Risk Analysis Of Brown Rot Disease In Potato Seeds Caused by
*Ralstonia solanacerum* Race 3 in Sudan

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DEDICATION

TO MY FATHER WHO GAVE US HIS LIFE
TO THE SOUL OF MY MOTHER MAY HER SOUL REST IN PEASE
I wish to express my appreciation to my supervisor Dr. Mohamed Osman Idres for his great valuable information and advices during this study.

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5.2.1.1 The PRA area
5.2.1.2 Probability of entry of the pest
5.2.1.3 Identification of pathways
5.2.1.4 Probability of the pest being associated with the pathway at origin
5.2.1.5 Probability of survival during transport or storage
5.2.1.6 Probability of pest surviving existing pest management procedures
5.2.1.7 Probability of transfer to a suitable host
5.2.1.8 Conclusion on the probability of entry.
5.2.1.9 Probability of establishment
5.2.1.9.1 Survival in the soil
5.2.1.10 Probability of Spread
5.2.1.11 Ability to survive in the soil

5.2.2 Assessment of potential economic consequences
5.2.2.1 Direct pest effects
5.2.2.2 Social consequences
5.2.2.3 Environmental consequences
5.2.3 Conclusions of the pest risk assessment
5.2.3.1 Presence or absence in PRA area
5.2.3.2 Regulatory status in PRA area
5.2.3.4 Potential for establishment and spread in PRA area
5.2.8 Potential for economic consequences in PRA area
5.2.9 Conclusion of pest categorization
5.2.10.1 Risk Management Options

References
## LIST OF TABLES

| Table 1: Percentage of wilted Draga Variety plants inoculated with *Ralstonia solanacerum* | 34 |
| Table 2: Average height (cm) of potato plants Draga variety inoculated with *Ralstonia solanacerum* | 37 |
| Annex 1: The Republic of Sudan Ministry of Science and Technology Metrological Authority temperature (year 2006/2007) | 37 |
# LIST OF PLATS

<table>
<thead>
<tr>
<th>Plates</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate 1. Colonies of <em>ralistonia solanacerum</em></td>
<td>27</td>
</tr>
<tr>
<td>Plate 2. Production of acid from carbohydrates from left to right: lactose(L), maltose(M), Sucrose(S), Control(C).</td>
<td>28</td>
</tr>
<tr>
<td>Plate 3. Positive oxidase test</td>
<td>29</td>
</tr>
<tr>
<td>Plate 4. Negative starch hydrolysis test</td>
<td>30</td>
</tr>
<tr>
<td>Plate 5. Potato baling and longitudinally showing vascular brown ring</td>
<td>31</td>
</tr>
<tr>
<td>Plate 6. Wilted artificially inoculated plants right wilted plants, left healthy plants</td>
<td>33</td>
</tr>
<tr>
<td>Plate 7. Potato variety (TPS6) grown on infested soil (right) and non infested soil (left)</td>
<td>36</td>
</tr>
</tbody>
</table>
ABSTRACT

This work aimed to study the possible pest risk posed by the introduction of the brown rot disease in potato crop, caused by the bacteria *Ralstonia solanacearum* (Smith) Yabuuchi et al. (*Pseudomonas solanacearum*) Race 3 and to decide if it is quarantine pest or not. The bacterium was intercepted in a potato seed variety Pelleini consignment imported from France in 2006. Zalinge local potato variety previously collected from the local market reported to be infected by the bacterium.

A brownish discoloration of the vascular ring was detected in 80% of Pelleini tubers, when the tuber was cut into two halves across the stem end. When the tubers were slightly squeezed creamy fluid exudates was observed oozing from the vascular ring. The same symptoms were observed on infected Zalinge potato tubers. Growth of the bacterium for 24 hours on nutrient agar resulted in small, irregular, smooth cream coloured and translucent colonies. The bacteria were short rods, motile and negative to gram reaction. When the bacteria were isolated and injected in potato plants, typical symptoms of brown rot appeared in the inoculated plants, the plants ultimately wilted and died. The soil and plant debris mixed with the inoculums of *R. solanacearum* Race 3 were left under the sun during the summer months (May-November). Certified potato seeds were then planted in this soil. Neither the potato plants grown on neither infested soil nor those grown on non infested soil showed disease symptoms. The average plant height was 12.62 and 11.66 cm in infested soil and non infested soil, respectively. No bacterium was re-isolated from the plants. This study revealed that the disease can not over summer in central Sudan environmental conditions where the average maximum temperature reached during that season was 46 °C.

A thorough pest risk analysis was conducted, the probability of introduction was high as the pest can be introduced with plant material intended for planting (seed potato) from Europe or with private individuals carrying infected material from neighboring countries i.e. Egypt, Saudi Arabia, Eritrea and Ethiopia. From this study survival in the soil is rated low risk. Survival of the bacterium in irrigation water is rated high. The study concludes
that *R. solanacearum* Race 3 should be classified as a quarantine pest in Sudan. Seed potato has to be imported from pest free areas only and the ban of ware potato from neighboring countries has to continue
هيئة هذه الدراسة إلى تحليل مخاطر إصابة محصول البطاطس بمرض العفن البني الذي تسببه البكتيريا. 

تحديد ما إذا كانت أفة حجرية أم لا تم الكشف عن Ralstonia solanacerum Race 3.

وفي السودان عام 2006 م في تقاوي مستوردة من فرنسا في وجدت سابقاً إصابة بهذه البكتيريا في الصنف المحلي زانجي. والذي تم تجميعه من السوق المحلي. تلاحظ وجود حشرة بني شفاف في منطقة الأوعية الدائمة عند قطع الذرات المصابة إلى نصفين من جهة الساق في شكل حلقي بنسبة 80٪ منها. أما الذرات التي لم تظهر فيها تلك الأعراض عندما وضع في الحمض لمدة (2-3) أسبوع ظهرت بها نفس الأعراض. كذلك لوحظ خروج سائل كريمي لزج من الأوعية الدائمة للذرات المصابة وقد شوهدت نفس الأعراض في الصنف المحلي زانجي. عند زراعة البكتيريا في الوسط الغذائي للدجاج لمدة 24 ساعة ظهرت مستعمرات صغيرة غير منظمة ذات لون كريمي شفاف. ومن مميزات هذه البكتيريا أنها ذات شكل عصوي وسائلية لمصغة الغرام. تم عزل وتشخيص البكتيريا من الذرات المصابة وحققت في نباتات بطاطس سليمة حيث ظهرت أعراض مرضاً على الإصابة في النبات البني بوضوح وهي الذيل الغليان للنبات ومن ثم Ralstonia solanacerum Zaraعته. تم تم تجريبي النباتات المصابة مضاد إليها محلول بكتيريا موته. تم تعريض النباتات التي بها بقايا الذرات المصابة مضاف إليها محلول بكتيريا وللشمس طوال فترة الصيف من مايو إلى نوفمبر 2007م. ثم بعد ذلك تم القد التصوير في الصنف في السودان على بقاء البكتيريا في النبات. وقد كان متوسط درجة الحرارة 41 درجة م. وقد تمت النباتات بصورة سليمة. ولم تظهر أي أعراض للمرض مما يؤكد عدم احتمال بقاء البكتيريا في درجات الحرارة العالية في النبات. وكذلك لم تظهر أعراض في الكنثور الذي تم زراعته بنفس التقاوي في تربة مقسمة. وقد كان متوسط أطوال النباتات من 11.66 إلى 12.26 سنتيمتر للتربي الفرة السليمة والصينية. عند أجراء عملية عزل البكتيريا من تلك النباتات لم توجد البكتيريا. من هذه الدراسة يتضح عدم احتمال بقاء البكتيريا بعد الصيف في النبات في وسط السودان حيث تصل درجة الحرارة إلى الصيف 46 م. في هذه الدراسة تم عمل تحليل مخاطر لهذه الدراسة. وكانت النتيجة أن هناك احتمال كبير لدخول هذه الأفة عن طريق التقاوي المستوردة من الدول الأوربية أو مع النباتات المصابة بالبكتيريا Ralstonia solanacerum Race 3 وآثيوبيا. وكذلك هناك احتمال ضعيف لبقائها في النبات في درجات الحرارة التي تزيد عن 41 م. وانها سريعة الانتشار بعياً. لابد أن Ralstonia solanacerum Race 3 ونخصص من هذه الدراسة إلى أن البكتيريا تصنف كافعة حجرية في السودان. وإن تقاوي البطاطس لابد أن تستورد من المناطق الخالية من الإصابة. وكذلك استمرار حظر استيراد تقاوي البطاطس من الدول المجاورة التي يوجد بها هذا المرض.
CHAPTER ONE
INTRODUCTION

Potato (Solanum tuberosum L.), family Solanaceae is an important food crop which originated in the Andes Mountains in South America.

