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Marie Fiers

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UNIVERSITE DE BOURGOGNE
Unité Mixte de Recherche Microbiologie du Sol et de l'Environnement

THESE

Pour obtenir le grade de

Docteur

Discipline: Ecologie microbienne

par

Marie FIERS

le 21 Juin 2010

Origine des altérations superficielles du tubercule de pomme de terre:

De la microbiologie du sol à l'environnement pédo-climatique

Co-directeurs: Christian STEINBERG, Catherine CHATOT, Véronique EDEL-HERMANN et Yves LE HINGRAT

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Prof. Monica HÖFTE	Université de Gand, Belgique	Rapporteur
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Presented by

Marie FIERS

on 21st June 2010

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Supervisors: Christian STEINBERG, Catherine CHATOT, Véronique EDEL-HERMANN and Yves LE HINGRAT

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Prof. Monica HÖFTE	University of Gent, Belgium	Reviewer
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La science ouvre à l'esprit humain une voie infinie, et le lance, par une série d'étapes sans nombre, sur l'Asymptote de la Vérité.

Paul Bert (1933 – 1986)

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Résumé

La qualité de présentation d'une pomme de terre de consommation, *Solanum tuberosum* L., commercialisée en produit frais est devenue une exigence et un enjeu économique significatif dans les relations commerciales. Compte tenu du mode de reproduction par voie végétative de cette espèce, ces exigences sont également imposées au tubercule de semence. Organe de réserve et de propagation, le tubercule est produit sous terre, ce qui l'expose aux microorganismes telluriques et le rend potentiellement porteur d'altérations superficielles dont l'origine n'est pas encore clairement identifiée pour certaines d'entre elles.

L'objectif de ce travail est de recenser et de caractériser ces altérations superficielles du tubercule et d'en déterminer les causes. Après l'établissement d'une nomenclature et d'une classification consensuelles des défauts observables sur tubercule, deux hypothèses ont été formulées et testées: (1) les défauts sont d'origine pathogène et/ou (2) ils résultent d'une réponse de la plante à des stress environnementaux.

L'évaluation de la première hypothèse a mené à l'identification d'une grande diversité de microorganismes vivant à la surface des tubercules altérés. Leur pouvoir pathogène a été testé par une série de tests biologiques. Ceux-ci ont permis de reproduire les défauts sur des tubercules néoformés et de vérifier les postulats de Koch pour le champignon *Rhizoctonia solani* responsable de la formation de sclérotes. Pour bon nombre d'autres défauts visuels, aucune relation claire entre un microorganisme et une altération n'a pu être établie. Une étude de la structure des communautés microbiennes de la géocaulosphère de tubercules, altérés ou non, a démontré que les communautés fongiques et bactériennes se comportaient selon des dynamiques différentes au cours de la culture et en fonction de l'état sanitaire du tubercule de semence mais aucune relation de causalité n'a pu être mise en évidence. Il a par contre été noté une augmentation de la population de *R. solani* autour des tubercules altérés. La diversité des isolats d'origine française et européenne associés aux altérations des tubercules a donc été caractérisée. Elle révèle l'existence de relations phylogénétiques indépendantes de l'origine géographique et du cultivar hôte et suggère l'existence de d'événements génétiques fréquents et d'un brassage génétique entre les populations de *R. solani*. Le test de la seconde hypothèse a consisté à rechercher une implication potentielle de différents facteurs abiotiques dans la formation des altérations superficielles. L'analyse d'une enquête menée auprès d'agriculteurs a mis en évidence l'implication du pH, de certaines pratiques culturales, de la sensibilité de cultivars et de conditions météorologiques particulières dans l'occurrence de certains défauts.

Ce travail a permis de clarifier la nomenclature des altérations, de confirmer l'implication de *R. solani* dans l'apparition de certaines d'entre elles et d'envisager de nouvelles hypothèses quant à la formation de défauts suite à une réponse de la plante à un stress environnemental. Ainsi, une voie a été ouverte vers la résolution de la problématique posée par toute une filière professionnelle, responsable de la mise en marché d'un produit frais de grande consommation, et répondant aux exigences du marché en matière de présentation, qualité culinaire et, mode de production respectueux de l'environnement.

Mots clé: pomme de terre, tubercule, défaut superficiel, microorganisme, stress environnemental, pratique culturale, communauté microbienne, *Rhizoctonia solani*.

Abstract

The visual quality of fresh potatoes, *Solanum tuberosum*, became a dominant criterion and a significative economical issue in potato market. According the vegetative reproduction of this species, requirements for visual quality are also needed for potato seed tubers. As an organ for reserve and propagation, the tuber grows underground and is in contact with soil-borne microorganisms, making it potentially exposed to blemishes, for the majority of which the origin is still unclear.
The objective of this work is to make an inventory of those tuber blemishes, to characterize them and determine their causes. After the establishment of consensual nomenclature and classification of the blemishes, two hypotheses were formulated: (1) blemishes are due to pathogenic attacks and/or (2) they result from a response of the plants to environmental stresses.
The assessment of the first hypothesis allowed identifying a wide diversity of microorganisms living on the blemished tuber surface. Their pathogenicity was tested by several biological assays that allowed producing blemishes on progeny tubers and fulfilling the Koch's postulates for the fungus *Rhizoctonia solani* causing sclerotia. For many other blemishes no clear relationship was established between a microorganism and a blemish. A study of the microbial structure of the geocaulosphere of tubers blemished or not, showed that bacterial and fungal communities adopted different dynamics during the growing season and according to the sanitary status of the seed tuber, but no causality link could have been drawn. On the other hand, an increase of *R. solani* population around blemished tubers was observed. The diversity of strains of *R. solani* originating from France and from Europe and associated to the blemished tubers was characterized. The phylogenetic relationships between the strains were independent of the geographical origin and of the host cultivar, thus the existence of frequent genetic events and genetic mixing between the populations of *R. solani* was suggested.
Concerning the potential implication of different abiotic factors, a survey conducted with farmers showed the implication of soil pH, some cultural practices, including the choice of the susceptible cultivars and meteorological conditions on the occurrence of some blemishes.
This work made clear the blemish nomenclature, confirmed the implication of *R. solani* in the occurrence of some blemishes and suggested new hypotheses concerning the occurrence of blemishes as a plant response to a stressful environment. Thus, a path was opened toward the resolution of the issue asked by all the potato community, responsible for the marketing of a mass consumption fresh product and answering to market requirements related to visual and culinary qualities and to environmental friendly modes of production.

Keywords: potato, tuber, blemish, microorganism, environmental stress, cultural practice, microbial community, *Rhizoctonia solani*.

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Preface

Preface

Potato (*Solanum tuberosum*) originated in the Andes Mountains of South America where Andeans people used to eat it since millennia. Recent evidence suggests that potato was domesticated 10,000 years ago near Titicaca Lake, in an area that is now the border between Bolivia and Peru, and where the greatest diversity of wild species is still to be found. First record of potato in Europe dates back in 1573 in Spain but it is only in the early seventeenth century that it becomes an important food and came back across the Atlantic ocean to North America (Stevenson *et al.*, 2001).

Potato crop is the fourth main food crop in the world after maize, rice and wheat with 325 million tons produced in 2007 (Table 0.1). This is also the world's number one non-grain food commodity and is integrated in the international food system. Despite potato trading is not as well developed as cereal trading on the global markets, efforts are made to promote potato spread all over the world. The year 2008 was declared international year of the potato by the FAO (Food and Agriculture Organization). Indeed, potato can help fulfilling the first ONU's millennium development goal that aims at eradicating extreme poverty and hunger in the world. The potato produces more nutritious food more quickly, on less land, and in harsher climates than any other major crop. Up to 85 percent of the plant is edible human food, compared to around 50 % in cereals. Potatoes have good gustative and nutritional qualities; they are rich in carbohydrates, making them a good source of energy. A single medium-sized potato contains about half the vitamin C recommended daily intake - and contains a fifth of the recommended daily value of potassium (FAO, 2008).

Table 0.1 Potato production by area in 2007

	Harvested surface (ha)	**Quantity (tonnes)**	**Yield (tonnes/ha)**
Africa	1 541 498	16 706 573	10,8
Asia and Oceania	8 732 961	137 343 664	15,7
Europe (including Russia)	7 473 628	130 223 960	17,4
South America	963 766	15 682 943	16,3
North America	615 878	25 345 305	41,2
WORLD	**19 327 731**	**325 302 445**	**16,8**

Potatoes are grown in more than 100 countries, mainly in Asia (135 million tons) and Europe (130 million tons) (Table 1) (FAO, 2008), under temperate, subtropical and tropical conditions. This crop is from an agronomical point of view well suited to places where land is limited and labour can be abundant, conditions that characterize much of the developing world, although it is essentially a "cool weather crop". For that reason, potatoes are planted in early spring in temperate zones and late winter in warmer regions, and grown during the coolest months of the year in hot tropical climates. In some sub-tropical highlands, mild temperatures and high solar radiation allow farmers to grow potatoes throughout the year, and harvest tubers within 90 days of planting. In temperate climates, such as in northern Europe, it can take up to 150 days (FAO, 2008). As well as being a staple food, potato is also processed into French fries, crisps and is used for dried products and starch production. In some countries, potato is still used for animal feeding but this use is decreasing. In many countries in Asia, Africa, Central and South America, there is a need for high and stable potato production to meet increasing food demands from human population growth during the current period of environmental (including climate) change (Vreugdenhil, 2007).

Over the last 10 years, world potato production has increased at an annual average rate of 4.5 percent especially in developing countries. Potato consumption is declining in Europe and is increasing in developing world with a global average of 31.3 kg per capita and per year. France is the tenth potato producer in the world with 6.3 million tons and French people consume about 40 kg of fresh potatoes and 25 kg of transformed products per capita and per year. Main French potato production areas are located in the North (Nord-Pas-de-Calais, Picardie and Champagne-Ardennes), West (Haute-Normandie and Bretagne) and Center (Centre) of France. Total cultivated surface represents 170 000 ha in which 100 000 ha are used for fresh potatoes production, 25 000 ha for early potatoes, 15 000 ha for seed potatoes and 30 000 ha for starch production (FAO, 2008).

At the European level, France has the leadership for fresh potato production and is the first exporting country. The exports are made toward other European countries among which Spain, Italy, Belgium, United Kingdom, Germany, The Netherlands and Greece are the main partners (CNIPT, 2010). France also imports 116 000 tons of

fresh potatoes (mostly for processing) and a reduced volume of early potatoes coming from non EU-countries like Egypt, Israel and Morocco. Concerning seed potato production, France ranks at the third level after the Netherlands and Germany. The annual export volumes are between 80,000 to 100,000 tons towards European Union (Spain, The Netherlands, Belgium, Portugal, Italy, Greece, Germany, United Kingdom, and central Europe). Other destinations are North Africa, i.e. Algeria, Egypt, Morocco, and Tunisia and the Middle East (FNPPPT and GNIS, 2007). Professional seed organizations such as Bretagne Plants in Brittany, Comité Nord in the North and Grocep in the Center of France are also responsible for breeding new cultivars. Germicopa, located in Brittany, is one of the oldest European private potato breeder, seed producer and trader. All breeding companies select interesting traits of plants and aim at creating new hybrids that integrate as much positive traits as possible.

In the North-West zone of Europe, fresh potato consumption per capita has been progressively declining in the past twenty years. Recent studies about consumer's behavior concerning fresh potato acquisition showed that visual quality - shape and smooth aspect of the tubers - was the first choice criterion (59%) well before taste or culinary use. Soil particles on tuber surface may not stimulate the majority of the consumers to buy potatoes which look like dirty and have a poverty connotation. However, contradictory is the situation in the organic market where soil aspect of tubers may represent the authentic and natural connotation of the product (Danielle Rapoport Conseil, 2007; CNIPT and TNS-Sofres, 2008). In order to stimulate interest for the fresh product and satisfy clients' expectation, fresh potato professionals have put forward several inciting actions like washing potatoes before being conditioned. But this action has had a major drawback: it highlighted tuber blemishes previously hidden by the soil residues and lead to the downgrading of a significant part of the commodity.

Indeed, potatoes – whether they are food or seed- are submitted to numerous diseases caused by fungi, bacteria, nematodes or viruses. Most of them can affect all parts of the plant: foliage, berries tubers and root system, as well as other Solanaceous hosts. As a consequence, affected tubers used as seeds are a very powerful mean of further disease dispersal. Transportations of tubers between different countries are submitted to control and rules to avoid the spread of diseases. Sanitary quality levels are established for both branches of the potato industry, fresh

potatoes and seeds. In the certification scheme of seed potatoes, strict regulations for field and lot inspections are required for the sanitary quality depending on seed categories and the type of diseases. For example, tubers for basic seeds (class Elite) must have less than 5 % of their surface covered by symptoms of *R. solani* and if the disease percentage reaches 5 to 10 %, seeds are downgraded to lower categories (A or B). For quarantine diseases (EPPO, 2008b) such as cyst nematodes, wart, ring rot or brown rot, no symptoms nor parasitic agents are tolerated.

Because the potato crop is vegetatively propagated, the visual quality of tubers, intertwined with its sanitary status, is then a consecutive requirement for seed production since it is a pre-requisite for ware potato. Among superficial blemishes of the tuber, most of them are due to well known and studied pathogens but others, called atypical blemishes, have not yet known causes thus making cultural control very difficult and inefficient.

In order to help potato growers to better know the causes of these blemishes and find technical solutions for improving the potato quality, Bretagne Plants and Germicopa have launched a 3-year Program of Collaborative Research (PRC) with the financial support of the Regional Council of Brittany and in collaboration with the laboratory of Soil and Environmental Microbiology (MSE) at INRA (National Institute of Agronomical Research). The locally based industrial company, Végénov-Bretagne Biotechnologie Végétale (BBV), has also contributed to the project.

The objectives of this project are

- To propose clear and consensual terminology and classification of the different blemishes that can be observed on the tuber surface.
- To determine whether the blemishes are caused by microorganisms, abiotic factors, or both.
- To identify environmental friendly products controlling the blemishes and to optimize their use.

This manuscript presents the PhD work carried out to fulfill the two first objectives (Figure 0.1). The third one was treated by BBV.

A review of the well known pathogenic microorganisms of the potato is carried out. Data about the most favorable development conditions, their ecology and the methods used to detect and quantify them are necessary to set up efficient methods for disease control. Moreover, since the use of pesticides is limited in order to prevent environmental pollutions, the need of alternative control methods is

becoming stringent. A review of the current available bibliography is presented in the Chapter 1 of this manuscript. This chapter is to be submitted to Agronomy for Sustainable Development.

Two consecutive sampling campaigns were led to collect tubers presenting blemishes in the main French - seed as fresh - potato production areas. Typical and atypical blemishes on tuber surface were observed, named and classified in different categories. This classification is the subject of Chapter 2 and was submitted to Plant Pathology (Fiers *et al.*, submitted). Concerning the determination of the blemish causes, two hypotheses were suggested. First, the blemishes have a pathogenic origin. This hypothesis was tested by isolations of microorganisms living on the tuber surface and pathogenicity tests. This study appears in chapter 3 and is published in European Journal of Plant Pathology (Fiers *et al.*, 2010). In parallel, Chapter 4, submitted to FEMS Microbiology Ecology, treats of the effects of stresses caused by the microbial communities living in the nearest surroundings of the tubers, tested by molecular methods. As *Rhizoctonia solani* appeared as an indeniable component of the biotic factors, chapter 5 aims at characterizing at the intraspecific level the structure of the populations of *R. solani* in France, compared with those from other countries. The second hypothesis suggests that the blemishes result from environmental stresses leading to a reaction of the plant. A survey was carried out with several French potato producers to determine the soil traits of the fields and the cultural practices. The results are described and discussed in chapter 6. Eventually, the results of this work are discussed and prospects are suggested.

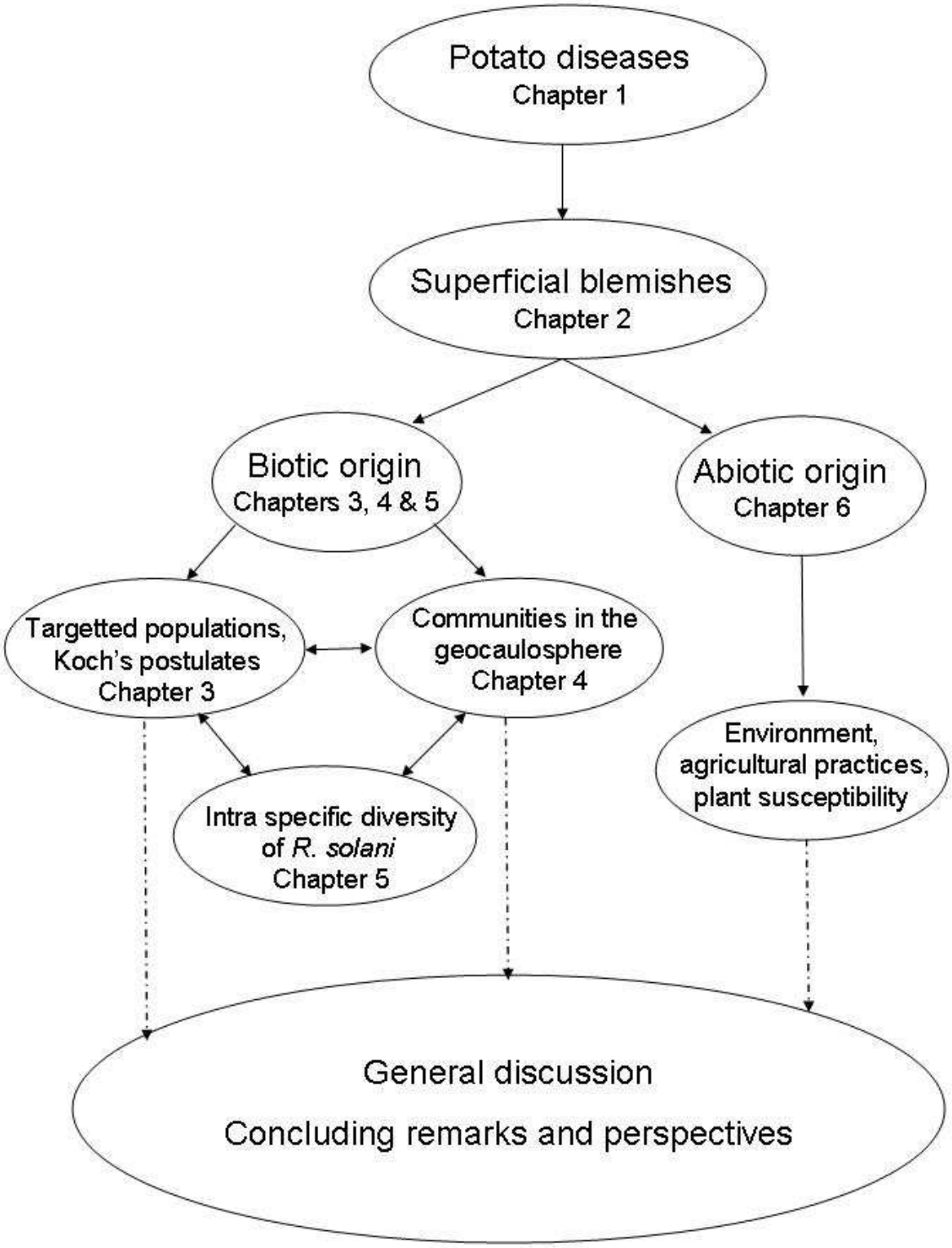

Figure 0.1 Diagram of the thesis outline

Chapter 1

Potato soil-borne diseases - A review

Chapter 1

Potato soil-borne diseases - A review

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Abstract

Potato crop is the fourth main food crop in the world and it will certainly feed a big part of the global population in the next years. The economical outlets for this crop are great; however, numerous diseases either soil-borne or air-borne can cause huge losses in the production. Worldwide about 40 soil-borne diseases affect potato and cause severe damages especially on tubers, the economically most important part of the plant. The occurrence and development of soil-borne diseases depend on very diverse factors affecting either the pathogen or the plant. Favourable conditions for potato disease development are frequently the same as the conditions needed for potato growth: temperature between 10℃ and 25℃, h igh humidity, medium pH etc. Adapted cultural practices such as a rotation longer than 4 years, minimum soil tillage, an adapted delay between dehaulming and harvest, dry and cool conditions for tuber storage are good ways to control potato diseases. In most cases, potato pathogens develop specific survival forms, dissemination ways and host penetration methods. The genetic variability of the pathogens implies the use of adapted diagnostic and control methods. Decision support systems developed to predict yield losses allow choosing good control methods such as the use of healthy seeds, adapted pesticides, cultural practices and biological control agents for each potato diseases. The complexity of the interactions between a pathogen and its host, influenced by biotic and abiotic factors of the environment, make the control of the diseases very difficult. However, deep knowledge of pathosystems allows setting up integrated pest management systems allowing the production of healthy and good quality potatoes.

