from the Ghanaian CSPWD strain and the lethal yellowing phytoplasma from Mozambique based on signature sequences on its secretion protein gene sequence. This allowed the development of a PCR-RFLP diagnostics that would support the local disease surveillance programs. The publication of these results can be found in the Annals of Applied Biology journal "Detection and differentiation of the coconut lethal yellowing phytoplasma in coconut-growing villages of Grand-Lahou, Côte d'Ivoire", doi:10.1111/aab.12333 (Annex 2).

In regards to the seed transmission, so far all attempts to detect the CILY phytoplasma through PCR from the F1 and F2 generations have failed. However, seed transmission has been proven for other phytoplasma diseases of tomato, alfalfa and sesame, so the possibility of seed-borne transmission of phytoplasmas cannot be ruled out. Nevertheless, the follow-up of the seed transmission trials conducted at CNRA has been included in the post-project action plan. Moreover, in order to prevent any risk of disease spread due to a not controlled seed exchange among farmers, field schools have trained 1,960 farmers and around 700 families on how to establish their own coconut nurseries for seed supply.

Disease-resistant candidate cultivars to be included in the rehabilitation plan identified from resistance trials in pilot farms.

One classic strategy is to identify disease resistant cultivars of coconut palms but information on coconut genotypes is sparse. Since Côte d'Ivoire houses one of five International Coconut Genbank repositories in the world, obtaining plant material from 56 palms in the collection was performed in an effort to determine their genotypes. The goal was to determine those cultivars genetically closer to SGDxVTT to identify candidates that could be tested for resistance. SGDxVTT was found tolerant to CSPWD in Ghana in the past ten years, and is currently used for replanting in areas of CSPWD mid and low pressure in Ghana. This is the first report of a large scale genotype analysis of *Cocos nucifera* cultivars. It opens the door to future experiments to determine resistance profiles of these cultivars and eventually, identify true resistant cultivars to plant in the fields.

Nine promising cultivars/hybrids: NVS, NJM, NVM, NBO, NJG, NVT NRM, NVE and NRC, were identified as the candidates to be included in the post-project long-term resistance trials (minimum 3 years) in Côte d'Ivoire and Ghana to assess for resistance or tolerance to CILY in pilot farms in Grand-Lahou. The resistant cultivar and the closest genetic relative, **GVSXGVT** was highly recommended. Due to its novelty and impact

for the coconut research and further control of lethal yellowing diseases, the writing of a full paper on the ddRAD results is under completion to be submitted to the PLOS One journal. Pilot farms include two in Adjadon, one in Noumouzou, one in Badadon, and the control at the Marc Delorme Station, CNRA (plot N°152) have already been planted with such varieties, and are ready to perform the resistance assessment trials. Varieties NVS, NJM, NVM, NBO, NJG, NVT NRM, NVE, NRC and GVSXGVT have been already selected for the long-term resistance trials, and included in the post-project action plan. Those with better performance will be selected for the rehabilitation plan to be implemented in Grand-Lahou after 3 years of assessment of resistance trials.

Updates on potential biocontrols

Illumina-based microbiome profiles from CILY phytoplasma-infected and non-infected coconut palms supported those obtained by 'dilution-to-extinction' high-throughput culture. Previously and newly bacterial and fungal endophytes identified were confirmed. Those included *Bacillus, Burkholderia, Pseudomonas, Enterobacter, Streptomyces, Trichoderma, Penicillium, Fusarium, Aspergillus, Candida, Cryptococcus, and Purpureocillium*. New Illumina-based testing confirmed the most abundant bacteria identified by V4 sequencing in all samples as *Erwinia, Enterobacteriaceae, Bacillus, Acinetobacter, Serratia* and *Pseudomonas*. The full paper on the results from the Illumina-based microbiome assessment is under completion to be submitted to the Phytobiome journal "Illumina-based analysis of the bacterial microbiome in coconut palms affected by lethal 2 yellowing disease in Côte d'Ivoire". Cultures are available for further field testing of their biocontrol potential. Results on the diversity of bacterial and fungal endophytes associated with coconut palms affected and not-affected by CILY are under currently review in the African Journal of Biotecnology journal (Annex 27). African Journal of Biotechnology_AJB_27.03 "Diversity of bacterial and fungal endophytes from coconut palms infected and non-infected by the coconut lethal yellowing phytoplasma in GrandLahou, Côte d'Ivoire".

Moreover, the study of the bacterial microbiome in both male and female specimens of the potential vector *Nedotepa curta* using Illumina-based sequencing confirmed the presence of the new bacterial endosymbiont *Pectobacterium*, which becomes a powerful tool to explore for future endosymbiont control of the insect vector populations in Grand-Lahou. The writing of the full paper on these results is under completion to be submitted to the PLOS One journal "Characterization of the bacterial community associated with the potential leafhopper vector of the Côte d'Ivoire lethal yellowing phytoplasma". For the very first time on phytoplasma research, a new species of parasitoid within the genus Anagrus (Anagrus nedotepae) was identified parasitizing the eggs of the potential vector Nedotepa curta. This opens more feasible and practical ways of vector control, and a new source of income for farmers due to the very fast and easy-to-implement massive rearing of the parasitoid. The paper on the new parasitoid identified was just accepted in the Zootaxa journal "Anagrus nedotepae sp. n. (Hymenoptera: Mymaridae), an egg parasitoid of Nedotepa curta (Hemiptera: Cicadellidae), a pest of coconut palm in West Africa (Annex 47).

Results from the poultry manure assessment.

The first observations towards the end of the project on the poultry manure assessment conducted by CNRA were provided. At present, the CILY-affected coconut have no more than 15 nuts/tree/year compared to a non-affected palm that would yield 104 nuts/tree/year, 240 g of copra/nuts and 3.5 t of copra/ha/year. Palm trees treated with poultry manure show an increase in the number of flowers from 8 to 10 per tree and per inflorescence. It suggests that with the application of the poultry manure, coconut palms affected by the disease could reach half of the standard yield measured for a non-CILY affected palm, which represents a considerable gain for producers. The follow-up of the poultry manure assessment was specified within the post-project action plan.

Profitability analysis of intercropping coconut with plantain and maize.

Towards the end of the project, the estimation of the profitability index was based on the cumulative balance over the four years of the net cash-flow of coconut-to-food crops compared to the pure coconut crop. It showed that the association for 4 years of the coconut crop was more profitable with the plantain (833 950 F CFA/ha) than with the maize (123 000 F CFA/ha). The advantages of the association of plantain or corn with the coconut crop are that the income generated by the associated crops allowed to cover the operating expenses until the coconut came into production. The follow-up of intercropping trials has been also include in the post-project action plan.