Potato is the fourth most important food crop in the world after wheat, rice, and maize. Its production represents half of world’s annual output of all root tubers (CPC, 2005). Worldwide, tubers of the potato crop are used for direct human consumption (48%), processing (11% of which 2% is for the production of starch), vegetative propagation (13%), stock feed (20%) and the remaining 8% is waste (CPC, 2005). Potato production in developing countries rose steadily from 28 million tons in 1963 to some 141 million t in 2003 (FAO STAT, 2003). Hence, world potato output rose from 270 to 311 million ton in the period 1963-2003. China, which produces over 67 million tons, is the world's largest potato producer (CPC, 2005).

Potato was introduced in the Sudan in 1920’s (Abdalla and Al Shafie, 1983). Main production areas are River Nile State, Khartoum State, southern Darfur state and Northern State. The area under production rose steadily from 39,000 feddan in 2000 to 48,000 in 2004 then dropped to 36,870 feddan in 2007 (Al Basheer, 2008). The drop was attributed to the heavy infestation of one of the most important production area with Orobanche ramosa. Production also increased from 3600 tons in 2000 to 272,000 tons in 2000 to 336,000 tons in 2004.
Potato is normally propagated vegetatively by small, 40-100 g; tubers, called 'seed tubers' or 'seed potatoes'. It can also be propagated by pieces of tuber ('seed pieces') or by true seed. The seed rate (tubers) ranges from 1.5-4.0 t/ha. The first problem facing growers in developing countries is obtaining supplies of healthy planting material of a suitable cultivar at an acceptable price. In many countries there are no provisions for local propagation of tubers and the import of seed potato is expensive and poorly organized. In the Sudan, tubers are spilted diagonally from heel to the tail but not completely separated into two, and then kept in the dark for 4-6 days to enhance growth before sowing.

Potato in the Sudan is grown under irrigation except for the local cultivar Zalinge which is grown in Gebel Mara in Western Sudan as a rain-fed crop. The cultivar is grown continuously for more than 50 years, the origin of the cultivar is not known. The potato crop grown in the Sudan arises from seed potato of different quality and sources. Before 1989 all certified seeds were mainly the cultivar Alfa which was annually imported from the Netherlands. Since then seed potato imports were allowed from different sources to fill the gab in potato seeds. The used cultivars are Desiree, Draga, Diamant, Frisia, Spunta, Avonova, Pellini, Sinora, Avnda and Mondial (Department of Horticulture Ministry of Agriculture and Forests personal communication 2007).

The amount of imported certified seeds has increased from 200 tons in 1975/76 to 2600 tons in 1991. Recently seed potato imported through Port Sudan seaport increased from 730 tons in 2001 to 5344 tons in 2005 (Plant Quarantine Department Statistics, 2006).
The first report of Potato pathogens in Sudan was by Tarr, (1956). He reported *Alternaria solani* from Torit, *Rizoctonia solani* from Shambat, *Fusarium oxysporum* from Kagelu; *Stysanus stemonites* from the Northern Province and rarely *Phytophthora infestans* from Equatoria.

Recently the pest and disease list of potato in Sudan was compiled by Plant Pathology Section of the Plant Protection Directorate (Plant Pathology Section, 2003). The list included powdery mildew pathogen *Leveillula taurica* from Shambat and *Macrophomina phaseolina* from Gezira (ARC, 1986). They also reported *Alternaria alternata; A. tenuis* and the common scab pathogen, *Streptomyces scabiei* from Gezira.

Many viruses were reported to infect potato and have been assessed by El Hassan and Ibrahim in 1989.

*Ralstonia solanacearum* (Yabuuchi et al., 1996) other synonyms are *Xanthomonas solanacearum* var. *asiatica* (Smith) is a serious disease of potato worldwide. The bacterium was reported on Zalinge potato from Gebel Mara area in Western Sudan by Rehan, (1979). The bacterium was never reported from potato growing areas in central Sudan.

In 2006 *R. solanacearum* was detected in a consignment of 70 ton of seed potato variety Pellini imported from France. This was the first report of the pathogen from imported seed potato. Being an important quarantine disease of potato it requires much attention on its introduction
with seed potato. This study was designed to investigate the risk of introduction, establishment and spread of *Ralstonia solanacearum* in potatoes in the Sudan.
CHAPTER TWO
LITERATURE REVIEW

2.1 History and distribution

_Ralstonia solanacearum_ (Smith 1896) Yabuuchi _et al._ 1996,
Synonyms: _Bacterium solanacearum_ (Smith) Chester; _Burkholderia solanacearum_ (Smith) Yabuuchi _et al._ (1992) and _Pseudomonas solanacearum_ (Smith) Smith is widespread in tropical, subtropical and warm temperate areas throughout the world. Its occurrence has now also been reported from temperate zones. In particular, race 3 was reported from a number of European countries in the 1990s (OEPP/EPPO, 1961; Jance, 1996). The disease brown rot of potato incited by _Ralstonia solanacearum_; was reported as isolated incidents in 1995 from France and from the Netherlands in the early 1990s and several outbreaks in 1995 (EPPO, 2005).

2.2 Host Range

_R. solanacearum_ as a species has an extremely wide host range, but different pathogenic varieties (races) which within the species may show more restricted host ranges. Over 200 species, especially tropical and subtropical crops, are susceptible to one or other of the races of _R. solanacearum_. Worldwide, the most important host plants are: _Lycopersicon esculentum_ (tomato), _Nicotiana tabacum_ (tobacco), _Solanum melongena_ (aubergine), _Solanum tuberosum_ (potato), _Musa_ spp.
(banana), *Musa paradisiaca* (plantain) and *Heliconia* and *Tectona grandis* (teak).

Within the EPPO region, race 3 has a limited host range including potato, tomato and the weed *Solanum dulcamara* and considered to have potential for spread.

Other host crops of Race 3 are: *Anthurium* spp., *Arachis hypogea* (groundnut), *Capsicum annuum* (bell pepper), *Gossypium* spp. (cotton), *Ipomoea batatas* (sweet potato), *Manihot esculenta* (cassava), *Ricinus communis* (castor bean) and *Zingiber officinale* (ginger). Tree species *Hevea brasiliensis* (Rubber), *Tectona grandis* (teak) and *Casuarina cunninghamiana* were reported susceptible to Race 3 of *Ralstonia solanacearum*.

Many weeds are alternative hosts of the pathogen. *Solanum cinereum* in Australia (Graham and Lloyd, 1978), *Solanum nigrum* and, in rare cases, *Galinsoga parviflora*, *G. ciliata*, *Polygonum capitata*, *Portulaca oleracea* (for example, in Nepal; Pradhanang and Elphinstone, 1996) and *Urtica dioica* have been reported as weed hosts for race 3 (Wenneker et al., 1998). *solanum. nigrum* and *solanum. dulcamara* are primary wild hosts for race 3.