Introduction

Potato crop, the world's number one non-grain food commodity, is the fourth main food crop in the world after maize, rice and wheat, with 325 million tons produced in 2007. Potatoes are grown in more than 100 countries, mainly in Asia (135 million tons) and Europe (130 million tons) (FAO, 2008). They have good gustative and nutritional qualities and can be grown under various climates. This is the reason why FAO (Food and Agriculture Organization) has declared the year 2008 the international year of the potato Indeed, potato can help fulfilling the first ONU's millennium development goal that aims at eradicating extreme poverty and hunger in the world. However, potato (*Solanum tuberosum*) crop can suffer more than 40 pests and diseases caused by insects, nematodes, viruses, bacteria and fungi. Those pathogens are air-borne or soil-borne and cause damages on all parts of the plant. In this review, we will focus on soil-borne fungi, bacteria and nematodes (Table 1.1, Figure 1.1).

Diseases caused by viruses or viroïds provoke generally foliar symptoms: leaf distortion, mosaic, crinkling, leaf and vein necroses, dwarfing and leaf rolling. Only some viruses - tobacco rattle virus (TRV), potato mop-top virus (PMTV), potato virus Y (PVY^{ntn}) and tobacco necrosis virus (TNV) - can cause damages on tubers such as blemishes or rots in tuber flesh (Table 1.1). They will not be considered in this review because they are not originating from soil-borne pathogens but depend on aphids, fungi or nematodes to be transmitted.

Soil-borne diseases affecting potato crop can be divided into two groups depending on symptoms: symptoms damaging tubers and those damaging other parts of the plant (Gudmestad *et al.*, 2007).

Diseases affecting stems or roots affect the crop development and may lead to a reduction of the yield (Table 1.1). Stem lesions can be watery and they develop to the stem pith with (stem rot) or without the formation of sclerotia (black leg, white mold). Other lesions can appear like more discrete light brown lesions but nevertheless they affect the yield of the crop (skin spot, stem canker). Soil-borne pathogens (*Phoma* leaf spot, *Verticillium* wilt) sometimes cause aerial symptoms like necroses or chloroses occasionally associated with wilting and rolling (bacterial ring

rot). Finally root lesions, mainly caused by nematodes feeding on the roots lead to necroses or rots. Nematodes feeding sites are good entry points for other soil microorganisms, which increase the destruction of the roots.

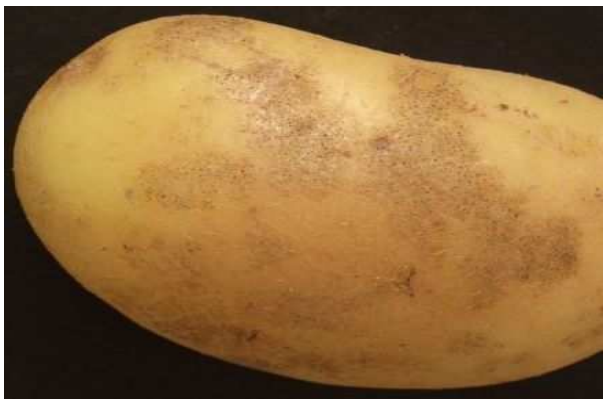
a

FNPPPT – Y. Le Hingrat

b

INRA – D. Mugniéry

c



FNPPPT – Y. Le Hingrat

d

FNPPPT – Y. Le Hingrat

Figure 1.1 Symptoms caused by some potato soil-borne diseases, a. Black leg (*Pectobacterium* spp.), b. Root-knot nematode (*Meloidogyne incognita*), c. Black dot (*Colletotrichum coccodes*), d. Potato virus Y (PVY).

Among diseases affecting tubers, symptoms can be divided into three categories: galls, blemishes and rots (Table 1.1). Galls causes outgrowth and tuber deformation. The most frequent galls are known as powdery scab, wart, common and netted scabs, root-knot nematode and false root-knot nematode.

Blemishes affect only the tuber skin but they became economically important since consumers' habits have changed and tubers are washed before selling. Blemishes can appear on the tuber surface as spots called black dot, black scurf, skin spot or powdery scab) or as areas of atypical appearance presenting a more or less

pronounced scabby (common scab) or silver (silver scurf) aspect. Rots, which affect the tuber flesh more deeply, include different types such as dry rots, soft rots (charcoal rot, leak, bacterial soft rot, black leg, and stem rot), flesh discoloration (pink rot) or vascular ring discoloration (ring rot, brown rot, *Verticillium* wilt, *Fusarium* dry rots). Dry rot diseases also damage stored potatoes.

Potato becoming a more and more important foodstuff in the world, it is essential to control diseases, which cause direct yield losses and decrease of farmer's incomes due to downgrading the quality of affected tubers. Therefore, knowledge about the pathogens as well as factors influencing disease severity is needed to set up efficient control strategies. Before reviewing the different causes of occurrence and development of the main soil-borne potato diseases it is important to recall the concepts of soil inoculum potential and soil suppressiveness which describe the complex interactions between the soil, the pathogens and the plant.

Plant diseases result from the compatible interactions between a susceptible host plant and a pathogen. These direct interactions are important but should not outshadow the key role of environmental factors, which influence these interactions and thereby disease incidence or severity. In contrast to aerial diseases, the soil-borne diseases are induced by pathogens which are embedded in the soil matrix. Thus, the soil interferes in many ways in the relationships between and among microorganisms, pathogens and host plant. It can even modify the interaction among microorganisms themselves. In some soils, disease incidence or severity commonly remain low in spite of the presence of the pathogen, a susceptible host plant and favourable climatic conditions. They are called disease suppressive soils (Messiha *et al.*, 2007; Steinberg *et al.*, 2007). Soil suppressiveness to diseases depends on the pathogen itself - its inoculum density and its intrinsic aggressiveness - and also on different soil factors, including both biotic and abiotic components.

In the first part of this paper, the influence of abiotic factors on disease severity will be reviewed. Then the characteristics of the inoculum and its relationships with the rest of the microbiota will be considered. Finally, risk assessment models, decision support systems and control strategies based on collected data will be discussed.

Table 1.1 Potato soil-borne pathogens.

Pathogen	Disease	Host range	Main symptoms						Pathogenicity test[a]	Distribution	Ref
			TUBERS			OTHER PARTS					
			Gall	Blemish	Rot	Stem lesions	Leaf lesions	Root lesions			
FUNGI & OOMYCETES											
Colletotrichum coccodes	Black dot	**Moderate**: 35 hosts from 13 families including *Cucurbitaceae*, *Fabaceae* and *Solanaceae*		X		X		X	X	Worldwide	4, 8
Fusarium spp.	Fusarium dry rots	*F. sambucinum:* **Wide:** potato, hop, leguminous plants, cereals *F. coeruleum:* **Wide**: potato, cereals and many other hosts			X				X	Worldwide	9
Helminthosporium solani	Silver scurf	**Potato**		X					X *	Worldwide	10
Macrophomina phaseolina	Charcoal rot	**Wide**: 284 recorded hosts both cultivated and wild			X					America, Europe, Asia	
Phoma andigena var. andina	Phoma leaf spot	**Narrow**: potato*, S. goniocalyx, S. medians, S. phureja*, tomato, solanaceous weeds					(X)			South America (Bolivia and Peru)	
Phoma spp.	Gangrene	*P. exigua* var. *exigua:* **Wide** *P. exigua* var. *foveata:* **Narrow**: Potato and some weeds			X				X	North America, Europe, Asia, Oceania	11
Phytophthora erythroseptica	Pink rot	**Narrow**: potato, tomato, spinach, and tulip			X				X	Worldwide	12
Polyscytalum pustulans	Skin spot	**Narrow**: Solanaceous species		X		X			X	Europe, North America, Oceania, Asia	13
Pythium ultimum var. *ultimum*	Leak	**Wide** including many crops			X				X	Worldwide	14, 30
Rhizoctonia solani AG3	Black scurf / Stem canker	**Narrow**: Solanaceous species		X		X			X	Worldwide	15
Rosellinia sp.	Rosellinia black rot	**Wide**: plants in over 63 genera in 30 families			X			(X)		South America, Africa	
Sclerotinia sclerotinium	White mold	**Wide**: approximately 400 species of dicots			X	X					
Sclerotium rolfsii	Stem rot	**Wide**: cultivated and wild plants including ferns			X	X			X*	Worldwide	16
Spongospora subterranea	Powdery scab (PMTV vector)	**Wide**: Solanaceous species, cabbage and related species	X	X				X	X	Worldwide	17, 28, 32
Synchytrium endobioticum	Wart	**Potato**	X						X *	Worldwide	18
Thecaphora solani	Thecaphora smut	**Narrow**: Solanaceous species, *Datura stramonium*	X		X				X	South America and Mexico	5, 27
Verticillium dahliae and *V. albo-atrum*	Verticillium wilt	*V. dahliae:* **Moderate**: artichoke, bell pepper, cabbage, cauliflower, chili pepper, cotton, eggplant, lettuce, mint, potato, strawberry, tomato, watermelon, etc *V. albo-atrum* **: Narrow**: alfalfa, hops, potato			X	X	X		X*	Worldwide	2, 19
BACTERIA											
Clavibacter michiganensis var. sepedonicum	Ring rot	**Narrow**: potato, sugar beet, tomato, eggplant			X		X		X	Worldwide	20
Clostridium spp.	Bacterial soft rot	**Wide**: animals and plants			X					Worldwide	

P. atrosepticum,P. carotovorum, Dickeya spp.	Black leg, soft rot	*Pectobacterium* spp. and *P. carotovorum*: **Wide:** potato, rapeseed, sugar beet, chicory witloof, carrots, radish, weeds *P. atroseptica:* **Narrow:** potato tomato, cabbage, weeds *Dickeya* spp.: potato, ornementals, maize, chicory witloof, tomato, weeds			X	X	X		X	Worldwide	21, 29, 33
Ralstonia solanacearum	Brown rot	**Wide**: plants in over 200 species in 28 families			X	X			X	Asia, Africa, South America (probably worldwide)	22
Streptomyces scabiei, *S. acidiscabiei*, *S. europeiscabiei*.	Common and netted scab	**Moderate**: potato, beets, radish, rutabaga, turnip, carrot, parsnips, etc.	X	X				X	X*	Worldwide	23, 31
NEMATODES											
Belonolaimus longicaudatus	Sting nematode	**Wide**: vegetables (carrot, corn, crucifers, beans, potato, etc.), fruits (citrus, strawberry, etc.), agronomic crops (cotton, peanut, sorghum, soybean, etc.), turf grasses and forest crops						X		North America	
Ditylenchus destructor, D.dipsacii	Potato rot nematode Stem and bulb nematode	**Wide**: almost all plants, feed also on soil fungi Potato, onions, pea, beans, rye			X			X		Europe, Africa, America	3
Globodera pallida, Globodera rostochiensis	Potato cyst nematode	**Narrow**: potato, tomato, eggplant, wild solanaceous weeds		X				X	X	Worldwide	3, 24
Meloidogyne spp.	Root-knot nematode	**Wide**: about 2000 species (Solanaceae, Cucurbitaceae, leguminous plants, carrots, scorsoneras, lettuces, chicory witloofs, artichokes, Swiss chards, celery, etc.	X					X	X	Worldwide	3, 25
Nacobbus aberrans	False root-knot nematode	**Wide**: potato, *Brassica oleracea*, *Capsicum*, carrots, cucumbers, lettuces, *Opuntia* spp. and other Cactaceae, sugarbeet, tomato, etc.	X					X		America	1,2,3
Paratrichodorus and *Trichodorus* spp.	Stubby-root nematode (TRV vector)	*Paratrichodorus* spp : **Wide**: alfalfa, azalea, boysenberry, vegetables, corn, tomato, potato, onion, wheat, sugarcane, rice, grasses, etc. *Trichodorus* spp.: **Wide**: trees, shrubs, crops, turf grasses		X				X		Europe, North America	
Pratylenchus spp.	Root-lesion nematode	**Wide**: a lot of fruit trees, some citrus fruits and cereals, ornamental plants, crops (potato and vine)						X	X	Worldwide	26
VIRUS	**Means of transmission**										
Tobacco Necrosis Virus (TNV)	Mechanical, *Olpidium brassicae*	**Narrow** : potato, tobacco, bean, tulip		X	X					Worldwide	2
Potato virus Y (PVY) ntn	Aphids, sap and contact	**Narrow** : potato, tomato, tobacco		X	X		X			Worldwide	2, 6, 7
Tobacco Rattle Virus (TRV)	Stubby-root nematodes	**Wide** : potato, gladiolus, lettuce, sugar beet, tobacco, tulip, etc.		X	X					Europe, Japan, New Zealand, North America, Russia	2, 7
Potato Mop-Top Virus (PMTV)	*Spongospora subterranea*	**Narrow** : mainly Solanaceous species		X	X	X	X			Andean region, Canada, China, North Europe, Japan	2, 7

1. Inserra et al. 2005 **2**. Stevenson et al. 2001 **3**.Vreugdenhil 2007 **4**. Tsror 2004 **5**. Mordue, 1988 **6**. Ghazala & Varrelmann, 2007 **7**. FNPPPT, GNIS, 2000 **8**. Aqeel et al., 2008 **9**..Peters et al., 2008a **10**. Cunha & Rizzo, 2004
11. Bain et al., 1987 **12**. Vico et al., 1997 **13**. Perez et al., 1994 **14**. Peters et al., 2004 **15**. Woodhall et al., 2008 **16**. Garibaldi et al., 2006 **17**. Nakayama et al., 2003 **18**. Hampson et al., 1997 **19**. Ochiai et al., 2008 **20**.Nissinen, 2000
21. Franco Cardoza et al., 2007 **22.** Park et al., 2007 **23**. Lambert et al., 2006 **24**. Pylypenko et al., 2008 **25**. Vovlas et al., 2005 **26**. France & Brodie, 1995 **27**. Andrade et al., 2004 **28**. Hims &Preece, 1975 **29**. Bradbury, 1977 **30**.Stamps, 1978
31. Zhao et al., 2008 **32**. Merz & Falloon, 2009 33. Hélias, 2008

a Stars mean that the Koch's postulates have been fulfilled for those pathogens.

I. Effects of abiotic factors on occurrence and development of soil-borne potato diseases

Soil abiotic components such as texture, organic matter content, pH as well as temperature and moisture greatly affect the behaviour of the pathogens and determine disease incidence or severity.

I. 1. Soil temperature

Temperature and moisture of the soil are obviously greatly dependent on the climatic conditions, but also of some cultural practices such as irrigation. Temperature is of major importance in disease development since it determines pathogen growth rate (Baljeet *et al.*, 2005), kind of symptoms (Bouchek-Mechiche *et al.*, 2000b) and geographical distribution of the diseases. Most of the potato pathogens can grow at soil temperatures between 10℃ and 25 ℃, the optim al potato growth temperatures (Table 1.2). However, gangrene, black scurf and powdery scab are favoured by mean temperatures below 15℃ (Baker, 1970; Gindrat, 1984; Harrison, 1997); on the contrary, black dot, black leg, stem rot and charcoal rot are favoured by temperatures above 27℃. Similarly, sting and root-knot nematode s reproduce better between 25℃ and 30℃ to 35℃ depending on the origin of th e populations.

