

BACTERIAL WILT MANAGEMENT:
A PREREQUISITE FOR A POTATO SEED CERTIFICATION
PROGRAM IN MALI

by

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of

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In

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November 2007

DEDICATION

To my son Sinaly Badra Thera
With my unconditional love in the blessing from God, as a symbol of my determination
to improve your courageous life of disablement.

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ABSTRACT

Potato is one of the most important cash crops in Mali and has been well adapted to the Malian cropping systems and food habit. Bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi et al., (1995) has become a major problem causing important damage in production. The Race and biovar of the bacterium present in Mali were not known at the start of this thesis research and the objective of this research was to identify the race/biovar and to evaluate integrated control methods with the aim to produce high quality disease free seed potato in Mali.

We have initially identified the biovar and Race of *R. solanacearum* affecting potato in Mali as Race 1 biovar 3. ELISA tests were used on soil and water samples to detect the presence of *R. solanacearum*. Three detection methods of the bacteria (bacterial streaming test, Agdia immunostrip test and Tetrazolium chloride media) in plant samples were compared; weeds and crops hosting the bacteria as sources of inoculum were identified using Agdia immunostrip test. Race was determined by hypersensitivity test, pathogenicity tests and by biochemical tests with disaccharides and alcohol hexoses. The level of soil infestation in soil samples collected throughout Mali was determined by growing tobacco plant susceptible to *R. solanacearum* Race 1.

ELISA tests showed the presence of *R. solanacearum* in irrigation water from Farako creek, Samogossoni well in Sikasso, and canal water in Baguineda. Other places such as Borko's soils were found free from the disease. Plant hosts identified as inoculum sources were *Commelina forskalaei* Vahl, tomato, egg plant and pepper. Out of the three techniques for bacterial detection in plant samples, the best detection was achieved with bacterial streaming test and Agdia immunostrip test. In varietal tests, three of the six varieties tested (Appoline, Claustar and Spunta) showed a better tolerance compared to Mondial, Daifla and Liseta in presence of high bacterial wilt disease pressure.

These results will be used to design and implement an integrated bacterial wilt control program in Mali.

INTRODUCTION

Bacterial Wilt and Potato in Mali

Bacterial wilt disease caused by *Ralstonia solanacearum* (Smith, 1895; Yabuuchi et al., 1995) is a devastating disease of a wide range of plants in tropical and warm temperate climates worldwide. In Mali, the disease has become a serious problem limiting potato production and has caused farmers to stop growing potatoes in many fields in the main potato growing area near Sikasso. Other crops affected by this disease in Mali include tobacco, tomato, pepper and eggplant. Critical knowledge regarding the epidemiology and identity of *R. solanacearum* Races/biovars in Mali is lacking and attempts to control this disease have been ineffective.

In Mali, potatoes are considered to be one of the most economically important vegetable crops and are becoming more important because they are considered to be a crop with high nutritional value, a high value cash crop, and a potential export crop for our farmers. According to IER (Institut de l'Economie Rurale), Malian production of potatoes was estimated at 50,000 tons in 2001 with a value of 7.0 billion CFA (Central African Francs) equivalent to an estimated US \$9.3M making Mali one of the most important producers of potatoes in West Africa (CAE, 2001). In the last 3 years bacterial wilt caused by *R. solanacearum* has become a limiting factor in production in the major production area near Sikasso and in other market garden areas while growing potatoes has become more popular, losses due to bacterial wilt have caused farmers to stop growing potatoes in many fields and potato production has dramatically decreased.

During the annual workshop in 2003 between IER researchers and the beneficiaries of research results, farmers in the Sikasso region agreed that their most urgent need from IER was to quickly find a solution to bacterial wilt, since potatoes provided food security for the poor rural and urban population and were important as cash crop.

The objective of this project is to discover ways to manage this disease at an economically acceptable level by understanding the biology and epidemiology of *R. solanacearum* under Mali's conditions. Critical knowledge needed regarding bacterial wilt management is the identification of the Race or Races and their biovars occurring in Mali. This work is critical since the Race/biovar determines the host range and the potential for control using resistant potato cultivars.

Races 1 biovar 3 and Race 3 biovar 2 cause problems in potato production nearly worldwide. In preliminary surveys (Thera, 2006), it appears that Race 1 is predominant in Mali. However it is important to thoroughly understand the Race situation in Mali so crop rotations can be planned as part of the integrated control strategy. In addition, it is critical to know what other crops or weeds host the bacteria and which inoculum sources are important in bacterial wilt survival between potato crops. Inoculum sources could include aquatic weeds, surface irrigation water (Biosca et al., 2005), symptomless weeds that transmit the disease (Hayward, 1991), susceptible solanaceous crops (tobacco, tomato, pepper, eggplant), leguminous host plants such as groundnut or French bean, monocotyledons host plants (banana, ginger) and some trees such as mulberry or eucalyptus. Another inoculum source is infested soils where bacteria can survive for long periods, in the absence of host plants (Genin and Boucher, 2002). Integrated management

tools such as crop rotation, and use of resistant varieties would be the backbone of management techniques to control this disease since Mali is a material resource poor country that needs better sustainable agricultural techniques. In addition, techniques such as the use of well-done composts to get suppressive soils (Ouedrago, 1994) may have applications in Mali.

Another critical tool for managing this disease is the use of disease free seed potatoes. If farmers plant infested seed either from their own saved seed or seed imported from Europe all management techniques known will fail. Growers currently save some of their own seed but have seen the increased production potential when they use certified disease-free seed imported from Europe. While imported certified disease-free seed has provided increased yields and quality compared to farmer saved seed, its cost is often nearly 50% of the production value (Kelly et al., 2005). Also, Race 3 biovar 2 of *R. solanacearum* is endemic in Europe and to date this Race is not known to occur in Mali, therefore importation of European seed potato is not desirable without a high quality phytosanitary program for imported seed.

Other problems with European source seed are the cultivars which are available are not always well adapted to Mali, there is a dormancy problem associated with seed imported for the November/December planting season and seed imports are not always timely relative to the optimal planting season. For these reasons, the vision of the Government of Mali is to create conditions for high quality seed potato production in Mali to reduce production cost, and to improve yield and profit for producers.

Currently, the Malian government with the assistance of United State Agency for International Development (USAID) is working on the conditions needed in Mali to

develop a certified pathogen-free seed potato production in Mali (Kelly et al., 2005). Understanding the Race/biovar situation, the host range of Malian isolates, the epidemiology of bacterial wilt and identifying tolerant and adapted potato varieties are vital to the development of the Malian seed potato and commercial potato production industries.

The major constraint in potato production in Mali as in most of the developing countries is the lack of adapted varieties and availability of high quality disease-free, economical seed. Farmers, public research and extension services such as IER, IPR and non-government organizations such as Science Outil et Culture International (SOC), and Association Malienne d'Appui Techniques aux Tons Villageois (AMATEVI) have attempted to produce seed potatoes in the last ten years without high success with the result farmers are still importing seed. The reason to that unsuccessful attempt was the lack of separation between seed potato production areas that are free from bacterial wilt from table potato production areas. Also the low capabilities to ensure quality control through the production chain, and the weak organization to scale up the production of seeds have been implicated in the unsuccessful development of a Malian seed potato industry (Coulibaly et al., 2002). Critical needed improvements are the establishment of national quarantine program, national certification and standards relative to varietal purity and freedom from pathogens. For that, rapid diagnostics tests for virus and bacteria adapted to Malian conditions should be available.

At the request of USAID Mali, a study was completed regarding the development of a regional center for disease-free seed potato production in Mali (De Greef, 2003). This report indicates that while the consumption of potatoes is rising, in 2005 Mali was

still importing more than 1,200 metric tons of seed potatoes from Europe. De Greef also indicated that the biotechnology laboratory of IPR in Katibougou has the capability for tissue and meristem culture critical to the production of disease-free potato seed stocks; however Mali does not have the infrastructure to produce seed stocks beyond this nuclear stage. Farmers dedicated to only seed production are needed, with farms isolated from food potato production regions and the phytosanitary infrastructure to certify freedom from pathogens. Today after gold and cotton, potato (seed or table potato) has the possibility to be the third important exportation product if more attention is given to the sector (Dicko, 2006).

In the agricultural politic, according to the “Cadre Stratégique de Lutte contre la Pauvreté” (Political Strategy to Reduce Poverty), Mali is engaged to support any production channel that generates profit, promotes exportation or replaces importation (CSLP FINAL, 2002).

From the regional Food Agriculture Organization FAO-ICA Workshop, titled “*Getting Agriculture Moving in the New Millennium: The Empowerment of African Farmers*”, held in Nairobi in March 2002 and attended by Mali, one of the recommendations was to sustain farmers groups in strengthening their technical, economic, financial, and organizational capacity (Ag Keratane, 2004). It is clear that the Government of Mali supports farmer’s activities in order to improve farmer’s standard of living and to develop the general economy.

Another study done by Tyner et al., in 2002 recommended to USAID to invest in the multiplication, dissemination, and demonstration of improved variety seeds. “To maintain high quality standards, field multiplication to scale up the production of seed is

an important step of seed production because of the possibility of tubers contamination through infestation by pathogens present in soil or in close environment” (CILSS, 1998).

To prevent contamination from pathogens during the multiplication, important measures of detection, identification of pathogens as well as sanitation and exclusion measures should be taken. Examples of pathogens are viruses, bacterial diseases, fungi and nematodes that could become endemic. For that, special precautions have to be taken while selecting the areas for seed multiplication.

One of the objectives of the CFA franc devaluation was to improve the income of farmers by producing high quality products able to compete with imported products in local markets or in regional markets. The horticulture sector was thought then to respond positively to the devaluation by competing with high quality product (CILSS, 1998).

According to Nathan-MSI Group, 2002, potato crops could offer to Mali an exceptional opportunity to improve its economy and farmer’s income through competitive markets. Among West African countries Mali is already favored for seed production since it has the highest yields of commercial potato in West Africa and is the biggest potential market. To improve its competitiveness, Mali will have to improve the quality of its potato and reduce the price through an efficient management of diseases (Nathan-MSI Group, 2002). Now it is clear that Mali needs to organize and promote its in-country disease free-seed potato production.

In the context mentioned above, this research master’s thesis project has the following objectives:

Overall Objective: To setup an integrated management program to control the bacterial wilt of potato in Mali.

Specific Objective 1: Identify the Race(s)/ biovar (s) of *R. solanacearum* infesting potatoes in Mali,

Specific Objective 2: Describe the epidemiology and the pathogen distribution so that an effective, integrated management program including resistant varieties for certified, disease-free seed potato production in Mali can be established.

LITERATURE REVIEW

Potato History and Crop Situation in Mali

Potatoes (*Solanum tuberosum* L.) originated from the high mountainous Andean regions of South America were introduced to Europe in approximately 1570 AD by the Spanish. In the early 1700s, potato production as a crop was found in North America, many Asiatic countries and most European countries (Stark and Love, 2003). Potatoes are reported to be introduced for the first time as a crop into Africa by European colonists (FAO, 1990). Worldwide, more than 290 million tons of potatoes are produced annually making potato the fourth ranked crop among all food crops in total production (CIP, 1984); United States is the fifth largest producer among the 130 countries that grow potatoes. (CIP, 1984). Reasons for the importance of potato in world food production are its high nutrition value and ability to produce high yield under a wide range of conditions in less time and using less space than other food crops (CIP, 1984). Recently potato production has migrated into tropical and sub-tropical areas where production is expanding rapidly. This expansion is because of the high productivity compared to the other major crops, consumer demand and high profitability (CIP, 1984).

Potato Crop in Mali: History and Trends.

The Catholic missionaries introduced potato for the first time in Mali in 1920, (Kleene 1983). Since that time the crop has been well integrated in the Malian's production system, because of its nutritional quality and the profitability for producers in both domestic markets and export markets in the Ivory Coast, Ghana, Burkina Faso, Benin, Togo and Senegal following the devaluation of the CFA (Dicko, 2006). Prior to

the devaluation of the CFA these countries use to import potato from Europe; following the CFA devaluation these countries began importing potatoes from Mali since the Malian product is fresh and less expensive than imports from Europe.

The crop has been cultivated by farmers from Kayes to Sikasso in 1938, in Segou (1940) and finally in Kidal in 1955-1960 (Coulibaly et al., 2002). First considered as a luxury food, the crop became popular after the drought of 1972-74 and 1983-86 and had its entry in the food habit of the Malians where it is now well integrated in the agricultural, nutritional and commercial customs (Coulibaly et al., 2002). Since then potato is one of the most important horticultural crops and production has expanded rapidly in Mali during the last two-decades. Conditions such as irrigated land, available labor and high yield potential favor the expansion of the crop in Mali. Average yields of 23 tones/ha compared to yields of 15-20 tones/ha in Senegal and Guinea or 6 tons in Nigeria demonstrate the advantage that Malian producers have over other West African producers (Kelly et al., 2005).

Zones of Production

Although potato is now growing everywhere in Mali, the main production areas are:

Sikasso Located in the southern part of the country with annual rainfall of 1,000-1,200 mm, the Region of Sikasso has a population of 1,780,042 covering about 6% (71,790 km²) of the territory of Mali and borders three countries in the sub region – Guinea-Conakry, Côte d'Ivoire and Burkina Faso (Kelly et al., 2005). Because of its climatic and environmental conditions, the region of Sikasso has agriculture as one of the main economic activities with production of cotton, corn, tubers, fruits and vegetables,

and cereals being major enterprises. Sikasso is considered to be the granary of Mali. Production of potato, pepper, eggplant, ginger and banana is widespread and all these crops are susceptible to *R. solanacearum*.

Approximately 105 villages produce more than 1680 ha of potato per year (Kelly et al., 2005). Potato is produced two times a year with a peak period from mid-December to the end of February and a less important rainy season production from June to September that is limited to hillsides (Coulibay et al., 2002). Irrigation water is taken from shallow surface wells 1-3 meters deep and from creeks. The production is all done through handwork. As mentioned by Vanderhofstad in 1999 the climatic conditions in raining season are not favorable to potato growth and result in poor tuberization and pathogens proliferation, especially bacteria that are difficult to control. To overcome these problems Vanderhofstad suggested the choice of free-draining soil, soil treatment with insecticide, and planting on ridges to provide better drainage.

Bamako and Baguineda This production area near the capital of Bamako is under expansion by a cooperative organization; The Government program in that area is to provide land and irrigation water from the canal sources from the Niger River. Farmers rent irrigated plot land and pay after harvesting. Farmers grow rice in rainy season and in the dry season, raise vegetables such as tomato, onions, cabbage, okra, melon, eggplant, cucumbers, melons and others vegetables in rotation. Several vegetables such as tomato, pepper, lettuce, carrot, parsley, squash, melon, eggplant, okra, cabbage and tobacco and potato are grown in rotation in Bamako. Irrigation water from shallow wells is also used in some areas remote from the Niger River.

Kati and Koulikoro Area Near Bamako Localized at 15 km from Bamako, Kati belongs to Koulikoro region and has a population of approximately 100, 000 people. Fruits and vegetables production is considered to be the main economic activity. Kati is second only to Sikasso in production of solanaceous crops, especially tobacco. Other crops susceptible to *R. solanacearum* such as peanut, tomato, eggplant and pepper are widely produced. Production is limited to the dry season, especially from November to February, although water availability can be often a limiting problem. Like in Sikasso farmers use water from shallow wells and creeks to irrigate their crops. Farm work is done by hand. Bamako is the primary market (Potato Atlas <http://research.cip.cgiar.org/confluence/display/wpa/Home>)

Other Areas In addition to these main production areas, potato is produced countrywide in small gardens, and the biggest tubers are produced in the desert area of Kidal and GAO regions (PotatoAtlas, <http://research.cip.cgiar.org/confluence/display/wpa/Home>). Farmers are mostly smallholder with production area smaller or equal to 2 ha. The family members do all the work. Hand labor is used in all operations including pulling irrigation water from shallow wells or creeks.

Production

While in Sikasso two crops per year are possible, only one production season is possible in the remainder of Mali. Seed is imported from Holland and France through "Sikassoise", a private trading company. The cultivars Bintje, Sahel, Spunta are the most commonly grown (potato Atlas <http://research.cip.cgiar.org/confluence/display/wpa/Home>). Farmers also try to keep

tubers after harvesting for next fresh season growing or directly use it as seed during the rainy season to get earlier production or earlier seed (Coulibaly et al., 2002). Although several diseases are present, they were not considered too important until the appearance of the devastating bacterial wilt.

The diseases identified as present on potato in Mali in the inventory of vegetables diseases in Mali by Thera in 2000 were Black leg *Erwinia carotovora* pv. *Atroseptica* (Van Hall), Fusarium wilt caused by *Fusarium oxysporium* (Schltdl.) and *Fusarium solani* (Sacc.); early blight caused by *Alternaria solani* (Ellis and G. Martin), and the bacterial wilt caused by *R. solanacearum*. In storage *Fusarium* dry rot caused by *Fusarium solani* (Sacc) and soft rot caused by *Erwinia carotovora* subsp *carotovora* (Jones) are common.

Sclerotium white mold caused by *Sclerotium rolfsii* (Sacc.), was identified in field plot at the IER station of Farako near Sikasso, in 2006 by Barry Jacobsen. No official report is found on the disease in Mali.

Rhizoctonia solani (Kuhn) and *Pythium* stem rot caused by *Pythium ultimum* var. *ultimum* (Trow) and identified on tomato are suspected on potato also.

In 2002, Coulibaly et al., in the evaluation of the sanitary quality of their seed multiplication materials reported the presence of viral diseases such as PVX, PVY, PVS; as bacterial disease they reported *R. solanacearum* and *E. carotovora* while *Fusarium* sp was the only fungal disease reported. They also confirm in the same report that root knot nematode population (*Meloidogyne* sp.) seems to be very low or not present at all while the potato tuber moth, *Phthorimaea operculella* was never reported in Mali.

Besides bacterial wilt the major problem is post-production storage for both table potato and seed potato. Although locally adapted storages have been built for farmers to store for a best price on the market; cold storage is still needed for longer-term seed potatoes. Seed potatoes need high humidity storage at approximately 5°C for 6-8 months (Coulibaly et al., 2002). The production of Kati is sold in Bamako market while Sikasso provides markets throughout Mali and exports to other West African countries. During the 2005-2006-potato production season in Sikasso, more than 10 000 families (>15000 people) produced 50,000 metric tonnes generating more than 6 million CFA (Dicko, 2006).

Potato is best adapted to temperate growing conditions (Stark and Love, 2003). Since 1979, the CIP (Centro Internacional de la Papa) has collaborated with many national programs in tropical regions to resolve problems of potato production in tropical environments, key factors for success are thought to be the use of good quality of seed so that vigorous plants could emerge earlier to cover the ground; and the use of adapted, resistant varieties to control the bacterial wilt disease through appropriate agronomic practices (CIP, 1984). Two major potato sector constraints at the farm level are the high costs of imported seed and storage prior to marketing the harvested crop or for seed production. The IPR/IFRA biotechnology laboratory has successfully used tissue culture technology to produce potato micro tubers *in-vitro* that can provide the foundation seed for the development of an integrated approach in the production chain of disease-free seed potatoes in Mali (Partenariat 80, 2005).

Seed Potato: Needs and Tentative Production

According to the work done by Partenariat 80, (2005) potato could offer more economical and social opportunity through improvement of the food security and diversification of the nutritional diet choice; In addition to all of this, beneficial aspects of growing potato could also bring to the producers economical advantages such as job opportunities and more sources of income. But the potato production process is hindered by the lack of high quality seed potato at the opportune moment and also its high price (more than 50% of the production cost (Kelly et al., 2005). At present, approximately, 1500 metric ton of seed potato representing 96% of total need in seed is imported from European Union every year without any competition (De Greef, 2005). The reality of seed potato importation is that seed often arrives from Europe too late for optimal planting and limits crop production to once a year. Seed imported for the November/December planting time is still dormant and this results in poor stand establishment. Prices and amount of imported varieties are not stable and varieties imported from Europe are not well adapted to the tropical conditions that need day neutral cultivars. Other difficulties are associated with the high cost price (\$40-\$60/25 kg-\$72-94/100lbs) of European seed. Because of cost, farmers cannot buy the amount of seeds they really need so they cut each seed tuber into small seed pieces each with one eye. These seed pieces are much smaller than recommended for optimal production and the excessive cutting results in lower yield and increases potential for pathogen transmission since farmers do not use any treatment for their seed piece or seed cutting tools (Secor and Gudmestad, 1993).

“Production of locally grown seed potato with high quality and adapted to local conditions could improve significantly the economic of farmer” (Partenariat 80, 2005). While it is well known that there is a critical need for cheaper, higher quality seed potatoes in Mali and in the surrounding sub-region it is critical to understand that Mali will face stiff competition from European seed exporters. “In order to capture markets in Cote d’Ivoire, Nigeria and Senegal, Mali needs to improve the price competitiveness of its seed potatoes compared to Europeans sources” (Nathan-MSI Group, 2002). That will be possible only if Mali develops an effective phytosanitary program for management of diseases and sets up the quality control standards throughout the seed potato production chain.