Lists of host records have been recorded by Kelman, (1953) Bradbury, (1986) Persley, (1986) and Hayward, (1994) but the original reports, gathered over many years, vary greatly in reliability. Few reference strains from reported host plants have been deposited in publicly accessible culture collections to support the authenticity of records.
2.3 Impact

*Ralstonia solanacearum* is the most serious pathogen of solanaceous plants in tropical regions and can cause serious losses in temperate regions. Accurate data on yield losses and further economic impacts in the Sudan are not available. A method to determine disease severity and yield losses for brown rot in potato has been described by Elphinstone (1989). New high-yielding but susceptible cultivars in place of older tolerant varieties may cause problems in areas where the disease is endemic (Weingartner and Shumaker, 1984).

Many factors influence disease incidence and yield loss. Records of disease incidence available demonstrate great variation because of influence of different factors such as location, environmental conditions and altitude. In a study in India on sesame, *Ralstonia* wilt incidence was significantly correlated with mean temperature, rainfall and relative humidity during the crop growth period (Hazarika and Das, 1999). In a study on the effects of physical soil properties it was found that sandy loam soil with a high sand content and low silt or clay content, with low water-holding capacity, was unfavorable for the pathogen and wilt incidence. Elevated disease levels were expressed in clay soils with high water-holding capacities (Keshwal *et al*., 2000).

Greatest economic losses have been reported on potato, tobacco and tomato. In potato heavy losses were reported from the South Atlantic and Gulf Coast states of the USA (Kelman, 1953). Extensive
losses of potato were reported in Greece (Zachos, 1957). In Israel, losses were heavier in the spring potato crop than the autumn crop, because of the higher growing temperatures in spring (Volcani and Palti, 1960). In Burundi losses of 64.1% were reported in seed potato (Berrios and Rubirigi, 1992). In India losses ranged from 30 -70% (Sinha, 1986). In the Philippines, there were average losses of 15% in tomato, 10% in aubergine and Capsicum, and 2-5% in tobacco (Zehr, 1969). In the Amazon basin in Peru, banana plantations have been seriously affected with rapid spread of the pathogen in previously unaffected plantations (French and Sequeira, 1968). In India, there are sometimes total losses in tomato crops. Bacterial wilt also appears to be very common in wild and cultivated turmeric (Curcuma spp.) in Thailand and Indonesia (Thammakijjawat et al., 1999). Bacterial wilt caused by Ralstonia is also a problem in ginger (Zingiber officinale); it was present in 80% of 310 fields surveyed in Himachal Pradesh, India (Sharma and Rana, 1999), and severe losses were reported from Thailand (Titatarn, 1985).

*R. solanacearum* has been intercepted regularly from rhizomes exported for cut flower production in Europe. The disease may cause serious indirect losses when quarantine measures entail restriction movement of, or destruction of plant products (Hyde *et al.*, 1992; Tuin *et al.*, 1996).

### 2.4 Losses in Potato

Multiplication by cutting seed potato seriously increases the risk of high losses. Cut seed potato increased disease incidence by 250% and reduced yield by 40% (Vijayakumar *et al.*, 1985). Tuber rotting averaged 10%, reaching 50%, in stored potatoes in Nepal (Shrestha, 1996).
Complete crop losses in small holdings in Nepal resulted from poor cultural practices including using seed from affected crops for subsequent plantings (Gurung and Vaidya, 1997). In Venezuela, in the period 1992-1996, *R. solanacearum* was found in most localities between 1100 and 3000 m above sea level, but was not found in localities at altitudes greater than 3000 m. Bacterial wilt disease incidence increased from 22% in 1992 to 37% in 1996 with disease incidence varying between 5 and 75%. Biovar 2 of race 3 was present in greatest frequency than biovar 3 and in most of the affected areas for potato (Garcia *et al*., 1999).

### 2.5 Losses in Tomato

In tomato hybrids, field grown in Taiwan for the fresh market, bacterial wilt incidence was 15-26% in improved tolerant hybrids compared to 55% in other hybrids (Hartman *et al*., 1991). In India, an investigation on the effect of time of infection showed that disease incidence; measured by plant mortality and plant yield; diminished with age of the plant at the time of inoculation. Maximum losses were recorded during the summer seasons (Kishun, 1987).

### 2.6 Groundnuts and Other Crops

In Vietnam, infection in groundnut was most severe in dry land cropping systems, especially on sandy soils along riverbanks, and on uplands (Hong *et al*., 1994). Tolerant varieties are infected by the pathogen but are not affected and can produce high yields (Liao *et al*., 1998). Bacterial wilt in groundnut (Race 1, biovars 3 and 4) is widespread in China. Annual disease incidence ranges from 4 to 8% on resistant cultivars. Pathogenicity varies between regions. The disease is generally being more serious in southern provinces where losses of up to
20% were common (Yeh, 1990; Tan et al., 1994). Disease severity mostly increases if *R. solanacearum* is found in association with root nematodes. In tobacco, nematode infestation leads to greater susceptibility to bacterial wilt (Chen, 1984). When bacterial wilt of teak was initially recorded during the 1980s in Kerala, India, it appeared to be of little consequence. However, the incidence of the disease has increased over the years, both in nurseries and plantations. *Ralstonia solanacearum* causes mortality of bare-root and root trainer seedlings raised in high rainfall areas. Young plantations raised in waterlogged sites in areas of high rainfall (>3000 mm per annum) are more seriously affected. In these areas, the incidence of disease varied from <1% to ca 20% (Sharma et al., 1985). Synergistic interactions between *R. solanacearum* and *Meloidogyne javanica* have been reported by Sitaramaiah and Sinha, (1984) Pathak et al. (1999) and Verma et al., 1997).

### 2.7 Biology

*R. solanacearum* does not behave as a single bacterium with a uniform biology and host range, but as a complex of variants, variously described as groups, races, biovars, biotypes, sub-races and strains. The different classifications of *R. solanacearum* have caused a considerable amount of confusion in the literature. Buddenhagen et al. (1962) distinguished three races on the basis of pathogenicity:

**Race 1**: Affecting tobacco, tomatoes, potatoes, aubergines, diploid bananas and many other solanaceous crops and weeds. Race 1 growth temperature optimum is high (35-37°C).
**Race 2:** Affecting triploid bananas (causing Moko disease) and *Heliconia* spp., with high growth temperature optimum (35-37°C).

**Race 3:** Affecting mainly potatoes and tomatoes without a high virulence on other solanaceous crops, with lower temperature optimum (27°C). Other host plants are the weeds *S. dulcamara*, *S. nigrum*, *S. cinereum* (in Australia); the composite weed *Melampodium perfoliatum* (in Costa Rica) and *Pelargonium hortorum*.

Two additional races affecting *Zingiber officinale* and mulberries (*Morus* spp) were also distinguished (Buddenhagen, 1986), but their status is still unclear.

Hayward (1964) distinguished four biotypes (biovars) by their ability to produce acid from several disaccharides and sugar alcohols. Mulberry strains have been described biovar as 5 (Buddenhagen, 1986). These biotypes do not correlate with the races of Buddenhagen *et al.* (1962). Only race 3, the potato race, is equivalent to biotype II (Hayward, 1983). Races and biovars have been classified into two 'main groups' according to a restriction fragment length polymorphism (RFLP) analysis (Cook and Sequeira, 1988; 1994). Asian strains of race 1 (biovars 3, 4, 5) were clustered as a group.