Table 1.2 Favourable climatic conditions for potato soil-borne diseases development

Pathogen	Disease	Optimal Temperatures (℃)	Optimal level of humidity		Optimal light duration			References
			low	high	Continuous	12 : 12 h (light : darkness)	Darkness or short day	
FUNGI & OOMYCETES								
Colletotrichum coccodes	Black dot	25-30; optimum: 27	X (whc < 50%)	X (storage)			Colonization, sclerotia	Davet, 1970; Lees, 2003; Tsror, 2004
Fusarium spp.	Fusarium dry rots	15-20	X (9.2 % whc)	X (27.9 % whc)		Mycelial growth		Tivoli, 1983; Kong et al., 2006
Helminthosporium solani	Silver scurf	15-32	X	X (sporulation)				Adams et al., 1987; Errampalli, 2001
Macrophomina phaseolina	Charcoal rot	> 30		X (RH > 52%)	Mycelial growth	Pycnidia production, mycelial growth	Germination tube elongation	Gindrat, 1984; Vishwa and Sarbhoy, 1989; Muthukrishnan et al., 1995; Somani, 1996; Amadioha, 1999; Mehta et al., 2006; Chowdary and Govindalah, 2007
Phoma andigena var. andina	Phoma leaf spot							
Phoma spp.	Gangrene	5-18; optimum: 10		X	Pycnidial and conidial productions	Pycnidial production		Fox et al, 1978; Gindrat, 1984; Bång, 1989; Coelho et al., 1997; Lo et al., 2000
Polyscytalum pustulans	Skin spot	15-30		X (waterlogged soil)				Salas, 2000
Pythium ultimum var. *ultimum*	Leak	5-20		X (storage)				Hide, 1987; Vico et al., 1997
Phytophthora erythroseptica	Pink rot	20-30		X (RH 95% in storage)				Lui, 2003
Rhizoctonia solani AG3	Black scurf / Stem canker	10-18	X (45 % whc)	X	Sclerotia formation			Baker, 1970; Hide and Firmager, 1989; Xu et al., 1997; El Bakali et al., 2006; Panka et al., 2007
Rosellinia spp.	Rosellinia black rot							
Sclerotinia sclerotinium	White mold	15-27	No effect of RH					
Sclerotium rolfsii	Stem rot	25-35; optimum: 30	X (30% whc)		Sclerotia production		Mycelial growth, sclerotia production	Chowdhury et al., 1993; Prithiviraj et al., 2000; Blum et al., 2002; Gupta et al, 2007

Spongospora subterranea	Powdery scab	Tuber galls: 12-15 Root gall: 17		X Constant dampness		Harrison et al., 1997; Graaf et al., 2005; Merz and Falloon, 2009
Synchytrium endobioticum	Wart	12-18	X			Hampson and Coombes, 1997; Stachewicz, 1998
Thecaphora solani	Thecaphora smut	5-20		X		EPPO, 1990; Sepulveda et al., 2000; Wale et al., 2008
Verticillium dahliae and *V. albo-atrum*	Verticillium wilt	22-26; optimum: 25		X (a_w = 0.995)	Sporulation	Jong Tae, 2001; Santamarina & Rosello, 2006
BACTERIA						
Clavibacter michiganensis var. sepedonicum	Ring rot	10-20		X		Wolf et al., 2004
Clostridium spp.	Bacterial soft rot			X		Suyama, 1990
Pectobacterium spp., *P. atrosepticum*,*P. carotovorum*, *Dickeya* sp.	Black leg, soft rot	*P. atrosepticum*: 15-25 *P. carotovorum*: 20-35 *Dickeya* spp.: 25-35		X		Jaggi, 1991; Serfontein, 1991; Vries, 1993, Latour et al., 2008; Hélias, 2008
Ralstonia solanacearum	Brown rot	23 (temperate strains) 30-35 (tropical strains)		X (whc 60%)		Shekhawat and Perombelon, 1991; Sunaina et al., 2000; Tomlinson et al., 2005
Streptomyces scabiei, *S. acidiscabiei*, *S. europeiscabiei*	Common and netted scab	Common scab: 19-24 Netted scab: 13-17	X			Adams, 1987; Bouchek-Mechiche et al., 2000, Pasco et al., 2005; Panka et al., 2007
NEMATODES						
Belonolaimus longicaudatus	Sting nematode	25-35	X (RH 7%)			Robbins and Baker, 1974
Ditylenchus destructor	Potato rot nematode	20-37; optimum: 21		X (RH 41-66%)		Mugniery and Phillips, 2007; Shojaei et al., 2006
Globodera pallida, *Globodera rostochiensis*	Potato cyst nematode	10-28	No effect of soil humidity			
Meloidogyne spp.	Root-knot nematode	*M. incognita* 25-32 *M. hapla*: 25-30 *M. chitwoodi*: 20-25	X (30% whc)			Stevenson et al., 2001;Chandel et al., 2002; Pandey et al., 2002. Wu et al., 2006
Nacobbus aberrans	False root-knot nematode	10-25; optimum: 20				Anthoine et al., 2006
(Para)trichodorus spp.	Stubby-root nematode					
Pratylenchus spp.	Root-lesion nematode	Optimum: 21		X		Jauhari and Lal, 2001; Pudasaini et al., 2007

RH: relative humidity
whc: water holding capacity

I. 2. Soil moisture

Soil moisture which depends on the climate and cultural practise is also determined by the soil texture (see below). In the literature dealing with interactions between soil moisture and potato diseases, many different terms are used to characterize the water soil content.

Soil moisture content, moisture-weight percentage and water holding capacity (whc) are used to evaluate the volume of water contained in soil. It is generally expressed as a percentage of the soil's dry weight. Other publications refer to water activity which is a dimensionless quantity (between 0 and 1) describing the amount of free water in soil for biochemical reactions. Water activity, which depends on soil texture, is related to moisture content in a non-linear relationship known as a moisture sorption isotherm curve.

High soil moisture due to abundant rainfalls, poor drainage, heavy soils or irrigation, influences disease development and the opening of the lenticels which are further entry points for soil-borne pathogens into the tuber (Helias, 2008). Several diseases, especially bacterial diseases, are enhanced by high moisture content (Table 1.2), but few diseases are favoured by low levels of moisture. This is the case for black dot, some dry rots induced by *Fusarium* spp., stem rot, wart, common scab, and sting and root-knot nematodes. High soil moisture generally has indirect effects which might favour disease severity. This is the case of flooding that provokes oxygen depletion and CO_2 enrichment resulting in an increase of *Spongospora subterranea* (powdery scab) development (Harrison, 1997). In some cases, the influence of soil moisture on disease severity is not clearly demonstrated. Depending on the studies, black scurf, stem canker, silver scurf (*Helminthosporium solani)* and *Thecaphora* smut (*T. solani)* are either positively or negatively correlated with soil moisture (Adams *et al.*, 1987; Hide and Firmager, 1989; Sepulveda *et al.*, 2000; El Bakali and Martin, 2006; Wale *et al.*, 2008). Conversely, high relative humidity during storage of tubers has always a negative impact (Table 1.2).

I. 3. Soil texture

The soil texture described the relative percentage of sand, loam and clay contents. Most of fungal diseases are enhanced in light sandy soils (Table 1.3). Conversely, it is generally accepted that clay soils favour bacterial activity (Marshall, 1975; Alabouvette *et al.*, 1996) explaining that clay or heavy soils are conducive to bacterial soil-borne diseases (ring rot, soft rot, brown rot and netted scab). Concerning nematodes, no general rule can be drawn up as some species are more prevalent in heavy soils (root-knot nematodes) and other species in light soils (sting nematodes). Soil texture also influences soil structure, through the distribution of different pore sizes, determining the actual living space for bacteria, fungi and predators. It also influences the water activity; water retained in pores of narrow diameter being less available for organisms that water present in big pores.

Table 1.3 Favourable edaphic conditions for potato soil-bornediseases development

Pathogen	Disease	Optimal soil texture		Optimal soil pH	Optimal soil nutrient concentrations	Optimal organic matter content	References
		Mainly sandy or light soils	Mainly clay or heavy soils				
FUNGI & OOMYCETES							
Colletotrichum coccodes	Black dot	X		6 -7	Low nitrogen level		Kang et al., 2003; Nitzan and Tsror, 2003; Tsror, 2004
Fusarium spp.	Fusarium dry rots	X		*F. solani* > 5.3 *F. roseum* : No effect	High Fe level Low Ca, borax and P levels	variable	Combrink et al., 1975; Tivoli et al., 1987; Tivoli et al., 1990; Alabouvette et al., 1996
Helminthosporium solani	Silver scurf	X	X				Lennard, 1980; Lutomirska, 2004
Macrophomina phaseolina	Charcoal rot	X		6.5			Singh and Kaiser, 1994
Phoma andigena var. andina	Phoma leaf spot						
Phoma spp.	Gangrene	X		3.8 – 5.6		2.9 – 7.6 ‰	Tivoli et al., 1987
Polyscytalum pustulans	Skin spot						
Pythium ultimum var. *ultimum*	Leak						
Phytophthora erythroseptica	Pink rot			No effect		No effect	Vivoda et al., 1991
Rosellinia spp.	Rosellinia black rot						
Rhizoctonia solani AG3	Black scurf / Stem canker	X		High?		High	El Fahl and Calvert, 1976; Rudkievicz et al., 1983; Lutomirska and Szutkowska, 2005
Sclerotinia sclerotinium	White mold					High	
Sclerotium rolfsii	Stem rot	X		~ 6.5	High nitrogen, organic carbon and low phosphorus and potassium levels		Sheoraj et al., 2007; Banyal et al., 2008
Spongospora subterranea	Powdery scab	X organic or over irrigated soils	X poorly drained soils	4.7 – 7.6	High aluminium level		Zambolim et al., 1995; Van de Graaf et al., 2005; Gilchrist et al., 2009; Merz and Falloon, 2009

Synchytrium endobioticum	Wart		X	variable			Hampson, 1985; Hampson and Coombes, 1997
Thecaphora solani	Thecaphora smut				High salt level		EPPO, 1990
Verticillium dahliae and *V. albo-atrum*	Verticillium wilt	X		6-9	High Ca, low K, Mg and total soil C level	Low	Baard and Pauer, 1981; Höper and Alabouvette, 1996; Davis, 2001
BACTERIA							
Clavibacter michiganensis var. sepedonicum	Ring rot		X				Moffett and Wood, 1984
Clostridium spp.	Bacterial soft rot						
Pectobacterium spp., *P. atrosepticum*,*P. carotovorum, Dickeya* spp.	Black leg, soft rot	X Black leg	X Soft rot		Low Ca concentration		Zielke et al., 1974; Lücke, 1975; Lambert and Manzer, 1991
Ralstonia solanacearum	Brown rot	X (Messiha et al., 2007)	X	variable	No ammonium intake		Hsu, 1991; Schekhawat and Perombelon, 1991; Michel and Mew, 1998; Yi et al., 1998; Keshwal et al., 2000; Muller et al., 2004
Streptomyces scabiei, *S. acidiscabiei*, *S. europeiscabiei*	Common and netted scab		X	5.2 -7	Low Mn level		Rudkievicz et al., 1983; Alabouvette et al., 1996; Loria et al., 1997; Milosevic et al., 2005; Lazarovits et al., 2007
NEMATODES							
Belonolaimus longicaudatus	Sting nematode	X					Mashela et al., 1991
Ditylenchus destructor	Potato rot nematode						
Globodera pallida, Globodera rostochiensis	Potato cyst nematode	X		6.1	Low nitrogen level	High	Pelsmaeker and Coomans, 1987; Ruijter and Haverkort, 1999; Trifonova, 2001
Meloidogyne spp.	Root-knot nematode	X	X	7.5		High	Kumar and Vadivelu, 1996; Kandji et al., 2001; Pandey et al., 2002; Melakeberhan et al., 2004
Nacobbus aberrans	False root-knot nematode						
Paratrichodorus and *Trichodorus* spp.	Stubby-root nematode (TRV vector)	*T. similis* and *P. pachydermus*	*T. primitivus*	low	High level of Fe		Barbez, 1983; Spaull and Cadet, 2001
Pratylenchus spp.	Root-lesion nematode		X	variable	Low level of Fe		Pelsmeaker and Coomans, 1987; Spaull and Cadet, 2001

I. 4. Soil pH

Disease development is also influenced by the soil pH linked to soil nutrient availability (Table 1.3). Soils with extreme pH values are often highly suppressive to several plant diseases (Höper and Alabouvette, 1996). However, pH fluctuations resulting from amendments influence pathogens and disease development. Decreasing pH increases the availability of phosphorus, nitrogen and aluminium ions and decreases potato cyst nematode, brown rot and common scab damages respectively (Mulder *et al.*, 1997; Michel and Mew, 1998; Ruijter and Haverkort, 1999; Mizuno *et al.*, 2003). On the contrary, addition of urea in soil induces a very large increase in pH and a good control of *Synchitrium endobioticum*, the fungal pathogen causing wart (Hampson, 1985).

Soil organic matter is both the substrate for and the result of microbial activity. In addition, together with clay, organic matter affects soil structure and thus moisture content and aeration. The quantity of organic matter in a soil has an effect on the appearance and the development of diseases but its quality is also an important point which has been too poorly addressed (Alabouvette *et al.*, 1996).

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Most physico-chemical factors are not independent one from the others, which makes experiments and data interpretation very difficult. Soil texture can affect humidity, soil amendments impact on pH and all those factors influence availability of chemical elements. Thus, the pathogenic inoculum present either in the soil or on the tuber surface has to find the optimal climatic and edaphic conditions to develop.

II. Effects of biotic factors on the occurrence and development of soil-borne potato diseases

II. 1. Autecology of pathogens

1. 1. Inoculum sources, survival and dissemination pathways

The survival of soil-borne pathogens during periods without potato crop depends on their ability to resist to unfavourable conditions. Most of them survive in soil under the form of resistant structures able to directly infect the new host crop. Some pathogens can also survive as saprophytes on host crop residues or on alternative hosts during winter. Finally, inoculum can also be introduced in the field by the seeds; it is called seed-borne or tuber-borne inoculum. Inoculum sources are diverse and for any disease several inoculum sources can play a role (Table 1.4). Soil-borne fungi produce different conservation structures. *Fusarium* spp. forms chlamydospores resistant to adverse conditions, *Rhizoctonia solani, Verticillium* spp., *Sclerotinia sclerotinium* overwinter as sclerotia. Bacteria can survive over winter with favourable moisture, temperature and soil type (Ficke *et al.*, 1973; Bradbury, 1977; Loria *et al.*, 2008). Nematodes can survive and persist in soil as protective cysts surrounding the eggs (*Globodera* spp.) or as juveniles in host roots (*Meloidogyne* spp.) (Qian *et al.*, 1996; Wharton and Worland, 2001).

In absence of resistant structures and of efficient saprophytic abilities, some pathogens need alternative hosts to survive in absence potatoes. These alternative hosts frequently belong to the Solanaceous family and act as a long term reservoir of the pathogen (Chang *et al.*, 1992; Tomlinson *et al.*, 2005).

Fungal dissemination occurs frequently as spores (conidiospores, chlamydospores, pycnidiospores, sporangiospores, oospores and zoospores) or mycelium transported by water (rain, irrigation, and flow in soil), by soil adhering to farm equipment or introduced by contaminated seed tubers (Zambolim *et al.*, 1995; Stevenson *et al.*, 2001; Bae *et al.*, 2007). Moreover, some pathogens liberate mobile dissemination forms such as zoosporanges. Zoospores of *P. erythroseptica*, *S. subterranea* and *S. endobioticum* are responsible for short distance dissemination of these pathogens (Wharton *et al.*, 2007; Merz and Falloon, 2009). Adult nematodes such as *P.*

penetrans are able to migrate on quite long distances better than do larvae (Pudasaini *et al.*, 2007).

1.2. Relationship between inoculum density and disease severity

Although there is not always a clear and linear relationship, the severity of the disease generally increases with an increasing level of inoculum (Table 1.4). Sometimes a minimum inoculum threshold is needed to initiate the disease development. This is the case for potato cyst nematode (Samaliev *et al.*, 1998). Conversely, the disease severity of black dot does not increase any more beyond a maximum threshold of inoculum density (Nitzan *et al.*, 2008). In fact as stated above, the relationship between inoculum density and disease severity greatly depends on the environmental factors which determine the level of soil suppressiveness.

Table 1.4 Inoculum sources and correlation between inoculum density and soil-borne potato diseases severity

Pathogen	Disease	Inoculum source	Correlation between inoculum density and disease severity (max or min thresholds)	Ref
FUNGI & OOMYCETES				
Colletotrichum coccodes	Black dot	Soil > Seed tuber	Disease severity remains constant above a **threshold** of soil-borne inoculum (0.5 to 1.7 g of inoculum per litre of soil)	Lees, 2003; Nitzan et al., 2008
Fusarium spp.	Fusarium dry rots	Soil, seed tuber	**Positive correlation** (> 10^4 conidia $\cdot ml^{-1}$ for *F. sulphureum* > 105 conidia $\cdot ml^{-1}$ for *F. coeruleum*)	Tivoli et al., 1987; Stevenson et al., 2001; Cullen et al., 2005
Helminthosporium solani	Silver scurf	Seed tuber, soil	**Negative correlation**	Lennard, 1980; Bains et al., 1996; Geary and Johnson, 2006
Macrophomina phaseolina	Charcoal rot			
Phoma andigena var. andina	Phoma leaf spot			
Phoma spp.	Gangrene	Seed tubers> Plant residues		Adams, 1980; Tivoli et al., 1987; Carnegie, 1991
Polyscytalum pustulans	Skin spot	Seed tuber		Salas et al., 2000
Pythium ultimum var. *ultimum*	Leak	Seed tubers; crop debris, dust in store and soil		Wale, 2008
Phytophthora erythroseptica	Pink rot	Soil	**Positive correlation** (> 10 propagules $\cdot ml^{-1}$)	Triki et al., 2001
Rhizoctonia solani AG 3	Black scurf / Stem canker	Sclerotia on seed tubers, in soil and in plant residues	**Positive correlation**	Rahman et al., 1996; Tsror and Peretz-Alon, 2005
Rosellinia sp.	Rosellinia black rot			
Sclerotinia sclerotinium	White mold	Soil, seed tuber		US Canola Association
Sclerotium rolfsii	Stem rot		**Positive correlation**	Rahman et al., 1996
Spongospora subterranea	Powdery scab	Soil, seed tuber, manure	**No significant / positive correlation** (≥100 sporosori $\cdot g^{-1}$ soil)	Zambolim et al., 1995; Graaf et al., 2005, Nakayama, 2007; Merz and Falloon, 2009
Synchytrium endobioticum	Wart	Soil, seed tubers	**Positive correlation** (1/25 sporangium $\cdot g^{-1}$ soil)	Hampson et al., 1994; Baayen et al., 2005

Thecaphora solani	Thecaphora smut	Seed tuber, soil, infested plant parts		Mordue, 1988; Wale et al., 2008
Verticillium dahliae and *V. albo-atrum*	Verticillium wilt	Soil microsclerotia, infected plant residues	**Positive correlation**	Nicot and Rouse, 1987; Mol and Scholte, 1995; Vallad et al., 2004
BACTERIA				
Clavibacter michiganensis var. sepedonicum	Ring rot	Seed tuber, soil, equipment	**No significant correlation**	Nelson, 1982; Westra et al., 1994
Clostridium spp.	Bacterial soft rot			
Pectobacterium spp., *P. atrosepticum,P. carotovorum, Dickeya* spp.	Black leg, soft rot	Mainly seed tubers but also soil, water, insects	**Positive correlation** (> 10^3 cell per tuber)	Naumann et al., 1974; Perombelon, 2000; Hélias et al., 2008
Ralstonia solanacearum	Brown rot	Seed tuber, soil, water		Hsu, 1991
Streptomyces scabiei, *S. acidiscabiei*, *S. europeiscabiei*	Common and netted scab	Seed tuber and soil-borne	**Positive correlation**	Wilson et al., 1999; Wang and Lazarovits, 2005
NEMATODES				
Belonolaimus longicaudatus	Sting nematode			
Ditylenchus destructor	Potato rot nematode	Seed tuber or soil		
Globodera pallida, Globodera rostochiensis	Potato cyst nematode	(cysts in) soil or soil-carrying seeds /seedlings/ equipment	**Positive correlation** (> 2 eggs $\cdot g^{-1}$ soil)	Samaliev et al., 1998; Anaya et al., 2005
Meloidogyne spp.	Root-knot nematode	(Eggs or larvae in) soil or soil-carrying seeds/seedling/ equipment	**Positive correlation** (> 0.5 to 0.64 eggs $\cdot cm^{-3}$ soil)	Mohsin et al., 1989; Nagesh, 1996; Vovlas et al., 2005
Nacobbus aberrans	False root-knot nematode	Seed tuber		Franco et al., 1992
Paratrichodorus and *Trichodorus* spp.	Stubby-root nematode (TRV vector)	Soil or soil-carrying vector	**Positive correlation**	Perez et al., 2000
Pratylenchus spp.	Root-lesion nematode	Soil	**Positive correlation** (> 0.4 eggs g^{-1} soil)	Holgado et al., 2009

1. 3. Mechanisms of infection

Potato plants are essentially composed of cellulose, a very solid polymer and tubers are enveloped in a protective covering called periderm made of a suberin biopolymer providing the primary barrier against disease, insects, dehydratation, and physical intrusions for the potato tuber (Lulai, 2001). Soil-borne pathogens of potato have various ways to penetrate the host plant and break physical barriers. They enter the roots, young sprouts, underground stem, stolons or tubers. Some pathogens cannot infect intact tuber periderm or lenticels and penetrate through wounds (Stevenson *et al.*, 2001; Taylor *et al.*, 2004) whereas other pathogens can penetrate either directly by mechanical and/or enzymatic degradation of the host's cells or through natural openings (stomata, lenticels, eyes) (Table 1.5).