The successive multiplication from disease-free nuclear stocks through certified production in fields is needed if farmers are to have economical high quality, disease-free seed to plant their crops. Production that avoids these steps will allow for the multiplication of pathogens in saved seed with the result of reduced yields and quality. (Partenariat 80, 2005). For the West Africa seed producer a short-term scheme for seed production starts with production of tissue culture plantlets or minitubers tested for freedom from pathogens, then small-scale production of tubers in the field followed by larger scale field production. At each step plants and tubers are tested for freedom from pathogens. It is critical to return to tubers from tissue culture derived plantlets every 3-4 generations. According to Slack (1993), the majority of seed potato producers, organize the production of basic stocks “into a strict limited generation schemes designed to flush out seeds stocks within a prescribed number of years” and that because the probability of contamination induced through tubers seed borne diseases increase with each new

generation of field production. This system requires technical support from a biotech laboratory to produce nuclear seed stocks and seed producers adopt regulations and strict sanitary measures (Partenariat 80, 2005). In addition, it is well established that seed potato production must be in a different region than table stock production. Therefore, a complementary task to success of this project will be to develop a market for Malian seed potatoes and to identify a disease-free seed production area with experienced farmers. (De Greef, 2005).

Bacterial Wilt Situation

Bacterial Wilt Worldwide

Elphinstone (2005) has recently reviewed the literature on bacterial wilt caused by *R. solanacearum*. The bacterial wilt pathogen *R. solanacearum* is present on all continents throughout wet tropic, sub-tropics and warm temperate regions of the world. This disease is of international concern and is being addressed by collaborative research between national researchers; and by International Agricultural Research Centers such as CIP working on potato (Hayward, 2005), ICRISAT working on peanut (Hayward, 2005) and AVRDC working on tomato, pepper and eggplant (Hayward, 2005). Publications on *R. solanacearum* until 1997, have dealt with finding resistance through breeding and selection (24%), pathogen host range, diversity, and distribution (22%), disease management and control (18%), pathogenicity and host pathogen interactions (17%), biological control (10%), detection and diagnosis (4%) and finally epidemiology and ecology (3%) (Elphinstone, 2005). In terms of host crops studied, 35% of publications have dealt with tomatoes, 24% with potato, followed by eggplant (11%), tobacco (9%), *capsicum* (7%), groundnut (3%), banana (3%), and ginger (2%), the remaining 5%

included many other host crops and ornamentals (Elphinstone, 2005). “While developed countries did focus their research on fundamental questions of the genetic basis of virulence, pathogenicity, and regulation of gene expression, developing countries primarily addressed work on disease resistance, management, many aspects of epidemiology and biology” (Hayward, 2005). This situation certainly favors the disparity in the availability of information between the two important Races and 3 biovars present on potato: Race 1 biovar 3 or biovar 1 and Race 3 biovar 2. More research work has been done on Race 3 biovar 2 since that is the Race present in both tropical highland climates and in warm temperate climates characteristic of most developed countries. Still more attention should be paid to Race 1 that occurs exclusively in tropical or sub tropical regions where soil never freezes. Race 2 biovar 3 is a novel variant of the pathogen adapted to cooler environments where soils freeze (Timms-Wilson et al., 2001). While Race identification is based on host crop the biovar is determined through the oxidation test on disaccharides and hexose alcohol. Race 1 is found on diverse crops, and the others Races (2, 3, 4 and 5) are specific on a few crops (Denny and Hayward, 2001).

Table 1: Geographical Distribution of Host Range and Biovars of *Ralstonia solanacearum* (Smith) Yabuuchi et al., Worldwide

Race	Host Range	Geog. Distribution	Biovar
1	Wide	Asia, Australia, America, Africa	3, 4, 1
2	Banana, Other Musa sp	Caribbean, Brazil, Phillipines	1
3	Potato, some other solanaceas, Geranium and a few other species	Worldwide except Canada and United State	2
4	Ginger	Asia	3, 4
5	Mulberry	China	5

(Table from Denny and Hayward (2001) modified by Daughtrey, (2003) reprinted now with modification by Thera)

Bacterial Wilt in Developing Countries and Africa

Bacterial wilt is one of the most economically important bacterial diseases in developing countries; its presence on crops such as banana, tobacco, peanut, tomato, eggplant, pepper and potato is a big concern for developing countries farmers where these crops are important cash crops. Bacterial wilt was identified by as Moko disease in Peru on plantains and banana (Buddenhagen and Kelman, 1964; French and Sequeira 1970). Bacterial wilt caused by *R. solanacearum* Race 1 has been reported in China on pepper, sweet potato, peanut, ginger, eggplant and more over (Ren et al., 1981; He et al., 1983 and He, 1997); in South Africa on eucalyptus by Countinho et al., in 2000, and in Brazil on eucalyptus (Keane et al, 2000). Biovar 3 was identified on tomato in Nigeria by Adebayo in 2005. The Race 3 biovar 2 was reported by Hakiza et al., in 2000 to be with late blight, one of the major constraints in potato production in Uganda due to the lack of good quality seed from improved varieties. *R.solanacearum* is known to be present in most countries in Asia, Latin America and the Caribbean and many countries in Africa. While the disease has been reported in Mali (Thera, 2000; Coulibaly et al., 2002) no identification as to race or biovar has been published.

Bacterial Wilt in Mali

Bacterial wilt was officially reported for the first time in Mali in 1999 on tomato in the Ouelessebougou area and on potato in the Sikasso area (Thera, 2000). The disease is present on tomato, potato, eggplant and pepper; also inoculations on tobacco and peanut have been positive (Thera, 2006). From this moment the disease has been reported every year in the main production areas of potato and tomato in Sikasso, Koulikoro, Kati, and Baguineda and has been also reported to be the most important disease on potato and

other Solanaceous crop (tomato, pepper, eggplant) production by farmers in Sikasso. No literature exists as to the Races or biovars found in Mali. Critical research needs are biochemical and genomic characterization of Malian strains in comparison with other tropical adapted strains and to determine the biological and epidemiological characteristics of Malian strains. Loss of 100% of the potato production during successive years in some villages of Sikasso have caused farmers to stop growing potato in many fields; the same thing now happens at Baguineda irrigated area on both tomato and potato.

In Sikasso farmers cultivate many of the crops recognized as hosts for *R. solanacearum* species worldwide; they are tomato, eggplant, and pepper in rotation with potato some time. They also grow banana, peanut and ginger; Sikasso being the sole area of ginger production in Mali. Even though bacterial wilt has never been officially reported on ginger in Sikasso, there is a strong possibility of transmission between potato and ginger since biovar 3 has been reported on ginger (Kumar et al., 2004). In addition to the identification of Race 4 biovar 4 done by Tsuchiya in 2004 on Zingiberaceae plant in Japan, the disease was also reported on ginger, in India, China, Japan, Indonesia, the Philippines, Hawaii and many other ginger-growing countries and the strain found in India is reported as Race 1 biovar 3 (Kumar et al., 2004).

In Kati, potato is produced in rotation or side-by-side with tobacco (Thera, 2006) and sometimes with peanut crop which is also a host for *R. solanacearum*.

Important Diseases Found on Potato Crop in Mali

Viral Diseases

A few viruses have been reported on potato crops in Mali. Strict quarantine measures are important especially in seed production area for stopping the entry of harmful viruses. Even they are not very important a low incidence of PVS (Potato Virus S), PVX (Potato Virus X) and PVY (Potato Virus Y) have been reported in the Sikasso region (Coulibaly et al., 2002). Symptoms like leaf roll have been seen some times without any confirmative diagnostics.

Potato Virus S PVS is a very common viral disease found worldwide; transmitted through seeds in latent infection; it can induce losses up to 20% or more if mixed with other PVX or PVM (Stevenson et al., 2001). Symptoms vary with strains of virus, cultivars and weather (Burton, 1996). Typical symptoms are slight deepening of veins, rugosity of leaves and stunting of plants. While certain cultivars show mottling, bronzing and necrotic spots on upper leaves after infection, other infected plant can appear just healthy (Coulibaly et al., 2002). Disease is mainly transmitted mechanically through seeds, cutting, and injuries to foliage and leaf-to-leaf contact. Aphid transmission is low; true seed transmission is not reported. Some varietal resistance reported has been overcome by graft transmission (Stevenson et al., 2001).

Management is done through the use of indexed seeds stocks, tolerant and resistant varieties and sanitation measures.

Potato Virus X Also found worldwide, PVX is the most widespread of potato viruses. The principal symptom of the disease is a mild mosaic; however some strains can

cause a severe and rugose mosaic in some cultivars. Severe infection results in dwarfing plant and reduction in the size of leaflets and extensive top and tuber necrosis; when coinfecting with PVA or PVY, a severe mosaic infection will develop (Stevenson et al., 2001). The pathogen is also called the “healthy plant” virus with reason since many strains cause latent infection on cultivars that remain symptomless and spread the disease easily. This disease is transmitted mechanically through foliar contact, cultivation, spraying, seed tuber cutting and other handling. No transmission in true seed has been reported.

The disease is best controlled by using PVX-free-seed and sanitation measures.

Potato Virus Y Distributed worldwide, PVY is amongst the most important viral diseases affecting potato. There are several known strains including PVY-o, PVY-n, and PVY-ntn. Symptoms depend on the strain of the virus, the cultivars and the nature of the infection either primary or secondary (Burton, 1996), going from mild mosaic to severe foliar necrosis and finally death of infected plants (Stevenson et al., 2001). Transmitted mechanically through contact between tubers, plant leaves, and more importantly through transmission by aphids, PVY also infect many solanaceous, some Chenopodiaceous and Leguminous plants (Stevenson et al., 2001).

Management is done through controlling aphid vectors; using certified disease free-seed potato and mineral oil to reduce the spread of the disease; also observing strict sanitary measures will avoid the transmission through equipment and other movements; use plant resistant cultivars.

Potato Leaf Roll Virus Transmitted by aphids, PLRV is a phloem-limited virus that occurs worldwide causing important losses of yield and quality. Symptoms first

appear as primary infection on young leaves that stand upright and roll upward, particularly at the leaf base, while the symptoms of the secondary infections are a rolling of the lower leaves with plant often stunted, upright and pale green to yellow. Net necrosis is particularly evident in some cultivars. *Physalis floridana* (Dubern Jean) and *Datura satramonium* (L) develop systemic interveinal chlorosis and are used diagnostically as indicator host. The virus is transmitted by migratory winged aphids that spread the disease between fields over long distances while nonwinged aphids spread the virus from in field infected source plants to adjacent healthy plants. Infected tubers and volunteer potato plants can serve as primary source of inoculum; the virus is also transmitted from generation to generation, or introduced to new areas through tubers (Stevenson et al., 2001). Mainly present on solanaceous plants, the disease is also found on Amaranthaceous sp (Juss) plant (Burton, 1996).

Management: use PLRV free seeds potatoes, resistant cultivars and, roguing of infected plants; control aphids with systemic insecticides.

Fungal Diseases

None of the fungal diseases are as important as bacterial wilt and occur only sporadically and at present do not cause significant economic losses in Mali.

Pythium ultimum var. *ultimum* (Trow.) This fungal disease has been seen more on other solanaceous plants like tomatoes more than potato; its incidence is very low. The disease is caused by *Pythium ultimum* Trow and other *Pythium* spp. a soil-borne fungus, which enters in the tubers through wounds generally produced during harvesting or grading. Characteristic symptoms of the disease give to the tuber a cooked texture while a discolored water-soaked area appears around wound. Rotted tissue is spongy and

extremely watery (Sygenta, 2003). Diseased tissues are separated from healthy tissue by a dark line and affected tissues change their color from gray, brown to black when exposed to air (Stevenson et al., 2001). Conditions that favor the disease are harvesting while soil temperature is above 21°C; succession of extremely wet conditions and short periods of dryness during tuber maturation and finally low ventilation and temperature >20°C favor spread in storage (Sygenta, 2003).

Fusarium wilt- caused by *Fusarium spp* Fusarium wilt is caused by soil-borne fungi such as *Fusarium avenaceum*, *Fusarium oxysporium* and *Fusarium solani* f. sp *eumartii* (C. Carpinter) W.C. Snyder & H.N. Hans, that infects through plant roots and causes vascular wilt in field (Stevenson et al., 2001). Stunting, chlorosis and wilting of lower leaves are typical symptoms that are confusing with those caused by *Verticillium* wilt. Laboratory tests are needed for an accurate diagnosis.

Rhizoctonia solani (Kuhn) Characteristic symptoms of *Rhizoctonia solani* are brownish to black sunken lesions on underground stems and stolons. The dark browns to black masses of sclerotia found on the surface of the tuber are a conspicuous sign of the disease (Stevenson et al., 2001). The causal fungus overwinters on infected tubers, in soils and in crop residue. Severe cankering of stems and stolons occur when soil conditions do not favor rapid growth. The disease is important when tubers remain in the soil after the death of the vines. Repeating potato cropping and the presence of cool, moist soils (55° to 60°F) are favorable to disease development. (Sygenta, 2003).

White Mold caused by *Sclerotium rolfsii* Sacc. (Observed only in Sikasso) has been observed in Sikasso and likely occurs elsewhere since this fungus is common in

tropical regions on cotton and a wide range of other plants. Stems are first affected and the rot appears below soil surface; then plant shows wilt and yellowing; tubers can be infected also. White mycelium of the fungus with sclerotia appears on the infected portion of the diseased plant near the soil surface.

The causal fungus survives in soil as sclerotia and disease development is favored by hot temperatures.

Rotation and resistant varieties are practical ways to manage the disease (Davis et al., 2007).

Early Blight caused by *Alternaria solani* (Ellis and G. Martin) Early blight is present late in the season in the majority of potato production areas; the disease is not very important economically for Malian farmers.

Symptoms: small, dark spots develop on older lower leaves; blackish-brown lesions with a yellow halo appear on older leaves and stem. Initially circular, lesions can become angular between leaf veins when they grow (Stevenson et al., 2001). Lesions on tubers are dark and sunken, surrounded by a raised margin. The causal fungus survives between crop on infected plant debris, infected tubers in soils, and other hosts. Primary infection is from rain-splashed spores onto older leaves. The disease is promoted during periods of alternating wet and dry weather and high relative humidity. Twelve hours of leaves wet at 50°F are required for leaf infection. Tubers can be contaminated by the spores through mechanical injuries or in wet harvest condition (Sygenta, 2003).

Fusarium Dry Rot and Seed Decay caused by *Fusarium solani* Sacc. This disease is very important in storage in Mali, since farmers are using traditional adapted storage where temperatures are barely lower than 20°C.

Symptoms: externally, tubers may have sunken or wrinkled areas and an occasional white or pink fungal growth. Internally, tubers develop a crumbly dry decay ranging from dark brown to black. Infections occur when the tuber presents wounds (Stevenson et al., 2001).

In addition, cavities often develop in the rotted tissue that contains the white or pink fungal growth. A moist rot may occur if tubers are invaded by a secondary infection with soft rot bacteria (Sygenta, 2003).

Disease Cycle: *Fusarium* can be seed or soil borne; as seed borne it does enter through wounds during harvesting or handling. The disease spreads through infected tubers, crops debris and infested soils.

Storage temperatures higher than 50°F with wounding of tubers during harvesting promote the disease.

Bacterial Diseases on Potato in Mali

“In tropical regions the major bacterial pathogen of potatoes is probably *R. solanacearum* (synonym *Pseudomonas solanacearum*), rather than *Erwinia* sp. although *Erwinia* sp. are still regarded as a significant potato pathogen even in such climates” (Salmon, 1992).

Soft Rot Disease of Tubers and Stems Potato soft rot is caused by the common soil bacterium resident such as *Erwinia carotovora carotovora* (Jones) Bergey. This bacterium can grow between the temperatures of 32 and 90 degrees F, with optimal

growth between 77 and 86 degrees F (Gudmestad and Secor, 1993). Bacterial soft rot occurs on a wide range of crops and is one of the most severe post harvest diseases of potatoes worldwide. Loss may occur during storage, transit, and marketing or a decay of seed tubers in soil. Stems can be infected following injuries from hail, blowing soil or insects. No resistant varieties are known. Contamination of potato tubers occurs anytime they come into contact with the bacterium, most commonly during harvesting, handling or washing. The bacterium invades the potato tuber through wounds. Infections usually occur in tissues that have been weakened, or killed by pathogens or by mechanical damages. The presence of other pathogens and wounding favor the disease entry; Immaturity, warm tuber and storage temperatures, free water and low oxygen conditions promote the disease development. Temperatures above 80°F predispose to soft rot and temperatures less than 50°F can retard decay, the lower the temperature, and the better is the protection. Symptoms on tubers first appear as small, tannish, water-soaked spots on the surface, which rapidly enlarge and the tissue decomposes in a soft area on the surface of the tuber (Johnson, 1914).

Blackleg caused by *Erwinia carotovora* subsp. *atroseptica* (Van Hall) Dye (Synonym *Pectobacterium atrosepticum*) Blackleg is one of the most important bacterial diseases found in potato field in Mali after bacterial wilt especially in Sikasso region. Even with low incidence, the disease is important since the pathogen can be transferred to new tubers that develop symptoms during storage or serve as primary inoculum when used as seeds. Opportunistic pathogen with a pectolytic ability (Pérombelon, 1992), the Bacteria causes severe disease according on seed handling techniques, soil moisture, temperature at planting, environmental conditions, cultivars, infection level of

seed, and the presence of inoculum source such as irrigation water and cull piles (IPM, 1986). Symptoms are yellowing and severe rolling of the leaves followed by dark, inky black and slimy lesions above ground level of the stem (O'Brien and Avery, 1976). Lesions can also extend downward through stolons into developing tubers that will develop the disease by producing small and dark lesions (O'Brien and Avery, 1976). Daughter tubers apparently healthy were found to be frequently contaminated, suggesting internal contamination from mother tuber to progeny (Helias, 2000). The bacterium causing blackleg disease to potato is tuber-borne and survives poorly in the soil. Blackleg disease can cause severe economic losses to the potato crop primarily through decay of seed pieces and death of seedlings. However, the occurrence of blackleg depends very much on the growing conditions, particularly temperature and rainfall after planting (De Boer, 2004).

Nematodes

Even though nematodes are commonly identified on tomato in Mali none has been yet officially reported on potato in Mali; the presence of root knot nematode is suspected since tomato grows side by side or in rotation with potato crops and both crops are hosts for the majority of *Meloidogyne* sp.. Of the large numbers of phytopathogenic root-knot nematodes, only a few species are known to cause serious damages on potato (MacGuidwin, 1993). This author confirms that peanut root knot nematode is associated with potato and also that the presence of this nematode increases the severity of *R. solanacearum* in the Southern United States. Root knot nematodes are of great importance on potato in tropics`and sub tropics` areas (Stevenson et al., 2001). Neither *Globodera rostochiensis* nor *G. pallida* are reported in Mali; however these nematodes

are found in seed producing areas of Europe that supply seed to Mali and without any phytosanitary screening or quarantine program it is likely they will or have been introduced. Producing local seed potato will protect Mali from having this kind of pathogens easily spread through seed importation. The use of nematicides or other cultural techniques such as rotation, use of resistant varieties help to control nematodes. The effects of neem cake, a nutrient-rich organic material derived from neem seed, as biological control on plant-parasitic nematodes, was investigated by Abbasi et al., in 2005. In greenhouse trials, 1% neem cake (mass/mass soil) caused a 67%–90% reduction in the number of lesion (*Pratylenchus penetrans*) and northern root-knot (*Meloidogyne hapla*) nematodes in tomato roots grown in three different soils. In the field, 1% neem cake (mass/mass soil) reduced the number of lesion nematodes by 23% in corn roots and 70% in soil around roots.

Bacterial Wilt or Brown Rot Disease Caused by *R. solanaceum*

The pathogen is spread through latent infection of seed tubers, other propagation material which can be solanaceous plant or not; weeds hosts, irrigation water, crops debris, tools, and movement of infested soil. Contamination can also occur during grading (Elphinstone et al., 1986). Plants selected at low temperature and planted under favorable temperatures for the disease will then express the disease with devastating consequences (Countiho, 2005). The bacterium infects the plant through root injuries and is more severe in the presence of roots and root knot nematode species (MacGuidwin, 1993); According to Stevenson et al., on potato crop, the southern root knot *Meloidogne incognita* is found in tropics and sub tropic areas while *Meloidogne javanica* is dominant in Australia and Africa. Bacterial wilt disease is identified through ELISA Test,

Immunostrip test, pathogenicity test, hypersensitivity test and bacterial streaming (OEPP/EPPO, 2004; Danks & Barker, 2000).

Under hot humid conditions favorable for disease, complete wilting occurs and the plant will die. Severity of the disease depends upon soil temperature, moisture, soil type, host susceptibility and virulence of strains. High temperature (86-95°F) and high soil moisture are the most important factors associated with high bacterial wilt incidence and severity (Momol, 2003).

Economic Importance of the Disease

The economic impact of *R. solanacearum* worldwide is not well defined but the disease is known to cause important losses on a large range of economic crops worldwide. Although yield losses vary according to host, cultivars, climate, soil type, cropping practices and pathogens strains (Elphinstone, 2005), the disease can destroy entire harvest when conditions that favor it are met. Worldwide losses from bacterial wilt on potato crops are more than \$950 million per year (Allen, 2003)

Important economic losses are caused by plant death, decay of harvested tubers and the voluntary destruction of entire production when disease presence is suspected in quarantine area (Elphinstone, 2005; Hay 2001).

Disease incidence of up to 55% have been reported in fresh market tomato, in Taiwan causing over US\$12 million losses per year (Jaunet, 1999). In the southeastern USA where the disease is reported on tobacco, important economic losses in North and South Carolina were estimated at \$40 million while average yield losses of the same crop was less than 5% in Zimbabwe and ranged from 10 to 30% in Australia (Elphinstone, 2005). Evaluation of losses in plantain crop over 2 years exceeded \$570 000 in six

municipalities of one region in Columbia. In the southern part of Vietnam, yield losses reported on groundnut went up to 20% while in Uganda losses were up to 10%. Severe losses on ginger were reported in Thailand and India where 80% of the 310 fields were infested (Elphinstone, 2005).