Race 3 (biovar 2) has appeared in many fingerprint studies to be very homogeneous. When studies of South American strains of race 3 (biovar 2) were made, however, more variation was observed: a) “normal” strains found east of the watershed of the Andes and all over the world, b) strains that are biochemically different, until now only found west of the watershed of the Andes and c) strains that behave
almost as an intermediate between race 1 and 3 that occur in the lowlands of South America (also named biotype 2N or 2T). It is not known whether the types mentioned under b) and c) are also found in other parts of the world (Janse, 1991; Gillings and Fahy, 1994; Hayward, 1994). These findings, as well as the fact that resistance is found in the wild Solanum phureja (Sequiera & Rowe, 1969) indicate South America as the possible origin of race 3.

2.8 Survival and Spread

The bacterium can spread in soil, in which it survives for varying periods of time, and in irrigation (drainage) water. In tropical areas, many weeds have been shown to be alternative host plants. The slow rate of development of the bacterium on the weeds allows them to withstand infection, and so provide a bridge for the pathogen between crops.

Entry into plants is by way of injured roots, stem wounds or through stomata. Within the plant, the bacteria move in the vascular bundles, a process which is accelerated by higher temperature. Speed of movement is also dependent on the plant part colonized, for instance in tobacco bacteria move quicker in the stalk than in the roots (Ono et al., 1984). This is followed by colonization of the xylem (Xiao et al., 1983), where the bacteria adhere to the vessel walls or invade the lumen. They adhere by polar attraction to the cell surfaces and subsequently become localized at preferential sites of the mesophyll (Petrolini et al., 1986). Blocking of the vessels by bacteria is the major cause of wilting.

The disease is most severe at 24-35°C; it is seldom found in temperate climates where the mean temperature for any winter month
falls below 10°C. There are distinct temperature requirements for optimum disease development and reproduction for the different races (biovars) (Swanepol, 1990).

High soil moisture and periods of wet weather or rainy seasons are associated with high disease severity. Soil moisture is also one of the major factors affecting reproduction and survival of the pathogen. The most favorable soil moisture is -0.5 to -1 bar while -5 to -15 bar is unfavorable (Nesmith & Jenkins, 1985).

Slightly unfavorable weather conditions such as low temperatures influence symptom expression. In Kenya, certified and obviously healthy (but latently infected) potato seed tubers produced at altitudes of 1520-2120 m showed infection when planted at lower altitudes (Nyangeri et al., 1984). This was due to a latent infection of the tubers grown in an environment less favorable to the pathogen.

2.9 Detection and Identification

2.9.1 Symptoms

(A) Symptoms on potatoes

Foliage: The first visible symptom is a wilting of the leaves at the ends of the branches during the heat of the day with recovery at night; eventually, plants fail to recover and die.

As the disease develops, a streaky brown discoloration of the stem may be observed on stems up to 2.5 cm or more above the soil line, and the leaves have a bronze tint. Moreover epinasty of the petioles may occur. A white, slimy mass of bacteria exudes from vascular bundles which are broken or cut. This slime oozes spontaneously from the cut
surface of a potato stem in the form of threads, when kept in a beaker with water. Such threads are not formed by other bacterial pathogens of potato. This test is of a presumptive diagnostic value in the field.

Tubers: External symptoms may or may not be visible, depending on the state of development of the disease; furthermore, symptoms may be confused with those of ring rot due to *Clavibacter michiganensis* subsp. *sepedonicus* (EPPO/CABI, 1996). *Ralstonia. solanacearum* can be distinguished by the bacterial ooze that often emerges from the eyes and stem-end attachment of infected tubers. When this bacterial exudates dry, a mass of soil adheres to the tubers at the eyes. Cutting the diseased tuber will reveals a browning and necrosis of the vascular ring and immediately surround tissues up to 0.5 cm each side of the ring. A creamy fluid exudates usually appears spontaneously on the vascular ring of the cut surface a few minutes after cutting. In the case of ring rot the tuber has to be squeezed in order to press out a mass of yellowish dissolved vascular tissue and bacterial slime. Atypical symptoms on potato (necrotic spots on the epidermis), possibly caused after lenticel infection, have been described by Rodrigues-Neto *et al.* (1984).

Plants with foliar symptoms caused by *Ralstonia. solanacearum* may bear healthy and diseased tubers, while plants that show no signs of the disease may sometimes produce diseased tubers.

**(B) Symptoms on tomatoes**

The youngest leaves are the first to be affected and have a flabby appearance, usually at the warmest time of the day. Wilting of the whole plant may follow rapidly if environmental conditions are favorable for
the pathogen. Under less favorable conditions, the disease develops less rapidly, stunting may occur and large numbers of adventitious roots are produced on the stem. The vascular tissues of the stem show a brown discoloration and, if the stem is cut crosswise, drops of white or yellowish bacterial ooze may be visible (McCarter, 1991).

(C) Symptoms on tobacco

One of the main symptoms is unilateral wilting and premature yellowing. Leaves on one side of the plant or even a half leaf may show wilting symptoms. In severe cases, leaves wilt without changing colour and stay attached to the stem. As in tomato, the vascular tissues show a brown discoloration when cut open. The primary and secondary roots may become brown to black (Echandi, 1991).

(D) Symptoms on bananas

Moko disease, caused by *R. solanacearum*, is easily confused with the disease caused by *Fusarium oxysporum* f.sp. *cubense*. A clear distinction is possible when fruits are affected - a brown and dry rot is only seen in the case of Moko disease. On young and fast-growing plants, the youngest leaves turn pale-green or yellow and collapse. Within a week all leaves may collapse. Young suckers may be blackened, stunted or twisted. The pseudostems show brown vascular discolouration (Hayward, 1983).

2.10 Morphology

*R. solanacearum* is a Gram-negative rod, 0.5-1.5 µm in length, with a single polar flagellum. The positive staining reaction for poly-ß-hydroxybutyrate granules with Sudan Black B or Nile blue distinguishes
*R. solanacearum* from *Erwinia* species. In addition, *R. solanacearum* stains heavily at the poles with carbol fuchsin. Agar colonies are initially smooth, shining and opalescent, but become brown with age (Saddler, 1994).

**2.11 Detection and inspection methods**

The bacterium may be obtained from infected tubers or stems for staining purposes if a small portion of tissue is pressed onto a clean glass slide. Potato tubers can be visually checked for internal symptoms by cutting. Potato tubers suspected to be latently infected should be diagnosed in the laboratory. Appropriate laboratory methods to detect the pathogen, also in its latent form, are indirect immunofluorescence antibody staining (IFAS) and a pathogenicity test on tomato to confirm a positive IFAS result. Standard samples of 200 tubers per 25 ton of potatoes are taken for examination (Janse, 1988, EPPO, 1990). Recently, a very effective selective medium has been described by Engelbrecht (1994), which can also be applied for detection in environmental samples. ELISA and the polymerase chain reaction (PCR), based on 16S rRNA targeted primers, have also been used successfully. Biochemical tests, fatty acid analysis, RFLP and protein analysis can be used for identification purposes (Seal *et al.*, 1993; Seal and Elphinstone, 1994).

**2.12 Means of movement and dispersal**

The natural spread of most of the *R. solanacearum* races is very limited and slow. However, race 2, which causes Moko disease of banana, is known to be transmitted by insects and has a high potential for natural spread. Race 3 may spread more easily with surface water when infected *S. dulcamara* grows with its roots floating in water. The
bacterium may subsequently be spreaded to other host plants when contaminated surface water is used for irrigation (Olsson, 1976).

The main path for international spread is by latently infected seed potatoes and other vegetative propagating materials. Natural infection of true seed has only been firmly established for groundnut. There are a few reports of occurrence of race 1 in tomato, capsicum and abourigine seed (Persley, 1986; Kelman et al., 1994; Singh, 1995). Infections of potato tubers may be latent, due to unfavorable weather conditions, partly resistant cultivars or low virulence of certain pathogen strains. Tubers with latent infection are the most probable means of introduction into a new area.