Once they have penetrated the host, pathogens colonize plant tissues. Fungi grow through the parenchyma of the cortex and often reach the vascular vessels. *T. solani*, *S. endobioticum* and *Streptomyces* spp. penetration provokes hypertrophy of the colonized tissues resulting in galls. They grow in the plant, induce cell death and feed on them saprophytically. They secrete phytotoxins – for example thaxtomin produced by *Streptomyces* spp. - inducing the formation of several layers of suberized corky cells, creating a large lesion firmly integrated within the tuber skin (Stevenson *et al.*, 2001; Mulder *et al.*, 2008; Perez and Torres, 2008). Compared to common scab, powdery scab pustules formation is a relatively short process, at the end of which a single wound-cork layer remains that covers the entire lesion. After hardening off, this layer can be easily removed from the lesion without any damage of the underlying tissues (Delleman *et al.*, 2005). *C. coccodes*, *H. solani*, *P. pustulans*, *R. solani*, *S. subterranea* and *Streptomyces* spp. are responsible for several superficial alterations called blemishes. Colonization by those pathogens is usually limited to superficial layers of tuber periderm (Harrison, 1997; Stevenson *et al.*, 2001; Cunha and Rizzo, 2004; Lehtonen *et al.*, 2008a; Loria *et al.*, 2008) but they can colonize other parts of the plant until they reach vascular system. *Streptomyces* spp. responsible for netted scab blemishes have pathogenic mechanisms that are assumed to not implicate thaxtomin but rather a necrotic protein (Bouchek-Mechiche *et al.*, 2006).

Fungi and bacteria causing rots produce a wide range of hydrolytic enzymes such as cellulases, pectinases, xylanases and proteases (Olivieri *et al.*, 2004). They are

responsible for tissue maceration and cell death, after which the microorganisms have access to the nutritional resources of the dead plant tissues (Amadioha, 1997; Aveskamp *et al.*, 2008). *Pectobacterium* spp. develop an original pathogenic strategy based on quorum sensing, which utilise freely diffusible chemical signal molecules allowing pathogenic bacteria to synchronise the production of virulence factors and make the pathogenic attack more efficient (Liu *et al.*, 2008). Finally, nematodes attacking potatoes can be classified in two categories: ectoparasites and endoparasites. Ectoparasites nematodes (*B. longicaudatus*, *Paratrichodorus* spp. and *Trichodorus* spp.) are mobile and feed on potato roots in the area of cell division and elongation without penetrating the root (Stevenson *et al.*, 2001; Mugniéry, 2007). The endoparasitic nematodes of potato, *D. destructor* and *P. penetrans* are migrating endoparasites; they feed from cell to cell into the host, whereas *Globodera* spp., *Meloidogyne* spp. and *N. aberrans* are sedentary endoparasites, they induce specialized feeding sites in plant roots. *D. destructor* and *P. penetrans* penetrate underground parts of the plant, feed on the cortical cells and migrate into the roots, destroying cell after cell. *G. pallida*, *G. rostochiensis*, *Meloidogyne* spp., and *N. aberrans* develop feeding cavities in host root, causing galls (Mugniéry, 2007).

Table 1.5 Genetic variability, strategies of conservation and attack of the pathogens and detection methods.

Pathogen	Disease	Genetic variability[a]	Conservation and overwintering	Main penetration ways	Detection methods[b]	References
FUNGI & OOMYCETES						
Colletotrichum coccodes	Black dot	6 or 7 VCG pathogenic for potato	At least 8 years at 10 cm depth in the soil as sclerotia	Mechanical	Q and RT- PCR , Fourier transform infrared (FT-IR)	Dillard and Cobb, 1998; Cullen et al., 2002; Heilmann et al., 2006; Erukhimovitch et al., 2007; Shcolnick et al., 2007; Nitzan et al., 2008
Fusarium spp.	Fusarium dry rots	**13 species**, especially *F. sambucinum* and *F. solani* var. *coeruleum* (15 VCGs)	Microconidia, chlamydospores and mycelium on plant debris	Wounds, enzymatic	Isolation and morphology , RT-PCR, PCR enzyme-linked immunosorbent assay, volatile profile	Tivoli et al., 1987; Ouellette et al., 1990; Stevenson et al., 2001; Oliveri et al., 2004; Cullen et al., 2005; Burlakoti et al., 2007; El-Hassan et al., 2007; Peters, MacLeod et al., 2008; Sharifi et al., 2008; Recep et al., 2009
Helminthosporium solani	Silver scurf		At least 4 years in the soil	Enzymatic	Classical detection methods, PCR	Bains et al., 1996; Errampalli et al., 2001; Martinez et al., 2004; Geary et al., 2007
Macrophomina phaseolina	Charcoal rot		Until 3 years under unfavourable climatic conditions as microsclerotia	Enzymatic		Dhingra and Sinclair, 1977; Amadioha, 1997
Phoma andigena var. andina	Phoma leaf spot					
Phoma spp.	Gangrene	**2 species**: *P. exigua* var. *foveata* and *P. exigua* var. *exigua*		Enzymatic	Conventional and RT-PCR	McDonald et al., 2000; Stevenson et al., 2001; Giebel and Dopierala, 2004; Cullen et al., 2007
Phytophthora erythroseptica	Pink rot	**One species** with few genetic variations		Enzymatic		Lucas and Pitt, 1974; Peters et al., 2004; Peters et al., 2005; Cullen et al., 2007; Taylor et al., 2008
Polyscytalum pustulans	Skin spot		7 years or more in soil as sclerotia	Mechanical	RT-PCR	Lees et al., 2009
Pythium ultimum var. *ultimum*	Leak		Many years in the soil and in the infected plant debris as oospores	Wounds	Conventional and RT-PCR, Conventional and RT-PCR	Cullen et al., 2007; Taylor et al., 2008
Rhizoctonia solani AG3	Black scurf / Stem canker	**One species** with 13 anastomosis groups pathogenic for potaotes		Enzymatic	Classical bioassays, PCR, immunochromatographic lateral flow	Tsror et al., 1993; Gilligan et al., 1996; Carling et al., 2002; Lees et al., 2002; Gvozdeva et al., 2006; Hughes et al., 2008
Rosellinia spp.	Rosellinia black rot	**3 species**: *R. bunodes*, *R. necatrix* and *R. pepo*		Enzymatic	Conventional and Scorpion-PCR	Stevenson et al., 2001; Schena et al., 2002; Ten Hoopen and Krauss, 2006
Sclerotinia sclerotinium	White mold			Mechanical		Wharton, Michigan potato diseases
Sclerotium rolfsii	Stem rot			Enzymatic		Madalageri et al., 1991; Ohazurike and Arinze, 1992; Ferreira and Boley, 1992
Spongospora subterranea	Powdery scab		For > 10 years in cold areas as cistosori	Mechanical	Classical methods, conventional and RT-PCR, ELISA	Zambolim et al., 1995; Stevenson et al., 2001; Graaf et al., 2003; Ward et al., 2004; Merz et al., 2005; Qu et al., 2006; Nakayama et al., 2007
Synchytrium endobioticum	Wart	**One species** with 43 pathotypes	> 30 years as zoosporangia	Mechanical	Conventional and RT-PCR	Boogert, 2005; Baayen et al., 2006
Thecaphora solani	Thecaphora smut		7 years or more in the soil	Mechanical	PCR	Andrade et al., 2004; Perez and Torres, 2008

Verticillium dahliae and V. albo-atrum	Verticillium wilt	**2 species**: *V. dahliae* (4 VCGs) and *V. albo-atrum* (VCG02 attacking potato)	≈ 63 months		Classical methods, PCR, Q-PCR	Nelson, 1984; Correll et al., 1988; Joaquim and Rowe, 1991; Platt and Mahuku, 2000; Tsror et al., 2000; Strausbaugh et al., 1992; Zhang et al., 2005; Atallah et al., 2007
BACTERIA						
Clavibacter michiganensis var. sepedonicum	Ring rot	**One species** with few genetic variation	≈ 18 months in plain soil	Enzymatic	Immuno-fluorescence antibody, staining (IFAS), (ELISA), RT-PCR; LMW RNA profiles	Nelson, 1984; Logan et al., 1987; Eichenlaub et al., 1991; Palomo et al., 2000; Smith et al., 2001; Stevenson et al., 2001; Vasinauskiene and Baranauskaite, 2003; Hukkanen et al., 2005; Gudmestad et al., 2009
Clostridium spp.	Bacterial soft rot	**Several species** among which *C. puniceum*		Enzymatic		Perombelon et al., 1979; Stevenson et al., 2001; Prescott et al., 2003
Pectobacterium spp., *Dickeya* spp.	Black leg, soft rot	**2 genera**: *Pectobacterium* spp. among which *P. atrosepticum* and *P. carotovorum* and *Dickeya* spp.	Overwintering possible (on crop debris or weeds) but varying between bacteria, seasons and areas	Enzymatic	Conventional and RT-PCR, isolation (CVP), volatile profile, biochemical tests, ITS-RFLP profiles, 16 S rRNA analysis, ELISA	Bradbury, 1977; Ouellette et al., 1990; Tsror et al., 1993; Hélias et al., 2000; Lazy and Lukezic, 2003; Atallah and Stevenson, 2006; Latour et al., 2008; Pitman et al., 2008; Hélias, 2008
Ralstonia solanacearum	Brown rot	**One species** with several biovars (1, 2, and 2T) and races (1 and 3) attacking potato	Water, weeds, (soil ?)	Enzymatic	Isolation, PCR , immunofluorescence and fluorescent in-situ hybridisation (FISH)	Hsu, 1991; Ronda et al., 1999; Rangaswami and Mahadevan, 2004; Messiha et al., 2007; Loria et al., 2008; Nouri et al., 2009; Smith and de Boer, 2009
Streptomyces spp.	Common and netted scab	**Common scab**: *S. scabies*, *S. europaeiscabiei*, *S. stelliscabiei, S. acidiscabiei, S. turguidiscabiei* and maybe some others. **Netted scab**: *S. reticuliscabiei* and some isolates of *S. europaeiscabiei*	Conidia	Enzymatic	Conventional and RT-PCR, RFLP, rRNA sequence analysis, carbon source utilisation, repetitive BOX profiles	Rudkiewicz and Sikorski, 1984; Bouchek-Mechiche et al., 2000; Flores-Gonzalez et al., 2008; Loria et al., 2008; Mulder et al.,2008; Zhao et al, 2008
NEMATODES						
Belonolaimus longicaudatus	Sting nematode			Mechanical	Centrifugal-flotation method, morphological detection	Crow et al., 2000
Ditylenchus destructor	Potato rot nematode		About 4 months in favourable conditions	Mechanical	Extraction in water, morphological identification, PCR-RFLP	Shojaei et al., 2006; EPPO, 2008; Ilyashenka and Ivaniuk, 2008
Globodera pallida, Globodera rostochiensis	Potato cyst nematode	**2 species**: *G. pallida* and *G. rostochiensis*	Until 8 years in the soil as cysts	Mechanical and enzymatic	Soil extraction and, morphological identification, allele-specific PCR	Wharton and Worland, 2001; Moxnes and Hausken, 2007; Achenbach et al., 2009; Reid, 2009; Rehman et al., 2009
Meloidogyne spp.	Root-knot nematode	**At least 7 species**: *M. hapla, M. chitwoodi, M. fallax* (Mediterranean soils), *M. arenaria*, *M. incognita*, *M. javanica* and *M. mayaguensis (Mediterranean and tropical soils)*		Mechanical and enzymatic	Morphometrics, host range, biochemical and molecular (RFLP) analysis	Hlaoua and Raouani, 2007; Melakeberhan et al., 2007; Mugniéry, 2007; Dietrich and Sommer, 2009; Ozarslandan et al., 2009
Nacobbus aberrans	False root-knot nematode			Mechanical	PCR	Franco et al., 1992; Atkins et al., 2005
Paratrichodorus and *Trichodorus* spp.	Stubby-root nematode (TRV vector)	**7 species** of *Paratrichodorus* spp. and **5 species** of *Trichodorus* spp.			Morphometric and molecular analysis	Riga and Neilson, 2005; Riga et al., 2007
Pratylenchus spp.	Root-lesion nematode	**11 species** of *Pratylenchus* spp.		Enzymatic	Morphometric and molecular (PCR-RFLP) analysis	Brown et al., 1980; Saeed et al., 1998;Stevenson et al., 2001; Mugniery and Phillips, 2007

a: VCG: vegetative compatibility group; AG: anastomosis group.
b: RT-PCR: reverse transcriptase polymerase chain reaction; Q-PCR: quantitative PCR; FT-IR: Fourier transformed infra red spectroscopy

1. 4. Genetic variability

A soil-borne disease can be caused by several species of pathogens belonging to a single genus, by one species, or even by a subgroup of a species. Each species or sub-species is adapted to particular conditions or variety. Knowledge of the genetic diversity of pathogens is useful for precise diagnosis and control of potato diseases.

Since *Erwinia* has been divided into 2 different genera, *Pectobacterium* and *Dickeya* (Helias, 2008), bacterial soft rot previously attributed to *E. carotovora*, *E. atroseptica* and *E. chrysantemi* is in fact one disease caused by several species belonging to different genera (Table 1.5). *Pectobacterium* spp. and *Dickeya* spp. are frequently associated with bacteria of the genus *Clostridium* which includes very numerous gram-positive, anaerobic bacteria. *C. puniceum* is one of the few well-characterized pectolytic clostridia isolated from rotting potato tubers (Stevenson *et al.*, 2001; Prescott *et al.*, 2003).

Within a same species, the pathogen may belong to different groups with various genetic, pathogenic and physiological traits leading to the characterization of races, biovars and, recently, genomovars - strains which are phylogenetically differentiable, but are phenotypically indistinguishable -, phylotypes and sequevars – one or several strains with a given sequence - (Nouri *et al.*, 2009). Fungi without sexual reproductive stage, such as *Colletotrichum* spp., *Fusarium* spp., or *Verticillium* spp., are classified in vegetative compatibility groups (VCGs). Within a VCG, hyphae belonging to different isolates can anastomose and form stable heterokaryons, whereas hyphae from isolates belonging to different VCGs can not. This mechanism is the only known mechanism of genetic exchange between individuals of asexual fungi (Hiemstra and Rataj-Guranowska, 2003). Hyphal anastomosis is also used to categorize the isolates of *R. solani* into anastomosis groups (AG). Presently 13 AGs have been described, several of which being divided into subgroups. Individual AGs are not strictly associated with a specific host or family of hosts, for example AG 1 with rice and AG 8 with cereals. AG 3 isolates, and more specifically isolates from the AG 3 PT subgroup, are often associated with potato diseases (Kuninaga *et al.*, 2000; Carling *et al.*, 2002). However it was shown, in Great Britain and France, that AG 2-1

and AG 5 can cause disease in potato crops but with a much lower incidence than AG 3 PT (Campion *et al.*, 2003; Woodhall *et al.*, 2007).

As a result of the genetic evolution of pathogens, new pathotypes are regularly discovered. Conversely, some populations such as *P. erythroseptica* and *C. michiganensis* subsp. *sepedonicus* vary slightly in pathogenicity and in genetic diversity suggesting a relatively recent introduction of a small founding population of the pathogen (Smith *et al.*, 2001; Peters *et al.*, 2005). Genetic evolution can be achieved by vertical or horizontal gene transfer. *Meloidogyne* populations originally did not possess the cell-wall-degrading enzymes required to invade host roots. Although the mechanism of horizontal gene transfer remains largely elusive, it has been speculated that a gene coding for a cell-wall-degrading enzyme was horizontally transferred from a rhizobial bacterium to the nematode and was kept in the genome of the nematode by strong selection pressures representing important initial steps facilitating the invasion of plants by nematodes (Dieterich and Sommer, 2009). By genetic evolution, pathogens can adapt to the different environmental conditions they are submitted to. This enables them to skirt control measures and continuously forced farmers to use new control methods.

1. 5. Diagnosis and detection methods

Rapid detection of plant parasitic pathogens enables to set up adapted control measures and to avoid disease expansion and yield losses, even if the infestation level is low. Classical detection methods begin with visual observation and characterization of symptoms followed by identification using morphologic traits for nematodes (Crow *et al.*, 2000; Riga and Neilson, 2005; Melakeberhan *et al.*, 2007; Mugniéry, 2007) or isolation on selective media for fungi and bacteria. Carbon source utilisation, sugar degradation and production of specific enzymes allow the biochemical identification of bacteria (Flores-Gonzalez *et al.*, 2008; Pitman *et al.*, 2008). However, these classical methods are often not accurate enough to distinguish different strains or pathovar of the same species. Molecular biology based-diagnosis and detection methods are expected to complement classical diagnosis. The most developed detection methods are based on polymerase chain reaction (PCR), which amplify DNA regions specific of the pathogen of interest (Table 1.5). Being even more sensitive than classical PCR, RT-PCR is currently among the

most powerful methods for the diagnosis of pathogens in complex environments. The quantity of a given pathogen can be measured by quantitative PCR that quantify pathogen ARN initially present in a sample. Intraspecific identifications such as fingerprinting methods - Restriction Fragment Length Polymorphism (RFLP) or Amplified Fragment Length Polymorphism (AFLP) – are used for more accurate identification of pathovars or races of bacteria, fungi or nematodes (Abeln *et al.*, 2002; Cullen *et al.*, 2007; Flores-Gonzalez *et al.*, 2008; Pitman *et al.*, 2008). Fluorescent in-situ hybridisation (FISH) or stable low-molecular-weight (LMW) DNA profiles were developed to detect *R. solanacearum* and *C. michiganensis* var. *sepedonicum*, respectively (Ronda *et al.*, 1999; Palomo *et al.*, 2000). Immunological techniques such as immunochromatographical lateral flow, Enzyme-Linked Immunosorbent Assay (ELISA) and immunofluorescence are based on the recognition of specific markers at the surface of pathogenic cells to detect and identify the pathogens (Ronda *et al.*, 1999; Merz, 2005; Hughes, 2008). Fungal pathogens display typical infrared spectra that differ from the spectra of substrate material such as potato; they can be early and rapidly detected by Fourier transform infrared (FT-IR) microscopically-based technique (Erukhimovitch, 2007). Finally, monitoring of normal and disease-induced volatile profiles in stored potatoes or of the light reflected from plant in fields are valuable techniques to detect stress and thus pathogenic infections (Ouellette *et al.*, 1990; Heath *et al.*, 2000).

II. 2. Interactions between microorganisms, organisms and pathogens

Potato pathogens are not the only microorganisms living in the potato surroundings. A huge microbial biomass is associated and interacts with potatoes. About 10^7 bacteria colony forming units (CFU) per g of soil live in the potato rhizosphere and potato geocaulosphere which is the volume of soil surrounding the tubers (Lazarovits *et al.*, 2007). The structure of microbial and nematode communities in the geocaulosphere varies according to the plant age and other factors related to cultivar, nutritional status, biotic and abiotic stresses, etc. (Al-Hazmi *et al.*, 1993; Krechel *et al.*, 2002; Ferreira *et al.*, 2008; Desgarennes *et al.*, 2009; Manici and Caputo, 2009).