Potato is one of the crops most infected by bacterial wilt in tropical and temperate areas, worldwide. Also damage is more important because the bacteria not only attacks the plant stem but also attacks the tuber that is able to transmit the disease. “Bacterial wilt of potato has been estimated to affect some three million farm families using 1.5 million ha in 80 countries with global damages estimated to exceed 950 millions annually”(Walker and Collion, 1998).

Taxonomic Position

Kingdom: Proteobacteria

Class: Neisseriae

Order: Burkholderiales

Family: Burkholderiaceae (Lemay et al., 2003)

Morphological Aspect of the Bacteria

“*R. solanacearum* is a strictly aerobic, gram negative, non-spore forming, non capsulated, and nitrate-reducing, ammonia-forming, aerobic, rod-shaped bacterium (Stevenson et al., 2001). The bacterium does not hydrolyze starch, nor readily degrade gelatin. It is also sensitive to desiccation and is inhibited by low concentration of sodium chloride in broth culture. Optimum growth generally is between 28 to 32°C except certain Race 3 strains pathogenic to potato that are able to grow at lower temperature” (Stevenson et al., , 2001).

On tetrazolium chloride (TZC) media the bacteria is fluidal, presents irregular shape and white with pink centered colonies (Kelman, 1954).

In sterile distilled water the pathogen can be stored for years at room temperature; while long storage in liquid media and the lack of oxygen can induce the loss of virulence characterized by a morphological change of colonies. Virulent colonies appear fluidal, irregular in shape, white with pink center after 36 to 46 hr of growth on TZC media. Avirulent colonies are uniformly round, smaller, and dark red (Kelman, 1954.).

All *Ralstonia* species share common physiological properties such as chemoorganotrophic nutrition, aerobic metabolism, absence of fermentation, and absence of photosynthesis, inability to fix nitrogen, and capacity for growth on a large amount of organic substrates.

The *Ralstonia* species can be similar in phylogeny and chemotaxonomic properties, and different in pathogenicity, host relationship and other phenotypic properties (Denny and Hayward, 2001).

Classification

A traditional classification of *R. solanacearum* shows great phenotypic and genotypic diversity. To describe its intraspecific variability, binary classification systems are used. The pathogen is divided into five Races based on host range and six biovars based on its ability to use each of three disaccharides and or each of the three hexose alcohols (Horita et al., 2001). The use of restriction fragment length polymorphism (RFLP) allowed division of the species into 2 groups correlated with the geographical origin of strains: the ‘Americanum’ division contains biovar 1, 2 and N21 strains whereas the “Asiaticum” division comprises biovars 3, 4 and 5 strains. The sequence analysis of the 16S rRNA gene, the 18S-23S rRNA gene intergenic spacer region, the polygalacturonase gene and the endoglucanase gene have confirmed these two divisions and revealed a

further subdivision including Indonesian isolates (Horita et al., 2001); The 2 geographic divisions that are «Division 1- biovars 3,4,5 primarily from Asia and «Division 2. Biovars 1, 2,N2-primarily from Americas but its taxonomy is still considered in transition. Poussier et al., in their research work in 2000, have confirmed the work of Li and Wang (2000) on the presence of two divisions in the species *Ralstonia* that may represent subspecies. But they concluded that the “Americanum” and the “Asiaticum” designation of these divisions as proposed by Cook and Sequeira (1989) in relation with the geographical origin of strains could be reconsidered since their analysis revealed an African biovar 1 subdivision, which may have its own center of genetic diversity, and has its evolutionary origin, in Africa. The genetic diversity among a worldwide collection of 120 strains of *R. solanacearum* was assessed by restriction fragment length polymorphism (RFLP) analysis of amplified fragments from the hypersensitivity *hrp* gene region (Cook and Sequeira, 1989). Five amplified fragments appeared to be specific to *R. solanacearum*. Fifteen different profiles were identified among the 120 bacterial strains, and a hierarchical cluster analysis distributed them into eight clusters. Each cluster included strains belonging to a single biovar, except for strains of biovars 3 and 4, which could not be separated. However, the biovar 1 strains showed rather extensive diversity since they were distributed into five clusters whereas the biovar 2 and the biovar 3 and 4 strains were gathered into one and two clusters, respectively (Poussier et al., 1999). Distribution of strains into genetic clusters did not appear related to biovar or geographic origin in considering randomly amplified polymorphic DNA (RAPD) and repetitive extragenic palindrome-PCR (rep-PCR) or composite data. Although strains

were more dissimilar based on RAPD data than on rep-PCR data, the two techniques gave complementary results for strain clustering (Jaunet and Wang, 1999).

The new classification is now based on phylogenetic information because the current Race and biovar classification was though inadequate for being based on phenotypic factors (Poussier et al., 2000).

“Based on phylogenetic data, the system divides the species complex into phylotypes (phylogenetic grouping of strains), sequevars (group of strains with endoglucanase or mutS gene sequenes diverging by <1%), and clones (group of strains exhibiting the same genomic fingerprint)” (Olson, 1976).

Table 2: New Classification for *Ralstonia solanacearum* (Smith) Yabuuchi et al., based on Phylogenetic Information

Taxonomic Level	Taxonomic Equivalent	Nomenclature
Species	Species	<i>R. solanacearum</i> complex
Phylotype	Subspecies	Phylotype Phylotypes I, II, III, IV
Sequevar	Intraspecifics group	Sequevar 1-23
Clone	Clone lines	

Phylotype I= Division I-biovars 3,4,5

Phylotype II+ Division II includes biovars 1, 2 and 2T-contains Race 3 potato worldwide and Race 2-banana

Phylotype III-strains from Africa-biovars 1 and 2T

Phylotype IV-biovars 1, 2 and 2T Indonesia, Australia and Japan

Here species complex are divided into phylotypes (phylogenetic grouping of strains), sequevars (group of strains with endoglucanase or mutS gene sequenes diverging by <1%), and clones (group of strains exhibiting the same genomic fingerprint) (Fegan

and Prior, 2004). Cook et al., (1989, 1991) in their characterization of *R. solanacearum* through the use of restriction fragment length polymorphism RFLPs found 33 RFLP group in 62 strains of bacteria forming two major divisions:

The division I that contains all members of Race 1 biovars III, IV and V whereas division II includes all members of Race 1 biovar I and Races 2 and 3. In addition, division II contained five distinct subdivisions corresponding to Race 1 biovar I, Race 3 and three subdivisions of Race 2. From the RFLP data, it was deduced that the pathogen had evolved as two separate groups, perhaps as the result of geographical isolation and it was postulated that members of division I had evolved in Australasia whereas division II originated in the Americas. The extremely wide host range of Race 1 was also reselected in the varied RFLP pattern of that race but no correlation could be made between host specificity and banding pattern. Further investigations by Cook and Sequeira (1994) using an additional 102 strains showed 12 more designed RFLP groups.

In the study of the diversity of *R. solanacearum* causing bacterial wilt on ginger and other hosts in India, the use of REP-PCR (Repetitive Extragenic Palindromic-polymerize chain reaction) and RFLP-PCR (restriction fragment length polymorphism-polymerize chain reaction) as molecular tool could cluster the highly pathogenic isolate in a cluster at 100% similarity coefficient in conformity with their host origin and biovar (Kumar et al., 2004).

Guidot et al., (2007) in their study investigated the gene distribution among strains of *R. solanacearum*, paying particular attention to the status of known or candidate pathogenicity genes. Based on the use of comparative genomic hybridization on a pangenomic microarray for the GMI1000 reference strain, they did define the

conditions that allowed comparison of the repertoires of genes among a collection of 18 strains representative of the biodiversity of the *R. solanacearum* species.

The results established the long coevolution of the two replicons that constitute the bacterial genome. They also demonstrated the clustering of variable genes in genomic islands. Most genomic islands are included in regions with an alternative codon usage, suggesting that they originate from acquisition of foreign genes through lateral gene transfers. Other genomic islands correspond to genes that have the same base composition as core genes, suggesting that they either might be ancestral genes lost by deletion in certain strains or might originate from horizontal gene transfers (Guidot et al., 2007).

Genus

Based on the belonging to the rRNA homology group II, the presence of distinct and separate subclusters proved through the analysis of the 16SrRNA genes sequences and additional chemotaxonomic data allowed Denny et al., (2001) to confirm the establishment of the genus *Ralstonia* to accommodate *R. solanacearum*, *Ralstonia pickettii*, and *Ralstonia eutropha* by Yabuushi et al.

Genomic Organization of the Bacteria

In genomic studies the bacterium is found with a bipartite genome structure. Salanoubat et al., (2002) presented the complete genome sequence and an analysis of strain GMI1000. The 5.8-megabase (Mb) genome is organized into two replicons: a 3.7-Mb chromosome and a 2.1-Mb megaplasmid. Both replicons have a mosaic structure providing evidence for the acquisition of genes through horizontal gene transfer. The genome encodes proteins associated with a role in pathogenicity. *R. solanacearum* is a b-

proteobacterium belonging to a group of bacteria whose genomic organization is not well characterized yet. The unique representative of this group for which the complete genome has been already sequenced is *Neisseria meningitides* (Salanouba et al., 2002).

Pathogenicity

Molecular studies concerning bacterial pathogenicity's factors towards plants have focused on a limited number of bacterial species that represent the taxonomic diversity of principal Gram-negative plant pathogens causing the most common diseases. This bacterium has an unusually wide host range, and offers a unique opportunity for the analysis of virulence factors (Salanouba et al., 2002). *R. solanacearum* has been studied intensively both biochemically and genetically, and has been recognized as a model system for the analysis of pathogenicity. Well adapted to life in soil and water in the absence of host plants, the pathogen provides a good system to investigate functions governing adaptation to ecological niche. Swimming motility also is thought to be the most important contribution to bacterial wilt virulence in the early stage of host plant invasion and colonization (Trans-Kersten et al., 2001).

From the study conducted by Salanouba et al., in 2002, it is known that a large majority of the genes encoding pathogenicity functions are part of the core genome, being a status in agreement with their base composition and codon usage that fit the general pattern of characteristics of the species. This suggests that pathogenicity is an ancestral trait in *R. solanacearum* with genes that vary from strain to strain (Salanouba et al., 2002). The phylogenetic analysis based on the distribution of the (type III secretory system) TTSS effector genes revealed an important degree of congruence with the rest of the genome (Guido et al., 2007). This distribution suggests the possibility for these genes

to have two different origins: either they are ancestral or ancestrally acquired pathogenicity determinants that follow the same evolution pattern as other genes or they were independently acquired in the different phylotypes during the evolution and were never exchanged between phylotypes (Guido et al., 2007).

Pathogene Biology and Mode of Infection

R. solanacearum is a soil-borne pathogen that naturally infects roots. It exhibits a strong and tissue-specific tropism within the host, specially invading, and highly multiplying in, the xylem vessels.

R. solanacearum Race 3 may have originated in the temperate highland regions of Peru and Bolivia (Van der Wolf and Perombelon, 1997). The term race is based on host range, while biovar is based on biochemical tests. *R. solanacearum* Race 3 biovar 2 is adapted to lower temperatures than what is found for the other *R. solanacearum* races (Van der Wolf and Perombelon, 1997).

Highly phytopathogenic, the bacterial cells penetrate the xylem vessels and spread throughout the plant establishing foci of infection (Timms-Wilson et al., 2001); virulence factors that the bacteria use in host colonization are the lytic enzymes, the extra cellular polysaccharides, endoglucanases and endopolygalacturonases are critical factors in colonization and cause the rot and disintegration of the tissue (Timms-Wilson et al., 2001). It is known according to Saile et al., (1997) and confirmed by Timms-Wilson et al., in 2001 that extracellular polysaccharide production contributes to the biomass of colonizing bacteria, causing a rapid wilting of infected plants. Wilting is generally followed by vascular discoloration of roots stems and tubers. When the level of the bacteria becomes high, white ooze can exudates from stem and tubers. According to Van

Elsa et al., (2005), “*R. solanacearum* has sophisticated machinery for plant tissue’s invasion and presents a capacity to grow at very low substrate concentration as well as to convert to a viable but non-cultivable VBNC form at low temperature”.

Pathogen Ecology

Race 1 (biovars 1, 3, 4), are pathogenic on potato and on a broad host range and restricted to tropical areas, while Race 3 that has a narrow host range (potato and tomato) and a lower optimum temperature occurs in cool upland areas of tropical regions and warm temperate areas; due to the low temperature optimum, Race 3 or biovar 2 is the causal organism of bacterial wilt on European potato crops (Wenneker et al., 1999).

Epidemiology

The detection of the *Ralstonia* inoculum source is a very important step to set up an efficient management of the disease, especially early detection in plants or weeds that had no symptoms and serve to spread the disease.

Pathogen Distribution

R. solanacearum is highly heterogeneous species containing hundred of distinct strains differing in natural host range, geographic distribution, biochemical and genetic characteristics and is found worldwide (Stevenson et al., 2001; Grover et al., 2006). The disease is favored by high temperatures and generally limited to areas without frozen soils, being particularly severe in the tropical and subtropical areas (Agrios, 2005). Poussier et al., (1999) affirmed that *R. solanacearum* is one of the most important diseases in tropical, subtropical and warm temperate regions worldwide and also stated the possibility for the disease to occur in cool temperate areas. This diverse species differs in

host range, geographical distribution, pathogenicity, epidemiological relationships, and physiological properties. Analysis of genetic diversity is important for understanding the distribution of strains worldwide and prevention of their further dissemination (Horita et al., 2001). The European strain that has been compared by Timms-Wilson et al., (2001), with strains from other races did show considerable genetic homogeneity with them; that could explain the occurrence of a new bacterial variety adapted to cooler temperatures. Known to be a limiting factor for potato crop in Asia, Africa, South and Central America and also in the southeast of United States from Maryland to Florida, the disease has been recognized by CAB International and by EPPO in 1999 to be widely distributed in the entire world as follows:

EPPO Region: Belgium, Germany, Hungary, Netherlands, Spain, Canary Islands, United Kingdom, England, and Lebanon.

Asia: Bangladesh; China (Fujian, Guangdong, Guangxi, Hebei, Jiangsu, Taiwan, and Zhejiang); India (Himachal Pradesh, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal); Indonesia (Java); Iran, Japan (Kyushu), Nepal, Pakistan, Philippines and Sri Lanka.

Africa: Burundi, Egypt, Kenya, Libya, Reunion, South Africa, and Zambia (CABI/EPPO, 1999). According to Elphinstone (2005), in Africa, *R. solanacearum* Race 1 strains have been reported in at least 21 countries; biovar 2 Race 3 has been reported on potato in Burundi, Egypt, Ethiopia, Kenya, Libya, Reunion, Rwanda, South Africa, Tanzania and Uganda. Isolates collected from potato in Kenya, Nigeria and Cameroon were found to have the biovar 2T phenotype. An interesting question is whether this strain was introduced on potato seed from S. America or Europe.

South America: Argentina, Bolivia, Brazil (Goias, Parana, Pernambuco, Río Grande do Sul, Santa Catarina, Sao Paulo), Chile, Colombia, Peru, Uruguay (CABI/EPPO, 1999).

Central America and Caribbean: Costa Rica, Guadeloupe and Mexico.

Oceania: Australia (New South Wales, South Australia, Victoria), Papua New Guinea.

In that distribution, Race 1 is reported on many economically important hosts, Race 2 on banana and others Musaceae and Race 3 on potato and other solanaceae (Elphinstone, 2005), Race 4 is reported on ginger (Kumar et al., 2004) and Race 5 on Mulberry (OEPP/EPPO, 2004) and Morus (OEPP/EPPO, 2004)

The predominant strains found in North America fall within the phylotype II that mainly affect tobacco, potato and tomato in the southeastern USA and Race 3 biovar 2 recently reported for the first time in USA on greenhouse geranium in Wisconsin and South Dakota. These geraniums were originally shipped from Kenya. This phylotype is reported to be present in several countries of Central America and the Caribbean and also in South America (Elphinstone, 2005). The phylotype I (biovar 3 and 4) strains have been reported from at least 20 Asian and Middle Eastern countries. These phylotype I strains are widely spread and affect an important range of economics crops across Asia. Race 1 is reported in 11 states in India (Elphinstone J, 2005). Race 3 biovar 2 is reported in nine Asian countries, in which the distribution is either given as restricted or unknown (Elphinstone, 2005).

The biovar 1 strains isolated in southern Africa from Angola, Madagascar, Reunion Island and Zimbabwe have been shown to form a new subdivision within *R.*

solanacearum now designated Phylotype III. Therefore 3 of the 4 known phylotype (I, II, III) are present in Africa (Elphinstone, 2005).

Levels of genetic variability differ greatly among the three main Races. Strains of Race 3 have a restricted host range, mostly affecting potato, and appear to be more genetically diverse in the potato's region of origin than in other parts of the world. Comparison of Race 3 populations isolated from potato pointed to a high level of genetic diversity in South America, while Kenyan populations consisted of only one major clone. This suggests that the bacterium has been introduced in Kenya. In contrast, Race 1 strain highly diverse in several tropical areas, as demonstrated by solanaceous crops in the West Indies, groundnut in Malaysia and various hosts in Australia (Jaunet and Wang, 1999) could be originated from tropical areas.

In Mauritius bacterial wilt is found particularly on potato, tomato, eggplant, capsicum and other crops including ginger, bean and groundnut as well as ornamentals such as anthurium (*Anthurium andreaeanum* Grp. Sierra) (Dokun et al., 2000).

According to Fouche et al., in 2006, biovar 3 has been identified as pathogen on eucalyptus in South Africa, Congo Republic and Uganda. PCR-RFLP based on hypersensitivity response and pathogenicity) *hrp* gene region has proven to be a reliable diagnostic technique to enable researchers for a rapid identification of the pathogen Race/biovar.

The bacteria possess a high degree of genetic variability. This genetic variability further translates to its versatility in exploiting various niches and hosts. The pathogen furthermore is easily spread through irrigation water, contaminated tools and other farm implements, through seed or other planting materials and in some instances, by insects

and by movement of infested soils (Denny, 2001). In India where biovar 3 and 4 are present on ginger, biovar 3 is more frequent because of its versatility to adapt to varying environmental conditions and its ability to be less influenced by the vagaries of soil edaphic factors (Kumar et al., 2004)

A very high variability of the genome of *R. solanacearum* was observed in field and clonal isolates in the studies done by Grover et al., in 2006. This situation could be the reason of the importance of the host range and the difficulty in breeding sustainable resistance.

Plant Host

Found on more than 200 hosts, *R. solanacearum* biovar 3 infects a wide range of crops and plants on which symptoms are not always observed. Pradhanang et al., in 2000 reported the presence of the bacteria on 29 natural hosts different from potato and tomato and it was possible to artificially infect 28 other hosts.

In Japan, bacterial wilt disease caused by *R. solanacearum* has been reported mainly for solanaceous crops including tomato (*Lycopersicon esculentum* Mill), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), eggplant (*S. melongena* L.), and sweet pepper (*Capsicum annuum* L.). To date, more than 34 species in 18 families of plants have been reported as hosts in Japan. Several workers have studied the classification of Japanese strains of *R. solanacearum*. However, the systematic relationship among strains is still poorly defined (Horita et al., 2001).

Studies that were done by Opina and Miller (2005) in Philippines have shown the presence of Race 1 biovar 3 and 4 on eggplant. Serological test such as ELISA and ImmunoStrip were performed and consistent in detecting *R. solanacearum* in

symptomatic plant tissue, compared to culturing on TZC media. culture on TZC seems likely to be less sensitive due to contamination by other microorganism or to plant condition. The result of the test showed 69.5% of similarity between the two serological test in the dry season and 96.2% in the wet season. Immunostrip test seems to be a slightly more sensitive than ELISA test. (Opina and Miller, 2005).

Ginger recognized to be one of the few monocots affected by *R. solanacearum* in tropical countries has been reported in India, to be affected rather by biovar 3 than biovar 4 (Kumar et al., 2004).

In North America, biovar 1 is found on tobacco, tomato, and potato; it is also found on certain ornamentals such as sunflower although these were not considered to be economically important (Elphinstone, 2005). Biovar 2 Race 1 has been suspected to occur in banana while biovar 2 Races 3 have been reported for the first time on geranium produced in greenhouses in Wisconsin grown from cuttings imported from Kenya (Elphinstone, 2005).

South America: Race 3 biovar 2 is reported on potato and tomato; Race1 biovar 3 is reported on tomato, potato, sweet pepper and tobacco. And the Race 2 biovar 1 is one of the most important diseases affecting banana and plantain; it has been also reported on the ornamental plant, *Heliconia ssp* (Elphinstone, 2005).

Race 3 biovar 2 is the only one reported in Europe on potato and the aquatic wild weed *Solanum dulcamara*; although occasional reports have been done on Race 1 strains on imported ornamentals, they are not known to have ever established (Elphinstone, 2005).

In Africa, Biovar 2, 2T Race 3 is reported on potato and tomato in high land areas of East Africa; biovar 3 occurs on tobacco, potato, tomato and pepper. Cases of bacterial wilt are also reported on groundnut, eucalyptus, geranium and bean. Biovar 2 is reported on plantain banana and banana, biovar 4 on ginger and biovar 5 on mulberry.