2.13 Survival

Most plant pathogenic bacteria are usually closely associated with their living host plants and temporarily in infected host plants debris. They survive for relatively brief periods in soil or other environments where competition is with active saprophytic populations. *Ralstonia solanacearum* is one of the few plant pathogenic bacteria for which there is evidence of survival in soil. Many weeds have been shown to be alternative hosts that maintain an on-going source of inoculums for the pathogen between crops. Race 3 survived for 2-3 years in Australia under bare fallow or pasture. Host plants debris, latent infected tubers and deeper soil layers were the most important means for survival (Graham and Lloyd, 1979; Graham et al., 1979).
2.14 Pest Risk Analysis (PRA)

Pest Risk analysis is a process of investigation, evaluation of information and decision making with respect to a certain pest that starts once it is known or determined that this pest is a quarantine pest (FAO, 2004).

Subsequently an evaluation of the potential of introduction of the pest into the country is done. With identification, determination and evaluation done, the process culminates with decision making to avoid or reduce the probability of entrance or establishment of the pest into the country.

Pest Risk Analysis (PRA) is done to protect the country’s agriculture from damages that can be caused by harmful (quarantine) pests which can be brought in along with imported commodities.

PRA evaluates the likelihood of the entry, establishment, or spread of a pest and the associated potential biological and economic consequences (SPS, 1994 Annex A)
3.1 Biochemical characteristic

3.1.1 Carbohydrates utilization

This test was performed using Ayer,s basal, synthetic medium or peptone free medium (S.A.B, Manual of Methods, 11, 14, 1944)

Mgso4,7H2o 0.02g
Kcl 0.02g
(NH4)2 .H2PO4 0.025g
Agar 20mg

100 ml (distilled water + bromthymol blue)

The medium was adjusted to PH 7.2 by the addition of 0.1 N soda sterilized in the autoclave at 20 lb. for 15 min.

Four carbohydrate compounds, namely, glucose, Sucrose, maltose and lactose were separately added at concentration of 1% to the above medium. The mixture was then autoclaved, redistributed to tubes and inoculated with the test isolate. One tube for each isolate was used and similar tubes were left uninoculated as control. Treated and control tubes
were incubated at 27c for 7 days and any color change of the medium were observed and recorded.

3.1.2 Oxidase

The oxidase activity of the isolate was detected by rubbing a small loop full of 24-old culture on a filter paper impregnated with 1% aqueous solution of tetramethyl-p-phenylene diamine dihydrochloride reagent. The color development as a result of the reaction was observed and recorded. (Hyward1964).

3.2 Starch hydrolysis

The isolate of pillni brown rot grown on nutrient agar starch (NAS) to test their ability to hydrolysis starch. Plates of NA medium containing 0.2% soluble starch per liter were prepared and streaked with the bacterium isolate under test. Inoculated plates was incubated at 27c for 5days. Then was flooded with lugol’s iodine solution. Development of hydrolysed areas was observed following the procedure of Downson (1957).

3.3 Biological Material

(A) Plant Material

The plant material used in this study were potato tubers variety Pellini imported from France, and variety Draga imported from the
Netherlands and tubers of the local Zalingi potato collected from Jebel Mara, Sudan.

(B) Symptoms of brown rot in the tubers

A sample of two hundred potato tubers was selected at random from a 20 ton container suspected to be infected with *Ralstonia solanacearum*. Then, twenty tubers were selected at random and were cut into two halves longitudinally across the stem end, to observe the presence of the brownish discoloration in the vascular ring which characterize the disease. The cut tubers were slightly squeezed to accretion the presence of any oozing substance. The tubers were then incubated at 30°C and 90% humidity for 2-3 weeks for detection of any latent infection in the tubers (Martin and French, 1985).

3.4 Isolation of the Bacterium

(A) The medium used

Oxoid Nutrient Agar (NA) was used for the isolation of the bacterium and the medium is composed of the following ingredients; Peptone (10 g), Beef extract (3 g), Sodium chloride (5 g), Agar (10 g) and one liter of sterilized distilled water. The PH of the medium was adjusted to 7.2. and was then sterilized by autoclaving for 15 min at 121°C.

(B) Isolation from the tubers

Five potato tubers were washed with water, air dried, alcohol flamed and cut into two halves with a sterilized knife. Tuber tissue weighing one gram was aseptically obtained from the vascular ring
showing brown discoloration and soaked in tubes containing distilled water for 24 hours. Bacterial isolation was made by streaking on replicated Nutrient Agar (NA) plates following dilution of the bacterial suspension……..folds.

After isolation pure cultures of the bacterium were made. All plates were then incubated for 24 hour at 28°C for observation of the growth and characteristics of the bacterium.

**(C) Maintenance of pure cultures**

Stocks of pure cultures of the bacterial isolate were maintained under mineral oil on potato dextrose agar (PDA).

**3.5 Detection of Brown Rot Disease Symptoms**

The soil used was collected from north Omdurman where the potato is usually planted. The soil was sterilized and used to grow potato tubers.

**(A) On naturally infected potato tubers**

Twenty tubers of each of the varieties Zalinge and pellini potato were sown in mid. February 2007 in clay pots containing the sterilized soil to observe infection of brown rot disease. The plants were observed for two month for the detection of brown rot symptoms.

**(B) On Pellini potato plants**

Fife tubers were taken randomly from Pellini potato imported from France; they were sown in winter of 2007 in the same soil used before to grow Zalinge potato plants. The plants were observed for two month for the detection of brown rot symptoms.
(C) Pathogenicity test

On November 2007 twenty healthy tubers of the variety Draga imported from the Netherlands were planted in 25 cm plastic pots filled with sterilized soil. The plants grew very well and gave very dense foliage. The potato plants were then inoculated with the inoculums prepared from the pure cultures isolated from diseased Pellini potato. The inoculums were prepared from 3-5 days old virulent culture grown on PDA in 500 ml flasks. Bacterial suspension was made by adding 100ml sterilized tap water to the culture flask and gently agitated until the bacterial growth was suspended in the water. The suspension was then diluted by the addition of 300 ml of distilled water. The plants were inoculated when they were 14-16 cm high and with 6-7 expanded leaves, using the stem puncture technique. The technique was performed by forcing a sharp syringe into the stem and a drop of bacterial suspension was placed in the axel of 3rd and 4th expanded leaves below the stem apex (Winsted and Kelman, 1952). Ten potato plants were used for this test and similar numbers of plants were left as control.

The plants were watered every day with 500 ml except when wilting was so severe that the soil remained wet. Symptoms development and disease incidence were recorded up to 8 weeks after inoculation. While plant height was recorded periodically for five weeks post inoculation.

3.6 Persistence of the bacterium in the soil

Contaminated soil with the bacterium and infected plant debris was left under direct sunlight during the period from May to November 2007 to test the ability of the bacterium to over summer under Sudan weather.
conditions. Then certified seeds of potato variety (TPS6) imported from the International Potato Centre in Lima, Peru were sown in the infested soil and observed for symptoms of brown rot. Ten plants were selected at random and the number of leaves and plant height were also recorded at 3 and 5 weeks after emergence.
4.1 Characteristics of the bacterium on Nutrient Agar

Growth of the bacterium for 24 hours on Nutrient Agar resulted in small, irregular, smooth cream colored, translucent colonies (Plate 1). Under the microscope the bacteria were short rods, motile and negative to Gram reaction.

4.2 Carbohydrates oxidation

Acid was produced oxidatively by the bacteria in Ayer's basal synthetic medium supplemented with bromthymol blue after 7 days incubation at 27°C from glucose, sucrose, lactose and maltose. This was observed by the change of the blue color of the indicator at pH, 7.2 to the yellow color indicating the drop of the pH due to acid production (Plate 2).