Research partnerships, governance, research ethics and use of results

The partnership with CSIR-OPRI has been strengthened since CSIR-OPRI has been a key factor during the hands-on training-course and technology transfer sessions for the LAMP-PCR. Ghana developed a LAMP-PCR method for the CSPWD phytoplasma, and with their support, a new LAM-PCR method, specific for the CIYL phytoplasma has been developed for the in-field used and early confirmation of the disease in field surveys and resistance trials. The LAMP-PCR will be used during the 3-year post-project action plan for the screening of CILY phytoplasma in resistance trials, and in other coconut-growing areas of Grand-Lahou and Côte d'Ivoire.

CSIR-OPRI has also provided guidelines and advice for the development of the rehabilitation plan in Grand-Lahou, for the performance of the insect caged transmission trials, and the establishment of the resistance and intercropping trials in Grand-Lahou. CAGEF from the Univ. of Toronto was a strong partner for the endophyte identification, microbiome studies and data analysis from the genotyping and ddRAD studies. Collaboration was also strengthened with the Univ. of Torino, Italy on the identification of endosymbionts within the potential vector.

Partnerships were also established between the UNA entomologist, Sporometrics and the Typhlocybine specialist, Dr. Dietrich from the Illinois Natural History Survey, University of Illinois, who is keen to provide Dr. Eric Kwadjo with a short hands-on training in his lab for the identification of Typhlocybine species. A new partnership was also established between the UNA entomologist, Sporometrics and Dr. Serguei Triapitsyn from the Department of Entomology of the Univ. of California, USA, who is keen to provide Dr. Eric Kwadjo with a hands-on training on the identification and mass rearing of the new Ivoirian parasitoid identified.

Collaboration has been strengthened with the Centre for the Analysis of Genome, Evolution and Function (CAGEF) from the University of Toronto, particularly with Dr. Pauline Wang and Dr. David Guttman. Dr. Wang has been one of the trainers for the two training-courses held at the University of Toronto for the Ivoirian partners. The cooperation links between Sporometrics and the University of Toronto have provided new knowledge on the identification of cultivable fungal and bacterial endophytes with promising biocontrol potential. Both the Ivoirian and Canadian teams have strengthen their collaboration links, particularly through the training opportunities, as well as with the coconut farming community in Grand-Lahou through the involvement in Plant Clinics, Field Schools and Women Coconut Fairs.

An Evaluation Visit was conducted by Sporometrics and UNA to CSIR-OPRI in Ghana from November 27th to 30th 2016 to assess the setting-up of the long-term resistance trials that will run in parallel in Grand-Lahou. Six new varieties (four 'tall' and two 'dwarf' types) were introduced from the CNRA Genebank to test their resistance to CSPWD: Palu tall (GDO3), Pilipog green dwarf (NVP5), Madang Brun dwarf (NBN), Sri Lanka tall (GSL), Takome tall (GDO1), and Tonga tall (GTG). The six varieties were planted in June/July 2016 at two sites of high CSPWD pressure in the Western Region (Nkroful), and Central Region (Asebu) using a completely randomized block design with two replications (RCBD). The rest of the varieties (provided by CNRA) will be planted for the 2017 rainy season as part of the post-project plan.

4.3 **Objective 3.** To strengthen local capacity to diagnose, implement and disseminate control strategies by coconut-producing community, through the involvement of natural and social scientists, authorities, policy makers, stakeholders and farmers, empowering women's role in addressing gender inequity in Grand-Lahou.

Progress on field schools, plant clinics, Women Groups and Women Coconut Fairs during this period and the entire project implementation can be found in sections 3.1 and 3.2. An update on the dissemination of policy briefs can be also found in section 3.3. It is noteworthy to mention the attendance of the Canadian Ambassador in Abidjan, and representatives of the Ministry of High Education and Scientific Research, the Major and Prefect of Grad-Lahou, and Government and University organizations to the plant clinic held at Braffedon in April 2016.

New transfer technology: LAMP-PCR specific for the CILY phytoplasma.

A new hands-on training-course was held at UNA from November 21st to 25th 2016. This training-course was to transfer the LAMP-PCR technology developed for the specific in-field detection of the CILY phytoplasma with specific primers and probes designed by CSIR-OPRI and Sporometrics on the ribosomal protein gene of the CILY phytoplasma. The training-course was attended by 18 project members, including MSc (6) and PhD students (3) from UNA and CNRA, who have now the expertise to transfer knowledge and technology to other local Universities, research institutions and Ministries (Annex 26). The use of the LAMP-PCR will allow the early in-field detection of the disease, and will enable farmers and stakeholders to take actions at the very early stages of CILY to prevent disease spread.

Attempts to engage the local private sector to fight CILY

Attempts were made by UNA and ANADER to engage the local private sector and disseminate the project outcomes in order to support farmers, especially women in finding potential new market and commercialization opportunities.

UNA visited private enterprises such as COCOSOL, FIBRIVOIRE, HUTROCI, and COPROIL. UNA and ANADER agreed with representatives of each company to identify opportunities of training for field school's teachers on usage and processing of coconut material, so the knowledge could be properly transferred to farmers through field schools.

An inventory of the coconut processing companies active in Cote d'Ivoire with the potential to be engaged in the fight against CILY was developed by UNA (Annex 48). These companies have been included in both the rehabilitation and post-project action plans to find new approaches to effectively manage CILY and prevent its spread to other coconut-growing areas in Grand-Lahou and Côte d'Ivoire. The companies include SICOR, CAIMPEX, COPROIL, COCSOL, FIBRIVOIRE, HUTROCI and the PETIT PRODUCTEUR D'HUILE DE COCO. An inventory of coconut vendors and suppliers in Canada was recommended to include as part of the post-project action plan.

Dr. Innocent conducted along with Sporometrics, an Evaluation Visit to UNA and CNRA during November 7 to 11th 2016. During this visit, the teams organized a visit to the sole private entrepreneur that produces coconut oil in Grand-Lahou. Project members learned the process adopted by the entrepreneur and the equipment required for a low-cost production of coconut oil. A visit of the entrepreneur to farmers' field schools in Grand-Lahou was coordinated through UNA as part of the post-project action plan.