In Australia (Oceania), biovar 1 Race 2 was reported in nursery on imported Heliconia; biovar 2 Race 3 was also reported on potato and in the tropical rainfall area, Race 1 biovar 3 is reported to be endemic on a large range of solanaceous crops such as tomato, potato, eggplants, capsicum and tobacco or on ornamental crops such as palms, heliconias, lilies, and more others; some trees have been also identified with the disease even though the effect was less important: they are neem, black sapote, custard apple and eucalyptus (Elphinstone, 2005; CABI/EPPO, 1999)

Also *Solanum dulcamara* (bittersweet) and *Urtica dioica* are found to be important aquatic weed that host the pathogen in Europe (Wenneker et al., 1999).

Pathogen Survival

Although considered to be a soil borne vascular pathogen, the bacteria is reported to have a poor survival ability in soil, but it can survive on roots of alternate hosts, undecayed infected plant tissues, volunteer tubers from precedent crops or in deeper layer of soil where they do not confront the antagonism from other soil microorganisms (Wenneker et al., 1999). This bacterium may also survive by colonizing the rhizospheres of non-host plants (Wenneker et al., 1999).

Several studies done by Wenneker et al., in 1999 to understand the epidemiology addressed the detection of natural infection of *R. solanacearum* of aquatic and riparian weeds. The results showed the pathogenicity of the bacteria on the aquatic weeds *Urtica*

dioica and *Solanum dulcamara* under high infection pressure and high temperature after artificial inoculation. An also heavy contamination was observed in surface water associated with these aquatic weeds. The bacterium was not isolated from the roots of *Bidens frondosa* and *Lycopus europaeus*. But even though no symptoms were observed, the pathogen was extracted from bittersweet root with a confirmation of systemic infection in some cases. The pathogen was first detected from *U. dioica* roots without any systemic infection; however under heavily used waterways contamination, systemic contamination was found. Those situations indicate that the bacterium survives on the rhizosphere of *U. dioica* and can also induce systemic contamination. Wenneker et al., 1999 suggested that the presence of infested surface water with different weeds should be regarded as a continuous source of inoculum. For that a possible control of bacterial wilt might be achieved by local eradication of potential hosts and prohibition of using contaminated surface water for irrigation or by disinfection of tools through chemical treatment.

In their research to identify potential hosts of *R. solanacearum* in Nepal, Pradhanang et al., (2000) compared artificial inoculation to natural infestation in some common agricultural weeds and crops in UK and Nepal. As result, inoculation of summer weeds *Drymaria cordata* and *Polygonum capitata* did cause systemic infection sometimes without any symptoms, plus the root colonization by the bacteria. Natural infestation did not cause infection in winter weeds including *Cerastium glomeratum* and *Stellaria media* indicating that winter conditions in the high hills of Nepal does not allow infection. Only one crop did respond positively to the artificial inoculation while in natural infested conditions the bacteria infected none of the crops. Pradhanang et al.,

(2000) concluded that the role of non-solanaceous weeds on the persistence of biovar 2 of *R. solanacearum* in the environment might have not been well understood.

According to the study done by Carusco et al., (2005), *R. solanacearum* biovar 2 was found in a Tormes river in Spain confirming once again the importance of fresh water as a possible permanent reservoir and a vehicle for the transmission of the pathogen even during colder months; however seasonal variation on the pathogen abundance at different sites were observed showing the direct effect of temperature on *R. solanacearum* population. The lower the temperature, the lower was the *R. solanacearum* population. Van Elsas et al., (2000) investigated the survival of the same biovar in the field and in soil microcosm in temperate climates. They found that culturable population of the bacterium invariably show declines in field soil under prevailing conditions in temperate climates, however they had drastic populations reduction due to the effect of low temperature as well as drought in three different soils. *R. solanacearum* Race 3 is a soil borne pathogen, which persists in wet, deep soils to depths of over 75 cm and in reservoir plants (Van der Wolf and Perombelon, 1997). Even though it is adapted to low temperature, its survival is reduced by very cold temperature.

In experiments done by Stansbury et al., in 2001, *R. solanacearum* Race 3 biovar 2 population densities declined at 15-20 °C and were severely reduced at 4 °C. Also populations were reduced under severe drought and Race 3 biovar 2 was found to be more severe between 24-35 °C with the optimal temperature of 27 °C and with decreases in virulence when temperatures exceeded 35 °C or below 10 °C. In a study conducted in potato fields (Van Elsas et al., 2000), in regions such as Australia, England, Kenya, and Sweden the organism was not detected in previously diseased potato fields after two

years, suggesting that long-term survival in temperate regions is reduced (Van der Wolf and Perombelon, 1997). In another study the bacterium persisted for 12 months in potato fields (Van Elsas et al., 2000).

Pathogen Spread

“The high level of similarity among ginger strains from geographically and chronologically separated isolation indicated that the isolates of biovar 3 of *R. solanacearum* were a lineage of single virulent strains and inter state rhizome transmission could be one of the possible means of pathogen spread across the state” (Kumar et al., 2004). In potato, pathogen spread through infected seed tubers, weeds, irrigation water, contaminated equipment and infested soils occurs commonly (Hay, 2001) and sometimes insects or bacterial pathogens such as, *Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff) Davis et al., (Stevenson et al., 2001).

According to Deberdt et al., (1999) *R. solanacearum* and nematode populations usually coexists together in tropical and subtropical areas; the nematode feeding wounds produced on roots serve as entry for the bacteria thus the high correlation between the presence of root knot nematode and the level of bacterial infection. The presence of root knot nematodes increases more the disease compared to reniform nematode even in presence of tomato varieties that are normally resistant to bacterial wilt (Deberdt et al., 1999).

Disease Symptoms and Diagnostics on Primary Hosts

Potato

Plant wilting during the day is followed by night recovery. Leaves commonly have a bronze cast and epinasty on leaves and petioles is common (Smith et al., 1997). When plants become stunted and chlorotic, the lower stem present a streaked brown appearance (Smith et al., 1997) and vascular tissue turns brown (DEFRA, 2001). The presence of bacterial ooze from a cut made on stem placed in transparent water is a diagnostic key (Hay, 2001). Infected tubers show vascular necrosis and their eyes could secrete bacterial ooze also (Priou and Aley, 1999a). And the plant finally stops recovering and dies (Stevenson et al., 2001).

Cross sections of the stem reveal brown discoloration of the vascular system (Stevenson et al., 2001). Bacterial is observed by placing a piece of stem tissue collected from a symptomatic plant into a glass of water and watching for viscous streaming (Smith et al., 1997). If streaming is not immediately evident, tubers should be incubated for 3-4 weeks at 30 °C and the presence of ooze coming from eyes can be used to screen tubers for infection. This method is time consuming and may not show low infection rates (Priou and Aley, 1999). These diagnostic tools can only determine the presence of the bacteria; the above procedures only confirm the presence of bacteria, but do not provide information on the exact genus/species nor biovar present. Other techniques that do provide genus/species and depending on the assay, Race and biovar information include: semi-selective media (Englebrecht, 1994); immuno-fluorescence staining (IF); enzyme-linked immuno-sorbent assay (ELISA) (Robinson-Smith et al., 1995); molecular

analysis using polymerase chain reaction PCR (Seal et al., 1993; Ozakman and Schaad, 2002) and pathogenicity testing.

Tomato

The youngest leaves are affected and show a flaccid appearance when it is warm; When pathogen met a favorable environmental condition, the total wilt of the whole plant can occur. When conditions are not favorable to the pathogen, symptoms develop slowly showing stunting and adventitious roots on the stem. The vascular tissues of the stem and the tubers are discolored in brown and can present drops of white or yellowish bacterial ooze when cut in crosswise (OEPP/EPPO, 2004)..

Pelargonium

“Symptoms are wilting and chlorosis of leaves. Stems may blacken and eventually become necrotic. Internal vascular browning is often visible. In a later stage, leaves become brown necrotic and the whole plant desiccate and totally collapse in final stages” (OEPP/EPPO, 2004).

Tobacco

Diseased plant shows unilateral wilting and premature yellowing. Some time a half leaf may show wilting symptoms. In case of severe condition of the disease, leaves wilt without changing color and stay attached to the stem and a brown discoloration in the vascular tissues appears when cut open the stem (OEPP/EPPO, 2004).

Banana

On young plants, the youngest leaves turn yellow before collapsing. The rot is dry and brown on affected fruits; all leaves may collapse within a week, . The pseudostems will present a brown vascular discoloration (OEPP/EPPO, 2004).

Characterization and Identification of Races and Biovars

Before starting any control plan, it is crucial to identify first the Race and Biovar of the bacterium. Several methods to identify Races and Biovars of *R. solanacearum* are known; Galal et al., (2003) have done a comparative study on the identification of Races and biovars of some Egyptian isolates of *R. solanacearum*. They found that indicator plants such as tomatoe, banana and tobacco plus the ability of bacteria to use hexose alcohols or disaccharides are still the most accurate identification methods of *R. solanacearum* races and biovars. Although the new detection techniques have high accuracy, they still need reference isolates.

Races Determination

The pathogen species is subdivided into Races based on host range. Currently, polymerase chain reaction (PCR) is the primary means of definitive identification of pathogen Race. Before PCR, a tobacco hypersensitivity test, developed by Lozano and Sequeira (1970), was used to distinguish between Races 1, 2, and 3, the most economically important Races. The test is done using tobacco leaves by infiltrating bacterial cell into the parenchyma with a fine niddle; Leaf necrosis and wilting of tobacco plant after 8 days indicates Race 1; yellowing of infiltration area in 48 hours is Race 3 and hypersensitivity with white necrosis of the interveinal area in one day is Race 2.

Table 3: Tobacco Plant Reaction to Different Races of *Ralstonia solanacearum* (Smith) Yabuuchi et al., According to Lozano and Sequeira (1970)

Race		Reaction type
1	24 h	no visible symptoms
	36 h	dark brown lesion surrounded by a yellow zone
	60 h	Vessels discolored
	8 d	leaf wilting and yellowing
2	10-12h	hypersensitive reaction, infiltrated tissue glassy
	60 h	tissue thin, transparent, white necrosis
3	48 h	infiltrated tissue becomes yellow

R. solanacearum is also subdivided into biovars based on the utilization of the disaccharides cellobiose, lactose, and maltose and oxidation of the hexose alcohols, dulcitol, mannitol, and sorbitol (OEPP/EPPO, 2004).

Biovar Determination

When Alcalá de M. and Lara in 1995, did the characterization and identification of the bacteria biovars they recommended the procedure developed by the Bacteriology Committee of the American Phytopathological Society. Factors used were growth of colonies on NGAS, agar, carbonate of calcium dextrose, the production of levan, the growth on crystal violet polipectate media (CVP), the gelatin liquefaction, nitrate reduction, catalyses production, and the growth of the bacteria at 27°C, 37°C and 41°C; the oxidation of hexose-alcohols, and the use of disaccharides. They used methods such as Suslow KOH test at 3%, the production of fluorescent pigment on B of King media used by Fahy and Hayward, and the growth on Kado and Heskett's selective media D1 and D3. The different techniques they used showed through theirs result the characteristics of Biovar 2 and Biovars 3 of Race 3.

If appropriate, *R. solanacearum* isolates can be classed as different biovars by their acid production from three hexose alcohols and three disaccharides in Haywards basal media (Table 4).

Table 4: Classification of *Ralstonia solanacearum* (Smith) Yabuuchi et al., in Biovar through Biochemical Test Method.

Test	Biovar				
	[1]	[2]	[3]	[4]	[5]
Acid from:					
Hexose alcohols					
mannitol	-	-	+	+	-
sorbitol	-	-	+	+	+
dulcitol	-	-	+	+	-
Disaccharides					
cellobiose	-	+	+	-	+
lactose	-	+	+	-	+
maltose	-	+	+	-	+

R. solanacearum biovars are determined on the basis of their ability to produce acid from three hexose alcohols and three sugars (Hayward, 1964 and 1994; OEPP/EPPO, 2004).

Pathogenicity Test

Also the Race1, 2, 3 are identified on the basis of their pathogenicity ability on tomato plants or eggplants in addition to tobacco plants (Buddenhagen et al., 1962).

Table 5: Race Determination through Different Host Plant Reaction to *Ralstonia solanacearum* (Smith) Yabuuchi et al., According to Janse (1991).

Reaction in:	Races		
	1	2	3
Tomato/eggplant	Wilting	No reaction	Wilting
Tobacco cv. White Burley plants (stem inoculation)	Wilting	No reaction	No reaction
Tobacco cv. White Burley leaves (hypersensitivity test)	Necrosis (48 h) and wilting (7–8 days)	HR (12–24 h)	Chlorosis (2–8 days)
<i>Musa acuminata</i>	No reaction	Wilting	No reaction

*Race 4, pathogenic to ginger and a few other hosts and Race 5, pathogenic to mulberry only, not included.

Race characterization by the pathogenicity test or tobacco hypersensitivity test may not be highly reliable and instead can be deduced from the biovar and the natural host of origin.

To evaluate the bacteria's pathogenicity, Alcalá de M. and Lara (1995) used bacteria inoculum at 24-48 h of growth stage in sterile water solution at the concentration of $1,5 \times 10^8$ cfu/ml. Healthy plants of potato variety "Andinita", tomato variety "Rio Grande" and pepper variety "Jupiter" at 4 weeks of age were transplanted in disinfected soil. Kelman and Person (1961) suggested inoculation technique method was used by adding a drop of inoculum suspension on the axis of the third leaf under the plant apices through wounding. The control had the same treatment with distilled sterile water. 15 plants of each crop have been inoculated. Temperatures of $30,1^\circ\text{C}$ as maximal and $17,9^\circ\text{C}$ as minima were maintained during the experiment; disease symptoms were evaluated every day and at 10 days after inoculation disease evaluation was done by using the Winstead and Kelman (1952) severity scale of 0 to 5 by (where 0 is no symptoms and 5 is dead plants). From the diseased plant the bacteria was re-isolated on TZC media to confirm the presence or not of the characteristic colonies of the bacteria. The results showed the efficiency of this inoculation method since the inoculated plants wilted at 10 days and died at 15 days while the control plant did not show any symptom; by the same way the isolation of the bacteria from the diseased plant did grow on TZC media with the typical colonies of *R. solanacearum* while the isolation from the control did not grow on the TZC.

The pathogenicity test used by EPPO is done by inoculating with syringe preferably, to the third true leaf stage or slightly older with a suspension (10^6 cell mL^{-1})

of a 48-h nutrient agar/YPGA culture into susceptible tomato (Moneymaker) or eggplant (Black Beauty) plants (OEPP/EPPO2004). Incubation time should be more than two weeks at 25–28°C under high relative humidity conditions. Symptoms are wilting, epinasty and chlorosis, sometimes only stunting. The bacterium should be reisolated from plants by taking a section of stem or petiole above the inoculation point and placing it in sterile distilled water or 50 mM phosphate buffer, plating on SMSA media, and observing for typical colonies (EU, 1998; OEPP/EPPO, 2004)

According to Klement et al., (1990), techniques to inoculate vascular wilt pathogens into plant can be in order of importance the following: the stem pricking, the dropping of bacterial suspension inside wounded petioles and the immersion of cut root tips into bacterial suspension.

Inoculation Techniques

Inoculation of Cotyledons Through the use of wounding done by needle puncture or carborundum to introduce the pathogen; that technique is recognized to be very effective in different conditions.

Stem Inoculation Simple and commonly used the inoculation is achieved through the injection of bacterial suspension with a fine needle hypodermic syringe into the vascular tissue of the stem. The technique has been proven effective.

Inoculation through Petioles The technique consists to wounding the petiole at its attachment point to the stem and applying inoculum. The method is not always successful under dry conditions since the inoculum drop can dry before getting inside the plant.

Leaf Inoculation The inoculum is sprayed on the needle wounded leaves or applied onto leaves directly.

Root Inoculation through Infested Soils The method is an effective inoculation technique. Bacterial suspensions (50ml of $8 \times 10^6 \text{ cfu.ml}^{-1}$) of inoculum solution are used to water tobacco during transplanting. Winstead and Kelman, (1952) have used the root injury technique to inoculate tomato and tobacco plants in green house by applying bacteria suspension on cut lateral roots of 4-6 weeks old plants

Inoculation by Dipping the Roots in Bacterial Suspension The method consisting in dipping plant root in bacterial suspension before transplanting can be used in field and in green house. The method was explained by Klement et al., (1990). Wounding induced through cutting 1-2cm of root before transplanting will increase the probability of disease occurrence. The technique is effective however the incubation time is longer (one month).

Winstead and Kleman in 1952 found that younger plants respond faster to inoculation than oldest plant.

The latent period between inoculation and symptom's appearance going over two weeks some time is generally longer than most of the known bacterial disease; the situation is certainly due to the cause of the wilt depending more to the number of the bacteria present in the xylem rather than the toxicity produced by the bacteria (Klement et al., 1990).

Traditional Detection Methods of *R. solanacearum*

According to Alvarez (2004), the variable phenotypic and genotypic characteristics of the versatile *R. solanacearum*, that serve for its identification, require rather the use of complementary methods instead of using some few universal known methods. Alvarez confirmed that symptom recognition and a rapid test is enough to identify a known bacterial plant pathogen, while the diagnosis of truly unknown pathogens requires more investigation such as field observation, examination of plant tissues, and isolation of the pathogen, characterization, and proof of Koch's postulates.

Rapid development of genomic techniques by scientists to characterize the bacteria over the past decade has greatly simplified and improved pathogen detection and identification.

Primer sequences that have been already described can be used with the polymerase chain reaction (PCR) to identify many common plant pathogenic bacteria (Alvarez, 2004). Several methods have been developed to detect the bacteria *R. solanacearum*; among them the most commonly used is direct plating on selective media and enzyme-linked immunosorbent assay (ELISA) (Dittapongpitch and Surat, 2003).

Each method had advantages and disadvantages, but for a good detection of the pathogen, Galal et al., (2003) think that at least three detection methods such as using semi-selective culture media followed by using immunofluorescent staining test (IF) and an indicator plant (tomato) should be adopted.

Visual Inspection The visual inspection is very important for being the first step in identification of symptomatic disease. Very useful in seed potato field inspection its efficiency depends on the experience of the inspector to recognize the symptoms. The

common symptom are day wilting and night recovering at the beginning, plus vascular discoloration of stem at a few cm above the soil line followed some time by a bronzing of leaves. Generally leaves wilt without changing of color. A stem cut test shows the bacteria white ooze exudes coming out from vessels when placed in water. The same ooze can be observed from tubers eyes and stem-end-attachment. When masses of soil are clumped to the tubers eyes it is good to cut the tubers and check for bacterial ooze or vascular discoloration because when the bacterial exudates dries, a mass of soil may adhere to the tubers at the eyes. In postharvest inspection also it is important to section the tuber and look for vascular necrosis and white bacterial ooze (OEPP/EPPO, 2004).

Isolation and Detection of Latent Infections According to French et al., (1995), one of the main constraints to develop efficient strategies in controlling bacterial wilt disease has been the lack of accurate, simple and rapid methods to detect the pathogen in plant, soil, and water samples.

Traditional detection methods are based on the isolation of bacteria from seed or plant extracts through culturing on semi selective media, followed by colony identification by morphological and biochemical characteristics and pathogenicity tests.

Usually, *R. solanacearum* occurs in a latent form in temperate European countries when infected symptomless weeds such as *Solanum dulcamara* grow along waterways (Elphinstone et al., 1998; Janse et al., 1998; Wenneker et al., 1999). For isolation from that latent form, the SMSA media modified has been more effective in Europe (e.g. Elphinstone et al., 1998; Wenneker et al., 1999).

Isolation and Detection from Symptomatic Infections Isolation from symptomatic material can easily be performed using YPGA non-selective media or Kelman's

tetrazolium media. In some cases when secondary infections are present, isolation on selective media is necessary. A presumptive test in the field can be the water streaming test as described under disease symptoms or a serological agglutination test using a field kit in the form of a lateral flow device (Danks and Barker, 2000).

Detection Through Bacterial Streaming The bacterial streaming test is an easy and simple test useful to identify bacterial wilt. The technique consists of cutting the diseased plant stem just above soil line and placing the cut surface in a beaker with water. When white bacterial ooze is seen streaming out from the vascular bundles is diagnostic for *R. solanacearum*. Any other bacteria causing vascular infection in potato or tomato plants will not show this phenomenon (OEPP/EPPO, 2004).

Isolation Media: Identification of Pure Culture The morphological, physiological and biochemical characters of a pure culture can be checked on culture media. It can also be checked by the pathogenicity test described earlier. Sterile PB and a known isolate of *R. solanacearum* should be used as controls. With pure culture inoculum, typical symptoms should be seen within 4 days (OEPP/EPPO, 2004).

According to French et al., in 1995, the bacterium *Pseudomonas solanacearum* is very difficult to be isolate by inexperienced researchers. While the bacterium multiply readily in its host, it grows very slowly in vitro comparing to most of the pathogenic bacteria. In culture its rate of mutation is rapid so that the only practical way to store it is in water. For its detection and its biovar identification, specialized media can be used. Also are available now several molecular biology tools for a fast identification; these are serological and different PCR test (Alvarez, 2004).

Selective Media These media are used in phytobacteriology for disease diagnosis, indexing and epidemiological studies. The principle is preventing growth of certain groups of microorganisms while promoting growth of the desired (Rudolph et al., 1990).