4.3 Oxidase

A positive result of oxidase test produced by pillini isolate of the brown rot bacterium was indicated by the development of the purple color (Plate 3).
4.4 Starch hydrolysis

The brown rot bacterium pillini isolate did not hydrolyses starch (Plate 4)

4.5 Symptoms of brown rot
4.5.1 Brown rot symptoms observed in tubers

In 80% of Pellini tubers a brownish discoloration of the vascular ring was observed when the tuber was cut into two halve across the stem end (Plate 5). Tubers showing no brown discoloration of the vascular ring showed typical symptoms when incubated for 2-3 weeks. When the tubers were slightly squeezed a creamy fluid exudate was observed oozing from the vascular ring.
The same symptoms of brown rot were observed on infected Zalinge potato tubers.
Plate 1. Colonies of *R. solanacerum* on nutrient agar
Plate 2: Production of acid from carbohydrates from left to right
Lactose (L) .Maltose (M) Glucose (G) Sucrose (S) .Control (C)
Plate 3: Positive oxidase test
Plate 4: Negative starch hydrolysis test
Plate 5. Potato tuber haling and longitudinally showing vascular brown ring.
4.5.2 The effect of the bacterium on potato plants

4.5.2.1 On naturally infected Zalinge potato variety

The general observation was that the plants were stunted and with small leaves. One month after emergence 20% of the plants showed stunting, yellowing, then a brown color was observed on the leaves and the plants wilted and dried. Sixty percent of the plants showed symptoms of wilt and yellowing of the leaves. Twenty percent of the plants were healthy one month after emergence but after two month symptoms appeared on the lower leaves in all plants.

4.5.2.2 On naturally infected Pellini potato variety

After three weeks one plant showed symptoms of stunting and small leaves. The other four plants showed similar symptoms 37 days after sowing.

After two month, four plants displayed symptoms of wilt, yellowing and bronzing of the leaves. After 3 month, the four plants completely wilted and dried.

4.5.2.3 On Draga potato

4.5.2.3.1 Disease symptoms

After inoculation wilt symptoms appeared in one plant. After one week the leaves of 8 of the inoculated plants turned pale green then yellow and finally bronze in color. The old leaves of infected plants dried and eventually shed out. Results of pathogenicity test are presented in Table 1 and Plate 6.
Plate 6. Wilted artificially inculcated potato plants
Right wilted plants left healthy
Table 1: percentage of wilted Draga variety plants inoculated with *Ralstonia solanacearum*

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Non Inoculated plants</th>
<th>% Wilting</th>
<th>Inoculated plants</th>
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<tbody>
<tr>
<td>1</td>
<td>0%</td>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>2</td>
<td>0%</td>
<td></td>
<td>40%</td>
</tr>
<tr>
<td>3</td>
<td>0%</td>
<td></td>
<td>60%</td>
</tr>
<tr>
<td>4</td>
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<td>5</td>
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<td>6</td>
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<td></td>
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</tr>
<tr>
<td>7</td>
<td>30%</td>
<td></td>
<td>90%</td>
</tr>
<tr>
<td>8</td>
<td>30%</td>
<td></td>
<td>90%</td>
</tr>
</tbody>
</table>
4.5.2.3.2 The effect of infection on Draga plants height

Inoculation of Draga potato variety with *R. solanacearum* reduced plant height compared to the control plants Table 2. Five weeks post inoculation the plant height was reduced by 13.6%. As from week six no comparison was possible as severe wilting was observed in the inoculated plants and 90% of the inoculated plants wilted Plate 3. Reisolation of the bacterium from wilted plants confirmed pathogencity.

4.5.3 Effect of temperature in persistence of the bacteria in the soil

Neither the potato plants grown on infested soil nor the ones grown on non-infested soil showed disease symptoms Plate 9. The average plant height of plants was 12.62 and 11.66 cm for infested soil and non infested soil respectively. No bacterium was re-isolated from the plants. The average maximum and minimum temperature during this period is presented in Annex 1.
Plate 7. Potato variety (TPS6) grown on infested soil (Right) and non-infested soil (left)
Annex 1: The Republic of Sudan Ministry of Science and Technology
Metrological authority Temperature (year 2006/2007)

<table>
<thead>
<tr>
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<tr>
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<td>45.9</td>
<td>45.5</td>
<td>40.0</td>
<td>41.0</td>
<td>43.3</td>
<td>40.5</td>
<td>41.0</td>
<td>39.5</td>
<td>37.3</td>
<td>38.4</td>
<td></td>
</tr>
<tr>
<td>min</td>
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<td>24.0</td>
<td>22.5</td>
<td>22.0</td>
<td>20.0</td>
<td>21.2</td>
<td>20.0</td>
<td>18.5</td>
<td>14.0</td>
<td>11.5</td>
<td>9.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Average height (cm) of potato plants Variety Draga inoculated with *Ralstonia Solanacearum*.

<table>
<thead>
<tr>
<th>No. of Weeks post inoculation</th>
<th>average height (cm) of the inoculated plants</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Inoculated Plants</td>
<td>37.1</td>
</tr>
<tr>
<td>Non inoculated Plants</td>
<td>37.3</td>
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</table>
The detection of *Ralstonia solanacearum* on potato imported from France and recently from Germany revealed that risk of introduction into the areas free from this bacterium is high. The bacterium was also isolated from the local variety Zalinge. The occurrence of the bacterium on zalinge stocks have been identified earlier by Rehan, 19996. Zalinge stocks were reported to have high load (30 to 80%) of brown rot (Rehan, 19996). This due to their inferior quality of potato and the fact that they are infected with *Rhizoctonia solani* and loaded with viruses (El Hassan and Ibrahim, 1989) these stocks were never used as seed potato outside Jebel Mara area. This resulted in fact that the bacterium was not reported from other potato growing areas.

The pathogenicity of the bacterium isolated from the French seed potato was established by growing Draga variety on sterilized soil and inoculation with the bacterium. It was verified by the typical symptoms produced and re-isolation of the bacterium from infected plants. Susceptibility of Draga variety to bacterium was previously confirmed by Rehan, 1996 using Zalinge isolate.

This study revealed that the bacterium *Ralstonia solanacearum* might not over summer in central Sudan environmental conditions where
the average maximum temperature reached 46°C. The survival experiments of the bacterium in the soil with the different soil were conducted twice at two different temperatures 28°C and 15°C, to approximate temperature conditions in subtropical and temperate climates, respectively (Messiha 2006). The data obtained confirmed that race 3 biovar 2 is adapted to the lower soil temperatures occurring in temperate regions. Race 3 biovar 2 is most severe between 24-35°C with optimal temperature of 27°C and decreases in virulence when temperature exceed 35°C or fall below 10°C (Stansbury et al., 2001). The average maximum temperature of Khartoum State is over 37°C for ten months of the year. This might explain the result that potato plants grown in infested soil remained healthy for 8 weeks and the survival of Zalinge isolate in Gebel Mara area where the temperature is much lower because of the high elevation of the area (3042 m) confirm these results The average maximum temperature during autumn which is the growing season of potato in the area.usually 15-19°C.
5-2 PEST RISK ANALYSIS (PRA)

5.2.1 Assessment of the probability of introduction and spread of *Ralstonia solanacearum*

5.2.1.1 The PRA area

Areas of high production include Khartoum and River Nile States. The area around Khartoum, the capital of the Sudan, accounts for over 70% of the country's potato production (Geneif, 1986). Located at the confluence of the Blue and White Niles, Khartoum receives less than 300 mm of rain annually, practically all of it from May to October. Most production occurs on small farms of 0.25 to 5 hectares, mainly for subsistence and for sale in the capital (ARC, 1985).