Research partnerships, governance, research ethics and use of results

PIs from Sporometrics and UNA visited the IDRC Office in Ottawa on November 17th 2016 to meet with the Program Officers, discuss the project results, and get recommendations on the avenues to improve the post-

project action plan, and the grounds to submit the new research proposal for the extension phase. During the week of November 7th to 11th of 2016, the IDRC program Officer, Dr. Innocent Butare performed an Evaluation visit along with the PI from Sporometrics to assess the progress of the project milestones, and provide recommendations towards their successful completion. During the visit, Dr. Butare visited the villages of Badadon and Braffedon, severely affected by CILY. He was able to know the work done by field schools and Women Groups, and visit a local entrepreneur from the private sector, keen to contribute with UNA on the CILY research, and support farmers of Grand-Lahou. In fact, this entrepreneur was included as part of the post-project action plan. The IDRC-DFTAD project has allowed project partners to strengthen cooperation with international plant pathologists, phytoplasmologists, entomologists, molecular biologists, socio-economists, and social scientists from Ghana and the University of Toronto; the University of Bologna in Italy, which have collaborated on the culture of the CILY phytoplasma; the University of California on the identification of the new species of parasitoid for *N. curta*, and the University of Illinois for the taxonomical identification of the new genus and species, *Nedotepa curta*, the potential vector of the CILY phytoplasma.

Our project outputs comply with the IDRC's Open Access policy since the publications will take place with open access publishers or through open access repositories. Therefore, scientific results will be free from restrictions on use and re-use, original authors will be properly acknowledged and cited. Scientific results and outputs will be freely and openly available to public in order to accelerate research and facilitate access to knowledge.

5.0 Synthesis towards AFS themes

The overall project results have directly impacted the following AFS themes: *improving access to resources, and/or markets and income; and informing policy.*

Improving access to resources, and/or markets and income

This is directly linked to the creation of Women Groups and the establishment of cassava yards for women coconut farmers in coconut lands that have been wiped out by CILY. This is currently helping women to have an extra income source to support family income and nutrition, accessing training on cassava planting and maintenance, and it is also an approach to diversify agriculture in Grand-Lahou. The extension of the cassava yards to other villages of Grand-Lahou, and other coconut-growing areas of Côte d'Ivoire has been included as part of the post-project action plan. On the other hand, the recommendations on intercropping coconut with plantain and maize, as well as the application of poultry manure to the coconut plantations contribute to increasing productivity. Women Coconut Fairs allowed women coconut farmers to sell out their products not only in the Grand-Lahou villages, but also in Abidjan. The diversity of coconut products increased from 90 to 160 different coconut-derived products from 2015 to 2016. Women coconut farmers from Grand-Lahou learned the new uses of the coconut husk a potential source for small business from making huskbased items such as cleaning brushes, bags, floor mats. Women farmers also included new coconut-based products such as coconut cakes and coconut-based repellent. Field schools and plant clinics are also approaches to support families in fighting plant diseases, and allow farmers, particularly women to access technical support, training and advice. Young villagers are interested to become 'plant doctors' as they identified them as a new job option and income source.

Informing policy

The project developed approaches to engage farmers, stakeholders, and policy makers in fighting CILY, and empower women to support family income and nutrition. One example was the plant clinic held at Braffedon in April 2016. It was attended by the Canadian Ambassador in Abidjan, and representatives of the

Ministry of High Education and Scientific Research, the Major and Prefect of Grand-Lahou, and Government and University organizations. Policy makers were invited to visit the areas devastated by the disease, where they were able to dialogue with farmers and project members. Policy makers took part in the first presentation of the new disease management plan to farmers and extension agents through factsheets, hands-on demonstrations of insect field trapping, guidelines to remove alternative hosts and diseased palms, as well as the farmer field mini-guide. These have been translated in French and made available at COWALY. In addition, project members visited the Canadian Embassy in Abidjan to present the project results and ask for support for the post-project action plan. Policy makers have been also included as part of the post-project action plan. Three policy briefs have been sent to the Canadian Ambassador in Abidjan, the Ministry of High Education and Scientific Research, the Ministry of Agriculture, and the Major of Grand-Lahou.

6.0 Methodology

Throughout the project implementation, methods, techniques and experimental designs were utilized as planned, some of them are mentioned under 'Progress towards milestones'. For the assessment of the disease socio-economic impact, and the development of the econometric model these included genderresponsive surveys, interviews and questionnaires, quasi-experimental research design with Propensity Score Matching (PMS), quantitative impact evaluations methods like randomized evaluations, PSM, Double Difference (DD), Instrumental Variables, Regression Discontinuity (RD), pipeline approaches, and a multinomial probit model with Using Markovian Chain Analysis. Illumina-based sequencing coupled with a 'dilution-to-extinction cultivation' method was applied for the very first time for bacterial and fungal community studies from coconut palms and rhizosphere to identify endophytes and beneficial organisms able to enhance plant resilience as biocontrols or bioinoculants. This was also applied to potential insect vector to identify bacterial endosymbionts to use for long-term biocontrol strategies. All the training modules planned and scheduled for field schools were performed accordingly, including the questionnaires and interviews for plant clinics. A new faster method was included, genotyping-by-sequencing to combine with Illumina sequencing to identify those coconut cultivars genetically closer to resistant cultivars to Cape St. Paul Wilt disease used in rehabilitation programs in Ghana, which resulted in 9 promising cultivars currently planted in pilot farms in Grand-Lahou and Ghana for long-term resistant trials as part of the postproject action plan. New recommendations on application of poultry manure and intercropping with plantain were provided based on results from the original implemented experimental designs of the research project. Polymerase chain reaction assays such as nested, real-time, and loop-mediated isothermal amplification (LAMP), as well as, RFLP (restriction fragment length polymorphism), cloning and sequencing were used for phytoplasma detection, identification and characterization from coconut palms, non-coconut palms and Hemiptera, which allowed to identify phytoplasma mixed infections, as well as alternative hosts and the potential insect vector for the CILY phytoplasma, all included in a new disease management plan. One of the outcomes was the development of an in-field LAMP assay specific for the CILY phytoplasma, which only takes 30 min, and allows early disease detection before symptoms develop. Technology transfer and training was provided to project partners and agricultural extension agents on the new LAMP method. Project partners learned new cutting-edge and current technologies for phytoplasma research and diagnosis, including new methods to culture phytoplasmas from coconut palms, and LAMP, which is now revolutionizing the point-care approach to improve plant disease diagnosis, management and control.