Kelman's Tetrazolium Chloride (TZC) Agar Media (Kelman, 1954) is used to distinguish *R. solanacearum* among other bacteria during isolation and also to distinguish virulent colonies from avirulents mutant during culture purification. Virulent colonies are whitish while avirulent are red dark (Rudolph et al., Klement et al., 1990). According to Sands (1990), that media containing ten gr/l of peptone, one gr of caseine hydrosalate, fifteen of agar and five of glucose plus the 10ml of 2, 3, 5-triphenyltetrazolium, autoclaved and added to the cooler agar before pouring is a rich media carrying an indicator. Used with *R. solanacearum* it shows the difference between stable avirulent colonies that look dark red from the unstable fluidal virulent that is white with pink center. It is also used as identification test because of the specific characteristics of the virulent strain colonies. Sands also confirmed that the loss of virulence in culture is irreversible.

Soil Isolation Media (SMSA-E) According to Klement et al., (1990), a most simple and effective method to study phytopathogenic's soil bacteria is the use of dilution plating. The plant pathogenic bacterias do not compete well with soils microbe and can be out numbered if selective media is not used.

Detection of Latent and Symptomatic
Infections Using Serological and Molecular Techniques

Since the previously described tests require up to several weeks to confirm the identification of the pathogen there is a need for rapid, reliable tests to replace the costly and time-consuming culture and plant bioassays (Alvarez, 2004).

Another reason to develop more sensitive pathogen detection is the need to quarantine unhealthy propagation material in the field and storage when the absence of symptoms does not always mean the absence of latent pathogen population; to aid selection of resistant cultivars to *Ralstonia* and to evaluate the effectiveness of integrated control techniques on the disease incidence (French et al., 1995).

Pradhnanang et al., (2000) compared the sensitivity and specificity of various methods for routine detection of *R. solanacearum* in a sandy loam soil; the different methods used were detection on selective media, detection by tomato bioassay, detection by indirect ELISA, detection by conventional PCR, and detection by nested PCR. Each method has its own advantages and limitations. PCR has potential as a sensitive and specific detection technique for studying survival of *R. solanacearum* in soil. Indirect ELISA, useful in low-cost screening of large numbers of samples where populations exceed 10⁴ CFU/ml, lacked the sensitivity required for epidemiological studies and was potentially prone to giving false-positive reactions. Cultures on modified semi-selective media combined sensitive and specific detection with reasonable cost of application and provided accurate quantification of viable population of *R. solanacearum* in soil.

In conclusion, Pradhnanang et al., (2000) found that sensitive detection in field soil depend largely on sampling strategy and extraction methods, as well as on the detection method employed. PCR which had great potential as a sensitive and specific detection method has not been commonly used for field samples to detect *Ralstonia* because of its inconsistent results reported on plant and soil sample by Elphinstone and Stanford (Dittapongpitch and Surat, 2003). Those inconsistent results could be related to the inhibition of the enzymatic PCR reaction by various compounds present in plant and soil samples. To overcome this problem, a detection assay for *Ralstonia* was developed by Dittapongpitch and Surat (2003) combining immunocapture and the polymerase chain reaction (IC-PCR). Anti-*R. solanacearum* polyclonal antibodies were produced in a white female rabbit and Dynal super-paramagnetic beads were coated with purified immunoglobulinG (IgG). Those anti-target bacteria were applied to separate target microbes from the media prior to cell rinsing and further processing (Dittapongpitch and Surat, 2003). Rudi et al., (1998) cited by Dittapongpitch and Surat in 2003 modified this technique known as immunocapture or immunoseparation in 2003 by attaching the antibacterium polyclonal antibodies to magnetic heads before mixing them with samples. DNA extraction and PCR using *R. solanacearum* specific primers will follow that. This method significantly improves the detection of bacteria in naturally infested samples with low population levels (Dittapongpitch and Surat, 2003). Dittapongpitch and Surat (2003), developed an IC-PCR protocol by using polyclonal antibodies against *R. solanacearum* that evaluated its sensitivity by examining soil and weed samples collected in the major commercial tomato fields of North Eastern Thailand.

From fifty-five weeds plants collected from weed fallow field and tomato cropping fields, 28 were found positive to *Ralstonia* using the enrichment and IC-PCR (Dittapongpitch and Surat, 2003). All weeds except *Cyperus rotundus* are dicotyledonous; *Physalis minima*, *Amaranthus spinosus* and *Euphorbia hirta* were the most common weeds in the field with over 50% of each weed species yielding IC_PCR positive results. IC was used to separate and concentrate *R. solanacearum* from samples prior to PCR amplification. In the presence of low bacterial populations, the target bacteria need to be cultivated in an appropriate or selective media to enhance detection efficiency (Dittapongpitch and Surat, 2003). Unlike the soil suspension, positive IC-PCR results were obtained without enrichment from tomato plants, which were inoculated with 10^5 - 10^7 CFU/ml of *R. solanacearum* 1 day after inoculation. This result suggests that the natural compounds in tomato plants have minor effects on PCR. As the inoculated plants had no symptoms, this result suggested that enrichment and IC-PCR could be used to discover latent infection in tomato plants.

Schaad et al., (2003) confirmed that the “specificity of all immunoassays can be improved by using monoclonal antibodies and serological techniques can greatly reduce the time needed for diagnosis; however, the results should only be considered as presumptive since both false positives and negatives are possible”.

Priou et al., developed in 2006 a low-cost and easy-to-use technique for the sensitive detection of *R. solanacearum* in latently infected potato tubers and soil by post enrichment ELISA (B); different biovars and different origins (33 countries) worldwide were tested and successfully detected by antibodies produced at the International Potato Center (CIP). Isolates of *R. solanacearum* belonging to Bv1 and Bv2A were successfully

detected by double antibody sandwich–enzyme-linked immunosorbent assay (DAS-ELISA) at low population levels after incubation of soil suspensions for 48 h at 30°C in a new semi-selective broth containing a potato tuber infusion. *R. solanacearum* was successfully detected by post enrichment (nitrocellulose membrane-enzyme-linked immunosorbent assay) NCM-ELISA (tubers samples) and DAS-ELISA (soil samples) even at low population levels in both inoculated and naturally infected extracts. Post enrichment NCM-ELISA combines different advantages such as high sensitivity, low-cost easy-to-use, speed and does not require extensive laboratory equipment as postenrichment nested PCR (Priou et al., 2006).

Also the use of PCR-RFLP technique based on *hrp* (hypersensitivity response and pathogenicity) gene region has been proved to be a reliable diagnostic technique to enable researchers for a rapid identification of the disease by Fouche et al., in 2006.

An ELISA test is available in kit from Agdia including all of the components needed to run the test in minimal lab condition like we do have in Mali, while the ImmunoStrips for *Rs* including the ready to use sample extract bags of BEB1 buffer are easy to use directly in field that are far from the laboratory.

These detection techniques from Agdia are based on serological techniques using the capture of monoclonal antibody.

ImmunoStrips offer the laboratory technology of ELISA, but in an easy-to-use format that works in the field. The testing process involves simply grinding the suspect plant sample in the special bag that contains the extraction buffer (included with the kit) and inserting the ImmunoStrip into the bag. Results can be seen in 10-15 minutes. The choice of the detection method will depend on several factors:

- The choice of sensitivity needed,
- The sample to be tested (soil water or plant section or tuber)
- Experience of the user
- Laboratories facilities available
- Cost-benefit-ratio

“Because the host plant acts as an efficient enrichment media for selective multiplication of the pathogen in the absence of saprophytic bacteria, it is much easier to detect *P. solanacearum* in potato tissue or others hosts than in soil or water” (French et al., 1995).

Integrated Management

Important factors to consider in Integrated Management of this pathogen are the identity of Race and biovar. Also important is the identification of the area of distribution of the pathogen, to prevent its occurrence and limitate its spread to new areas.

The most efficient way to control the disease is preventing its introduction to a production area by effective quarantine and phytosanitary methods. After introduction of integrated management methods that incorporate cultural controls such as rotation with non-host crops, soil amendments, fallow periods, anaerobic flooding, use of disease free planting material and resistant cultivars are needed to effectively manage this disease. Biological and chemical soil treatment can also contribute to improve but will require the use of healthy-tested planting material and cultural practices including strict crop and storage hygiene.

In Uganda as indicated in the 1999-2000 CIP program report by Lemaga (2000), bacterial wilt can be satisfactorily controlled and potato yield increased by one of the following respectively from best to the least controlled:

-Combined use of clean seed of a less susceptible variety and improved cultural practices;

-Clean seed of less susceptible variety alone

-And improved cultural practices alone.

All the 3 options were economically beneficial compared to the traditional farmer package consisting in the use of farmer's varieties and seeds planted under their own cultural practices.

Crop rotation, good amendment and regular control of latent infection with ELISA tests should complement the efficiency of the best control.

Disease Free Seed Potatoes

The use of disease free seed potatoes is a good approach to improve crops productivity. In Mali in Pilot Unit, trials of farmers producing seed potato through the use of micro and mini-tubers gave average yield from 6 to 12 t/ha for micro tuber and 8 to 16 for mini-tubers. In spite of severe conditions the cost of the second generation is already below the price of the imported seed potato (Vanderhofstadt, 1999). But the seed quality still has to be improved if we want to make an advance in potato production. This result could be better if farmers were not growing seed potato where they use to grow their commercial potato. To maximize seed production and land productivity it is very important to separate seed production from food production and increase the disease free seed potatoes (Kinyua and al., 2005).

Crop Rotation

Crop rotation is a strong component of the management of bacterial wilt caused by *R. solanacearum*.

Kumar and Sharma (2004), found a significant reduction of bacterial population to 83% in maize-spinach-watermelon rotation and 80.6% of reduction with the ragi-French bean-okra rotation when compared to the initial population.

Alcala and Lara (1995) demonstrated that *R. solanacearum* can survive in soil during 161 days.

In CIP annual report by Lemaga (2000), the importance of crop rotation was also confirmed; however it was clear that while a single rotation can be sufficient for the production of food potato on mildly infested fields, rotation must be longer than two seasons if the soil is heavily infested. The useful crop for rotation adapted to their conditions can be beans, cereals, or sweet potatoes; to be more efficient a good amendment of soil, preferably a combination of organic and inorganic components should accompany the rotation.

Crop rotation reduces the inoculum build up of pathogens but is still difficult for farmers to fulfill because of the limitation of their lands and their ability to market crops produced. cereal crops, liliaceous and brassicaceous crops, legumes and cucurbitaceous crops are reported to be non hosts of *R. solanacearum* (Priou et al., 1999b)

Requirements for seed production are more stringent than those of table crop production; therefore seed potato production areas should not be cropped to any host plant.

Soil Amendments

Increasing the doses of well-done compost to get a suppressive soil for *R. solanacearum* is another possibility to control the disease as experimented in Burkina Faso by Ouedrago (1994).

In 2004, Islam and Toyota compared 3 types of soil: CF soil (amended with chemical fertilizers for 14 years), CF+FYM-soil (amended with chemical fertilizers and farmyard manure at 40 t/ha/year for 14 years) and FYM-soil (amended with farmyard manure at 400t/ha/year for 14 years). Tomato plants were grown to evaluate the degree of suppressiveness of bacterial wilt of tomato by the soil. More than 70% of tomato plants showed wilt symptom in the CF- and CF-FYM-soil after 30 days of cultivation whereas less than 10% of tomato plant wilted in the FYM-soil. The study demonstrated that bacterial wilt of tomato was suppressed in the poultry and FYM added soil due to higher microbial activity.

This result suggest that the suppression of bacterial wilt in the FYM-soil was due to the combined effect of biotic and abiotic factors associated with repeated application of a large amount of farmyard manure.

Alcala de M. and Lara (1995), after soil analysis found some correlation between the level of soil compost and the permanent presence of the bacteria that spend more time in soil with a low level of organic material. In their study on the effect of soil amendments on bacterial wilt incidence, Lemaga et al., demonstrated in 2001 that soil amendments can help to reduce wilt incidence and increase the yield; but the best result came from the combination of organic and inorganic fertilizers, when potassium was added with an organic source of nitrogen.

Also, Gorissen, et al., in 2004 demonstrated that the addition of pig slurry decreased significantly the population of *R. solanacearum* as well as reduced numbers of infected and diseased plants in the soil suppressiveness tests. On the other hand, they found that solarization of soil also decreased *R. solanacearum* survival but did not

enhance soil suppressiveness as measured by development of disease symptoms and plant invasion after 9 weeks. Combined soil solarization and pig slurry amendments showed an additive effect of both treatments. However healthy-looking plants, primarily from soils treated with pig slurry and solarization, incidentally revealed the latent presence of *R. solanacearum* in the lower stem parts.

The effects of compost addition and simulated solarization of soil on the survival of *R. solanacearum* biovar 2 strains 1609, as well as on the structure of indigenous soil bacterial communities, were analyzed by Schönfeld et al., in 2003. They found as result that solarization of unamended soil did not drastically affect *R. solanacearum* survival or plant invasiveness, while the addition of household compost resulted in enhanced *R. solanacearum* population decline rates, as well as reduced numbers of diseased plants in suppressiveness tests; the combination of solarization and compost addition yielded differential results between microcosms and the field. At the end of their study, compost amendment had clearly induced changes in microbial communities, which were detectable until the end of the experiment. Two major bands in DGGE (Denaturing Gradient Gel-Electrophoresis) analysis, affiliated with *Variovorax paradoxus* and *Aquaspirillum psychrophylum*, were associated with the compost amendment.

Islam and Toyota in their study in 2004, demonstrated that the bacterial wilt of tomato was suppressed in the poultry and FYM added soils, compared to non-treated soil, and higher microbial activity was likely responsible.

The work done by Messiha, in 2006 compared different techniques of soil treatment to reduce *R. solanacearum*. Survival and suppression were studied for soils differing in origin (Dutch versus Egyptian [sand versus clay]), and management (organic

versus conventional). Effects of amendment of conventional soils with NPK and organic soils with compost or cow manure were compared with non-amended controls. Brown rot infection was only slightly reduced in organically compared to conventionally manage sandy soils from Egypt, but organic management significantly increased disease incidence and pathogen survival in Dutch sandy and clay soils, which correlated with high dissolved organic carbon content in the organic Dutch soils. There was no correlation between disease incidence or severity and bacterial diversity in the potato rhizosphere in differently managed soils (as determined by 16S DGGE). NPK fertilization reduced bacterial wilt in conventional Egyptian soils but not in Dutch soils. Cow manure amendment significantly reduced disease incidence in organic Dutch sandy soils, but did not affect the bacterial population. However, cow manure reduced densities of *R. solanacearum* in Egyptian sandy soils, most probably by microbial competition as a clear shift in populations was detected with DGGE in these and Dutch sandy soils after manure amendment. Amendment with compost did not have a suppressive effect in any soil type. The absence of a disease suppressive effect of mineral and organic fertilization in Dutch clay soils may be related to the already high availability of inorganic and organic nutrients in these soils. This study shows that the mechanism of disease suppression of soil-borne plant pathogens may vary strongly according to the soil type, especially if quite different types of soil are used.

Andriantsoa et al., (2006), tested different cruciferous plant as amendments to control *R. solanacearum* and found that 50% of the total plant reduced the population of the bacteria. Data indicated that suppressive effects of the residues might depend on nutritional status and undefined factor in the soil but were not directly dependent on the

concentration of glucosinolates in the residues and *in vitro* tests showed that authentic isothiocyanates were inhibitory to the pathogen only at high concentrations (more than 2 $\mu\text{mole g}^{-1}$ soils).

According to the result obtained by Pradhanang et al., (2003), from their work on the effects of plant essential oils on *R.solanacearum* population density and bacterial wilt incidence in tomato, concluded that some essential oils including thymol did reduce the population of the pathogen in soil and decreased the disease incidence while the plant shoot and root weigh were increased.

A study conducted by Sharma and Barawdj in 2005 to evaluate the potential of AMF(Arbuscular Mycorrhizal Fungi) with five botanicals namely *Azadirachta indica*, *Carica papaya*, *Ocimum sanctum*, *Ricinus communis* and *Tagetes patula*, resulted in reducing nematode multiplication and increased tomato plant growth and yield.

The effects of neem cake, a nutrient-rich organic material derived from neem seed, on plant-parasitic nematodes, have been investigated in greenhouse trials by Abbasi et al., (2005). At the end of the experiment 1% of neem cake (mass/mass soil) caused a 67%–90% reduction in the number of *Pratylenchus penetrans* and *Meloidogyne hapla* (root-knot nematodes) in tomato roots grown in three different soils. The same experiment in the field with 1% of neem cake (mass/mass soil) reduced the number of lesion nematodes by 23% in corn roots and 70% in soil around roots (Abbasi et al., in 2005). In Mali neem trees are growing every where and and the flour obtained from the seed has been already effective in controlling soilborne diseases in seed bed (Noussourou et al., 2003). Neem can be a good alternative in our integrated management approach to reduce bacterial wilt.

Rouging and Sanitation

More attention should be given to rouging and sanitation, an important practice in seed production system to maintain seed quality. Growers usually just throw the infected plants into a well, a corner of the field or to a nearby canal increasing the possibility of contamination of soils and water. In addition to farmers, most of the technicians who have undergone agricultural training do not advice often the rouging, so that more information and exchange about rouging is necessary to improve the final product quality. Rouging is important in the reduction of inoculum to eradicate the disease when it is already present. Routine measures of hygiene should include the cleaning and disinfection of all handling equipment and the storage room for harvested tubers (OEPP/EPPO, 2006)

Field Inspection

Also important, is based on regular inspection in seed potato field, identifying visuals symptoms and routine sampling for lab testing. In the period of seed production all seed fields are regularly inspected to detect earlier diseases, especially quarantine diseases. *Ralstonia* being an important quarantine disease transmitted through soil water and plant has to be detected rapidly at field level so that its spread could be stopped before causing important losses.

Laboratory Testing

This activity is very important for an efficient monitoring of the disease. It concerns tissue culture and derived plantlets, micro and mini-tubers and tubers that have to be tested at different steps of seed production (laboratory, green house and fields); the testing methods can be PCR, ELISA, TZC media and others biochemical or physiological

tests. In South Africa, additional control measures by a special bacterial wilt committee have been established. They are in charge to visit suspected farm, giving advice on prevention and eradication methods; they also determine the distance surrounding the infection site and the area where potatoes cultivation should be prohibited (Mienie and Theron, 2005). The introduction of mandatory testing in South Africa allows detecting the presence of *R. solanacearum* in tubers that did not show any bacterial wilt symptoms during field inspection (Mienie and Theron, 2005). The impact of that test has been beneficial to their certification program by controlling the disease and stopping its spread. In Europe important quarantine measures have been organized by EPPO to stop the propagation between countries (OEPP/EPPO, 2004).

Finding Inoculum Reservoir Sources

The survival of *R. solanacearum* in soil and in aquatic weeds in irrigated rice were studied by Pradhanang and Momol (2001) through the monitoring of soil prior to planting potato, the detection of latent infection, monitoring of aquatic weeds while rice is growing and the monitoring of soil borne *R. solanacearum* before and after rice harvest. The Hayward Biovar (Hayward, 1991) determination method was used on a representative group of the bacteria strains collected from latently infected potato tubers before spring planting, from the basal stem of wilted potato plant, from the weeds during rice culture or from soil after rice harvest. *R. solanacearum* was diagnosed using standard PCR reaction. Results showed that from the total of potato found latently infected only 14% will show symptoms upon planting despite soil infestation; this result indicates that the spring season is not very conducive to the development of the bacteria. *R. solanacearum* was isolated from roots of 2 aquatic weeds. With the highest incidence

of infection in *Dopatrium* sp and the lowest in *Monochoria vaginalis*. Soil borne populations recovered before and after rice harvesting confirmed that the pathogen could survive under water for 3 months. The plot was infested by the Biovar 2A (in latent potato and isolated from soil after rice harvest. Biovar 3 was isolated from aquatic weeds and soil after rice harvest and biovar 4 was isolated from soil after rice harvest To conclude, the three biovars found in field can survive during irrigated rice culture and only the Biovar 2A was found in latently infected potato tuber or wilted plant indicating that potato is the natural host of Biovar2A (Pradhanang and Momol, 2001).

Use of Resistant and Tolerant Varieties

Reported by Grimault et al., (1993), the most effective method to control bacterial wilt is the use of resistant host plants. Genetic improvement by cultivar selection seems to be the best component in Integrated Management of the disease, specifically for Malians and other resources poor farmers, since the bacteria is difficult to control due to its large host range, its latent nature and the expense of chemical control. Identification or improvement of the resistance to Race 1, Race 3, and to both has been done already (Boshou, 2005). Still that resistance to Race 3 strains is expected to be more stable than resistance to Race 1 strains of *R. solanacearum* prevalent in the low land since strains of Race 3 are genetically homogeneous group (Priou et al, 2005). A good resistance levels has been identified from some species related to potato by Sequeira and Rowe in 1969, unfortunately such resistance was rather unstable across different locations according to difference between Races or biovars present in each locations. All varieties that seem to be resistant should be well checked to see if the disease is not latent because different

factors like dryness (low humidity in soil) could slow down the manifestation of the symptom while the disease is present (Alcala de M. and Lara, 1995).

The incorporation of resistance to potato cultivars is a major challenge to breeders. A progeny F1 developed by crossing lines of tomato from Taiwan, Philippine and Indonesia, derived from different bacterial wilt resistance sources did not show significantly higher resistance than its respective high parent (Hanson et al., 1998). Later in 1999 some encouraging results from studies on the genetic and the nature of resistance in *S. tuberosum*, suggested that wilt resistance was stable and identified through somatic hybridization and preliminary work on a population derived from the first backcross to *S. tuberosum* did indicate segregation for resistance (Laferriere et al., 1999); also Fock et al., (2005) concluded that the use of somatic hybrids was a valid way for introducing traits of resistance from wild species to cultivated potatoes.