Earthworms and nematodes favour pathogens mobility by transporting them through the soil (Jensen, 1978) (Table 1.6). Nematodes enhance potato diseases because they act as vectors of the pathogens. They also enhance the diseases either by facilitating the development of other pathogens – acting as mechanical wound agents and providers of necrotic tissues for pathogen penetration or nutrition - or by benefiting of their attacks as opportunistic microorganisms (Jensen, 1978).

Table 1.6 Detrimental and beneficial associations of microorganisms with potato soil-borne pathogens

Pathogen	Disease	Organisms enhancing diseases	Organisms reducing diseases	References
FUNGI & OOMYCETES				
Colletotrichum coccodes	Black dot	*V. dahliae, S. subterranea*		Tsror 2004; Merz and Falloon, 2009
Fusarium spp.	Fusarium dry rots	*P. atrosepticum, Meloidogyne* spp. *D. destructor, S. subterranea*	*Serratia plymuthica, D. destructor*	Munzert et al, 1977; Jensen, 1978; Gould et al., 2008; Merz and Falloon, 2009
Helminthosporium solani	Silver scurf		*Acremonium strictum*, *Pseudomonas putida*, *Nocardia globerula*, *Xanthomonas campestris*	Elson et al., 1997; Rivera-Varas et al., 2007
Macrophomina phaseolina	Charcoal rot		*Trichoderma harzianum, Bacillus subtilis, P. aeruginosa*	Kumar and Khare, 1990; Gupta et al., 1999
Phoma andigena var. andina	Phoma leaf spot			
Phoma spp.	Gangrene			
Phytophthora erythroseptica	Pink rot	*S. subterranea*	*Enterobacter* sp., *E. cloacae,* Pseudomonas sp., *P. fluorescens*	Merz and Falloon, 2009; Schisler et al., 2009
Polyscytalum pustulans	Skin spot			
Pythium ultimum var. *ultimum*	Leak		*Pseudomonas fluorescens, Burkholderia ambifaria*	Li et al., 2002; Bardin et al., 2004
Rhizoctonia solani AG3	Black scurf / Stem canker	*G. rostochiensis, Meloidogyne* spp. + *V. dahliae, Pratylenchus neglectus* + *V. dahliae*	*Paenibacillus polymyxa, Bacillus licheniformis, P. fluorescens, Chryseobacterium gleum, Lysobacter enzymogenes, Streptomyces* spp., *Verticillium biguttatum* + *Gliocladium roseum* + *Azotobacter chroococcum, Trichoderma* spp., non pathogenic *Rhizoctonia* spp.	Scholte and Jacob, 1989; Krechel et al., 2002; Grosch et al., 2005; Back et al., 2006; Grosch et al., 2006; Santamarina and Rosello, 2006; Mahmoud et al., 2008; Wilson et al., 2008
Rosellinia sp.	Rosellinia black rot		*Trichoderma* spp.	Al-Chaabi and Matrod, 2002
Sclerotinia sclerotinium	White mold		*S. plymuthica, Penicillium* strain PY-1, *Gliocladium* sp., *Fusarium* spp., *Coniothyrium minitans*, *Trichoderma harzianum*	Phillips, 1989; Kamensky et al., 2002; Yang et al., 2008
Sclerotium rolfsii	Stem rot		*Bacillus subtilis* *Trichoderma* spp.	Kumar and Khare, 1990; Dey et al., 2004
Spongospora subterranea	Powdery scab	*C. coccodes*	*Trichoderma harzanium*	Merz and Falloon, 2009
Synchytrium endobioticum	Wart	earthworms		Hampson and Coombes, 1989

Thecaphora solani	Thecaphora smut	*Meloidogyne incognita*		Bazan de Segura and Carpio, 1974
Verticillium dahliae and V. albo-atrum	Verticillium wilt	*C. coccodes, Meloidogyne* spp. + *R. solani, P. neglectus+ R. solani, P. penetrans, G. rostochiensis, G. pallida*	*T. harzianum*, *Pseudomonas* spp., *Streptomyces* spp.	Jensen, 1978; Franco and Bendezu, 1985; Scholte and Jacob, 1989; Krechel et al., 2002; Rotenberg et al., 2004; Tsror, 2004; Santamarina and Rosello, 2006; Bharadwaj et al., 2008
BACTERIA				
Clavibacter michiganensis var. sepedonicum	Ring rot			
Clostridium spp.	Bacterial soft rot	*Pectobacterium* spp.		Perombelon, 1979
P. atrosepticum,P. carotovorum	Black leg, soft rot	*Clostridium* spp, *F. solani var. coeruleum*	*Bacillius spp., Pseudomonas* spp	Munzert et al., 1977; Perombelon, 1979; Dong et al., 2004; Bharadwaj et al., 2008
Ralstonia solanacearum	Brown rot	*G. pallida*	*P. fluorescens*, *P. putida*, *B. subtilis*	Jensen, 1978; Mahmoud, 2007
Streptomyces scabiei, *S. acidiscabiei*, *S. europeiscabiei*	Common and netted scab		Non pathogenic *Streptomyces*	Wanner, 2007
NEMATODES				
Belonolaimus longicaudatus	Sting nematode			
Ditylenchus destructor	Potato rot nematode			
Globodera pallida, Globodera rostochiensis	Potato cyst nematode	*V. dahliae*, *R. solani*, mycorhization	*V. dahliae, F. oxysporum, P. exigua*	Jensen, 1978; Wronkowska and Janowicz, 1989; Ryan et al., 2003; Back et al., 2006; Mugniery and Philipps, 2007;
Meloidogyne spp.	Root-knot nematode	*P. neglectus, R. solani, V. dahliae*	*Pseudomonas* sp., *Streptomyces* sp., *Rhizobium* sp.; *Bacillus megaterium* var. *phosphaticum B. penetrans*, *Glomus mossae*	IPC, 1978; Scholte and Jacob, 1989; Hafez and Sundararaj, 2000; Sankaranarayanan and Sundarababu, 2001; Krechel et al., 2002
Nacobbus aberrans	False root-knot nematode			
Paratrichodorus and *Trichodorus* spp.	Stubby-root nematode (TRV vector)			
Pratylenchus spp.	Root-lesion nematode	*V. dahliae, R. solani*		Scholte and Jacob, 1989; Saeed et al., 1998

The microbial or faunal interactions in the geocaulosphere are involved in disease suppressiveness of the soil. Two classical types of suppressiveness of soil are known. General suppression is related to the global activity of the whole microbial biomass in the soil. In contrast, specific suppression is due to the specific activity of certain individuals or groups of microorganisms (Alabouvette *et al.*, 1996; Weller *et al.*, 2002). For instance, *Serratia plymuthica*, *Pseudomonas* spp., *Bacillus* spp., *Streptomyces* spp. and *Trichoderma* spp. (Kumar and Khare, 1990; Kamensky *et al.*, 2002; Krechel *et al.*, 2002) are able to decrease the severity of several potato diseases (Table 1.6). They can be considered as biological control agents. Some biological control agents can act directly against fungal pathogens by enzymatic degradation of their cell walls (Kamensky *et al.*, 2002; Li *et al.*, 2002), by parasitism – as it seems to be the case against numerous nematodes- (Nunez-Camargo *et al.*, 2003; Papert *et al.*, 2004), by antibiotics production (Grosch *et al.*, 2005), by siderophore secretion that reduces the availability of iron needed by plant pathogens (Bharadwaj *et al.*, 2008) or by interfering with communication between pathogens, i.e. by degrading molecules involved in the "quorum sensing" mechanisms of *Pectobacterium* spp.(Dong *et al.*, 2004). Indirectly, biological control agents can lead to the plant strengthening and a better resistance to pathogen attacks by producing plant growth hormone or by inducing the production of plant defence molecules such as phytoalexins and PR proteins (Stevenson *et al.*, 2001; Larkin, 2008). Mycorrhizal fungi also have a beneficial effect; inoculation with arbuscular mycorhizal fungus suppressed tuber dry rot and reduced stem canker and black scurf (Bharadwaj *et al.*, 2008).

II. 3. Interactions between plants and pathogens

The major method to control potato diseases is to find resistant cultivars to a majority of pathogens especially since the use of chemicals is limited (INRA and Cemagref, 2005; Paillotin, 2008). Different levels of resistance towards most of the soil-borne potato diseases have been observed among potato cultivars. Wild species of *Solanum* provide excellent sources of disease resistance genes that may be introgress into *S. tuberosum* genome by interspecific crossing (Jansky and Rouse, 2003) (Table 1.7) and international structures such as the International Potato Center

in Peru are aiming at preserving the genetic diversity of native potatoes. Varieties of potato which contain colour pigments are more and more utilized in current breeding programmes because cultivars producing anthocyanins can provide better resistance to soft rot or other diseases compared to white/yellow flesh cultivars (Wegener and Jansen, 2007). Cultivars resistant to several diseases were obtained, but simultaneous resistance to all pathogens is very difficult to achieve. Moreover, for some diseases, new genotypes of pathogen appear regularly and overcome plant defence turning the former resistant cultivars into susceptible ones. Hence the levels and durability of field resistance are often highly depending on numerous abiotic and biotic factors still not well known neither controlled.

Table 1.7 Resistance of wild potato cultivars toward pathogens

Cultivar	Resistance	References
Solanum vernei	*Spongospora subterranea*	Merz and Falloon, 2009
Solanum acaule	*Clavibacter michiganensis* var. *sepedonicus*	Laurila *et al.*, 2003
Solanum commersonii	*Ralstonia solanacearum*	Kim-Lee *et al.*, 2005
Solanum bulbocastanum	*Meloidogyne chitwoodi*	Nitzan *et al.*, 2009
Snowder (*Solanum etuberosum* x *Solanum berthaultii*)	*Pythium ultimum* and *Phytophthora erythroseptica*	Salas *et al.*, 2003; Thompson *et al.*, 2007
Solanum brevideus	*Pectobacterium* spp.	Ahn *et al.*, 2001

Resistant potato cultivars resist to pathogenic attacks by plant defence reactions that generally lead to the production of suberin and anti-microbial agents, activation of defence genes and trigger hypersensitive cell death (Levine *et al.*, 1994) delaying the pathogen development in plant tissues until a wound periderm could form. Susceptible cultivars produce non-uniform deposits of suberin making them less performing against pathogens (Finetti Sialer, 1990; Ray and Hammerschmidt, 1998). The anti-microbial agents produced by potato can be glycoalkaloids (α-chaconin and α-solanine), phenolic compounds and phytoalexins, antimicrobial compounds produced by the plant after pathogen attacks (Okopnyi *et al.*, 1983; Lyon, 1989; Ray and Hammerschmidt, 1998; Zagoskina *et al.*, 2006; Baker *et al.*, 2008; Lerat *et al.*, 2009). Plants also produce inhibitors of virulence factors (Kim *et al.*, 2006). An other plant defence reaction called systemic acquired resistance (SAR) spreads a signal through the surrounding cells. It allows plants to become highly resistant to subsequent infection by the original pathogen but also by a wide variety of other

pathogens. For example, foliar SAR-inducing applications (benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester -BTH - and harpin) reduce the numbers of root-lesion nematodes (*Pratylenchus* spp.) and root knot nematodes, *Meloidogyne chitwoodi* by potato harvest (Collins *et al.*, 2006).

III. Effects of cultural practices on the occurrence and development of soil-borne potato diseases

Each technical choice made by the farmers concerning the way of growing potatoes plays a predominant role on the quantitative and qualitative yield at the end of the cropping period. All cultural practices may impact disease development.

III. 1. Rotations

The most traditional way to control diseases is to use crop rotations that include a non host plant that can "sanitize" the soil (Alabouvette *et al.*, 1996). Several studies show good results when potatoes are grown only one every three or four years (Table 1.8). The beneficial effect of crop rotation depends on the host range of the pathogen and its ability to survive in soil in the absence of its host plant thanks to dormant structures such as sclerotia or chlamydospores. Obviously, crop rotation must avoid including alternative hosts for the pathogen (Peters *et al.*, 2004). Susceptible weeds - such as hairy nightshade (*Solanum sarrachoides*) - have to be eliminated as they enable the pathogen to survive during the absence of the main host (Boydston *et al.*, 2008). Crop rotation can also fail to control highly specialized pathogens, such as *Globodera* spp. or *S. subterranea*. These organisms are able to survive for long periods, either saprophytically or as dormant structures, in soil, and a very low inoculum density is sufficient to induce disease (Samaliev *et al.*, 1998; Merz and Falloon, 2009). Rotations with potatoes can include very diverse crops (Table 1.8). If some of those crops are beneficial effect, other might favour pathogen development and should not enter in the rotation, or at teast not as the crop preceding the potatoes.

III. 2. Fertilization and amendments

Supplying plants with micronutrients and macronutrients can be achieved with organic or inorganic fertilizers, either through soil application, foliar spray, or seed treatment (Davis *et al.*, 1994; Panique *et al.*, 1997; Malakouti, 2008). Adapted fertilization and amendment allow strong and healthy crops, which are less attractive to pathogens (Khomyakov and Kostin, 1981). Fertilization may also indirectly favour diseases by enhancing foliar development that maintain high level of humidity needed for example for the growth of *Pectobacterium* spp. (Rousselle *et al.*, 1996). Amendments contribute to control diseases by modifying soil properties, especially pH (see I.2.) and microbial activities. That could result in specific suppression caused by the stimulated specific antagonistic populations or in general suppression caused by increased microbial activities or both (Lazarovits *et al.*, 2001; Steinberg *et al.*, 2007).

For some diseases, such as stem rot, organic fertilizers are more efficient than mineral ones in terms of disease suppression (Amitava and Maiti, 2006) (Table 1.8). Among organic fertlizers composts are known to have the capacity to suppress diseases, depending on their degree of maturity (organic matter content, microbial activities). The causal agents of disease suppression brought by compost amendment of the soil are complexes of bacterial and fungal populations, which invade the pile during the curing stage, although some residual activity is probably related to fungistatic compounds occurring in the composts (Raviv, 2008).

Table 1.8 Cultural practices favourable to reduce disease development

Pathogen	Disease	Favourable rotation	Favourable fertilisation & amendments	Tillage	Favourable planting, lifting, and harvesting methods	Pesticides	Cultural systems	Storage	Ref
FUNGI & OOMYCETES									
Colletotrichum coccodes	Black dot	**Long rotations (>5years)** **With** wheat, red clover, alfalfa, rye, maize, orchard grass, fallow, barley **Withou**t yellow mustard, soybean, spring canola		Mouldboard ploughing at 30 cm	Avoid water stress, Early harvesting Short interval between haulm destruction and harvesting	**Increased by** oxamyl **Decreased by** imazalil, tolchlofos-methyl, mancozeb, thiabendazole, fenpiclonil and propiconazole		Dry curing and/or temperatures below 5℃	Hide and Read, 1991; Andrivon et al., 1997; Denner et al., 2000; Esfahani and Bak, 2004; Glais-Varlet et al., 2004; Cwalina-Ambroziak and Czajka, 2006; Nitzan et al., 2006
Fusarium spp.	Fusarium dry rots	No monoculture, minimum 3 years of rotation **with** red clover	Composted manure	Minimum tillage	Early harvesting Short interval between haulm destruction and harvesting, wound healing	Chlorine dioxide, fenpiclonil and a mixture of thiabendazole and imazalil, mancozeb	Organic	Dry curing and/or low temperatures below 4℃	Khomyakov and Kostin, 1981; Polovny, 1995; Carnegie et al., 2001; Lui and Kushalappa, 2002; Carter et al., 2003; Olsen et al., 2003; Peters, 2004; Cwalina-Ambroziak and Czajka, 2006; Raviv, 2008
Helminthosporium solani	Silver scurf	Minimum 3 years of rotation **with** red clover		Minimum tillage	Small seed pieces and low planting density. Late planting and early harvesting,	Mancozeb, imazalil, prochloraz, chlorine dioxide, thiabendazole, fenpiclonil, benomyl		Dry conditions, and/or temperatures below 4℃	Lennard, 1980; Hide and Read, 1991; Firman and Allen, 1995; Carnegie et al., 1998; Carter et al., 2003; Olsen et al., 2003; Peters, 2004; Geary and Johnson, 2006
Macrophomina phaseolina	Charcoal rot					Captan, benomyl, copper oxychloride			Amadioha, 1998
Phoma andigena var. andina	Phoma leaf spot								
Phoma spp.	Gangrene				No evident effect of planting time. Early haulm destruction. Lifting at > 8 deg C	2-aminobutane, thiabendazole	Organic	Wet conditions and/or temperatures above 15℃	Meredith et al., 1975; Fox and Dashwood, 1979; Croke and Logan & Copeland, 1979; Ostergaard and Henriksen, 1983; Bang, 1989; Polovny, 1995; Carnegie et al., 1998
Polyscytalum pustulans	Skin spot				Early harvesting	Imazalil, prochloraz (seed), 2-aminobutane, benomyl, thiabendazole		Curing in dry conditions at high temperatures	Lennard, 1980; Hide and Cayley, 1987; Hide and Read, 1991; Carnegie et al., 1998;
Pythium ultimum var. *ultimum*	Leak		Composted manure		Planting in well-drained fields, harvesting in cool weather, minimizing damages,	Mefenoxam		Drying after harvesting	Raviv, 2008; Taylor et al., 2008