7.0 Project Outputs

• Project Video 'Fighting a lethal disease for farmers in Côte d'Ivoire'. (Annex 33)

A 12 min video has been developed and uploaded to COWALY. The video was launched at the Final Project Meeting held at CNRA on December 14th 2016. The video summarizes the most outstandig results and outcomes fo the project, and shows approaches to engage stakeholders and policy makers to continue the fight against CILY as part of the post-project action plan.

• Plant Clinics' Video. (Annex 32)

A 15 min video has been developed and uploaded to COWALY. The video summarizes the imapct of plant clinics in Grand-Lahou, and highlights the significance of these to mobilize farmers and stakeholders for disease awareness, and to tackle disease problems in coconut and over 20 other crops.

Rehabilitation plan (Annexes 30 and 31)

A rehabilitation was developed to relaunch the coconut sector and limit the spread of the disease into new coconut-growing areas within Grand-Lahou and throughout Côte d'Ivoire. The plan will start by replanting those coconut plantations devastated by CILY in selected farms. The plan will utilize those cultivars and hybrids confirmed as tolerant or resistant to CILY after three years of assessment of the resistance trials in Grand-Lahou and Ghana. The plan is available at COWALY in English and French.

• Post-project action plan (Annexes 44 and 45)

A plan was developed to provide a guide of actions to take after the project end to support the continuation of project activities that require long-term assessment, monitoring and follow-up to mantain the implementation of the communication and gender strategies previously developed. The plan has general and specific objectives, as well as indicates the specific action and responsible organization of the action. The plan "Post-project action plan" is available at COWALY in English and French.

- Policy Brief to control the movement of coconut germplasm in Grand-Lahou. (Annexes 11 and 12) The uncontrolled movement of coconut germplasm among farmers would speed up the spread of the disease into other coconut-growing areas, and would eventually lead to the collapse of the coconut industry in the country. This would dramatically reduce the exports of coconut and coconut oil from copra and would severely impact the national economy and the livelihood of the smallholder coconut farming sector of Cote d'Ivoire. There is as yet no evidence that the CILY phytoplasma is seed transmitted through to the seedling to cause disease in progeny palms. Nevertheless, farmers in Grand-Lahou have been trained on how to establish on-farm nurseries through field schools. Such nurseries will be the source of seedlings for farmers, and will help preventing unnecessary exchange of seedlings. The protocol for the establishment of nurseries in Grand-Lahou is provided. It is available at COWALY in English and French.
 - Protocol for the establishment of on-farm nurseries for a safe coconut germplasm movement in Grand-Lahou. (Annex 41)

Care must be taken in choosing the seedlings to start a plantation. High quality seedlings allow to sustain the coconut palms productive and economic for more long years in the field under extremely variable conditions. The protocol developed can be applied for both the Genebank and hybrid trials with recommendations on nursery site selection, seedbed and seedling maintenance, pricking, polybag nursery, etc. These nurseries are the supply source of coconut seedlings for farmers to prevent the uncontrolled exchange of seedlings among farmers. It is available at COWALY in English and French.

• New CILY disease management plan. (Annexes 7 and 8).

Recommendations on farm management including measures to identify and remove the alternative hosts of the CILY phytoplasma, and how to identified populations of the insect potential vector *Nedotepa curta* in the

coconut palms by stage and during their peak seasons are provided. It is available at COWALY in English and French.

• Posters, factsheets, flyers and brochures generated (Annexes 23, 38, 39, 40).

Oneposter and six factsheets were designed and generated as outcomes of the project that illustrate the different plant species identified as alternative hosts for the CILY phytoplasma, the distinct stages of the potential insect vector *Nedotepa curta*; the description of CILY symptoms, the protocols to establish on-site nurseries and to control the germplasm movement, and summaries of the disease management, and environmental and mitigation plans are provided to be disseminated in Grand-Lahou. It is available at COWALY in English and French.

Policy brief on good cultural practices in Grand-Lahou. (Annexes 13 and 14).

The knowledge of both insect vectors and alternative hosts contribute to develop a more effective control strategy for phytoplasma diseases such as CILY in Grand-Lahou. They may have epidemiological implications for CILY spread in coconut farms in Grand-Lahou. If not actions are taken in regards to the removal of those weeds that act as reservoirs for the CILY phytoplasma and to control insect populations, CILY will wipe out the coconut groves of Grand-Lahou more likely in the next five years, and that would be a niche for the emergence of phytoplasma diseases in other weeds and crops. Field schools and plant clinics should train the farmers and extension agents on how to recognize the alternative hosts and the potential vector. It is crucial that policy makers and stakeholders, including Women Groups support the farmers in Grand-Lahou to adopt and implement the present policy brief. It is available at COWALY in English and French.

• Environmental and Mitigation Plans (Annexes 28 and 29).

CILY has had an important environmental impact in the coconut-growing areas of Grand-Lahou. Environmental management and mitigation plans have been developed to minimize such impact in the coconut-growing villages of Grand-Lahou. The purpose of the plans are to help the Grand-Lahou community to build local adaptation capacity, engage in climate smart decision-making at the community level, and create a feedback mechanism to inform national policy through a bottom-up approach in addressing the short- and longer-term environmental impacts of CILY. The plans target resiliency recommendations and measures on irrigation (applicable in periods of severe drought), manuring, intercropping, moisture maintenance, plastic and metal waste management, removal of infected palms, and information dissemination. These will improve livelihoods of the poor-resource coconut farmers through a participatory community-level process that involve farmers, stakeholders and extension agents to prioritize resiliency measures and for post-project monitoring. It is available at COWALY in English and French.

Policy brief for the Environmental and Mitigation Plans (Annexes 9 and 10)

The coconut-growing areas of Grand-Lahou have been environmentally impacted by CILY since the late 1990's when the first symptoms were spotted in the village of Palmindustrie V1, and since its very first outbreak. Information from previous soil analysis and recent observations during the IDRC-DFATD project, and the records of environmental variables such as rainfall and temperature in Grand-Lahou were considered to develop environmental and mitigation plans directed to farmers, stakeholders, authorities, policy and decision makers and NGOs and the coconut farming community. It is available at COWALY in English and French.

 Detection and identification of the coconut lethal yellowing phytoplasma in weeds growing in coconut farms in Côte d'Ivoire. 2016. Canadian Journal of Plant Pathology, Vol 38(2): 164-173. http://www.tandfonline.com/toc/tcjp20/current (Annex 4).