In Uganda, Osiru et al., (2001), at the end of their study on the inheritance of resistance to tomato bacterial wilt and its implication for potato improvement in Uganda, found that two genes control resistance to bacterial wilt. Such results could lead breeders to strengthen the resistance in potato by using others solanaceous plant as sources of resistance. Since immune potato cultivars are still unavailable because of the high complexity of its resistance gene and the variability of its reaction in different environment, integrated disease management remains the primary control strategy of the wilt in this crop (Osiru and al, 2001).

Some work done by Ateka et al., (2001) in Kenya also showed that the disease spread was due to the use of infected seed and the accumulation of *R solanacearum* in soil (lack of rotation); also the contrasted results between cultivars inoculated in field and

cultivars inoculated in greenhouse suggest that resistance against *R. solanacearum* may be influenced by environmental factors.

Several investigations were done with tomato crop to find the source of natural resistance to the bacteria and it has been showed that several quantitative trait loci are involved in the control of the resistance in which loci on chromosome 6 play the predominant role. Wang et al., in (1999) identified a new locus on chromosome 12 that appears to be active specifically against a Race 1 Biovar 3 Pss4 bacterial strain endemic to Taiwan; to them it is doubtful that there is any tomato line carrying resistance to all strains of *R. solanacearum*. Tomato breeding programs are usually specific to certain geographic locations and hundred of different strains of the pathogen are present worldwide.

Jaunet and Wang (1999) after their study of the variation in genotype and aggressiveness of *R. solanacearum* Race 1 isolated from tomato in Taiwan could hypothesize that the location specificity of resistance observed in the worldwide evaluation could be related to the large variation in aggressiveness. Nevertheless studies initiated in Reunion Island to identify sources of resistance to *R. solanacearum* among *Lycopersicon* spp accessions against a strain belonging to r3 Biovar2 found all tomato accessions used in this study susceptible to the representative strain in Reunion Island and the result clearly demonstrate that the tomato germoplasm is highly susceptible to the Reunion R 3 Biovar 2 strain (Carmeille et al., 2006); so there is a need to pursue the search for resistance source in additional tomato material to facilitate resistance genes pyramiding and provide broad spectrum resistance to bacterial wilt in tomato.

The different result can indicate that the resistance breakdown of *R. solanacearum* could be related to environmental factor and pathogen diversity. In 1999 a worldwide field evaluation in 11 countries of a set of 35 resistant sources to bacterial wilt in tomato has clearly demonstrated existence of location-specific resistance in most sources (Wang et al., 1998).

Priou et al., (2005) at the end of their three evaluation on assessment to resistance to bacterial wilt in the advanced clones of potato from the CIP, found resistance to *R. solanacearum* Race 3 biovar 2 in thirteen clones from which five clones were, highly resistant. They also suggested that resistance in Race 3 strains to belonging to a genetically homogeneous should be more stable than Race 1 strains.

Use of Sanitary Measures

In developing countries such as Mali the respect of strict sanitary measures will improve significantly in controlling the disease spread. Because of the lack of information on the disease, farmers are using practices that are certainly at the origin of the disease spread and development. Two practices should be just stopped for the moment: the tuber cutting and the poor management of the crops debris. Over these two many other practices are not allowed in a good integrated management program.

Fortnum and Kluepfel (2005) demonstrated the impact of mechanization on the spread of bacterial wilt on tobacco in the southeastern of United States; it has been proved in the same way that mechanical harvesting of tomato or use of pruning tools on banana can transmit the bacteria from diseased plant to healthy ones and favor a rapid increase of the disease.

Quarantine Regulations

To secure the potato seed production and protect the crop against new diseases that are not present, Mali is required to have a strict quarantine program.

The success of quarantine procedures relies on the use of rapid and sensitive detection techniques (Lee and Wang, 2000). The development of specific and sensitive diagnostic test in Taiwan, adapted to their specific strains for an effective quarantine was suggested in 2000 by Lee and Yang to be a necessity; the same is valid for Mali that needs to strengthen Their quarantine program, by developing a rapid diagnostic test adapted to the bacterial strains present in Mali.

Hypotheses

The hypotheses being tested in this research activity are:

- The bacteria present in Mali are Race1, biovar 1 or 3 or some other Race/biovar.
- With a good management plan there is a possibility to produce high quality seed potato in Mali with the presence of *R. solanacearum*.

MATERIALS AND METHODS

Laboratory Research at Sotuba and Montana State University (2005-2007)

Detection Methods for *R. solanacearum*

Samples of irrigation water and fields soils were collected from different areas of potato production and tested with Agdia ELISA test to check the presence of bacteria; also soils and irrigation water were collected from areas where potato is not produced to determine the presence or not of the bacteria. Symptomatic plants samples were tested with the Immunostrip ready-to-use test and water and soil samples were tested with ELISA in the laboratory.

Four different techniques for detection of *R. solanacearum* such as the bacterial streaming test, selective media, the Agdia Immunostrip (Agdia, Elkhart, IN) and ELISA test (AgDia, Elkhart, IN) were used to confirm the presence of *R. solanacearum*

In the bacteria streaming test, segments of infected stems freshly collected from field were suspended for several minutes in a glass of clear water. White bacterial streaming from the vascular tissues at the cut end into the water was considered diagnostic as indicated by Elphinstone (2000, 2004).

The Tetrazolium Chloride (TZC) selective medium (Kelman, 1954) was used for culturing. Bacterial suspensions from bacterial streaming of suspect infected stem tissue were put in sterile phosphate buffer and streaked on the TZC media using a loop under sterile conditions. To obtain a pure culture of *R. solanacearum* a single colony with morphological characteristics of *R. solanacearum* (Rudolph et al., 1990) was transferred

to TZC media under sterile conditions to produce enough bacteria for inoculation and storage.

The AgDia immunostrip test and the ELISA (Enzyme Linked Immunosorbant Assay) test are serological techniques used in identification of *R. solanacearum* in suspected samples. The two tests can provide a rapid and effective diagnostic (Danks and Barker, 2000). While the immunostrip test is ready to use in field, the ELISA 96-well plate formats need enrichment before use and a laboratory condition after use, the ELISA is less expensive (Opina and Miller, 2005). Using monoclonal antibodies which are relatively specific in their reactivity to *R. solanacearum*, will improve the technique, but this technique is unable to differentiate races, biovars or virulence from avirulent (Rajeshwari et al., 1998)

Three of the methods described above, the bacterial streaming test, the selective media and the Agdia Immunostrip serological testing (Agdia, Inc., Elkhart, IN) were tested and compared for their efficiency to detect the presence of the bacteria in symptomatic plants.

Identification of Weeds and Crops Sources of Inocula

Symptomatic solanaceous crops and asymptomatic local weeds were collected around potato fields and potato irrigation creek borders in Sikasso, Kati, Koulikoro and Baguineda. In total 13 samples including 11 weeds, one pepper and one eggplant were tested in field just after collection with Agdia Immunostrip test.

Biovar Tests

In the biovar test, biochemical technique described by Hayward in 1964, were used to determine the biovar of the bacteria present in samples from Sotuba, Koulikoro,

and Sikasso. The ability of the bacterium to use hexoses alcohols such as dulcitol, manitol, sorbitol and disaccharides like maltose lactose and cellobiose was tested. Positive reactions were noted by the color changing to yellow because of the oxidation of the carbon source by the bacteria. The same media with distilled water to inoculate were used as negative controls. Each of the five biovars of *R. solanacearum* can be identified through the utilization of hexose alcohol and disaccharides. Pure Ayer Basal media also was used as the no-carbon-added control that should not show any color change (Denny and Hayward, 2001).

Experiments in Pots: Plant Inoculation in Sotuba and MSU (2005-2006; 2006-2007)

Hypersensitivity Test

Tobacco, tomato and peanut plants were grown in plastic pots of 30cm in diameter and 25cm in height in Mali, while at MSU the pots used for tobacco hypersensitivity were plastic and 17.5 cm in diameter. Those plants were used for the hypersensitivity test and pathogenicity test to confirm the biovar, the Race or the pathogenicity of *R. solanacearum* strains collected in Mali.

Tobacco hypersensitivity (Klement, 1963) was tested in greenhouse at MSU and at Sotuba Station in Mali. A bacterial suspension from a 48hr old TZC culture of different isolates, was used to infiltrate nicotiana tobacco cv xanthi leaves into the leaf lamella with a syringe (Klement et al., 1990) with hypodermic needle of BD 22G 1 and ½ from Becton Dickinson and Company Franklin Lakes N.J. to identify the Race of bacteria present in the sample from Mali. At Sotuba stations in Mali bacterial suspensions

isolated from different host crops were infiltrated as described previously. For the hypersensitivity test in 2005-2006, the bacteria concentration was not calculated.

In 2007, four different strains from frozen samples sent from Mali, which had shown positive reaction in immunostrip tests, were tested using the tobacco hypersensitivity test. The samples were grown on TZC selective media for 48 hr. The bacteria suspension was injected as described above. Sterile distilled water was used as control.

The four strains were:

Frozen 1(sample A): Isolated from tomato plant in Baguineda camp with a concentration of 1.6×10^8 /ml;

Frozen 2 (sample D): Isolated from tomato of Souban village in Koulikoro with a concentration of 2.5×10^8 /ml;

Frozen 3 (sample B): Isolated from potato at Sikasso 2006 with a concentration of 4×10^8 /ml;

Frozen 4 (sample C): Isolated from potato grown at Sotuba with a concentration of 1.5×10^8 /ml.

Pathogenicity Test

Pathogenicity tests were used as a confirmative test of a first diagnosis of *R. solanacearum* done at Sotuba in 2005-2006 and 2006-2007, and for the test of the virulence of the bacteria identified as *R. solanacearum*. Inoculations were done using tomato, tobacco and peanut (Galal et al., 2003). Stem inoculation through hypodermic injection with syringe (BD 22G 1 and ½ from Becton Dickinson and Company Franklin Lakes N.J.) was used to compare the pathogenicity of frozen and non-frozen bacteria at

Sotuba Station (2005-2006; 2006-2007). Tobacco, tomato and peanut planted in pots of 30cm of diameter and 25cm of high were inoculated and evaluated. Pure colonies of bacteria were grown overnight on TZC selective media; for the second night of growth the non-frozen bacteria were left in laboratory condition while the frozen were frozen at the temperature of -4 to 0° Celsius for 12 hour. Suspensions of the frozen and non-frozen bacteria colonies were prepared with distilled water. Five plants of each crop: tomato, tobacco and peanut were inoculated through stem injection with frozen and non-frozen bacterial suspension. The same experiment was repeated in the season 2006-2007 with only 3 repetitions at Sotuba Station. A randomized complete block design was used in the experiment and the evaluations were done on the number of transplanted plants, the number of established plants, the number of death plants and the number of plants wilted by bacteria at 40 days when all tomato and tobacco plants have already died and no more deaths were reported on peanut from that date.

Evaluation of the Level of Soil Infestation by *R. solanacearum*

To determine the level of soil infestation, infested soils were used in host range tests; five soils samples were collected from infested fields from Sikasso, Kati, Koulikoro and Baguineda production areas of potato and tomato in Mali. Three to five villages in each area were sampled because of the intensive production of solanaceous plants. A minimum of four farmers fields were sampled and mixed together for each village. Soils were collected from infested fields using a diagonal cross pattern to a depth of 15-20 cm.

Host assays were done by planting one-month-old tobacco from farmer local variety (*Nicotiniana tobacco*) plants into the different soil samples contained on pots (30cm diameter and 25cm height) and maintained in an outdoor area at Sotuba. Tobacco

was chosen as an indicator plant for Race 1 bacterial wilt identification (Janse, 1991). Twelve pots per village were filled with the mixed soil sample except Sikasso where 24 pots were used. The planted pots were watered and fertilized as necessary for 90 days in field condition and the number of wilted plant testing positive in the bacterial streaming test was recorded. Straw mulch was used between pots to reduce the potential of splash spread from soil. The experimental design was the randomized complete block design with 3 replication of 4 plants per village for the Baguineda, Kati, and Koulikoro samples and 6 replications of 4 plants for the Sikasso samples.

Field Research at Station and
Farmers Fields in Mali 2005-2006 and 2006-2007

Assessing of Resistance of Different European
Origin Potato Cultivars to *R. solanacearum* in Mali (2005-2006)

Six popular European origin potato cultivars were assessed for susceptibility to bacterial wilt at Sikasso, Sotuba, Baguineda and Kati (2005-2006). Cultivars used were Claustar, Appoline, Mondial, Daifla, Liseta and Spunta. These varieties are widely used by farmers in Mali. In these experiments plant establishment, disease incidence, tuber infection by *R. solanacearum* and yield was compared. The experimental sites were Sikasso, Baguinta, Sotuba and Kati, and were naturally infested with *R. solanacearum*. Plots used a 30 cm row spacing and 30 cm plant spacing with the planted area being 1.5m². The European big tubers were sectioned into different pieces each one having a sprout. In the same hole a total of two pieces were planted and twenty pieces were planted per plot. All plots were treated with the nematicide Diafuran (carbofuran,

Mitsubishi Chemical Corporation) at a dose of 6.0g/1.5m². The experimental design used was the randomized complete block design with four replications.

Assessing of Different Soil Treatments
on Disease Incidence caused by *R. solanacearum* in Mali (2006-2007)

Five different soil treatments were evaluated to compare their effect on the incidence of the disease in Sikasso, Sotuba, Baguineda and Kati. The experiment had 4 repetitions in a randomized block design. The potato cultivar used was the Star Burn planted in rows spaced 30 cm between rows and 30 cm between plants in the same row; two pieces of seed potato are planted in each hole. The experimental unit was 2m². In Sikasso and Baguineda five treatments were used. As a control the compost from cows manure and dried weeds decomposed at Sotuba experimental station during four months, was used at the doses of 2kg/m². The use of compost in that area is common farmer practice when they grow potato and tomato. The other 4 treatments were the nematicide Diafuran (carbofuran, Mitsubishi Chemical Corporation) at the dose of 5.0gr/m²; the nematicide Oncol (benfuracarb, Otsuka Chemical Co Ltd) at 3.0gr/m², Compost plus neem (0.5kg/m² of neem (powder from ground seed) plus 2.0kg/m² of compost), and neem alone (powder from ground seed) at the dose of 0.5kg/m². Experiments at Sikasso and Baguineda used the five treatments described, while at Sotuba station and at the Kati farmer field the neem plus compost was not used. Evaluations were the number of plants established, the number of wilted plants, the number of wilted by *R. solanacearum*, the number of tubers produced, the number of tubers infected by the bacteria and the yield.

Statistical Analyses

The paired t-test was used to compare the difference in wilting between plants inoculated with frozen bacteria and plants inoculated with non-frozen bacteria using EXCEL (Microsoft, 2003).

Two field experiments were conducted using totally randomized complete bloc design in 2006 and 2007 on varieties and soil treatment. Data in both experiments were analyzed in a nested design with sites as the main factor and soil treatment or varieties as subfactors. Four replications were used in both experiments. Statistical analysis was performed using the Statistical Analysis System (SAS), version 9.0. Varieties and soil treatment are considered as fixed factors while replications and sites were considered as random factors. The general linear model procedure was used to test for significance at the 5% level for varieties and soil treatments as well as their respective interaction with sites.

Analysis of variance was followed by means separation using Fishers protected least significant difference mainly $\alpha=0.05$ and sometime $\alpha=0.01$. The coefficient of variance was also recorded.

RESULTS

Laboratory Research at Sotuba and MSU (2005-2007)

From 2006 to 2007 all the lab experiments were repeated; from bacterial streaming test, to selective media and Agdia Immunostrip test; In 2007, the number of samples were increased in the studies of bacteria free areas with Agdia ELISA test for a future production of seed potato. Also we added the biochemical test for biovar identification to the laboratory experiments and experiments done in MSU and in Mali at Sotuba Plant Pathology laboratory.

Once again all the symptomatic samples collected from field were positive to the bacterial streaming test except the tobacco plant collected at Sonytieni; the same test was used in all the field experiments to confirm the presence of *R. solanacearum* on any diseased plant suspected to have *R. solanacearum*.

Detection Methods of *R. solanacearum*

Detection of *R. solanacearum* in Soil and Water Samples Soil and water samples were collected from main production areas such as Sikasso, Baguineda, Kati and Sotuba, suspected to be infested and from new prospective areas for disease free seed production. Samples were analyzed for the presence of *R. solanacearum* using the Agdia enrichment and ELISA procedure. Results are presented in Table 6.

From 18 samples collected, 11 samples corresponding to 61% were positive to *R. solanacearum* using ELISA test; more than 50% of positive results were coming from potato production areas; However, the bacteria has been detected in several areas which

do not produce potato. So it is very important to always tests soils and water when we plan to produce seed potato.

Sotuba soil is known to be heavily infested by *R. solanacearum* for being used for several years to produce tomato. Also the main source of irrigation in most of the irrigated production areas is the River Niger which has been found positive.

Some of the new places such as Ibissa chosen for not growing potato crops or not using the irrigation water from the Niger River are surprisingly positive. However it is known that Borko and Ibissa in the past time (ten years ago) did grow tobacco.

Table 6: Result of the Detection of *Ralstonia solanacearum* (Smith) Yabuuchi et al., in Soil and Irrigation Water Samples Collected from Infested Soils and Irrigation Water from Malian Production Areas of Sikasso, Koulikoro, Kati, Baguineda and Benfabougou and Non-Production Areas of Borkho and Ibissa using Agdia Enrichment and ELISA Procedure from 2006 to 2007 at Sotuba.

Detection of <i>R. solanacearum</i> in soil and water samples through Agdia ELISA Test in Mali	
Positive reaction to <i>R. solanacearum</i>	Negative reaction to <i>R. solanacearum</i>
Soil/Sotuba	Soil/Samogossoni/Sikasso
Water from canal/Baguineda	Soil/Farako/Sikasso
Water from well/Samogossoni/Sikasso	Soil1/Borkho (Mopti)
Water from creek/Farako/Sikasso	Soil2/Borkho (Mopti)
Soil1/Ibissa (Mopti)	Water/ Sonitieni/Kati
Soil2/Ibissa (Mopti)	Water/Diago/Kati
Soil/Koulikoro	Water/Ibissa/Mopti
Water/canal/Benfabougou/Mopti	Distilled water as negative control
Water/source/Benfabougou/Mopti	
Water/Niger River/Koulikoro	
Soil/ Sotuba as positive control	

The positive reaction to ELISA test shows the presence of the bacteria and the negative reaction to ELISA test means there is no bacteria present in the sample.

Comparison of Different Techniques of Identification In Table 7 are the results of comparison of bacterial streaming test, Agdia ImmunoStrip test and selective TZC media test on tomato and potato samples that showed symptoms of bacterial wilt. These data

demonstrated that there was 95% agreement between the streaming test and the Agdia immunostrips test. There was only 68% agreement between the selective media TZC and the bacteria streaming method. The bacterial streaming test is ideal to teach to subsistence farmers of Mali as a diagnostic key to confirm the presence of *R. solanacearum* in their fields and it did show the best performance in disease identification. In total, 25 samples were tested through the three identification techniques; 92% of total samples and 96% of symptomatic plants were positive to bacterial streaming, 88% of total samples and 90% of symptomatic plants were positive to immunostrip test and 68% of total samples and 70% of symptomatic plants were positive to tetrazolium chloride selective media test. Two samples were negative to bacterial streaming and to the rest of the test; one of them was a tobacco plant presenting a typical wilting symptom of bacterial wilt and the second was also tobacco plant, collected just five days after inoculation without any symptom of wilt. There was just one sample of pepper collected from Souban that reacted positive to bacterial streaming test and negative to the immunostrip test and the selective media test.

The bacterial streaming test worked well when disease symptoms were evident but is not useful before symptom development.

The Agdia Immunostrip test is a quick test for identification in field but the test can inform only on the presence of the bacteria but not the Race or biovar. The Agdia Immunostrip test was used in Mali at Sotuba and in USA, at MSU to detect the bacteria from the five frozen samples and the two inoculated tobacco plants sent from Mali; The TZC media is a selective media for *R. solanacearum* and can be used to detect pathogenic bacteria colonies through the presence of characteristic fluidal whitish colonies with pink

centers which can be enumerated as pathogenic. The colonies can then be used as pure colonies in carbon utilization tests to identify biovar.

The growth of the bacteria on TZC was similar for all the strains tested showing the whitish fluidal form with a pink center except potato from Sikasso that showed the dark fuchsia color of the colonies. The selective media test, however, did show the lowest performance in detecting the bacterium in symptomatic plants; we still think that the reason is due to the low concentration of the bacterium in the suspension used for the selective media test.

At Sotuba in 2006, all symptomatic diseased plants showed the positive reaction at least to one of the detection method tested, except tobacco. At MSU in 2007, from the seven samples tested five were positive to the immunostrip test. frozen bacteria isolated from pepper sample collected in Souban and tobacco plants inoculated just five days before sampling with no symptoms were negative to the immunostrip test. From the two samples, only the pepper from Souban was positive to bacterial streaming test in Mali.

After being positive to Immunostrip test, cultures of all isolates were done on Tetrazolium Chloride media at MSU. All did grow except frozen bacteria isolated from pepper from Souban and Tobacco plant inoculated five days before sampling.

Cultures A (frozen bacteria isolated from tomato from Baguineda Camp), C (frozen bacteria isolated from potato from Sotuba) and D (frozen bacteria isolated from tomato from Souban) were much similar in producing whitish colonies with pink center while B (frozen bacteria isolated from potato from Sikasso) was dark pink colored and the culture from tobacco plant E (inoculated one month before sampling) was more whitish with very little pink coloration in the center.