Phytophthora erythroseptica	Pink rot	3 years **with** barley and red clover			Planting in well-drained fields, harvesting in cool weather, minimizing damages	Mefenoxam, metalaxyl-m		Drying after harvesting	Peters et al., 2005; Al-Mughrabi et al., 2007; Taylor et al., 2008
Rhizoctonia solani AG 3	Black scurf / Stem canker	Minimum 3 years of rotation **with** wheat, alfalfa, ryegrass	Composted manure, straw	Minimum tillage, autumn ridging	Shallow planting (5 cm), high soil temperature, low planting density. Short time between haulm destruction and harvest.	**Increased by** 1,3-dichloropropene, aldicarb and ethoprophos. **Decreased by** pencycuron, chlorine dioxide, thiophanate-methyl, flutolanil , mancozeb, benomyl, thiabendazole Oxamyl soil treatments increase stem canker and decrease black scurf	Conventional		Johnston et al., 1994; Firman and Allen, 1995; Hide and Read, 1995; Lakra, 2000; Klikocka, 2001; Peters, 2004; Baljeet et al, 2005; Cwalina-Ambroziak and Czajka, 2006; Errampalli et al., 2006; Nitzan et al., 2006; Respiene and Minekiene, 2006; Zimmy et al., 2006; Henriksen et al., 2007; Raviv, 2008; Wilson et al., 2008
Rosellinia sp.	Rosellinia black rot								
Sclerotinia sclerotinium	White mold	4-5 years **With** : cereals, grasses **Without** : rapeseed, peas, beans			Irrigation management	Fluazinam, iprodione, thiophanate-methyl, fluazinam, boscalid			US Canola Association; Johnson and Atallah, 2006; Wale, 2008
Sclerotium rolfsii	Stem rot		Composted manure			Carbendazim (resistance), quintozene, mancozeb			Bisht, 1982; Solunke et al., 2001; Amitava and Maiti, 2006; Raviv, 2008
Spongospora subterranea	Powdery scab	Minimum 10 years, no pasture	No cow manure	No ploughing in spring	Late planting date in well-drained fields	Flusulfamide , fluazinam, mancozeb			Christ, 1989; Blum and Merz, 1993; Zambolim et al., 1995; Falloon, 1997
Synchytrium endobioticum	Wart	Very long rotation (30 years)	urea		Planting in well-drained fields	Carbamide = urea			Derevenko et al., 1981; Hampson, 1985
Thecaphora solani	Thecaphora smut	Long rotations				Carbendazim, thiabendazol, methyl bromide and dazomet			EPPO, 1990; Wale et al., 2008
Verticillium dahliae and *V. albo-atrum*	Verticillium wilt	3 years of rotation **With** red clover, Sudan grass, corn and **Withou**t fallow, rape, Austrian winter pea, oat, rye, mint, weeds	Ammonium lignosulfate	Minimum tillage		Mancozeb, captan, metam sodium, 1,3-dichloropropene, chloropicrine			Johnston et al., 1994; Davis et al., 1996; Soltani et al., 2002; Taylor, 2005; Tsror et al., 2005; Omer et al., 2008; Wale, 2008
BACTERIA									
Clavibacter michiganensis var. sepedonicum	Ring rot	**With** onion				Flusulfamide protects against Cms	Organic		Slack and Westra, 1998; van der Wolf et al., 2005; Respiene and Minekiene, 2006
Clostridium spp.	Bacterial soft rot		Neem leaf and seed aqueous extracts						Bdliya and Dahiru, 2006
Pectobacterium spp., *P. atrosepticum,P. carotovorum, Dickeya* spp.	Black leg, soft rot	No monoculture, rotation **with** wheat, red clover, barley or orchard grass	No over nitrogen		Planting in well-drained fields, roguing , and elimination of infected plants/tubers Limiting wounds	Chlorine dioxide, aluminium and bisulfite salts, naphtoquinone naphthazarin, kasugamycin, stable bleaching powder, streptocycline, benzoic acid, sodium benzoate, copper oxychloride + metalaxyl, metiram, copper oxychloride + cymoxanil, klorocin	Conventional	Early efficient and quick drying..after harvesting	Bushkova et al., 1981; Khomyakov and Kostin, 1981; Lewocz, 1992; Saleh and Huang, 1997; Karwasra and Parashar, 1988; Bartz, 1999; Olsen et al., 2003; Medina et al., 2004; Yaganza et al., 2004; Respiene and Minekiene, 2006

Ralstonia solanacearum	Brown rot	Without solanaceous plants. With barley and flax	Calcium superphosphate	4 deep ploughings after harvest		Tri-potassium phosphate, bleaching powder	Depends on the soil type	Kishore et al., 1996; Mahmoud, 2007; Messiha et al., 2007
Streptomyces scabiei, *S. acidiscabiei*, *S. europeiscabiei*	Common and netted scab	**With** lupin, soybean, winter rye or serradilla **Without**: sugar beet, carrots, pasture	Ammonium lignosulfate, potassium, phosphate, compost, swine manure	Subsoiling		**Increased by** oxamyl, 3% boric acid, streptomycin, streptomycin sulfate, daminozide, DL-ethionine	No effect	Meredith et al., 1975; Volovik et al., 1980; Hide and Read, 1991; Conn and Lazarowits, 1999; Park et al., 2002; Soltani et al., 2002; Chaudhari et al., 2003; Mizuno et al., 2003; Peters, 2004; Scholte, 2005; Respiene and Minekiene, 2006; Henriksen, 2007; Al-Mughrabi et al., 2008
NEMATODES								
Belonolaimus longicaudatus	Sting nematode	**Without** sorghm-sundangrass **With** cotton				1,3- dichloropropene		Crow et al., 2000; Crow et al., 2001; Perez et al., 2000
Ditylenchus destructor	Potato rot nematode					Oxamyl		Rojancovschi, 1994
Globodera pallida, Globodera rostochiensis	Potato cyst nematode	Long rotations **With** peas, flax, rye, oat or rye grass	phosphore		Avoiding dissemination from infected fields with equipment	Dimethyl disulphide, 1,3-dichloropropene, aldicarb, phoxim, A.C. 92100, carbofuran, A.C. 64475	Conventional	Cornejo Quiroz, 1977; Hague et al., 1982; Mulder et al., 1997; Trifonova, 1997; De Ruijter and Haverkort, 1999; Molendijk, 1999; Minnis et al., 2004; Coosemans, 2005
Meloidogyne spp.	Root-knot nematode	**With** cotton**,** or black fallow **Without** most crops (carrot, beat, salsify, red clover, cereals, vegetables,..)				Methyl bromide, metham sodium, dicloropropen-cloropicrin, metham sodium + 1,3-dichloropropene, fosthiazate + metam sodium, dimethyl disulphide	No effect	Molendijk, 1999; Crow et al., 2000; Carter et al., 2003; Coosemans, 2005; Hafez and Sundararaj, 2006; Charchar et al., 2007; Ingham et al., 2007
Nacobbus aberrans	False root-knot nematode				Planting in June or July	Abamectin and furateocarb, A.C. 92100, aldicarb, carbofuran, A.C. 64475		Cornejo Quiroz, 1977; Iriarte et al., 1999; Main et al., 2001
Paratrichodorus and *Trichodorus* spp.	Stubby-root nematode (TRV vector)	**With** beet, oats, grasses **Without** sorghm-sundangrass or velvetbean , maize, wheat, cabbage, rape, barley				Aldicarb (+ oxamyl), 1,3-dichloropropene		Barbez, 1983; Perez et al., 2000; Crow et al., 2001; Hafez and Sundararaj, 2006
Pratylenchus spp.	Root-lesion nematode	**With** wheat, ryegrass, **without** red clover				1,3-dichloropropene, oxamyl, fosthiazate, cadusafos, carbofuran	Organic	Philis, 1997; Johnston et al., 1994; Molendijk, 1999; Kimpinski et al., 2001; Carter et al., 2003

III. 3. Tillage management

Potato cultivation traditionally involves intensive soil tillage throughout the cropping period. Mechanical tillage, ridging and harvesting entail intensive soil disturbance and modify the environmental conditions especially the microbial characteristics of soil, both on quantitative and qualitative aspects (FAO, 2008; Vian, 2009). As an example, ploughing contributes to redistribute vertically the inoculum, which increases the probability of infection (Taylor, 2005). Over the last decades, there is tend to replace ploughing by techniques without soil inversion i.e. no tillage or superficial tillage, leading to efficient disease suppression (Klikocka, 2001; Peters *et al.*, 2004; Vian, 2009) (Table 1.8). Indeed, rotation and conservation tillage practices can improve disease suppression by enhancing the antibiosis abilities of endophytic and root zone bacteria (Peters *et al.*, 2003). Although minimum tillage is more beneficial for soil suppressiveness to disease, the plant growth and the macronutrient (N, P, K, Ca and Mg) contents in potato plant respond positively to a deeper soil caused by ploughing (Boligowa and Glen, 2003; Nunes *et al.*, 2006).

III. 4. Planting, haulm destruction, lifting and harvesting

Planting, dehaulming, lifting and harvesting are decisive for disease expression (Table 1.8). For example, low planting density increases the yield per plant because the foliage has more space for growth. Also, sparse plants are less exposed to the attacks of disease than plants at high densities (Milic *et al.*, 2006). Diseases can be reduced by adjusting planting, dehaulming and harvesting dates and cultivation of early-tuberizing cultivars combined with pre-harvesting desiccation of haulms and treatment of seed tubers with chemicals (Sikka and Singh, 1976). Black scurf development on tubers has a positive correlation with the curing period (time between haulm destruction and harvest) because infection on tubers continues in the soil even after haulm destruction (Lakra, 2000).

III. 5. Pesticides

Pesticides are commonly used to control various pathogens altering potato tubers. They can be applied as soil fumigant (fumigants such as carbamates are not allowed in some European countries), sprayed or powdered directly on seed tubers after harvest or applied as granular (Hide *et al.*, 1995; Tsror *et al.*, 2000; Errampalli *et al.*, 2006). The chemicals have to be carefully chosen, since pathogens can adapt and become resistant (Table 1.8). Thiabendazole-resistance was detected in *F. avenaceum*, *F. culmorum*, *F. equiseti*, and *F. sporotrichioides* (*Fusarium* dry rot) (Ocamb *et al.*, 2007), in *Polyscytalum pustulans* (skin spot) (Carnegie *et al.*, 2008) and in *H. solani* (silver scurf). Mefenoxam-resistance is known for *Phytophthora erythroseptica* (pink rot) populations (Taylor *et al.*, 2006) and numerous treatments of carbendazim select resistant mutants of *Sclerotium rolfsii* (stem rot) (Solunke *et al.*, 2001). Moreover, the use of numerous chemicals is nowadays regulated and many of them are no longer permitted in France.

III. 6. Organic farming versus conventional agriculture

Organic farming relies on agricultural techniques that exclude the use of chemical pesticides and recommend organic fertilization. As a result, the soil and tuber environment is quite different from the one caused by conventional practices and may induce disease suppression (Table 1.8). To reduce disease incidence or severity, the best adapted cultural system depends on the pathogen to control and varys strongly according to the soil type (Messiha *et al.*, 2007). It has been reported that farmers who switch from conventional to organic system faced critical pest or disease problems during a transition period of about 5 years but managed to control soil-borne diseases on the long-term (Bruggen and Termorshuizen, 2003). However, organic farmers generally faced more sanitary problems than conventional farmers.

III. 7. Handling and storage

Inappropriate manipulation of tubers at harvest or during storage can provoke wounds that increase diseases such as black dot, *Fusarium* dry rots, silver scurf, gangrene, leak, pink rot, black leg and soft rot (Meredith *et al.*, 1975; Hide, 1994; Vanvuurde and Devries, 1994; Salas *et al.*, 2000; Marcinkowska *et al.*, 2005; Peters *et al.*, 2008b) (Table 1.8). Significant measures of managing potato diseases include: avoiding mechanical damage to potatoes during harvesting, shipping and sorting, curing the harmed parts thereby preventing infection and disease onset, avoiding manipulation of cold potato since potato tubers are more sensitive to injuries when cold, avoiding the exposure of table potato to light, and continuously providing stored potatoes with fresh air (Milosevic and Alovic, 2006; Scheid, 2006). Most of the storage diseases decrease when the tubers are cured in dry conditions and stored at temperature below 4 or 5 ℃, except gangrene (Table 1.8). However, tubers stored in a dry atmosphere show greater weight losses than tubers stored in a humid atmosphere, despite having less infection (Lennard, 1980).

IV. Diseases management

IV. 1. Risk assessment and decision support systems

Disease occurrence and development influenced by abiotic and biotic factors are difficult to predict. However, their prediction would be very useful to assess disease risk and consequently the potential yield loss and to choose the best disease control strategy. Current methods to evaluate yield losses are based on predictive models which commonly assign a value or score to each risk factor, such as cultivar resistance, inoculum density, cultural practices and environmental factors. The maximum score that can be assigned to each factor depends on the relative importance of the factor in determining the disease. For example, cultivar resistance is considered to be a major determinant of powdery scab severity, so this factor has a higher score than the zinc content of soil, which is thought to be less important (Burgess and Wale, 1994). Assessment of the risk for each factor and for each

disease is performed by bioassays in fields or in growth chambers under controlled conditions. They are generally costly, laborious and time consuming.

Tolerant cultivars, that host pathogens without expressing symptoms, are a particular risk factor in potato production as they can maintain and increase the inoculum level in fields (Merz and Falloon, 2009). A tolerance threshold of the crop has to be determined. It takes into account the relationship between inoculum density and disease incidence or severity according to cultivar resistance (Table 1.4).

A score can also be attributed to each cultural practice in the equation of the model since they have various impacts on yield losses. For example, incidence and severity of *Verticillium* wilt decrease with long rotations (Johnston *et al.*, 1994), but mint as a previous crop increases *Verticillium* wilt (Omer *et al.*, 2008). Consequently, in the equation of the model, rotation length will be negatively correlated to yield losses whereas mint as previous crop will be positively correlated to yield losses due to *Verticillium* wilt.

On the same pattern, some predictable environmental factors such as nutrient content and soil pH can be scored. However, abiotic environmental factors are difficult to predict. For example, at planting time, rainfall and temperature conditions occurring at the critical growth phase of the disease are almost impossible to foresee. As climatic conditions cannot be predicted at middle term, models of risk assessment are less reliable. However, no factor alone has a dramatic effect on the disease; and the beneficial reduction of a disease is usually achieved by the sum of optimized factors (Harrison, 1997).

Mathematical modelling including all the data related to the environmental factors and to the results concerning plant resistance appeared to be helpful to evaluate risk, to overcome the scaling gap between bioassays in growth chamber and field application and to simulate scenario based on crop management (Janvier *et al.*, 2007).

Calculation of yield losses enables to identify a damage threshold and to determine the time at which disease control must be initiated. Indeed, yield loss threshold and economic threshold are different. Economic threshold is frequently higher than yield loss threshold; because up to a certain point, losing yield is less penalizing for farmers than spending money to avoid it. Calculation of economic thresholds beyond which control of diseases is profitable takes into account a damage function drift to

potato yield, pathogen population density and crop selling prices. For example, application of control measures is found to be beneficial at an initial density of *G. rostochiensis* higher than 8 eggs and larvae g^{-1} soil, while the damage threshold is at 2 eggs g^{-1} soil (Samaliev and Andreev, 1998). Economic thresholds allow taking short-term strategic decisions such as choice of the cultivar, cultural practices, timing of crop establishment, seed treatment, planting density etc. and long-term strategic decisions such as define research priorities, design the breeding programs or develop integrated pest management strategies (Savary *et al.*, 2006) (Figure 1.2). Predicting models are used by farmers as decision support systems (DSS) and generally provide a theoretical yield to be obtained at the end of the cropping period, a monitoring of pest populations and comments and advices in order to increase the theoretical yield as much as possible (Been *et al.*, 2005; Jorg *et al.*, 2006). Some DSS are able to send real time alerts to farmers when several risk factors are combined and when control measures have to be taken immediately (Dubois and Duvauchelle, 2004). DSS are environmental and farmer friendly as they enable to increase economical yields by applying the right chemical doses at the right time and when disease pressure requires it, in order to reduce unnecessary environmental pollutions and treatment cost.

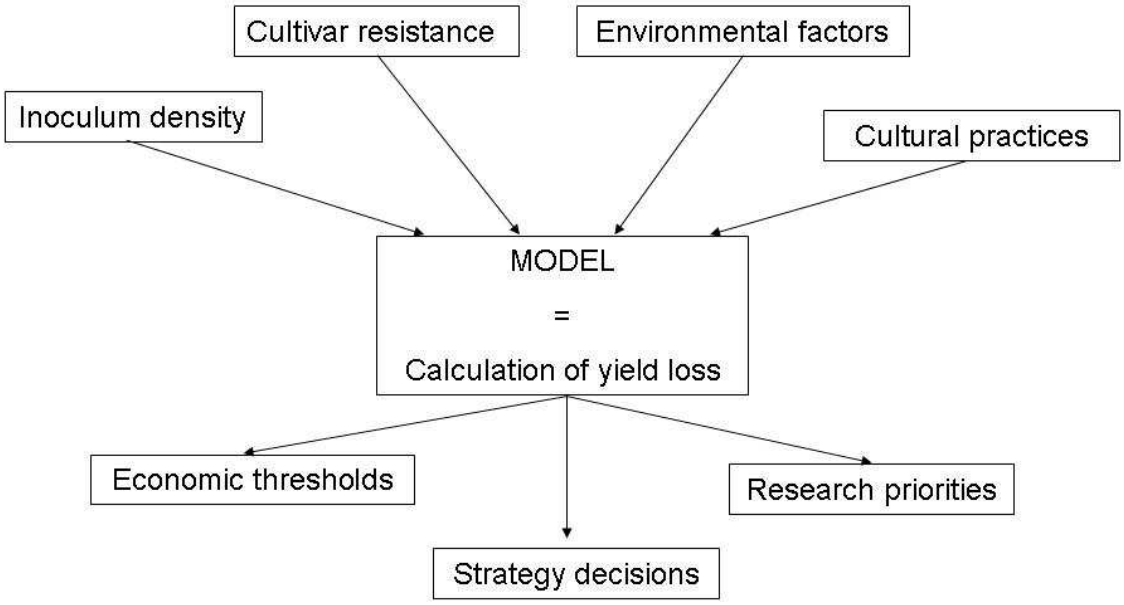

Figure 1.2 Input and output parameters of yield loss calculation models

IV. 2. Control methods

Ways to control diseases are evolving since the use of chemicals is supposed to be reduced. In many cases, the most efficient long-term strategy is to use resistant cultivars when available. Otherwise, management strategies consist either in exclusion, avoiding contact between plant and pathogens, or by pest eradication, and leading to complete elimination or partial reduction of pathogen populations.

For the potato crop which is multiplied vegetatively, exclusion methods begin with the use of healthy tubers. Many soil-borne pathogens can be carried on by seed tubers and the use of certified seed potatoes is a major way to control or restrict the movement of pathogens of potato crops (Andrade *et al.*, 2008). Seed certification programs aim at warranting seed tuber quality to potato producers and favour the diffusion of genetic progress. The certified seed production process may be 8 to 10 years long. Strict rules established by the national regulation institutions (i.e. National Potato Council in USA or GNIS - SOC (Groupement national interprofessionnel des semences - Service officiel de contrôle) in France) have to be respected and the seeds are regularly inspected for bacterial, viral, and fungal diseases, as well as varietal purity and identity. Each country is free to apply more or less severe rules. Certification systems have been developed in most of the seed producing countries to cover the production of certified seed potatoes free from pathogens and pests (McDonald, 1995; Grousset and Smith, 1998; Sahajdak and Uznanska, 2003). An international project of commercial and phytosanitary minimal guidelines (CEE-ONU S-1) is in progress. It is intended to serve as a minimal base consensus between the various standards established at "regional" levels (EU, NATTO, etc) (UNECE).

Eradication strategies aim at eliminating an established pathogen from plant propagation material or production sites. Eradication methods involve the use of pesticides, adapted cultural practices or biological control. Application of fungicides and nematicides are protecting strategies (see section III. 5. and table 1.8) whose application time and doses can be advised by DSS. However, pesticides are sometimes inefficient against pathogens, such as *P. carotovorum* (Latour *et al.*, 2008), or their use is limited by environmental regulations. Consequently, alternative methods based on adapted cultural practices have to be recommended (see section III and tables 1.3 and 1.8). Some crops either susceptible or resistant may serve as baiting-crop, for example, resistant potato cultivars cropped just before the main

potato crop decreased black scurf (Scholte, 2000). Likewise, alfalfa can be used to avoid TRV transmitted by stubby root nematode, as this crop is a host for stubby-root nematode but immune to TRV (Stevenson *et al.*, 2001). Cultivar precocity can be used to avoid some diseases. Since black dot and charcoal rot damages occur late in the growing season, early cultivars are generally recommended to control these diseases (Stevenson *et al.*, 2001). When a disease is established in a production site, its spread must be avoided as much as possible. All diseased plants have to be eliminated or burned and tools should be properly disinfected before use in another field (Salas *et al.*, 2000; Latour *et al.*, 2008).

Natural interactions of plants and microorganisms with the pathogens are used as biological control to protect potato crops. There is a continuum from a conducive soil to a suppressive one (Alabouvette *et al.*, 1996) what means that in each soil, almost each pathogen can be potentially controlled by other microorganisms either by a specific antagonism or by competition with total microbial biomass (see section II. 2. and table 1.6). Appropriate agricultural practices, thanks to the DSS should stimulate this potential to enhance or to maintain the soil suppressiveness to potato diseases.