A recent survey of coconut farms in Grand-Lahou yielded percentages of infection over 85% for the Côte d'Ivoire lethal yellowing phytoplasma disease. Given the highly weedy status of most of the farms surveyed,

plant species growing within and in the periphery of the coconut farms were assessed for the Côte d'Ivoire lethal yellowing phytoplasma to identify potential alternative host species. A total of 288 samples from thirty five botanical families were collected. Total DNA was extracted and tested by nested PCR and sequencing assays with primers targeting the 16S rRNA and the translocation protein (secA) phytoplasma genes. Plants from five families yielded amplicons of expected size; and their both SecA and 16S rDNA sequences exhibited over 99% of identity with that of the Côte d'Ivoire lethal yellowing phytoplasma identified from the coconut farms previously surveyed. Plant species from families Poaceae, Verbenaceae, Plantaginaceae, Phyllanthaceae and Cyperacea were identified as hosts of the Côte d'Ivoire lethal yellowing phytoplasma and may have epidemiologic implications for disease spread in coconut farms in Grand-Lahou.

 Socio economic impact of CILY on coconut farm families in Grand-Lahou. 2016. African Journal of Agricultural Economics and Rural Development, Vol 4(9): 463-479.
 www.internationalscholarsjournals.org. (Annex 20).

Côte d'Ivoire lethal yellowing (CILY) disease has devastated coconut plantations over the past ten years in the Grand-Lahou in the south littoral of Côte d'Ivoire. This paper used primary data collected from 338 coconut farming households of Grand-Lahou to assess the socio-economic impact of CILY for smallholder farmers. Using the propensity scores matching method, the Average Treatment effect on the Treated was calculated to assess the impact of the disease on the household. To characterize the welfare of coconut farming households, five socio-economic parameters where estimated; income, food expenses, non-food related expenses, health and school expenses. Food and non-food related expenses, including health expenses increase for CILY-affected households compared to their counterfactual. Inversely, households' total income and spending children's schooling expenses decreased. The survey data evidenced the inequality of gender roles in Grand-Lahou, emphasizing the limited access to resources for women farmers. Based on the findings, recommendations were made to address gender inequities and to promote awareness for smallholder farmers in preventing disease spread; and approaches for better education access. Informing farmers that CILY is associated with an increase of food- and non-food related expenses may encourage their engagement at mitigating the impact of the disease and improving disease management.

 Detection and differentiation of the coconut lethal yellowing phytoplasma in coconut-growing villages of Grand-Lahou, Côte d'Ivoire. 2017. Annals of Applied Biology ISSN 0003-4746 doi:10.1111/aab.12333. (Annex 2).

Surveys for the Côte d'Ivoire lethal yellowing (CILY) phytoplasma were conducted in eight severely CILY-affected villages of Grand-Lahou in 2015. Leaves, inflorescences and trunk borings were collected from coconut palms showing CILY symptoms and from symptomless trees. Total DNA was extracted from these samples and tested by nested polymerase chain reaction/RFLP and sequence analysis of the 16S rRNA, ribosomal protein (rp) and the translocation protein (secA) genes. The CILY phytoplasma was detected in 82.9% of the symptom-bearing palms collected from all the surveyed villages and from all the plant parts. Trunk borings were recommended as the most suitable plant tissue type for sampling. Results indicate that the CILY phytoplasma may have a westward spread to other coconut-growing areas of Grand-Lahou. CILY phytoplasma strains infecting coconut palms in the western region of Grand-Lahou exhibited unique single nucleotide polymorphisms on the rp sequence compared to the strains from the eastern region. Moreover, single nucleotide polymorphisms on the SecA sequence distinguished the CILY phytoplasma from the Cape St. PaulWilt Disease phytoplasma in Ghana, and the Lethal Yellowing phytoplasma in Mozambique.

• Field schools and plant clinics: effective agricultural extension approaches to fight the coconut lethal yellowing disease and improve livelihoods of smallholder farmers in grand-lahou, côte d'Ivoire.

International Journal of Agricultural Extension and rural Development. 2017. Vol. 5 (3), pp. 545-555. (Annex 19).

Field schools and plant clinics are key extension tools to fight the lethal yellowing disease of coconut that is severely impacting the livelihoods of thousands of smallholder farmers in Grand-lahou, Côte d'Ivoire. Field schools have trained 1,960 men and women farmers on coconut farming, marketing and disease management. Six Women Groups have been created to support women in establishing cassava yards as an alternative food and cash crop. Plant clinics have mobilized circa 600 producers that are willing to pay consultation services to plant doctors. Young farmers foresee plant doctors as a new local job source. The number and diversity of coconut products sold by women farmers through Women Coconut Fairs increased 64% from 2015 to 2016 enabling women to sell and marketing, and to identify small business opportunities. Sex-disaggregated surveys revealed the main gender issues and major constraints associated with the coconut production chain in Grand-Lahou. Field schools and plant clinics are effective approaches to engage farmers, stakeholders and policy makers, and to empower women. These may be also the most suitable platforms to discuss and encourage the implementation of low-cost local interventions on behalf of the farming community.

• Economics of land use dynamics in coconut plantations of Grand-Lahou, Cote d'Ivoire. Land Use Policy journal. LUP 2016 1283. Under review. (Annex 21).

This paper uses a multinomial probit model with random effect based on a theoretical land use model to predict the spatial distribution of land use within the department of Grand-Lahou in Côte d'Ivoire where coconut plantations have been devastated by the Cote d'Ivoire lethal yellowing (CILY) disease. The model used a sample of 9432 grids covering the land map of the department. Using Markovian Chain Analysis based on Markov transition probability matrix, the spatial distribution of land use was predicted for the next 30 years. The results showed a significant conversion of small coconut areas (< 50 % of a grid) to large areas (50 – 75 % of a grid) before the outbreak of CILY. However, this expansion was halted when the disease outbreak hit; followed by a slight conversion of large coconut areas to other agricultures land uses. It was also found that conversion of urban areas (> 50 % of a grid) to other land uses will continue over time. A clearly dynamic use of land characterized by an increase of urban areas with an expansion of coconut plantations, mostly towards the continental non-CILY affected zone of Grand-Lahou is predicted. To prevent the spread of disease to these non-CILY affected areas, research and gender-responsive extension services focused on smallholder coconut farmers should be reinforced to fight CILY disease.