Table 7: Result of the Comparison of Bacterial Streaming Test with the Immunostrips Test (Agdia, Elkhart, ID) and the Tetrazolium Chloride Selective Media with Tomato, Tobacco and Potato Samples Showing Symptoms of Bacterial Wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi et al., and Collected from Sikasso, Baguineda, Koulikoro, Kati Production Areas and Sotuba Station in Mali from 2005 to 2006 at Sotuba and MSU.

Collection site of Samples	Crops/cultivar	Bacterial Streaming	Agdia Immunostrip	Selective Media
Baguineda	Tomato	+	+	--
Sonityeni	Potato/ Claustar	+	+	+
Sonityeni	Tobacco	-	-	--
Sotuba	Potato, cv unk	+	+	+
Kogniba	Tomato	+	+	--
Sikasso I	Tomato	+	+	+
Sikasso II	Tomato	+	+	--
Farako	Potato Claustar	+	+	+
Farako	Potato Appoline	+	+	+
Sikasso 1	Potato Mondial	+	+	+
Sikasso2	Potato Mondial	+	+	--
Sikasso3	Potato .Mondial	+	+	+
Koulikoro Sb	Tomato	+	+	+
Sotuba Station	Potato	+	+	+
Baguineda Cp	Potato	+	+	+
Baguineda Knb	Tomato	+	+	+
Baguineda	Potato	+	+	-
Baguineda	Tomato	+	+	+
Baguineda Cp	Frozen isolate from tomato	+	+	+
Souban	Frozen isolate from tomato	+	+	+
Sikasso	Frozen isolate from potato	+	+	+
Sotuba	Frozen isolate from potato	+	+	+
Souban	Frozen isolate from pepper	+	-	-
Sotuba	Tobacco 30 d inoculation	+	+	+
Sotuba	Tobacco 5 d inoculation	-	-	-

Unk is unknown; + is positive reaction to the test; -is negative reaction to the test; d is days; Cp is camp; Knb is Kogniba and Sb is Souban; cv is cultivar

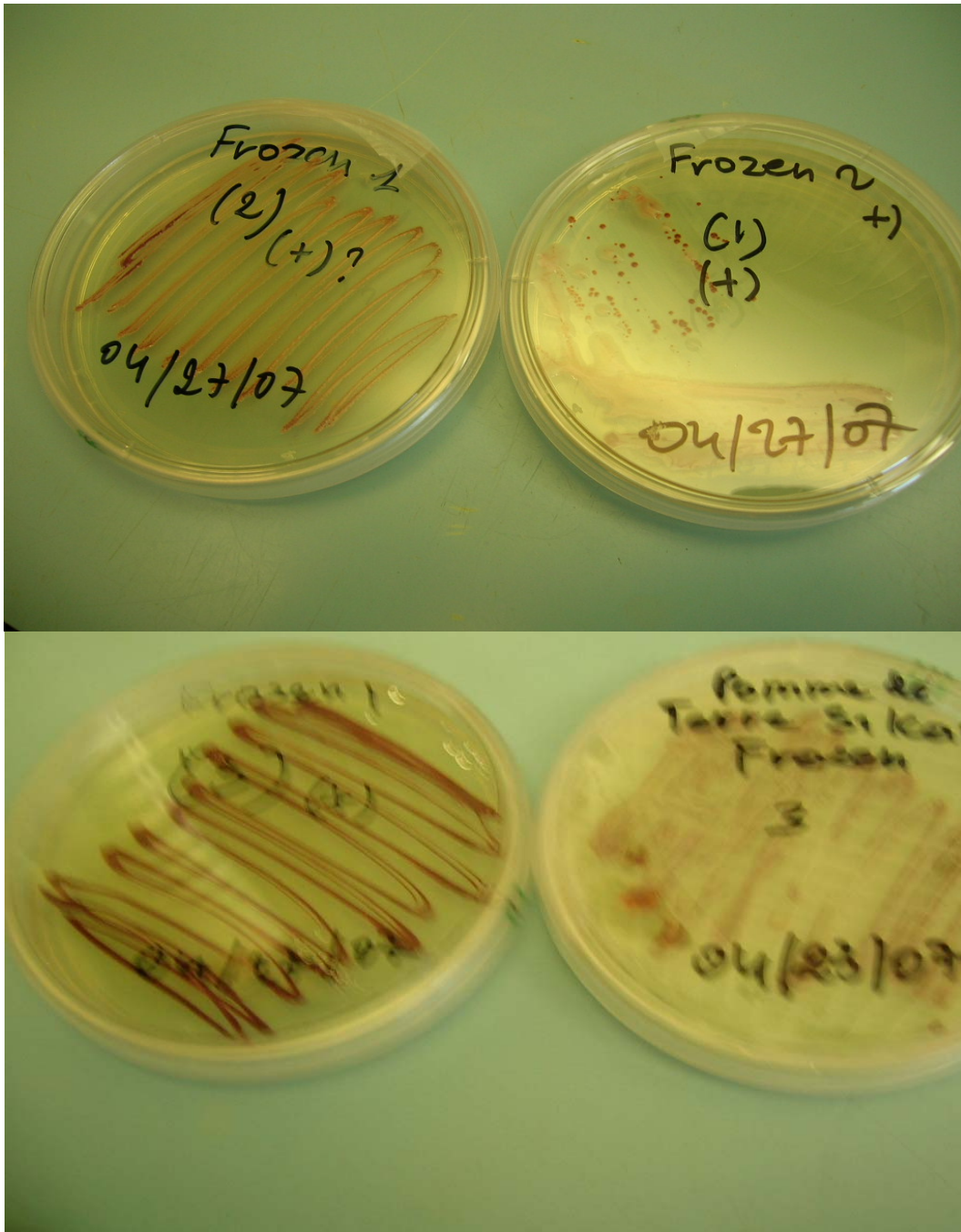


Figure 1: Comparing the Difference of *Ralstonia solanacearum* (Smith) Yabuuchi et al., Colony Coloration on the Selective Media TZC of Isolates from Potato Collected at Sikasso Mali (frozen 3+) Presenting a Dark Fuchsia Color and from Tomato Collected at Souban Mali [frozen 1 (2+)] and Isolate from Potato Collected at Sotuba [frozen 2 (1+)] Presenting Both a Whitish Coloration with a Pink Center.

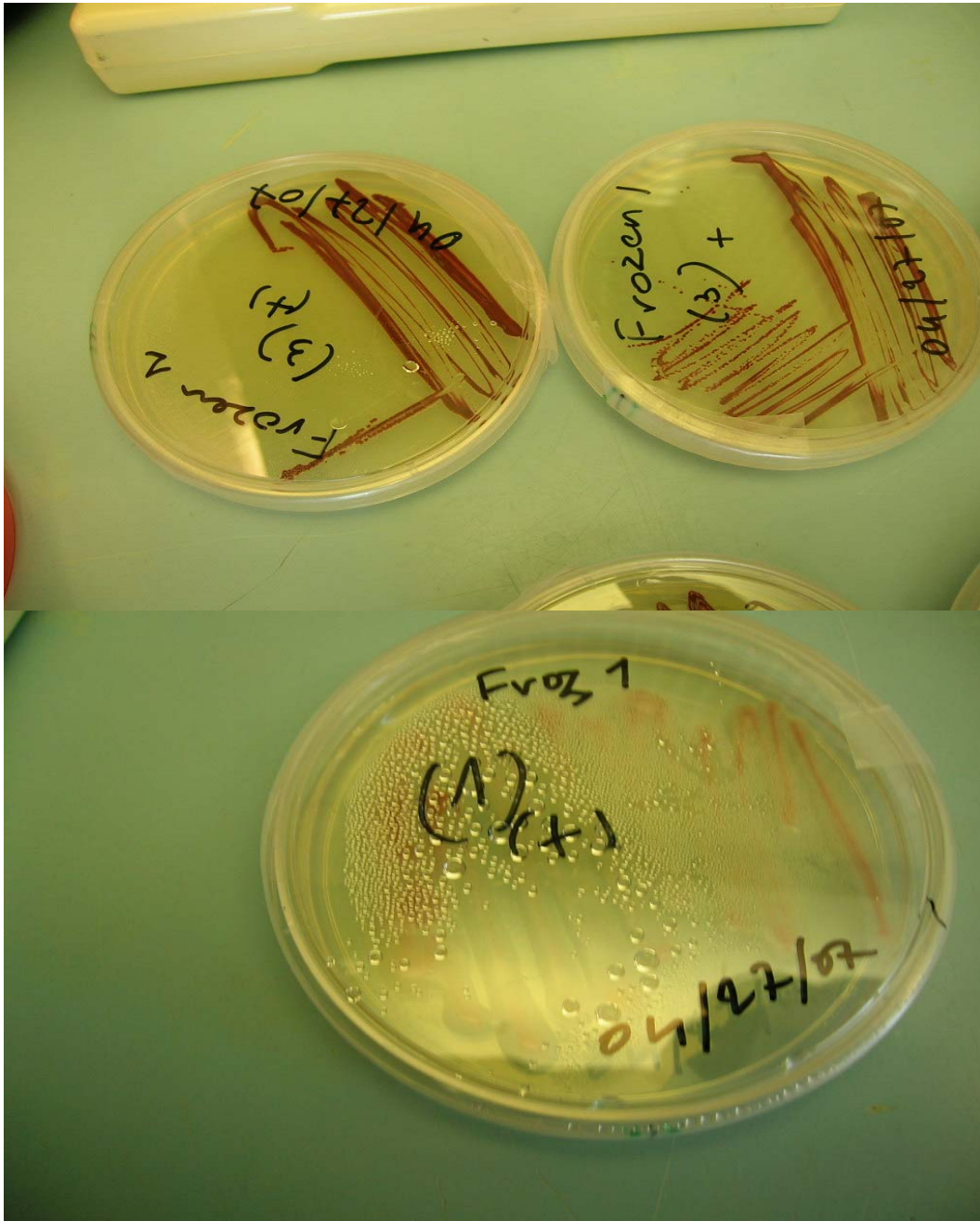


Figure 2: Comparison of the Difference of *Ralstonia solanacearum* (Smith) Yabuuchi et al., Colony Coloration on the Selective Media TZC of Isolate from Potato Collected at Sikasso Mali [frozen 1(3+)] Presenting a Dark Fushia Color and Isolate from Tomato Collected at Baguineda Mali [frozen 1 (1+)] Presenting a Whitish Coloration with a Pink Center.

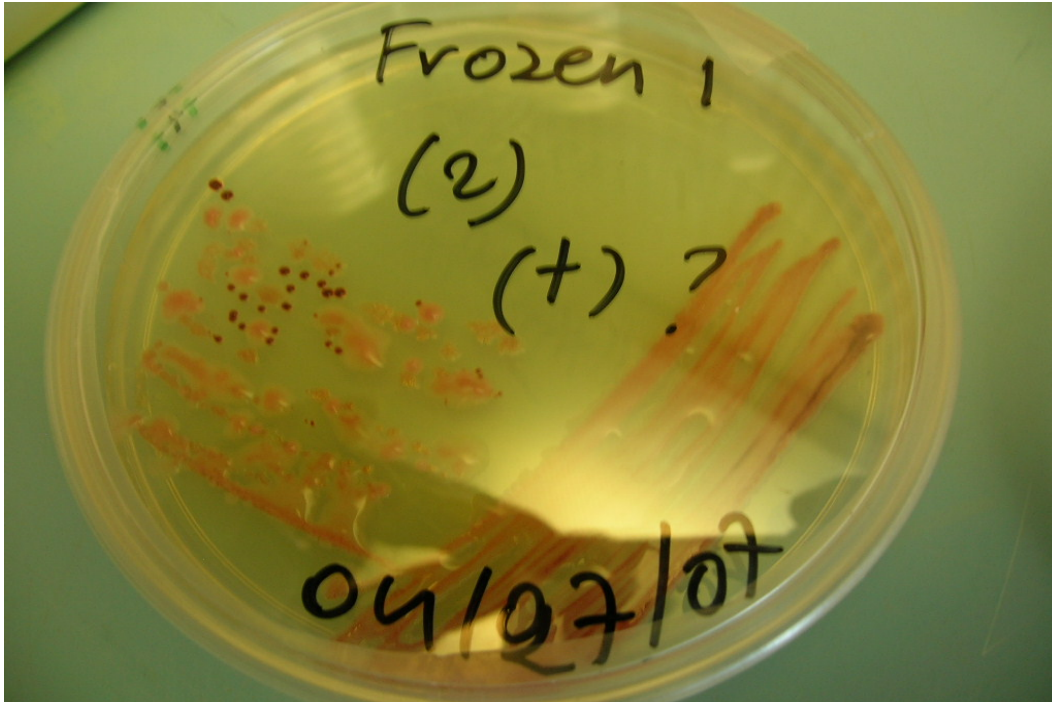


Figure 3: Technique of Single Colony Isolation to Obtain Pure Culture of Bacteria *Ralstonia solanacearum* (Smith) Yabuuchi et al., on the Selective Media TZC

Identification of Weeds and Crops Sources of Inocula

Between the thirteen samples collected at Sikasso, Kati, Koulikoro and Baguineda; eleven local weeds were collected in the river border and two solanaceous plants were collected near potato fields. From the asymptomatic weeds collected from Sikasso, Kati, Baguineda et Sotuba, only one sample was tested positive: *Commelina forskalaei* (Vahl), collected on the humid border of the creek Farako at Sikasso without any wilting symptom. The two solanaceous plants, eggplant and green pepper showing wilting symptom, collected respectively, in Sikasso and Bamako were tested positive to *R. solanacearum*.

Biovar Tests

The biochemical test for biovar identification is based on the ability of the bacteria to oxidize the carbon source added to Ayers basal media; each biovar has its specific carbon source to oxidize; in our case the biovar test in both Mali and Montana showed approximately the same result. All disaccharide and all hexose alcohols were oxidized and did change color from green to yellow; the strain collected in Sikasso on potato took more time to induce color changing on maltose and dulcitolose while at MSU the color change was slow with the 3-hexose alcohols.

In the two situations at Bamako or at MSU, the oxidation of the three disaccharides and three hexose alcohols was achieved in the normal time except the isolate from potato collected in Sikasso that spent two more weeks to complete its color changing.

Inoculation of 3 disaccharides (maltose, cellobiose and lactose) and 3 hexose alcohols did give color changing before 7 days of isolates A, C and D; while isolate B (frozen bacteria of potato from Sikasso) was very slow in changing color and after 21 days still not totally change color with the 3 hexose alcohols. Complete color changing was obtained after 5 weeks. That is also the only strain that was kept on TZC media in refrigerator for one year; the color on TZC dark pink, was different from all the strains; the bacteria certainly did mutate; however the bacteria showed a brown necrosis in hypersensitivity test. These results means that the hypersensitivity test is not 100% reliable or the bacterium was having some kind of mutation.

Table 8: Result of Comparing the Reaction of Different Isolates of *Ralstonia solanacearum* (Smith) Yabuuchi et al., from Mali in their Capacity to Produce Acid with Three Disaccharides and Three Hexose Alcohols for Biovar Identification in 2007 Season at Sotuba and at Montana State University.

Biochemical Test	Samples Origins									
	Tomato/Souban		Potato/Sotuba		Tomato/Ba+		Potato Sikasso		Tomato/Bag++	Pepper/K
	Mal	MSU	Mali	MSU	Mali	MSU	Mali	MSU	Mali	Mali
Disaccharides										
Maltose	+	+	+	+	+	+	?	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	+
Hexose Alcohols										
Dulcitol	+	+	+	+	+	+	?	?	+	+
Sorbitol	+	+	+	+	+	+	+	?	+	+
Manitol	+	+	+	+	+	+	+	?	+	+

The sign + is the oxidation reaction of disaccharides and hexose alcohol with color changing into yellow. The sign? means that the oxidation took 2-3 weeks more to have a total color changing. Bag+ is Baguineda camp and Baguineda++ is Baguineda Kogniba. Koul is Koulikoro.

Experiments in Pots: Plant Inoculation Through Infested Soils at Sotuba and Montana State University (2005-2007)

Tobacco Hypersensitivity Test

Very important in the identification of the Race of the bacteria *R. solanacearum*, the tobacco hypersensitivity test was negative: no white necrosis with any of the four strains tested. Symptoms of brown necrosis with yellow halo were observed on all the leaves inoculated with bacteria while no change was noticed on leaves inoculated with distilled water; and that is according to the confirmation of the presence of Race 1. All the four strains tested through hypersensitivity test were negative to the test and did show

the brown necrosis that is positive for Race 1. Necrosis appeared five-six days after leaf inoculation and only Race 1 will affect tobacco.

Table 9: Comparing Hypersensitivity Reactions of Indicator Tobacco Plant through Leaf Infiltration with *Ralstonia solanacearum* (Smith) Yabuuchi et al., Cell Suspension from 48 old TZC Cultures of Four Different Strains Sent from Mali to Montana State University in 2007.

Origin of isolates	White necrosis or simple chlorosis: hypersensitivity reaction: Race 3	Brown necrosis: pathogenic on tobacco: Race1	No necrosis, no chlorosis: non pathogenic on tobacco plant
Frozen bacteria isolated from Baguineda Camp tomato	-	+	-
Frozen bacteria isolated from Souban tomato	-	+	-
Frozen bacteria isolated from potato of Sikasso	-	+	-
Frozen bacteria isolated from potato from Sotuba	-	+	-
Distilled water	-	-	+



Figure 4: The negative Response of Tobacco to Hypersensitivity Test through Leaf Inoculation is shown in the Figure above. Brown Necrosis Surrounded by Yellow Halo 5-6 days after Inoculation Confirming the Presence of Race 1 after Infiltration of Bacterial Suspension of *Ralstonia. solanacearum* into Leaf Mesophyle with Hypodermic Syringe at MSU.

Pathogenicity Test

Pathogenicity tests through stem inoculation of peanut, tomato and tobacco was done during two years; the results obtained are in the following tables.

No difference has been seen between non-frozen and frozen in tobacco and tomato inoculation; there is no difference either between tobacco infection and tomato infection. In contrast there is a significant difference between peanut inoculation with frozen and non frozen bacteria and also difference between peanut infection and both tomato and tobacco infection. Non-frozen seems to be a little more virulent than frozen one and pathogenicity seems to be especially related to the species of the crop.

Tobacco and tomato seems to be more susceptible than peanut to our Malian strain.

Table 10: Comparison of the Pathogenicity of Frozen and non-Frozen *R. solanacearum* Isolates on Tomato, Tobacco and Peanut at Sotuba Station in 2005-2006 40 Days Post Inoculation.

Crops Inoculated	Inoculum Conditions	Percentage of Infection
Peanut	Frozen	13.33c
	Non-Frozen	43.33b
Tomato	Frozen	100a
	Non-Frozen	100a
Tobacco	Frozen	100a
	Non-Frozen	100a

Alpha=0.05; means with the same number do not have significant difference; bacteria was frozen for one night in the laboratory; infection was determined through bacterial streaming test on symptomatic plants.

Evaluation of Soil Infestation Level

In Table 11 we have the percentage of wilted plant by village and by area of production; Soils from Sikasso area followed by Baguineda and Kati did produce respectively the higher percentage of bacterial wilt. Actually Baguineda soils in some

specific area such as Sebela appear to be the less contaminated. It is important to know that in Sikasso, the soil was collected from these villages that produce potato in the hillside in rainy season and the production from that season is used as seed in the plains in the fresh season production. That condition favors the disease spread and could also explain why Sikasso has the higher percentage of tobacco wilting caused by *R.*

solanacearum. In Table 11 all the villages that presented a higher percentage of wilt are tomato (Massala, Baguineda, Dogura, Kokun) or potato producers (Kati Mission, Zangaradougou, Bambadougou and Niagansoba) since a long time and it is obvious that they now have their soils infested. To avoid that situation, crops rotation should be introduced in potato production system and help to reduce the inoculum level in the soil.

Table 11: Comparison of the Percentage of *Ralstonia solanacearum* (Smith) Yabuuchi et al., Infected Tobacco Planted in Soils Collected from Different Villages of Malian Potato Production Areas in 2005-2006 at Sotuba (Mali).

Soil sample from	Percentage of wilted tobacco plants/ Villages providing Infested soil samples					Average percentage of wilted tobacco plants in%
Baguineda	Baguineda	Kokun	Dugura	Kogniba	Sebela	
	50	50	50	25	0	35
Kati	Kati Mission	Diago	Garo	Sonitieni	Massaman bougou	
	50	25	33.33	33.33	25	33
Koulikoro	Tolomadio	Massala	Fougadougou	Manabougou	Kayo	
	16.67	60.00	8.33	33.33	8.33	25
Sikasso	Zangaradougou	Bambadougou	Niaganssoba	W/i	W/i	
	100	100	95.45			98

W/i means without information

The percentage of wilted plants among numbers of transplanted plants is evaluated first per village and then per production area. Sikasso is the biggest area of potato production and also the most infested soil. The higher soil contamination with

98% of wilted plants is observed in the soil collected from the hillside fields used in rainy season to grow potato.

In Kati the higher percentage of wilt is at Kati mission where they grow the most important part of potato with tomato; gardens are small so farmers barely rotate and the water used for irrigation is surface water. The water from Diago and Sonitieni have been tested negative to *R. solanacearum* through ELISA test; that confirms the main inoculum sources in these two villages are soils, seeds or other crops host such as tobacco largely produced in that area.

Since bacterial wilt has been very limiting in tomato production also the high level of wilt is related to the importance of tomato production in the village of Massala.