Another approach consists in applying biocontrol agents. However, the choice of a biological control agent must take into account the potential risks to human health. Even if *Serratia grimesii* and *Burkholderia cepacia* decrease dry rot and black scurf and stem canker respectively, they can cause human infections and are not recommended for biological control (Table 1.6) (Grosch *et al.*, 2005; Gould *et al.*, 2008). Moreover, indirect control such as strengthening of potato plants by mycorhization increases tuber yield and allow an integrated management of potato cyst nematode and root-knot nematode (Sankaranarayanan and Sundarababu, 2001; Ryan *et al.*, 2003). Biological control may also include the use of natural toxic compounds for pathogenic agents. Fumigation of essential oils is studied to control dry rot, gangrene, black scurf and stem canker (Bang, 2007). Fish emulsion and crushed crab shell are used against *V. dahliae*, *V. albo-atrum* and *S. endobioticum* respectively (Hampson and Coombes, 1995; Abbasi *et al.*, 2006). Soil can be disinfected from pathogens by biofumigation or solar heating or both. For example, Brassica crops used in crop rotations and as green manures have been associated with reductions in soil-borne pests and pathogens. These reductions have been attributed to the production of volatile sulfur compounds through the process of

biofumigation, and to changes in soil microbial community structure (Janvier *et al.*, 2007). Composting is also a sanitizing method which combines temperature, time and toxic compounds to control potato diseases. The composts the most frequently used on potato crop are organic wastes (sludge, manure, tea, etc.) that have undergone long, thermophilic, aerobic decomposition. The most effective compost composition and combinations of temperature and time have to be determined for each pathogen. As it decreases the pathogenic population or favours microbial enrichment of the soil, compost has generally a positive or no effect on disease suppression, and only rarely a disease stimulating effect (Termorshuizen *et al.*, 2006). Sanitization is also performed on tubers before planting by hot water (Janvier *et al.*, 2007) or during storage with chemical treatments at high temperatures (Secor *et al.*, 1988). However, heating may damage tubers resulting in fewer sprouts. Biocontrol can also be performed by disrupting pathogens molecular pathways. *P. carotovorum* quorum-sensing mechanism is controlled by a quorum-quenching strategy aiming at interrupting the quorum-sensing by using compounds or organisms able to cause interferences in the bacterial signal (Latour *et al.*, 2008). Finally, it is also possible to enhance plant defence reactions against soil-borne pathogens by foliar spraying with different inducers such as salicylic acid, di-potassium hydrogen phosphate and tri-potassium phosphate (Mahmoud, 2007).
The different methods that were presented above are not items that have to be taken at random. Their combination generally gives better results than each of the method applied alone.

Decision support systems developed to predict yield losses allow choosing good control methods such as the use of healthy seeds, adapted pesticides, cultural practices and biological control agents for each potato diseases.

Conclusions

If a disease results from the interaction between the plant and a pathogen, its severity is influenced by soil abiotic and biotic factors affecting the plant, the pathogen, or both (Alabouvette *et al.*, 1996). Biotic and abiotic factors are not independent, the abiotic factors modulating the biotic ones. They act both on the disease epidemiology, that means the environmental conditions which make the

plant growing and the pathogen, present or latent on the crop, causing or not the disease. Moreover, some unfavourable factors for a given disease can be favourable to another. The multifaceted interactions between plants, pathogens and their environment make disease management complex since controlling every factor occurring in the disease development is quite impossible. Potato producers have to aim at limiting contact between plant and pathogens by using for example healthy seeds. Moreover, pathosystems are continuously changing since the pathogens genetically adapt to their hosts or to the environmental conditions implemented by human activities or not. In a system whose parameters vary continuously, the control strategies have to be adapted to each situation at every time.

This review aimed at being as exhaustive as possible about the factors impacting the occurrence and development of the soil-borne potato diseases. To our knowledge, such a work putting in relation numerous potato diseases and comparing their development conditions, the ecology of the causal pathogens and their abiotic and biotic interactions responds to a clear demand from both scientists, extension services, breeders, and farmers. Studies dealing with potato diseases frequently consider only one or few diseases at the same time. Thus, this review constitutes by itself a decision support system since the optimal factors limiting disease development are listed. Nevertheless, the data collected here deal more with diseases known in developed counties and those which cause severe economical losses. Knowledge about minor diseases such as *Phoma* leaf spot, *Rosellinia* black rot and *Thecaphora* smut are extremely rare, probably because these diseases occur in very isolated areas. *Phoma* leaf spot was recorded only in Bolivia and Peru, *Rosellinia* black rot was described in South America and Africa and *Thecaphora* smut in South America and Mexico.

Moreover, soil-borne diseases are difficult to study because soil is a complex environment in which numerous interactions occur and where detection of pathogens is not easily performed. However, researches on those diseases could be beneficial at long-term in case they would spread throughout the world. It would have been rather complex to consider air-borne diseases in addition to soil-borne diseases of potato. However, air-borne diseases such as late blight caused by *Phytophthora infestans* and early blight caused by *Alternaria solani* are responsible for huge economical losses and have to be considered with as much attention as soil-borne

diseases. Finally, since few years, importance of potato tuber quality raised in developed countries where tubers are washed before selling. Indeed, washing tuber makes visible some superficial blemishes that were previously hidden by adhering soil. Consumer's habits changing, blemished tubers cannot be sold anymore and the losses take seriously damaging proportions for potato market.

The previous considerations acknowledge the fact that the plant disease problem can be reduced in short term thanks to solid knowledge in epidemiology and pathogens ecology, but in longer term control strategies must be adapted with the constant evolution of pathosystems.

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Chapter 2

Review - Nomenclature and classification of potato tuber blemishes

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Abstract

Since most of ware potato tubers are washed before selling, tubers blemishes are an important cause for downgrading and rejection of tuber lots. Tubers may be affected by a diversity of superficial blemishes among which typical and atypical blemishes can be distinguished. Typical blemishes are those for which the causal agents are known. They are more studied than atypical blemishes for which the causal agents are still unknown, sometimes putative and not yet scientifically identified. Researchers, growers and technical staff dealing with such blemishes all over the world use different terminologies to describe each of the blemishes. Besides major tuber diseases like silver scurf, black dot and powdery scab, for which common names of symptoms are usually well defined and consensual, there is a wide range of atypical blemishes and some typical ones for which the terminology is more disparate and may induce confusion and misunderstandings. Physiological mechanisms involved in the appearance of atypical blemishes are reviewed; this highlights the scarcity of available data on the subject and the need for carrying out complementary studies based on a consensual nomenclature. After a critical worldwide bibliographical review of the most commonly used terminology of tuber blemishes, we put forward a proposal for a new nomenclature harmonizing common names for each kind of blemish.

Introduction

Since several decades potato (*Solanum tuberosum* L.) crop area increases especially in developing countries. Some 325 millions tons of tubers are produced annually in the world (FAO, 2008). In western Europe, most fresh potato lots are washed before selling (Commission of the European Communities, 2007): this radical procedure has positively prevented a too harsh decline of the consumption of table potatoes but has had an adverse effect of highlighting tuber skin defects. As a consequence, visual quality of tubers has become a very important cause of rejection or downgrading of potatoes leading to potential important economical losses for farmers.

Potato tubers are subjected to a very large range of blemishes, which generally affect only upper layers of the tuber periderm and do not damage the inner part nor the nutritional quality of the potatoes. On one hand, typical blemishes are those that are largely studied and which have usually known causal origins, for example, common scab, netted scab, powdery scab, black scurf, black dot and silver scurf that are caused by thoroughly identified bacteria or fungi. Consequently the symptom terminology is consensual though with the exception for potato scabs (common, netted scab and russet scab). On the other hand, atypical blemishes, because still of unknown origin, might be frequently observed but they have been very rarely investigated (Perraton, personal data, 1996 and Campion *et al.*, 2003). Since ware tubers are washed in order to remove adherent soil, there has been a constant feedback from growers and packers about a number of skin defects which do not appear to be associated with any of the typical blemishes, but which lead as likely to rejections or downgrading. These atypical blemishes are distributed on the tuber surface as more or less big patches of various aspects: shallow, irregular, and scaly, crackled or rough (Jouan, 1997; Stevenson *et al.*, 2001; Sexton, 2003; Selman *et al.*, 2008).

Literature shows that terminologies concerning atypical blemishes as well as some typical blemishes like potato scabs differ according to the aspect of the blemish and the country where they are observed. It also appears that atypical blemishes are sometimes falsely attributed to pathogens causing other similar blemishes. Since several years, these issues are discussed among researchers and end-users; they highlight a lack of knowledge on the biology and symptomatology of those potato

blemishes. Thus, the establishment of a consensual classification nomenclature of blemishes for all the scientific community is a prerequisite for further scientific investigation. At first, this paper briefly reviews the disorders leading to the alteration of the periderm. Then, based on the literature, as well as shared experience on assessment of tuber blemishes in collaborative research (Fiers *et al.*, 2010) and extension projects (FNPPPT *et al.*, 2008), it suggests a consensual nomenclature describing the main superficial blemishes, with special emphasis on the atypical blemishes of potato tubers.

Physiological disorders involved in the formation of atypical blemishes

The mechanisms underlying the appearance of the blemishes are not well known, but the most important lack of knowledge concerns the atypical blemishes. The formation of atypical blemishes on the potato surface implies histological modifications and cellular disorders as a result of the plant response to an attack. Atypical corky blemishes forming polygonal lesions (Figure 2.1.A) appear to be the result of one part of the tuber surface not growing as fast as the rest of the tuber (Sexton, 2003). These polygonal lesions may be due to localized stress that only affects one area of the tuber, while the rest of the tuber continues to grow and expand normally. They may occur when a stress uniformly slows down the growth of the whole tuber, but after the stress ends, the resumption of growth is not uniform across the tuber. If one area of the tuber surface fails to recover from the stress as fast as the rest of the tuber, it will grow slower than the surrounding tissues. If the tuber does not have a mechanism to respond to that stress, then polygonal lesions could develop within the tuber structure. Several authors suggest that polygonal lesions could be due to unfavourable environmental conditions, i.e high temperature, high organic matter content, high soil moisture or fertilization (Stevenson *et al.*, 2001; Turff, 2002; Selman *et al.*, 2008). The fungus *R. solani* was cited as well as responsible for the formation of polygonal lesions. The fungus growing on the tuber surface would delay the growth of the underlying tissues, which may result in deformed tubers. The pattern of the lesions on the skin would be related to the pattern of the branching hyphae of the fungus. Some of these hyphae may still be present on newly harvested tubers (Mulder *et al.*, 2008).

Corky cracks and tuber cracks (Figures 2.1.G and 2.1.R) are also quoted as physiological reactions to a growth stress, either abiotic (fluctuation of soil moisture, herbicide) or biotic (disease, e.g., *R. solani* etc.). The main cause of tuber cracks is an irregular water uptake during the growing season. During a period of drought, the tuber stops growing. A water uptake induces a rapid rehydratation of the tuber. The internal turgor pressure becomes stronger than the peridermal resistance. The periderm breaks and heals progressively afterwards (Stevenson *et al.*, 2001; FNPPPT *et al.*, 2008).

Dry core blemish (Figure 2.1.S) is almost always allocated to *R. solani*, but it seems that the blemish only appears when the tuber is mechanically or naturally wounded. Wireworms might be the main cause of natural wounding making fungal invasion easier (Keiser, 2007).

It is likely that an unclear identification associated to a fuzzy terminology may explain the lack of publications about the mechanisms inducing corky spots and star-like lesions (Figures 2.1.H to 2.1.J).

The majority of atypical blemishes affect the periderm integrity. Some of these blemishes, such as polygonal lesions, corky cracks or dry core are similar to the scars observed on tuber after they have been wounded. Tuber wound responses and wound-healing involve many biological processes, the most important of which being wound-induced suberization. The tissue distribution of suberized cell walls in plant suggests that suberization occurs wherever and whenever the plant needs to form a physical barrier (Franke and Schreiber, 2007; Pollard *et al.*, 2008). As the wounded tuber tissue begins to heal, it forms a closing layer where the walls of existing parenchyma cells become suberized. In conjunction with the formation of the closing layer, a wound periderm forms under the closing layer (Lulai, 2007). The similarity between corky blemish and healed wound suggests that similar cellular mechanisms are induced by plants. Wounding, stress and/or invasion of plant tissues by pathogens elicit an oxidative burst of superoxide, hydrogen peroxide and hydroxyl radicals at the wound surface. These reactive oxygen forms play a role in protecting host tissue. Wounding activates superoxide radical formation that may have a role in the suberin synthesis (Kumar *et al.*, 2007). In addition to this putative role i.e. polymerization of phenolic monomers in lignin/suberin synthesis, the reactive oxygen forms serve as (a) signaling molecules for upregulating defence-related genes, (b) anti-microbial agents and catalysts for cross-linking cell wall proteins, and (c)

molecules triggering hypersensitive cell death to contain infection (Levine *et al.*, 1994; Simon-Plas *et al.*, 2002; Kumar *et al.*, 2007).

Data about physiological processes resulting in atypical blemishes are rare however the understanding of these processes is needed to control tuber superficial alterations. Doubtless, the first step toward a full comprehension of these mechanisms is to remedy the confusing terminology of blemishes and to clearly identify, describe and name typical and atypical blemishes.

Critical analysis of current terminology for superficial blemishes on potato tubers

Morphological descriptions and pictures of blemishes have been published in several books and guides (Radtke and Rieckmann, 1991; Stevenson *et al.*, 2001; FNPPPT *et al.*, 2008; Mulder *et al.*, 2008; Wale *et al.*, 2008). However depending on the authors, common names and descriptions of the blemishes differ. This induces ambiguities and misunderstandings between the different actors along the potato production chain whether they are scientists or extension workers (Table 2.1).

We firstly review typical blemishes, whose causes are known, and whose terminologies are straightforward. Albeit, they have been fully described in the literature, in practice they may be confused one with the others because of the similarity of symptoms. As an example, black dot caused by *Colletotrichum coccodes* (Figure 2.1.A) and silver scurf caused by *Helminthosporium solani* (Figure 2.1.B) may be misinterpreted by inexperienced eye because of the similarity of the discoloured patches they form on tuber surface. Black dot causes diffuse grey-brown spots with black microsclerotia, whereas silver scurf blemishes are clear silvery spots with very fine black punctuations and induce a separation of the periderm (El Imane-Collet, 1993; Stevenson *et al.*, 2001). Inspection with a hand lens (10 x) will quickly differentiate the regularly spaced black dots from the bunched threads of silver scurf. Though dealing with a pathosystem thoroughly studied (Merz and Falloon, 2009), one may still mistakes between some types of powdery scab lesions caused by *Spongospora subterranea* (Figure 2.1.M) and some common scab symptoms caused

by some strains of *Streptomyces* (Figures 2.1.N, 2.1.O, 2.1.P) simply because they produce visually similar puffy pustules (Jouan, 1997).

Secondly, typical blemishes, whose causes are known, but whose terminologies are unclear are considered. The confusion may come from the different common names used for the same typical blemish or from the same name used for different blemishes. Diseases caused by *Streptomyces* spp. are the most striking example. In Europe, and especially in France, the term common scab refers to at least two different diseases caused by *Streptomyces* spp. (Table 2.2): i) raised scab, a raised blemish sometimes looking like craters on tuber surface (Figure 2.1.O), and ii) netted scab, a symptom forming corky network (Figure 2.1.D) (Bouchek-Mechiche *et al.*, 2000b). Netted scab was only described in European countries. In North America or Asia, the term common scab describes one disease including several types of lesions. They are round to star-shaped, clearly defined corky lesions called pitted scab (Figure 2.1.N), raised or erumpent scab (Figure 2.1.O) and shallow scab (Figure 2.1.P) (Goyer *et al.*, 1996; Takeuchi *et al.*, 1996; Wanner and Haynes, 2009). Russet scab (Figure 2.1.E) has only been described in the USA (Loria *et al.*, 1997), in Japan (Oniki *et al.*, 1986), in Canada (Faucher *et al.*, 1993), in India (Khanna *et al.*, 2000) and in Finlande (Kreuze *et al.*, 1999). Russet scab and netted scab are visually very similar blemishes, which can be sometimes considered as one unique blemish (Natsume *et al.*, 2005). However, netted scab tends to form a network at the tuber surface, while russet scab does not. Moreover, russet scab and netted scab differ in several characteristics such as cultivar susceptibility, root attack, and optimum soil temperature and are, therefore, considered being different diseases caused by different *Streptomyces* species (Loria *et al.*, 1997; Kreuze *et al.*, 1999; Bouchek-Mechiche *et al.*, 2000b). The different kinds of potato scabs are caused by different species of the *Streptomyces* genus. *S. scabies*, *S. europaeiscabiei*, *S. stelliscabiei, S. acidiscabiei, S. turguidiscabiei,* and maybe some others cause common or pitted scab (Stevenson *et al.*, 2001; Mulder *et al.*, 2008); *S. reticuliscabiei* and some isolates of *S. europaeiscabiei* cause netted scab (Bouchek-Mechiche *et al.*, 2000a), and some isolates belonging to *S. aureofaciens* group cause russet scab (Faucher *et al.*, 1993; Kreuze *et al.*, 1999).