 Identification of a newly described member of the tribe Erythroneurini as a potential vector of the Côte d'Ivoire lethal yellowing phytoplasma in coconut palms sole or in mixed infection with a 'Candidatus Phytoplasma asteris'-related strain. Crop Protection. Under review. (Annex 3).

Côte d'Ivoire lethal yellowing (CILY) phytoplasma is severely affecting the south coconut littoral of Grand-Lahou. Phytoplasmas are transmitted by phloem-feeding Hemiptera, although a number of those have been reported as potential vectors for palm lethal yellowing-like diseases in Central America, the Caribbean, and Mozambique, no vector capacity has been proven yet for them, except for *Haplaxius crudus* in Florida. Over 300 Hemiptera specimens were collected by using sweep net and hand-made aspirators from coconut palm fronds in six villages of Grand-Lahou. Eight major families were identified including Aphrophoridae, Achilidae, Derbidae, Flatidae, Membracidae, Pentatomidae, Tropiduchidae, and Cicadellidae, the latter being the most abundant throughout the surveyed villages. Specimens were subjected to taxonomic identification and total DNA extraction. PCR assays with primers targeting the 16S rRNA and the translocation protein secA genes yielded PCR amplicons from 216 out of 296 (73%) of the tested specimens of a newly identified cicadellid leafhopper, *Nedotepa curta* Dmitriev. PCR amplicons were purified, cloned and sequenced. The 16S rDNA and secA sequences from *N. curta* showed a 99% sequence identity with those of the CILY phytoplasma identified in coconut-growing villages of Grand-Lahou, which suggested N. curta as a potential

vector for the CILY phytoplasma. Phytoplasmas of group 16SrI 'Candidatus Phytoplasma asteris'-related were identified from CILY phytoplasma-infected coconut palms and N. curta specimens from the Badadon and Yaokro villages, and from the weeds Dalbergia saxatilis and Baphia nitida from Badadon, indicating their presence in mixed infection with the CILY phytoplasma.

 Diversity of bacterial and fungal endophytes from coconut palms affected by the lethal yellowing disease in Grand-Lahou, Côte d'Ivoire. 2017. African Journal of Biotechnology. Accepted. (Annex 27).

The diversity of bacterial and fungal endophytic communities was assessed from coconut palms affected and not affected by the Côte d'Ivoire Lethal Yellowing (CILY) disease, as well as, infected and non-infected by the CILY phytoplasma. Bacterial and fungal endophytes were isolated from leaves, trunk and rhizosphere samples collected from two CILY-affected villages of Grand-Lahou, Braffedon and Yaokro, by using a high-throughput 'dilution-to-extinction' method coupled with PCR and sequencing with primers that amplified both the 16S ribosomal RNA and intergenic transcribed spacer (ITS) genes. The bacterial endophytic community in Braffedon was significantly more diverse than that from Yaokro, and conversely, the fungal endophytic community was more diverse in Yaokro than that in Braffedon. No differences were found on the diversity levels of the endophytic communities between the different CILY disease stages and symptomless palms from Yaokro or Braffedon. Bacterial and fungal endophytes commonly found in both villages included *Burkholderia*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Streptomyces*, as well as, *Aspergillus*, *Candida*, *Cryptococcus*, *Fusarium*, *Penicillium*, *Purpureocillium* and *Trichoderma*. Results provide the basis to further investigate the effect of these endophytes against the CILY phytoplasma in order to design new strategies for the effective management of CILY in Grand-Lahou.

• Anagrus nedotepae sp. n. (Hymenoptera: Mymaridae), an egg parasitoid of Nedotepa curta (Hemiptera: Cicadellidae), a pest of coconut in West Africa. Zootaxa. Accepted. (Annex 47).

A new Afrotropical species of *Anagrus Haliday* (Hymenoptera: Mymaridae), *A. nedotepae* Triapitsyn sp. n., is described and illustrated. The type series was reared from parasitized eggs of the leafhopper *Nedotepa curta* Dmitriev (Hemiptera: Cicadellidae) on leaves of a coconut palm in the course of a survey for the natural enemies of *N. curta* in Côte d'Ivoire, as part of the international project on coconut. This peculiar leafhopper, which was recently described from Ghana (Dmitriev 2016), is and economically important pest of coconut palm in West Africa, particularly being a suspect vector of its yellowing-like diseases. Thus, the parasitoid may be of potential importance for natural biological control of this pest. Notes on its biological traits are also provided.

 Characterization of the bacterial community associated with the leafhopper potential vector of the Côte d'Ivoire coconut lethal yellowing phytoplasma. Phytobiome to submit.

The aim of this work was to characterize the bacterial diversity associated to the leafhoppers vector of the coconut phytoplasma. This study was done by means of Illumina sequencing of the regions V3-V4 of 16SrRNA gene. Moreover, qualitative PCR reactions with specific primers for major leafhopper symbiont clades were performed on whole insect and on specific body parts (head, thorax, and abdomen) to assess their presence, prevalence, and localization. The most abundant orders identified (i.e. those present at least in one of the samples with percentages >5) included Actinomycetales, Flavobacteriales, Bacillales, Lactobacillales, Clostridiales, Rhizobiales, Enterobacteriales, Pseudomonadales, and Xanthomonadales.

• Côte d'Ivoire coconut palm lethal yellowing disease -associated phytoplasmas: molecular and biochemical characterization of strains isolated in cultures. *PLOS One. To submit*.

The Côte d'Ivoire coconut lethal yellowing disease (CILY) is severely affecting the coconut village area of Grand-Lahou. This has already caused the loss of over 400 ha and is currently threatening another 7,000 ha

along the south coastal littoral. A rapid method for isolation, purification and characterization of the CILY phytoplasma was established. CILY phytoplasma strains were isolated in either solid or liquid special media from 14 samples of leaves, inflorescences or trunk borings collected from twelve palm trees, which were showing symptoms of disease stages 1, 2 and 3. All symptomatic samples yielded the presence of the CILY phytoplasma (group 16SrXXII-B) by PCR with universal phytoplasmas primers that amplify the 16S rDNA. One symptomless palm also collected was negative for phytoplasma testing and isolation. Colony morphology, PCR and sequencing analyses revealed the presence of ribosomal groups, 16SrI-B, 'Candidatus Phytoplasma asteris', 16SrV-A 'Candidatus Phytoplasma ulmi', and 16SrXII-A 'Candidatus Phytoplasma solani' in mixed infection with the 16SrXXII-B phytoplasma. Trunk borings proved to be the most suitable plant organ for phytoplasma isolation, showing the typical phytoplasma-like colonies. These findings indicated that mixed phytoplasma populations occur, and could lead to difficulties for colony purification and characterization. Furthermore, biochemical tests including urea and arginine hydrolysis, and glucose fermentation were carried out for 16SrI and 16SrXII-A phytoplasma isolates. Results confirmed both phytoplasmas as urea negative and glucose and arginine positive. To our knowledge, this is the first isolation in artificial media of phytoplasmas from coconut palms affected by CILY and their first biochemical characterization.