Field Research in Stations and Farmer's Field in Mali (2005-2007)

Assessing Resistance of Six Varieties to *R. solanacearum* in Mali (2005-2006)

Six popular varieties imported from Europe were tested for their resistance to bacterial wilt caused by *R. solanacearum* in fields known to be infested by the bacterium. Experiments were conducted in station at Baguineda and Sotuba and farmers field at Kati. Results are shown in the following table. The varieties most tolerant to bacterial wilt seem to be Appoline, Claustar and Spunta. The lowest incidence of bacterial wilt in these varieties is shown at Sotuba.

Table 12: Comparison of Bacterial Wilt Incidence in Six Imported Potato Varieties Used by Farmers in *Ralstonia solanacearum* (Smith) Yabuuchi et al., Infested Soils of Kati and Sotuba in 2005-2006 at Mali.

VARIETIES	SOTUBA	KATI	BAGUINEDA
Mondial	5.50 A	2.50	7.00 A B
Liseta	1.50 B	2.25	5.25 B C
Daifla	0.75 B	1.75	10.00 A
Claustar	0.50 B	1.50	3.00 C
Spunta	0.25 B	0.75	3.25 B C
Appoline	0.00 B	1.50	6.25 B C
Mean	1.42	1.66	5.79
CV %	96.44	59.32	40.40
Signification 5%	**	n.s	**
Sites x Varieties	**		

CV is the coefficient of variation; ** is highly significant; * is significant; ns is non-significant. At alpha=0.05, there is no significant difference between varieties in disease incidence. Disease incidence was determined through visual symptoms and bacterial streaming test of symptomatic plants.

Means with the same letter are not significantly different. Means with different letters are significantly different.

At Sotuba a significant difference appeared between Mondial and the other varieties in the incidence of the bacteria. Mondial was the most susceptible while there was no statistical difference between Liseta, Daifla, Claustar, Spunta and Appoline. Liseta was the second most important susceptible after Mondial.

At Kati there was no significant difference between the varieties but Mondial still continued to show the highest number of wilted plants followed by Liseta.

In Baguineda, there was a significant difference between the varieties; the higher incidence is seen with Daifla followed by Mondial followed by the rest of the varieties. Significant difference was seen between Liseta, Claustar, Spunta and Appoline. Also it

appears that there is an interaction between the sites and varieties. The results obtained from different varieties were significantly different between experimental sites.

Assessment of Different Soil Treatments on Disease Incidence Caused by *R. solanacearum* in Mali (2006-2007)

Tables 13 and 14 present results of the effect of different soil treatment techniques to reduce the incidence of *R. solanacearum*. Three biological techniques are compared to nematicides since *Meloidogyne* sp. was found in some of the experimental fields after growing tomato several years. To maintain the sustainability and durability of these techniques in farming system, they were chosen to be simple and easily adopted by farmers. Kati and Sotuba that have the same treatment are analysed together while Baguineda and Sikasso also sharing the treatment are analysed together,

Table 13: Comparison of the Effect of Compost, Neem Kernel Flour and the Nematicides Dafuran and Oncol as Soil Treatments on the Incidence (number of wilted plants) of Bacterial Wilt in Potato Fields at Kati and Sotuba.

VARIETIES	SOTUBA	KATI	MEAN
Compost	1.00	0.50	0.75
Dafuran	0.00	0.50	0.25
Neem	0.50	0.75	0.62
Oncol	0.00	1.50	0.75
Control	1.25	1.25	1.25
Mean	0.55	0.90	0.725
CV %	172.48	117.85	138.79
Signification 5%	n.s	n.s	ns
Sites x Soil treat.	n.s		

CV is the coefficient of variation; ns is non-significant. At $\alpha=0.05$, there was no significant difference between varieties in disease incidence. Disease incidence was determined through visual symptoms and bacterial streaming test of symptomatic plants. Means with the same letter are not significantly different.

In the combined analysis of Kati and Sotuba, no significant difference was seen in bacterial incidence between the different soil treatments at Sotuba and Kati, neither a significant difference between the treatments nor between sites hosting the experiment.

Table 14: Comparison of the Effect of Compost, Neem Kernel Flour, Neem Kernel Flour Plus Compost and the Nematicides Dafuran and Oncol as Soil Treatments on the Incidence (number of wilted plants) of Bacterial Wilt in Potato Fields at Baguineda and Sikasso.

Soil treatment	Baguineda	Sikasso	MEAN
Compost	3.00	1.25	2.12
Dafuran	0.75	1.75	1.25
Neem	0.25	1.75	1.00
Neem+compost	1.00	1.00	1.00
Oncol	1.25	1.25	1.25
Mean	1.25	1.35	1.30
CV %	108.56	43.29	80.37
Signification 5%	n.s.	n.s.	n.s.
Sites x Soil treat.	*		

CV is the coefficient of variation; ns is non-significant. At $\alpha=0.05$, there was no significant difference between varieties in disease incidence. Disease incidence was determined through visual symptoms and bacterial streaming test of symptomatic plants. Means with the same letter are not significantly different.

In the combined analysis of Sikasso and Baguineda, no significant difference was seen in the bacterial incidence between the different soil treatments. Here there is a significant difference between the treatments and between the sites hosting the experiment. The result of the same treatment did vary according to the experiment site.

DISCUSSION

Laboratory Research at Sotuba and MSU (2005-2007)

Detection Methods for *R. solanacearum*

Detection of *R. solanacearum* in soil and water samples The results obtained from detection of the bacterium through ELISA tests showed that the bacterium is easily found in soil and water of areas where potato or tomato are produced.(Table 6)

The ELISA tests worked well with distilled water as the negative control and the positive control as Sotuba soil known to be infested, and tested positive to ELISA test one year previous. The ELISA test can allow one to detect infection in all areas selected for seed production before starting, since some of the areas where potato or tomato are not produced actually were found positive for *R. solanacearum*. Also, the contamination of irrigation water could be explained by the fact that farmers just discard the diseased plants in wells or creeks as we observed in Sikasso. More investigation is needed on the presence of aquatic weeds being able to disseminate the disease through irrigation water, as found in United Kingdom (Elphinstone et al., 1998), in Netherlands (Janse et al., 1998), in Sweden (Olson 1976) and in Spain by Caruso et al., (2005). So weeding should be an important part of the integrated control of the disease since the bacterium is able to survive in asymptomatic weeds.and from now the detection of the disease should be one of the most important steps in any production of seed potato in Mali. Ground water, protected water should be preferred to river and any surface water. Rotation with crops that are not susceptible to the bacterium, crops such corn, cowpea, is a durable, easily

adopted management method. Soybeans and rice well-known and grown by Malian farmers will reduce *R. solanacearum* populations in soil. In Table 6 the detection of *R. solanacearum* in field soils and irrigation water samples (2005 to 2007) using Agdia enrichment and ELISA procedure was useful in demonstrating this phenomenon. The Sotuba soil was suspected to be contaminated with *R. solanacearum* because tomato crops grown there for the past three years showed bacterial wilt symptoms each year. Also the water from the Baguineda Canal being surface water from the Niger River and used for irrigation in one of the most important tomato production areas in Mali was found positive to the bacteria. In the Samogossoni water that is not surface water, the presence of *R. solanacearum* in the shallow well water was expected since farmers disposed of rotted potatoes in the well. Farmers commonly throw crop debris and rooted tubers debris in these shallow hand dug irrigation wells. In contrast no presence of the bacterium has been detected in the soil of Samogossoni that has been abandoned to potato production due to devastating losses in the previous years by bacterial wilt. It is possible that the bacterium at Samogossoni do not survive well in the soil or there is a more important way for the bacterium to spread. The presence of *R. solanacearum* in the shallow well water can confirm that idea. The water from Farako creek is also surface water that is easily contaminated since the creek crosses several villages that produce potato.

The results in Table 6 are important in our search for disease-free areas for seed production. Some results, such as the negative reaction of water in Diago and Sonityeni, prove that the inoculum source is effectively infested soils according to the results obtained below in the experiment using indicator tobacco plants transplanted into infested

soils. The positive reaction of Koulikoro soils to tests for *R. solanacearum* confirms expected results about soils infestation in the main areas of tomato production. Ibissa is one of the villages chosen for seed potato production after discussions with farmers, but the positive reaction of their soils will not allow them to be part of the seed potato production program in Mali. And finally, the positive reaction of the Niger River samples confirms the positive test in Baguineda canal last year. This is a big problem since that river is a main source of irrigation in Malian agricultural systems. Therefore, it is clear now that for seed production the irrigation water has to be protected (no surface water) or treated.

Comparison of Different Techniques of Identification In comparisons of bacterial streaming, TZC media culture and Agdia ImmunoStrip (table 7) for detection of *R. solanacearum* in suspected infected tissues of tomato tobacco and potato plant on 2005-2006, the result in the Table 7 indicated that some detection techniques worked better than others. Bacterial streaming works better (96% of symptomatic plant were positive) followed by immunoStrip test (90% were positive). Least efficient was the selective media test with 70% of symptomatic plants testing positive. These techniques may not be useable when bacterial populations are low such as with early infections or latent infection. All symptomatic plants collected and reported in Table 7 were positives to the streaming test except the tobacco.

According to Kelman (1954), the TZC media is used to distinguish *R. solanacearum* among other bacteria during isolation. Also when TZC media is used with *R. solanacearum* it shows the difference between stable avirulent colonies that look dark red from the unstable fluidal virulent that are white with pink center and finally, it is used

as an identification test because of the specific characteristics of the colonies of virulent strain (Klement, 1990). The colonies here were fluidal whitish with a pink center, proof of the presence of pathogenic bacteria as suggested by Klement (1990) except the strain collected from Potato at Sikasso which was dark pink..

Using this test after the bacterial streaming test is a good way to confirm the diagnosis, but sometimes samples that have been positive to bacterial streaming do not grow on TZC. In Table 7 Some crops have been tested positive to the bacterial streaming test, but the same did not grow on TZC. This may have been caused by the low concentration of *R. solanacearum* in the suspension we used or the poor quality of the culture.

By giving exactly the same result as the bacterial streaming test, the Agdia ImmunoStrip test confirmed the presence of the bacteria in the samples tested positive in the streaming test. This shows by the same process that the selective media TZC did not work as well as expected. In this test it seems that ImmunoStrip test has the same sensitivity as streaming test in diagnosing bacterial wilt disease.

The best way to be sure that you do have *R. solanacearum* is to use multiple types of traditional tests together or use molecular genetic tools for accurate identification.

Also, the difference in colony color compared to the other strains could be caused by a mutation or another atypical strain of the bacteria. That morphological difference could be related to some genomic difference between strains.

Identification of Weeds and Crops Sources of Inocula

The presence of an asymptomatic local weed (*Commelina forskalaei* Vahl) hosting the bacterium at Sikasso around Farako creek, is very important in the spread of

the disease in that area. More investigation is needed on checking on the presence of more aquatic weeds able to spread the disease through irrigation, as found by Carrusco et al., in 2000. As expected some solanaceous in Sikasso and Bamako such as eggplant and green pepper were found susceptible to *R. solanacearum*. There is a clear need to set up a rotation program to decrease the disease level in the production area. The presence of a local weed hosting *R. solanacearum* in Sikasso could explain why the Farako creek was tested positive to the bacterium in addition to the fact that farmers throw diseased plant in the creek, Not only susceptible solanaceous crops will spread the disease so a special attention should be focus on weeds control for an efficient management of the disease.

Biovar Test in Mali and MSU (Table 8)

The *R. solanacearum* strain isolated from potato from Sikasso was different from other Malian isolates of *R. solanacearum* in the oxidation color changing, after showing different growth on TZC.

The positive reaction of the different Malian strains of *R. solanacearum* to the three disaccharides and the three hexose alcohols allows us to report that the biovar of Race 1 found in Mali is the biovar 3 as sustained by Hayward (1964, 1994).

Cultures of the strains at MSU in 2007 on Ayers basal media without any source of carbohydrate did not induce color changing after five weeks. According to Hayward (1964), *R. solanacearum* is separated into biovars on the basis of the ability to produce acid from three hexose alcohols and three sugars. According to the CABI/EPPO report (1999), potato isolates are normally Race 1 (equivalent to biovar 1 or 4) or 3 (biovar 2); but our results did give us Race 1 biovar 3 for tomato and potato isolates. Biovar 3 is further found on potato in Asian countries.

By 3 weeks, all but one of the strains had already changed the color of the disaccharides and the alcohol hexoses. The strain isolated from frozen potato from Sikasso changed completely after 4-5 weeks. We already know that this strain in contrast to the others was kept for one year in refrigerator on TZC media. Compared to the other strains, the Sikasso strain did show morphological differences in color and form like avirulents strains as cited by Klement in 1990. From that study the Malian isolate of *R. solanacearum* found on potato and tomato has been identified as Race 1 biovar 3 for the moment. Further investigations are underway to determine the presence of other strains.

Experiments in Pots: Plant Inoculation in Sotuba 2006, 2007

Hypersensitivity Test on Tobacco With Different Malian Isolates (Table 9)

The result obtained in this experiment can confirm the statement made by Buddenhagen et al., in 1962 when they found that Race one is pathogenic on tobacco and produce wilting on tobacco; the plants of tobacco used for the experiment started to wilt one month after inoculation.

According to Janse, 1991, the results seen in Table 9 confirm the presence of Race 1 of *R. solanacearum* through the production of dark brown necrosis on tobacco leaves inoculated since Race 2 and 3 will not produce brown necrosis on tobacco. In our experiment the brown necrosis on leaves was surrounded by a yellowing halo, as cited in EPPO report (OEPP/EPPO, 2004). The necrosis appears at the 5th day and the wilting started for weeks after inoculation. Comparing to the table presented by Janse (1991), above in the literature review part on hypersensitivity test we can confirm with our result that we have for sure the Race one of the bacteria species.

In Mali, at Sotuba station hypersensitivity test on 10 plants of tobacco through inoculation by tiny syringe in the mesophyle of the leaf shows brown necrosis three days after inoculation; and wilt symptom on three plants after 2 weeks and death of the same three plants after for weeks. Once again the wilt of tobacco plant allows us to confirm that the Race of bacteria present in Mali is the Race 1, which is the only one able to wilt tobacco.

The Frozen strain tested at MSU and collected from potato at Sikasso had a positive reaction to the immunostrip test and a negative reaction to hypersensitivity test. However, coloration of the colonies on TZC was dark compared to the other strains and it did take more time to oxidize the carbohydrate sources in the biovar test. The bacteria collected from potato at Sikasso were frozen and kept for a year in refrigerator at Sotuba so we need to understand the reliability of the hypersensitivity test to identify Race.

Pathogenicity Test Inoculation of Three Crops with Frozen and non Frozen Bacteria (2005-2006)

The objective of these experiments (Table 10) was to compare the pathogen habit to induce disease in crops in normal condition and in frozen condition; Apparently there is no big difference between pathogenicity in frozen and non frozen bacteria as expected, only on peanut the frozen pathogens seems less pathogenic than the non frozen; but the pathogenicity seems to be dependent mostly on the specie of crop; it is higher on tobacco followed by tomato and minimal on peanut. Since the Race of bacteria identified in Mali is the tropical one that is not seen in temperate and tropical high land conditions the frozen bacteria should not induce pathogenity.

The confirmation of the first experiment by the second experiment result shows that one night of bacterial culture freezing do not affect much the pathogenicity; but we

do not know if that is because the bacteria is on culture media and what will be the difference if the bacteria freeze in natural environment.

Evaluation of Soils Infection Level

Indicator tobacco plants were transplanted into infested soils collected from the main areas of potato production throughout Mali. The explanation of the results summarized in Table 11 could be that Sikasso and Kati are the biggest potato production areas and year after year farmers do not practice a non-host rotation system. The same situation exists on tomato in Koulikoro area, so that in Koulikoro at Baguineda, where vegetables are rotated with rice culture every year, farmers could take advantage of alternating potatoes with rice. In 1974 Thung had recognized that flooding of the soil in rice production could have an adverse effect on the survival of *R. solanacearum*.

Pradhanang and Momol in 2001 will confirm the same by advancing the idea that in submerged rice during 3 months the bacterial population was reduced from 10^4 CFU per gr of soil to 10^3 CFU per gr of soil, even though some biovars were identified for the first time to be able to survive in that condition. Also two facts are evident in Table 11 first the bacterium is present in all the main production areas of potato in Mali and second the Race of pathogen present is the Race one for being able to induce wilting on tobacco plant.

In Sikasso area we got the maximum of wilted plants and since these three villages are the rainy season production area on hills side it is imperative to find resistant varieties for these contaminated soils and stop using the tubers produced in that area as seeds in the valley during the fresh season. The high level of the disease in Sikasso coincide with the fact that Sikasso region being the most important area of potato

production, was the first place to deal with the disease and since nothing has been done to manage the disease. Also in Sikasso farmers use the tubers harvested in the raining season from the hillside as seed potato in the field during the dry, fresh season, so the pathogen is multiplying easily in the traditional cropping system. That proves the necessity to introduce integrated management techniques in farmer's habit to reduce the pathogen population. The susceptibility of tobacco to the bacteria indicates the presence of Race one.

Comparison of Six Imported Varieties in Disease Condition at Sotuba, Baguineda and Kati (Table 12)

Between the six varieties tested for their susceptibility to *R. solanacearum* in farmers field at Kati, no difference significative is seen between the varieties but the lowest incidence were seen with Spunta and appoline while the highest was with Mondial and Liseta; At Sotuba Mondial with the highest incidence was significantly different from all the varieties and Appoline showed no bacterial wilt incidence. At Baguineda there was a significative difference with the highest incidence noticed with Daifla at first place followed by Mondial in second place, followed by liseta, Appoline and Spunta in third place; the lowest incidence was seen with Claustar. Here it is evident that the experiment site did influence the behavior of the different varieties; while Mondial was the highest incidence at Kati and Sotuba and the second highest at Baguineda, the highest at Baguineda was Daifla. Appoline, Claustar and Spunta are the only one appearing between the lowest incidences with or without siognificative difference at Sotuba, Kati or Baguineda. So we can already start to advice farmers in the sites of high infestation to use the less susceptible varieties that are Spunta, Calustar and Appoline; farmers should not grow Mondial, Liseta or Daifla in high infestation areas such as Sikasso on the hillside

especially in rainy season. Sotuba did show the lowest incidence with Appoline showing zero wilted plant.

It seems that the resistance to bacterial wilt here is not genetic but depends on other conditions like environmental condition, farmer's experiences. Comparing the different experiments held in different areas of production we can say that the variety Appoline, Spunta, and Claustar showed nice performance everywhere; Taking in count the environmental condition we should check about the possibility of any relation between varietal performance and environmental conditions in Mali.

Soils Treatments at Sikasso, Sotuba, Baguineda and Kati (Table 13, 14)

At Kati and Sotuba as observed in Table 13, there was no significant difference at between the different treatments of soil. The lowest incidence was seen with Dafuran in the two places. Diafuran and Oncol did work at Sotuba where tomatoes used to grow infested by root knot nematodes; It is possible that with the presence of nematodes the disease incidence was higher and by using the chemical nematicide such as Oncol and Diafuran we decrease the disease level; Also neem considered to be biological nematicide did protect better against bacterial wilt after Oncol and Diafuran. The control did show the higher incidence in the experiment.

In Sikasso and Baguineda (Table 14) Even though there is no significant difference between factors in the same treatment, compost plus neem has shown the good performance and protection against bacterial wilt. Neem alone gave the lowest incidence at Baguineda while in Sikasso the lowest was neem plus compost. These experiments need to be repeated to confirm any of the results presented in this paper.

CONCLUSION

After several experiments in laboratories and fields and other surveys, we concluded that bacterial wilt disease is present in Mali. The only Race identified currently that is able to naturally infect tomato, potato, pepper and artificially infect peanut, and tobacco is the Race 1 biovar 3. The bacterium has been detected in asymptomatic weeds *Commelina forskaleei* collected at Sikasso in the riparian area of the Farako River close to potato production areas.

Although none of the varieties commonly used by farmers and tested for their reaction to the disease are resistant to *R. solanacearum*, some (Appoline, Spunta, and Claustar) did show a better tolerance to the disease. These varieties can be used in the integrated bacterial wilt control program along with rotation with non-host crops.

The different soil treatments tested did not show any significant difference, as we were expecting. The major problem with this experiment was the relatively low level of the disease severity. Therefore the same experiment will be repeated in the field to confirm or not the results we obtained. The identification of infested areas through water and soils analyses allowed us to choose the best locations for seed multiplication as an important step in disease-free seed production.

One of the most important concerns for potato producers in Mali is how to get healthy, adapted, and inexpensive seeds to improve their production. Setting up an integrated management program for bacterial wilt disease in Mali will not only reduce the infection level of the disease in infested areas but also avoid the entrance of diseases to the healthy areas identified for seed production.

From all the information available now, Mali can start a sound program of integrated management to control the *R. solanacearum* disease starting by producing disease-free seed potato. Because infested and healthy areas have been identified, all the factors eliminating the bacteria in seed production areas are now identified. These factors include the choices of disease-free areas, the use of free-of-disease and protected water, no use of surface water, the use of nuclear seed to start the seed production program, the use of resistant varieties, and an effective adoption of certification and quarantine programs.

In the actual situation of potato production in Mali one of the most important disease reported is the bacterium *R. solanacearum*; Mali has the advantage and the capacity now to control that disease and improve the production of potato. Local potato seed production will avoid the introduction of important quarantine diseases and nematodes reported in Europe from where Mali get actually more than 90% of its seed potato.

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