5.2.1.2 Probability of entry of the pest

Seed potato is mainly imported from the Netherlands but recently it was imported from France. According the European Plant Protection Organization (EPPO) isolated incidents were reported from France in 1995. The present interception confirmed the presence of the pest in France. As for the Netherlands isolated incidents were reported in the early 1990s and several outbreaks in 1995. The disease was not intercepted in seed potato imported from the Netherlands in the decade.
Introduction of ware potato from Egypt, Saudi Arabia, Ethiopia, Uganda, Kenya and Zaire can be a source of infection to the PRA area.

5.2.1.3 Identification of pathways

This study identifies:

(i) Plant material intended for planting (seed potato)

(ii) Private individuals carrying infected material, and

(iii) Soil associated with both seed and ware potato is identified as potential pathways for the entry of the pathogen into the PRA area. Only the pathways (i) and (ii) are analyzed in the pest risk assessment as, according to the document, the current regulations ban the import of soil.

This study considers that seed potato is the major pathway for the entry of the pathogen into the PRA area, especially in the case of latently infected (asymptomatic) tubers. The study considers that illegal entry by private individuals of potentially infected host plant material intended for planting or consumption could also be a potential pathway for the entry of the pathogen into the PRA area. Interceptions of illegally introduced ware potato are documented from the borders with Ethiopia, Eritrea and Egypt.

The current import ban on soil makes the probability of entry of the pathogen through soil material negligible as soil could represent a potential pathway for the entry of the pathogen into the PRA area.

5.2.1.4 Probability of the pest being associated with the pathway at origin

*Ralstonia. solanacearum* race 3 is classified by the Crop Protection Compendium as present in France according to the data from CAB

Janse and Wenneker (2002) reported that as a result of an extensive monitoring and control policy, the number of brown rot cases in the Netherlands has been reduced with approximately 75% over the past decade (1990 - 2000). However they reported that the intended complete eradication has still not been achieved and that the knowledge about the importance of several risk factors on brown rot prevalence is still poor.

In 2003 USDA/APHIS/PPQ, Center for Plant Health Science and Technology, Plant Epidemiology and Risk Analysis Laboratory in Raleigh, NC Pest Data Sheet listed the Netherlands as infested.

5.2.1.5 Probability of survival during transport or storage

The probability of survival of the pathogen during transport or storage is high. Potato seeds are usually transported and stored under cool conditions after curing, potatoes for seed or table stock are held at 38 and 40 F. (2 and 4°C) respectively), which do not affect the survival of the pathogen harbored inside the tuber.

5.2.1.6 Probability of pest surviving existing pest management procedures

Many trials have been carried out all over the world to control the disease without much success. No promising control of brown rot was
achieved using antibiotics (Habashy et al., 1993), soil fumigants (Weingartner and Shumaker, 1988), chemical control (Murakoshi and Takahashi, 1984) or breeding of resistant varieties (Hartman and Elphinstone, 1994; Mendoza, 1994; Fock et al., 2001; Lopez and Biosca, 2004). Moreover chemical control becomes much less an option due to the increasing demand for low-input and organically produced products since the last ten years because of fear of the hazardous effects of pesticides and chemical residues (Sylvander and Le Floc’h-Wadel, 2000; Parrott and Kalibwani, 2004).

The probability of the pest surviving pest management procedure was studied using the EPPO case. It revealed that the only way to control the bacterium is exclusion of infested area from production. Brown rot caused by *R. solanacearum* biovar 2, race 3 was found in the Netherlands for the first time in 1992 in one field of ware potatoes in the south of the country. The field was planted with uncertified seed of unknown origin. In the same year brown rot infections were found in Belgium and the UK, all in ware potatoes. In all of these cases no clonal links could be established. In September 1995, after two unusually warm summers the disease was found again, now in seed, of a local variety. The Plant Protection Service (PPS) of the Netherlands started a survey to trace the origin of the infection and a testing of all traded (national and export) seed, some 55,000 samples. The agreed EPPO method (Bulletin OEPP/EPPO Bulletin 20, 255-262, 1990) was used. 200 Samples of tubers/25 ton were sampled by extracting of heel ends, screened with immuno-fluorescence (IF) and confirmation of IF-positive cases with a host test on tomato or plating on selective medium in addition to
identification of the bacterium using IF, fatty acid analysis and a host test with pure culture. It was found that mainly one seed line (grower number) of the local variety was heavily infected and spread the disease to various locations in the country. In a number of cases no clonal links could be established and contamination by surface water, used for overhead irrigation during the warm summer remained the only explanation. When a small survey was initiated, in the neighborhood of these farms the bacterium was detected in water as well as in bittersweet (*Solanum dulcamara*), a solanaceous weed growing with its roots floating in water. In total 94 places of production were found to be contaminated, they were excluded from seed production.

In the same year France reported also a case of brown rot, where water apparently played a role. In 1996 again all seed to be traded was tested and now only 9 places of production were found to be contaminated. Again these were excluded from seed production. In the same year an extensive survey (15,000 samples) of surface water was performed. The bacterium was detected especially in the north of the country, explaining all cases where no clonal links were established.

Zones were determined where irrigation with surface water is forbidden. In this year it became clear that also in France, Belgium and the UK water played a dominant role and that there is a possible link to sewage plants of the potato processing industries and municipalities. Infections in the Netherlands and other Western-European countries may have been caused at some time in the past were by contamination of water by infected waste of consumption potatoes imported from
Mediterranean countries where the disease occurs. The Netherlands then affected a ban on the import of ware potato from infested plant material,

The pathogen is known to cause latent infections. Therefore, latently (asymptomatic) infected seed and ware potato most probably go undetected at border inspection. Based on the above, the study considers that the probability of the pathogen surviving existing management procedures is high.

5.2.1.7 Probability of transfer to a suitable host

*Ralstonia solanacearum* as a species has an extremely wide host range, but different pathogenic varieties (races) within the species may show more restricted host ranges. Over 200 species, especially tropical and subtropical crops, are susceptible to one or other of the races of *R. solanacearum*. Worldwide, the most important are: *Lycopersicon esculentum* (tomato), *Nicotiana tabacum* (tobacco), *Solanum melongena* (aubergine), *Solanum tuberosum* (potato), *Musa* spp. (banana), *Musa paradisiaca* (plantain) and *Heliconia* spp (ornamental plants). Within the EPPO region, race 3 has a limited host range including potato, tomato and the weed *Solanum dulcamara*, *S. nigrum* is considered to have potential for spread. Recently it has been found that race 3 also causes a wilting disease in geraniums (*Pelargonium* species). Audits conducted by countries such as the Netherlands and the United States have shown that geraniums imported from certain countries may be a potential pathway for introduction of this bacterium.

Of the above pathways of entry, the probability of transfer of the pathogen to a suitable host in the PRA area is high. The pathogen would
be able to spread from infected to neighbouring healthy host plants on cutting tools, surface water and soil adherent to shoes, machinery, etc.

The risk associated with infected ware potato is mainly with the waste (e.g. domestic waste, waste from chips industry etc) and its management.

Various means of transfer of soil-borne pathogens with soil are documented (e.g. surface water, shoes, farm machinery, etc) (Agrios, 2005). The probability of transfer of \textit{R. solanacearum} race 2 to a suitable host with soil is high.

\textbf{5.2.1.8 Conclusion on the probability of entry.}

The study concludes that the probability of entry of the pathogen into the PRA area is high on the conventional plant propagation material.

\textbf{5.2.1.9 Probability of establishment}

\textbf{5.2.1.9.1 Survival in the soil}

\textit{Ralstonia solancerum} is a soil-borne bacterium. In warmer climates research has shown that the bacterium is able to survive for extended periods in deep soil layers, in sheltered sites such as the roots of alternate hosts (e.g., weeds), in infected plant debris, or in volunteer potato tubers from previous crops. In temperate climates, survival of the bacterium in soil (i.e., without a host) for long periods is not considered very likely. However, studies in temperate Europe have shown that overwintering of the bacterium can occur in potato volunteers, or in the roots of certain weeds, mainly \textit{Solanum dulcamara}. 