• Detection of the Côte d'Ivoire lethal yellowing phytoplasma in mixed infection with cassava mosaic virus strains in cassava orchards of Grand-Lahou. Plant Disease. To submit.

Symptoms of yellowing, mosaic, curling, and size reduction of the leaves were recently observed by farmers in different cassava orchards located nearby the coconut-growing villages affected by the Côte d'Ivoire lethal yellowing. Such orchards were confirmed in the past as infected by the cassava mosaic virus. Leaf samples were collected from cassava plants showing symptoms and those symptomless, and from *Pueraria* sp., commonly growing on the cassava orchards, and Hemiptera specimens, captured with a sweep net and a hand-made aspirator. Total DNA was extracted from all samples collected and subjected to PCR and sequencing with primers generic for phytoplasmas and specific for the CILY phytoplasma (group 16SrXXII-B, 'Candidatus Phytoplasma palmicola'-related strains). Total DNA was also tested in a multiplex PCR for the African (ACMV) and Eastern Africa (EACMV) cassava mosaic virus strains. Thirteen out of 21 cassava plants were found positive for the CILY phytoplasma, while one Pueraria sp. sample and one insect sample were positive for a phytoplasma strain of group 16Srl 'Candidatus Phytoplasma asteris'. In addition, 11 cassava plants infected by the CILY phytoplasma were mixed infected with either or the combination of the ACMV/EACMV, as well as the sole insect specimen that was PCR positive. This finding indicates that cassava is a potential alternative host for both the CILY phytoplasma, and the 16Srl phytoplasma, which makes more complex the epidemiology and control of the diseases, and urged the implementation of more effective disease management strategies. This is the first report of cassava as a plant host for the CILY and 16SrI phytoplasmas, and their mixed infection with cassava mosaic virus strains. The presence of both 16SrXXII-B and 16SrI phytoplasmas in the cassava orchards nearby CILY-affected coconut farms, and used for the Women Groups as an alternative source of family food and income, pose a serious threat to the food security of the smallholder coconut farmers in Grand-Lahou.

• Illumina-based analysis of the bacterial microbiome in coconut palms affected by lethal yellowing disease in Cote d'Ivoire. Phytobiome. To submit.

Illumina-based sequencing was used to characterize the bacterial microbiome of coconut palms affected and non-affected by the Côte d'Ivoire Lethal Yellowing (CILY) disease, as well as, infected and non-infected by the CILY phytoplasma. Bacterial and fungal endophytes were isolated from leaves, trunk and rhizosphere samples from CILY-affected villages of Grand-Lahou. The most abundant bacteria identified were *Erwinia*, *Enterobacteriaceae* (unidentified genus), *Bacillus*, *Acinetobacter*, *Serratia* and *Pseudomonas*. These are the very first results on the study of the bacterial microbiome in coconut palms affected by a lethal yellowing

disease. Results provide the basis to further investigate the effect of these bacterial endophytes against the CILY phytoplasma in order to design new strategies for the effective management of CILY in Grand-Lahou.

Development of a ddRAD approach for SNP discovery and genotyping in Cocus nucifera L. PLOS One.
 To submit.

Genotyping was performed to sequence "RAD" or Restriction Associated DNA in 55 coconut cultivars and hybrids tested for resistance to the Côte d'Ivoire Lethal Yellowing phytoplasma to determine their genotypes. One cultivar, SGD X VTT, is known to be resistant to the Cape St. Paul Wilt disease that destroyed the coconut industry in Ghana in the last 20 years. Being able to identify closely related genotypes would provide candidates that could be tested for resistance. SNP data was extracted and used to perform a phylogenetic analysis. The resistant cultivar and the closest genetic relative was GVS X GVT. This is the first report of a large scale genotype analysis of *Cocos nucifera* cultivars. It opens the door to future experiments to determine resistance profiles of these cultivars and eventually, identify true resistant cultivars to plant in the fields.

• Detection of the Côte d'Ivoire lethal yellowing phytoplasma using a ribosomal protein gene based assay and an internal control based on a WRKY transcription factor gene of coconut. African Journal of Biotechnology. To submit.

The Côte d'Ivoire lethal yellowing (CILY) phytoplasma is impacting the livelihoods of thousands coconut smallholder farmers in Grand-Lahou. A rapid and robust diagnostic technique is crucial in efficient disease management. We established a phytoplasma loop mediated isothermal amplification (LAMP) and real time polymerase chain reaction (PCR) based on the sole ribosomal protein sequence of the CLY phytoplasma. The LAMP reaction was set at 65 °C and end point detection made using hydroxynaphthol blue (HNB) and agarose gel electrophoresis. Molecular typing of LAMP products were made with restriction enzyme HpyCH4 V. Conventional PCR with LAMP external primers and sequencing of amplicons was carried out. Real time LAMP was performed on the Genie II platform (Optigene Ltd., UK). An annealing curve analysis was programmed at the end of the incubation to check the fidelity of the amplicons. The phytoplasma positive samples produced typical ladder like bands on agarose gel, showed colour change from violet to blue with HNB and produced unique annealing peak at 85 ± 0.5 °C in the real time detection. Restriction digestion produced predicted size fragments. Sequencing and BLASTN analysis confirmed that the amplification corresponded to the CILY phytoplasma. LAMP PCR can be use in the field for the on-site screening of the CILY phytoplasma in CILY-affected farms, and it is also a tool for the screening of healthy seedlings.

• "Farmer field mini-guide". (Annexes 5 and 6).

This is a mini-book produced from research collaboration between Canada and Côte d'Ivoire to guide farmers and stakeholders on how to implement the new management plan for the lethal yellowing disease to prevent disease spread and help to save the coconut industry in Grand-Lahou. It is available in French and English.