5.2.1.10 Probability of Spread

The movement of the bacterium on cutting knives, in soil, or in contaminated irrigation water, may lead to rapid spread of the bacterium. The pathogen can, however, also be introduced into an area by planting infected potato tubers and it can be disseminated, and survive in contaminated surface water (Janse, 1996; Persson, 1998; Schans and Steeghs, 1998; Wenneker et al., 1998) and weed hosts such as *Solanum dulcamara*, *S. nigrum*, *Portulaca oleracea* in Europe (Elphinstone et al., 1998) *Rumex dentatus* and *Solanum nigrum* in Egypt (Farag et al., 2004). In most European findings, first introduction appeared not via seed but via surface water that became contaminated by industrial or household waste where infected potatoes were used. Volunteer plants or perennial weeds in temperate climates, weeds can be a reservoir and responsible for transmission of the pathogen through successive seasons (Janse, 1996; Lopez and Biosca, 2004). The pathogen can persist for a long time in soil, in infected host plant debris or by colonizing potato volunteer plants, alternative hosts or even non-host plants (Graham et al., 1979; Granada and Sequeira, 1983; Akiew and Trevorrow, 1994). When the disease establishes in a growing area in temperate zones it can survive for periods between 12 months to 3 years in the absence of a potato crop as shown for New South Wales in Australia (Graham et al., 1979). It is therefore crucial to understand the ecology of the organism and the factors that affect its survival and suppression in different soil types and climates.
5.2.1.11 Ability to survive in the soil

It was found that *R. solanacearum* survived least in loamy soil with relatively high organic matter content (4%) whilst survival was highest in soil with lower organic matter content of 2.0-2.5% (van Elsas *et al*., 2001). This was related to composition and/or activity of the soil micro biota by enhancing natural biological control capacity (van Elsas *et al*., 2005).

The soils where potatoes are generally grown in Sudan are of alluvial origin, ranging in texture from heavy clays to lighter silty and sandy loam. The silty soils are generally preferred for potato cultivation. In sandy and clay soil, with and without amendments, the pathogen was below detection limits (10^2 CFU g^-1 d.w. soil) within 5 months (Messiha 2006). The decline in colony forming units of *R. solanacearum* per g of soil was faster in sandy soils from either country than in clay soils from both countries.

The survival experiments with the different soils were conducted twice at two different temperatures 28°C and 15°C, to approximate temperature conditions in subtropical and temperate climates, respectively (Messiha 2006). The data obtained confirmed that race 3 biovar II is adapted to the lower soil temperatures occurring in temperate regions. Race 3 biovar 2 is most severe between 24-35°C (optimal temperature of 27°C) and decreases in virulence when temperatures exceed 35°C or fall below 10°C (Stansbury *et al*., 2001). The average maximum temperature of Khartoum State is over 37°C for ten months of the year and over 34°C for the remaining two months (December and
January). This might explain the result that potato plants grown in infested soil remained healthy for 8 weeks.

Messiha, 2006 also suggested that the low nutrient availability and high competition for nutrients in the soil samples used in her study may explain the failure of the pathogen to maintain itself for longer periods. This suggestion is supported by the longer survival periods in the Dutch soils used in this study, which had higher concentrations of dissolved organic carbon concentration (DOC) than Egyptian soils, indicative of higher nutrient availability and better survival chances for *R. solanacearum* (Messiha, 2006). The DOC concentrations were particularly low in the Egyptian sandy soil where the pathogen dropped to undetectable levels within 1-2 months, especially in the organically managed soil with a relatively high microbial activity and diversity.

Michel and Mew (1998) showed that a high pH (similar to the pH in the Egyptian soils) may have a deleterious effect on *R. solanacearum*. In addition, soil pH affects the availability of soil ions: a higher pH restricts the availability of many soil nutrients. Positive correlations were found between the availability of potassium, sodium, phosphorus, and nitrogen respectively and the 50%-reduction time and negative rate of decline of the pathogen, implying a slower decline at higher concentrations of these nutrients. Indeed, the soil with the highest (most negative) decline rate had the lowest available nutrient contents and the highest pH. However, these correlations may simply be coincidental, and are difficult to interpret.
5.2.2 Assessment of potential economic consequences

5.2.2.1 Direct pest effects

Production of potato in Sudan is around 336,000 tons in 2004, (Plant quarantine directorate personal communication 2007) this figure has increased considerably as farmers of the northern region are growing considerable area of potato. Serious yield and/or quality losses still occur in smallholdings, where pest and disease control measures are usually not undertaken due to their high cost. In the Netherlands the infected fields are excluded from production. When the farmer wants to return to seed production, he cannot grow potatoes on the infected field for 5 years. In the first 3 years he it only allowed either to have bare fallow with weeds and volunteer plants control as pasture or to plant cereals excluding corn. This process takes place under the supervision of the Plant Protection Service.

5.2.2.2 Social consequences

Potato industry provides direct and indirect jobs in the PRA areas. The additional control costs due to bacterial wilt may reduce the competitiveness of potato industry in the PRA area. As a result, employment may be reduced, causing negative social impacts. Since the disease is not readily controllable in smallholdings and private gardens, high yield losses and a potential disruption of subsistence production and consumption patterns would occur. This may also cause negative social impacts. As holdings are generally small (Geneif, 1986; ARC, 1985) the adverse social effects will be very high.
5.2.2.3 Environmental consequences

The environmental consequences will stem from the fact that the bacterium was well known to spread by water. Water will spread the pathogen to all known host and unknown host plants.

5.2.3 Conclusions of the pest risk assessment

5.2.3.1 Presence or absence in PRA area

*Ralstonia. solanacearum* was not reported from potato growing area in central Sudan.

5.2.3.2 Regulatory status in PRA area

*Ralstonia. solanacearum* race 3 is considered as a quarantine pest in the PRA area.

5.2.3.4 Potential for establishment and spread in PRA area

- The pathogen has a high potential for establishment and spread. Host plants of the genera *Solanum tuberosum* (potato), *Lycopersicon* (tomato), *Capsicum sp.* (peper), *S. nigrum*, *Portulaca sp.* and *Pelargonium* (geranium) are present in plantations, nurseries, private gardens and in the wild in the PRA area.

- Weeds that act as hosts and/or reservoir plants (*e.g. Solanum nigrum and Portulaca sp. are present in the PRA area*).

- The pathogen may spread in the PRA area by various means, such as infected plant propagation material, contaminated cutting tools and farm machinery, contaminated soil, surface water and root-to-root contact (Thwaites *et al.*, 2000).
The current study revealed that the pathogen did not manage to over-summer under Sudan conditions. van Elsas et al. 2001 reported that the major effect of temperature was found, with survival being maximal at 12°C, 20°C, and 28°C. Temperatures of 4°C, 36°C, or 44°C induced accelerated declines of the culturable cell numbers. The pathogen can evade from infected plants, survive in the soil environment (plant debris, reservoir plants, etc.) and cause disease through root infection.

5.2.8 Potential for economic consequences in PRA area

The establishment of the disease would affect potato and tomato production and marketing. Then *R. solanacearum* race 3 is considered to have a potential for economic consequences in the PRA. Nevertheless, the marketability of potato could be reduced and future export producing countries could be blocked.

5.2.9 Conclusion of pest categorization

*R. solanacearum* race 2 is not known to occur in the PRA area, has a potential for introduction and spread. The document concludes that *R. solanacearum* race 3 should be classified as a quarantine organism for the endangered area.

5.2.10.1 Risk Management Options

Seed Potato should be imported from pest free areas only.
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