8.0 Problems and Challenges

A 'Stakeholder Workshop' was planned to be held at UNA on the week of January 16th to 20th 2017 to engage the private sector for the post-project action plan. However, the unrest events occurring during January in the cities of Abidjan and Bouaké prevented this activity to take place. The events occurring during early January to mid-February were related to demobilised military soldiers – mainly former rebels from the decade-long conflict – who broke into police stations across the city seeking payment of money they believed they were owed by the government. Soldiers were looting weapons and producing heavy gunfire

before taking up positions at entry points into the city. Nevertheless, CNRA and UNA partners will joint efforts to meet with key local private companies, which has been included in the post-project action plan.

It is well known that obtaining a coconut cultivar or hybrid resistant or even tolerant to lethal yellowing can take long time given the experiences in Jamaica and Mexico, or in Ghana, which is yet under the implementation of their rehabilitation program. This was not possible to achieve from our project due to the very short length for the required research and assessment. However, the team was able to provide promising cultivars and hybrids to recommend for the long-term assessment of resistance to CILY in Grand-Lahou and Ghana. The follow-up of the resistance trials already established in 5 pilot farms has been included as part of the post-project action plan.

The project team has achieved the publication of several results in high impact factor and open access journals as stated by the 'IDRC Open Access Policy'. The number of publications achieved is higher than expected, but the reviewing process for most of these publications has taken longer than expected. Therefore, there is a number of manuscripts that have been long submitted but the team has not yet feedback of their final acceptance. Other manuscripts are currently under the completion of the writing process to be submitted. Due to this situation, the budget specified for 'Publications' under the 'Research' category was not fully used. This will be specified in the Final Financial Report.

Upon discussion with our IDRC Program Officers and following the Open Access policy for IDRC-funded project outputs beyond the funding period but resulting from the IDRC funding, we are providing the list of publications that are currently under review process, as well as the titles that are currently under completion for its soon submission. Hoping that our publication costs are deemed eligible expenses under the IDRC Open Access policy, we are providing the journal names, and the estimates of the publication fees. Upon receiving the journal notification of acceptance, Dr. Yaima Arocha Rosete will let the IDRC Program Officers know to coordinate the respective payment of the publication fees.

All titles have been included as project outputs

The publications' titles are the following:

- 1. Economics of land use dynamics in coconut plantations of Grand-Lahou, Cote d'Ivoire. (Land Use Policy, Annex 21). Under review process. Open Access publication fees: \$1,800 USD.
- 2. Identification and biology description of *Nedotepa curta*, potential vector of the CILY phytoplasma. (Crop Protection, Annex 3). Under review process. Open Access publication fees: \$2,500 USD.
- 3. Detection of the Côte d'Ivoire lethal yellowing phytoplasma in mixed infection with cassava mosaic virus strains in cassava orchards of Grand-Lahou. To be submitted to Plant Disease. Current open access publication fees are \$50 per printed page for the first six pages and \$80 for each page thereafter for APS members. Dr. Yaima Arocha Rosete is an APS member.
- 4. Côte d'Ivoire coconut palm lethal yellowing disease -associated phytoplasmas: molecular and biochemical characterization of strains isolated in cultures. PLOS One. To be submitted. Open Access publication fees: \$1,800 USD.
- 5. Characterization of the bacterial community associated with the leafhopper potential vector of the Côte d'Ivoire coconut lethal yellowing phytoplasma. (PLOS One). To be submitted. Open Access publication fees: \$1,495 USD.
- 6. Illumina-based identification of the bacterial microbiome of coconut palms affected by the Côte d'Ivoire Lethal Yellowing. (Phytobiome). To be submitted. Open Access publication fees: \$1,350 USD.

7. LAMP-PCR optimized for the specific detection of the Côte d'Ivoire coconut lethal yellowing phytoplasma (African Journal of Biotechnology). To be submitted. Publication fee \$650 USD.

9.0 Overall assessment and recommendations

The project team has achieved more than expected in such a short-time being dealing with culture-based issues, gender inequalities, particularly related to women farmers, as well as, adverse conditions of work in remote farms of the Grand-Lahou village area. The project implementation was well adopted by farmers, and stakeholders, and even policy makers such as the Major of Grand-Lahou and the Ministry of High Education and Scientific Research, and the Canadian Ambassador in Abidjan were involved in some of the project activities. We consider that we have developed approaches that are currently helping farmers, particularly women, to improve their family income and nutrition.

Administratively, we recommend to organize events like the Scaling-Up Workshop that was held in Nairobi, Kenya, as part of the inception meetings, so IDRC project grantees are able to get to know each other, as well as, apply new knowledge, suggestions and recommendations right from the beginning of the implementation of their projects.

10.0 Annexes

- 1. Annex 1_http://cowaly.com/wp-content/uploads/2014/09/Annex-1-Phytopatogenic-Mollicutes-2015.pdf "Analyses based on the 16S rRNA and secA genes identify a new phytoplasma subgroup associated with a lethal yellowing-type disease of coconut in Côte d'Ivoire".
- Annex2_http://cowaly.com/wp-content/uploads/2017/01/Rosete_et_al-2017-Annals_of_Applied_Biology.pdf "Detection and differentiation of the coconut lethal yellowing phytoplasma in coconut-growing villages of Grand-Lahou, Côte d'Ivoire", doi:10.1111/aab.12333
- 3. Annex 3_Crop Protection 2017 "Identification of a newly described member of the tribe Erythroneurini as a potential vector of the Côte d'Ivoire lethal yellowing phytoplasma in coconut palms sole or in mixed infection with a 'Candidatus Phytoplasma asteris'-related strain".
- Annex 4_Canadian Plant Pathology Journal 2016 Vol. 38, No. 2, 164–173, http://dx.doi.org/10.1080/07060661.2016.119104, "Detection and identification of the coconut lethal yellowing phytoplasma in weeds growing in coconut farms in Côte d'Ivoire" (Annex 4, http://cowaly.com/wp-content/uploads/2017/01/Detection-and-identification-of-the-coconut-lethal-yellowing-phytoplasma-in-weeds-growing-in-coconut-farms-in-C-te-d-Ivoire-1.pdf)
- 5. Annex 5_"Farmer field mini-guide" (http://cowaly.com/wp-content/uploads/2017/01/Annex-37_Farmer-field-miniguide-CILY-English.pdf).
- 6. Annex 6_"Farmer field mini-guide", http://cowaly.com/wp-content/uploads/2017/01/Annex-36-Farmer-field-miniguide-French.pdf),
- Annex 7, New disease management plan (http://cowaly.com/wp-content/uploads/2017/01/Annex-2 New-CILY-disease-management-plan English.pdf).
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