Table 2.1 Description of potato tubers main blemishes

Common name(s) found in the literature		Description of tuber blemishes		Cited causes	Possible confusion	References
Black dot		Diffuse brown spots with black punctuations called sclerotia on the upper surface		*Colletotrichum coccodes*	Silver scurf	Jouan, 1997; Stevenson *et al*., 2001; FNPPPT *et al*., 2008; Wale *et al*., 2008
Silver scurf		Clear silvery patches with very fine black punctuations and separation of the skin layers		*Helminthosporium solani*	Black dot	Stevenson *et al*., 2001; FNPPPT *et al*., 2008; Selman *et al*., 2008
Powdery scab		Whitish pustules releasing a brownish and powdery mass consisting of cytosori at maturity		*Spongospora subterranea* f. sp. *subterranea*	pitted and raised scab	Jouan, 1997; Christ, 2001; FNPPPT *et al*., 2008; Mulder *et al*., 2008; Merz & Falloon, 2009
Common scab	Pitted scab	Round to star-shaped, clearly defined lesions	Roughly, star-shaped, shallow, sometimes crater-like lesions	*Streptomyces scabiei, S. europaeiscabiei, S. stelliscabiei, S. acidiscabiei, S. turgidiscabiei S. reticuliscabiei*	Powdery scab, tobacco necrosis virus	Faucher *et al*., 1993; Bouchek-Mechiche *et al*., 2000a; Bouchek-Mechiche *et al*., 2000b; Stevenson *et al*., 2001; Mulder *et al*., 2008
	Raised scab / Erumpent scab		Raised lesions		Russeting, polygonal lesions, shallow scab, netted scab, tobacco necrosis virus	
	Shallow scab / Superficial scab		Superficial lesions		Russeting, polygonal lesions, raised scab, netted scab, tobacco necrosis virus	
Netted scab		Superficial net-like structures limited to typical polygonal scab plates often associated with necrosis of all underground parts of the potato plant, including roots		*Streptomyces reticuliscabiei, S. europaeiscabiei*	Russeting, polygonal lesions, russet scab	Loria *et al*., 1997; Bouchek-Mechiche *et al*., 1998; Bouchek-Mechiche *et al*., 2000a; Scholte, 2005; FNPPPT *et al*., 2008; Mulder *et al*., 2008

Russet scab	Superficial lesions irregular in their pattern and not associated with root necrosis	Strains belonging to *S. aureofaciens* group	Netted scab	Faucher *et al*., 1993; Kreuze *et al*., 1999
Black scurf	Sclerotia: superficial and irregularly shaped, ranging from small, flat, barely palpable blotches to large, raised lumps	*Rhizoctonia solani*		Jouan, 1997; Stevenson *et al*., 2001; Wale *et al*., 2008
Rough russeted skin / russeting	Rough or scaled skin, splitting or cracking of the tuber	Varietal traits		Okazawa & Iriuda, 1980; Jong, 1981
Skinning / scuffing / excoriation / skin-set	The tuber periderm is rubbed off, giving the tuber a scuffed or feathered appearance	Physiological, mechanical injury		Stevenson *et al*., 2001; Lulai, 2007
Elephant hide / alligator hide / fishy skin / turtleback / scurfy appearance / rhizoscab	Rough, irregular, netted, crinkled, scaly, crackled shallow, thick russeting sometimes located on a deformation of the tuber or in the form of star or patch	Unknown but contributing factors may include high temperature, variety, high soil organic matter content, excessive soil moisture and fertilization, and *R. solani* or *Streptomyces* spp.	Armillaria tuber rot	Stevenson *et al*., 2001; Turff, 2002; FNPPPT *et al*., 2008; Selman *et al*., 2008
Tuber cracks / growth cracks / tuber cracking / thumbnail cracks (air cracks)	Shallow to moderately deep fissures in the surface tissues of the tuber	Physiological disorder related to fluctuations in soil moisture and turgidity of the tuber or *R. solani*		Stevenson *et al*., 2001; FNPPPT *et al*., 2008; Selman *et al*., 2008
Dry core	Brown circles of 3 to 6 mm on the tuber surface and cavities up to several mm deep	*Rhizoctonia solani* and wireworms		Radtke & Rieckmann, 1991; Keiser, 2007; Wale *et al*., 2008
Enlarged lenticels	Small, raised, white bumps of corky tissue on the surface of the tuber	Excessive moisture or insufficient supply of oxygen	Scab lesions	Jouan, 1997; Stevenson *et al*., 2001; FNPPPT *et al*., 2008; Selman *et al*., 2008

Table 2.2 Nomenclature of potato scabs in the world and suggestion for a consensus

<table>
<tr><th colspan="4">Former nomenclatures</th><th colspan="3" rowspan="2">New nomenclature</th></tr>
<tr><th colspan="2">Europe</th><th colspan="2">America and Asia</th></tr>
<tr><td rowspan="4">Common scabs</td><td rowspan="3">Raised scab</td><td rowspan="3">Common scab</td><td>Pitted scab</td><td rowspan="6">Potato scabs</td><td rowspan="3">Common scab</td><td>Pitted scab</td></tr>
<tr><td>Raised scab</td><td>Raised scab</td></tr>
<tr><td>Shallow scab</td><td>Shallow scab</td></tr>
<tr><td>Netted scab</td><td colspan="2">Not described</td><td colspan="2">Netted scab</td></tr>
<tr><td colspan="2">Russet scab (in Finland)</td><td colspan="2">Russet scab</td><td colspan="2">Russet scab</td></tr>
</table>

Likewise, tubers may present different types of superficial rugosity which are described by very diverse terms. A rough or scaled periderm of the tuber might be referred as a blemish called rough russeted skin or russeting (Figure 2.1.K). This blemish is a genetic varietal characteristic (eg. cultivar Russet Burbank), even though some authors suggest that physiological causes could also intervene (Okazawa and Iriuda, 1980; Jong, 1981). Russeting differs from russet scab as it forms deeper and larger network on potato tubers. Another similar blemish induces a scuffed or feathered appearance of the tuber with a rubbed off periderm, called scuffing, skinning, excoriation or skin-set (Figure 2.1.L). This blemish is allocated to physiological or mechanical injury (Stevenson *et al.*, 2001; Lulai, 2007).

Thirdly, we focus on atypical blemishes, whose causes are unknown, and whose terminologies unclear. They are often allocated to typical blemishes, although the symptoms are not exactly identical and the causes are not clearly demonstrated through the fulfilment of Koch's postulates. Atypical blemishes include several kinds of blemish which often have corky appearance (Figures 2.1.F to 2.1.J) and, to some degree, are visually similar to the ones due to known pathogens, namely *Streptomyces* spp. or *R. solani*. This is particularly true for netted scab-like blemishes (Figure 2.1.F and 2.1.H). The most common names encountered in North-American publications are elephant hide or alligator hide – for russet-skinned cultivars, or fishy skin, fish scale, and turtleback – for smooth-skinned cultivars (Hart, 1971; Stevenson *et al.*, 2001; Turff, 2002; Selman *et al.*, 2008) (Table 2.1). More descriptive names are used in Europe to describe these atypical corky blemishes, like cork, desquamation, scurfy appearance or scabby lesions (Campion *et al.*, 2003; FNPPPT

et al., 2008). In some instances, these corky blemishes have sometimes been called rhizoscab because the occurrence of these atypical blemishes was suspected to be connected with *R. solani* (Jouan, 1997). Indeed, such symptoms are compared to the ones observed with tuber deformations and cracks (Figure 2.1.G) (Stevenson *et al.*, 2001). However, the implication of *R solani* has so far never been clearly demonstrated (Turff, 2002; Campion *et al.*, 2003).

In Europe, *R. solani* is also frequently associated with the occurrence of dry cores (Figure 2.1.S), which are restricted brown cavities up to several millimetres deep with a diameter of 3 to 6 millimetres (Radtke and Rieckmann, 1991; Keiser, 2007; Wale *et al.*, 2008). Still this blemish is not mentioned in the American compendium of potato diseases (2001). In addition, several other atypical blemishes are often mentioned by farmers and extension workers dealing with thin-skinned potato cultivars grown under irrigation schemes; theses blemishes have the appearance of scurfy tissues around the lenticels (Figure 2.1.C). This blemish called enlarged lenticels is known to be due to excessive moisture or insufficient supply of oxygen. However, it is sometimes allocated to *R. solani* or *Streptomyces* spp. because enlarged lenticels may resemble scab lesions, although they are smaller and lighter in colour (Stevenson *et al.*, 2001). Another interesting study case is the scab-like pale brown lesion with or without star-shaped cracks (Figure 2.1.J) which is attributed to tobacco necrosis virus (TNV) (Mulder *et al.*, 2008) whereas TNV tuber symptomatology has been illustrated by other authors (Radtke and Rieckmann, 1991; Jeffries, 1998) as being quite differently looking and whereas other authors have isolated, from such identical blemishes, *R. solani* from the fifth anastomosis group (Perraton, personal data, 1997). Moreover such blemishes can easily be confused with several types of scab caused by *Streptomyces* spp. At this point and because no complete Koch's postulate has been scientifically fulfilled, it is impossible to draw any conclusion about the causing agent whether it is TNV or *R. solani* or *Streptomyces* spp.

Further more, in recently published potato disease guide (Mulder *et al.*, 2008), atypical corky blemish called polygonal lesion (Figure 2.1.G) has been attributed as a consequence of *Armillaria* species infection : very few data are available about this pathosystem (Jones and MacLeod, 1937) and the fulfilment of Koch's postulate has probably never be attempted. By all means, this type of superficial blemish could be the result of any soil-borne microorganism, whether it is bacterial or fungal.

This survey about current terms used for designation of some typical and atypical blemishes highlights confusing situations and an obvious need of clarification. The following paragraph will endeavour to give a clearer and more structured nomenclature for classification of potato tuber blemishes.

Proposal of a nomenclature for potato blemishes

In order to resolve the nomenclatural problems about the common names of typical and atypical blemishes on potato tubers, we suggest the nomenclature hereafter, based on the visual aspect of blemishes rather than on their typical or atypical character. According to the naked-eye visual aspect, we can establish two categories of blemishes: the superficial blemishes and the raised ones (Table 2.3).

Table 2.3 Suggestion of a nomenclature for the superficial blemishes of potato tubers

Name(s) used in literature		New nomenclature			
Black dot		Superficial blemishes	Discolorations		Black dot
Silver scurf					Silver scurf
Enlarged lenticels			Corky	Typical	Enlarged lenticels
Netted scab					Netted scab
Russet scab					Russet scab
Elephant hide / alligator hide / fishy skin / turtleback / scurfy appearance / rhizoscab				Atypical	Polygonal lesions
					Corky crack
					Corky spots
					Star-like corky lesions with or without halo
Rough russeted skin			Rugosities		Russeting
Skinning / scuffing / excoriation / skin-set					Skinning
Powdery scab		Pitted or raised blemishes	Pustules	Powdery scab	
Common scab	Pitted scab			Common scab	Pitted scab
	Erumpent scab / Raised scab				Raised scab
	Shallow scab / Superficial scab				Shallow scab
Black scurf			Sclerotia		Black scurf
Tuber cracks / growth cracks / tuber cracking / thumbnail cracks (air cracks)			Tuber cracks		
Dry core			Dry core		

1. Superficial blemishes alter only the external layers of the periderm and occasionally the external cortical cells but they never deeply damage the tuber flesh. They include:

- Discoloration such as black dot and silver scurf lesions, which form discoloured spots on the tuber surface (Figures 2.1.A and 2.1.B).
- Corky blemish such as
    - Enlarged lenticel, small, initially white turning brown, corky lesion surrounding the lenticels, that are clearly described (Figure 2.1.C).
    - Netted scab (produced by *Streptomyces* spp.) which forms typical, very superficial and regular polygonal corky lesions (Figure 2.1.D)
    - Russet scab (produced by strains belonging to *S. aureofasciens* group), which is very similar to netted scab, with different conditions of development (Figure 2.1.E)
    - Atypical corky blemish (unknown origin) that can appear under different forms, we suggest to distinguish 4 categories:
        - Elephant hide, turtleback or scabby lesion (Figure 2.1.F), characterized by the polygonal framework it forms on the tuber periderm. We suggest using the term polygonal lesion to describe this blemish.
        - Corky crack (Figure 2.1.G) refers to corky lesions present on split tubers;
        - Corky spot, formerly named rhizoscab (Figure 2.1.H), is similar to polygonal lesions, but forms little corky patch instead of large corky plates. The term rhizoscab should not be employed henceforth because it suggests that *R. solani* might be responsible for this blemish, even though the Koch's postulates have so far never been fulfilled;
        - Star-like corky lesion with or without halo (Figures 2.1.I and 2.1.J);
- Rugosity including
    - russeting, which refers to a rough or scaled periderm of the tuber (Figure 2.1.K),
    - skinning, which describes the blemish quite similar to russeting but forming a detached periderm (Figure 2.1.L).

2. Pitted or raised blemish form recessing or projecting skin piece masses on the tuber (Table 2.3). They include:

- Pustules resulting from either
    - o powdery scab, which forms pustules releasing a brownish and powdery mass consisting of cytosori at maturity (Figure 2.1.M) or
    - o common scab. We suggest that the international scientific community should henceforth use the term common scab to describe pitted, raised or shallow lesions caused by *Streptomyces* bacteria. The general term common scab used in Europe, and referring either to netted scab or (raised) common scab, should be replaced by the term potato scabs (Table 2.2). Three different kinds of common scab blemishes can be distinguished:
        - ▪ pitted scab (Figure 2.1.N) is commonly used to describe those deep, sometimes star-shaped lesions and the term should be conserved;
        - ▪ raised scab (Figure 2.1.O) instead of erumpent scab, which is less frequently used;
        - ▪ shallow scab (Figure 2.1.P) instead of superficial scab which is a too general term.
- Sclerotia or black scurf, typical potato blemish appearing as black superficial and irregularly shaped raised lumps, resting pellets of *R. solani* mycelia tightly attached to the periderm (Figure 2.1.Q).
- Tuber crack (Figure 2.1.R) being shallow to moderately deep splits of the tuber and for which this general term can be kept to describe all the kind of cracks without suggesting their origin or their cause.
- Dry core (Figure 2.1.S), a brown deep lesion for which terminology is commonly accepted.

The suggested nomenclature aims at harmonising the terms used to describe the different blemishes of potato tubers and could help avoiding several mistakes that can be made in the fields when blemished tubers are observed.

Conclusion

As the globalisation extends and because more and more multinational research projects are set up, there is a need for harmonising the terminology for scientific community and to make sure that we talk the same language about potato blemishes. Scientific papers often focus on a single disease with one or several symptoms, whereas pluridisciplinary approaches are les frequent. Underlined is the problem of dealing with the potato overall pathosystems and more precisely the soil-borne pathosystems. By suggesting a detailed transversal view of potato blemish issue, this paper intends to make closer the points of view of scientific researchers and of more generalist persons dealing with potatoes such as growers, breeders, seed certification staff, etc. Reviewing the literature on this subject, it was obvious that a clarification of the terminology was necessary. This nomenclature is based on the study of the different terms used in the world to describe main potato blemishes. The terms the most widely employed and the richest in sense for the description of the blemishes were adopted and the terms which can bring confusions were eliminated. Furthermore, this article shows the need of knowledge about the histological and physiological changes induced by abiotic and/or biotic stresses causing atypical blemishes. It appears as well that several hypotheses have been proposed to determine the causes of the atypical blemishes but reproducing potato-soil-borne pathosystems under controlled conditions appeared to be difficult task thus the Koch’s postulates difficult to fulfill. The authors sincerely hope that this article will catch the attention of the potato community on this major economical problem of potato tuber blemishes and will facilitate future researches, the establishment of seed certification standards and the improvement of varietal assessment. Indeed, the determination of potato blemishes' causes often refers to known diseases while visual observations relate to symptoms of diverse origins. In the future, this new nomenclature will be optimized by being translated into several different languages, and will also be extended to other blemishes which are important for other potato areas, allowing the harmonization of potato blemish terminology in all the branches of the potato production systems.

Aknowledgements

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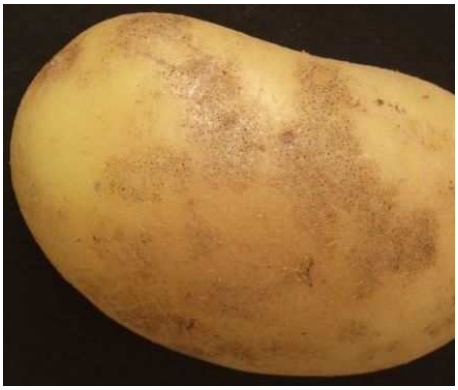
SUPERFICIAL BLEMISHES

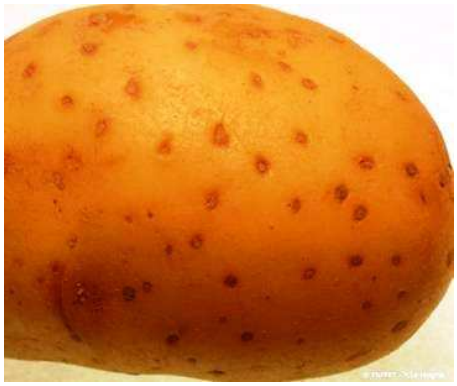
Discolorations



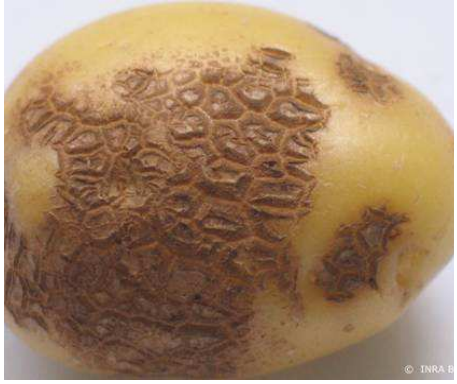
A. Black dot

B. Silver scurf

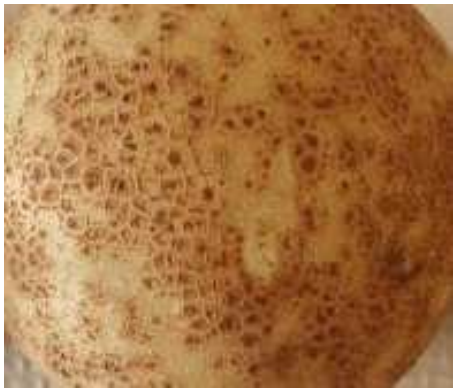
Corky blemishes



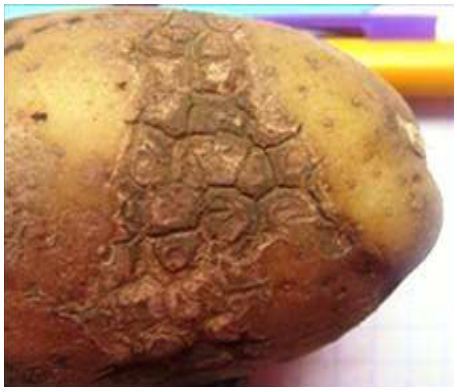
C. Enlarged lenticels



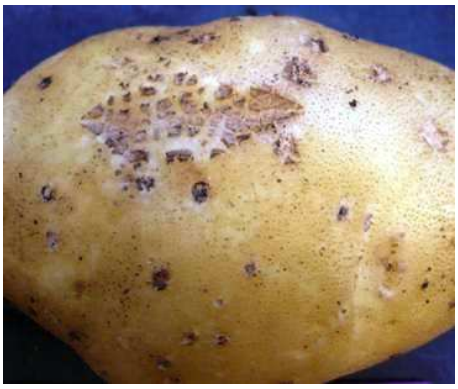
D. Netted scab



E. Russet scab



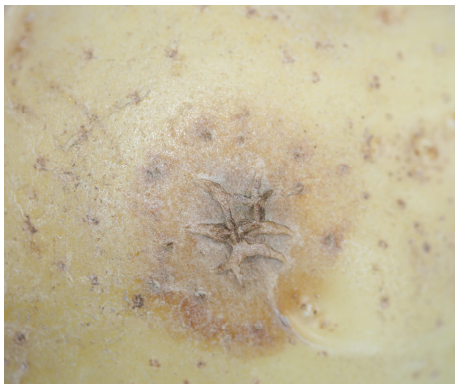
F. Polygonal lesions



G. Corky crack

H. Corky spots

I. Star-like corky lesions without halo



J. Star-like corky lesions with halo

Rugosities

K. Russeting

L. Skinning

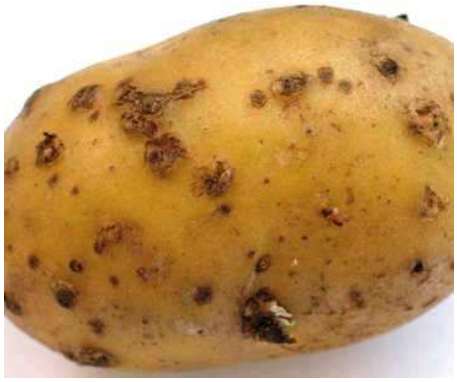
PITTED OR RAISED BLEMISHES

Pustules

M. Powdery scab

N. Pitted scab

O. Raised scab

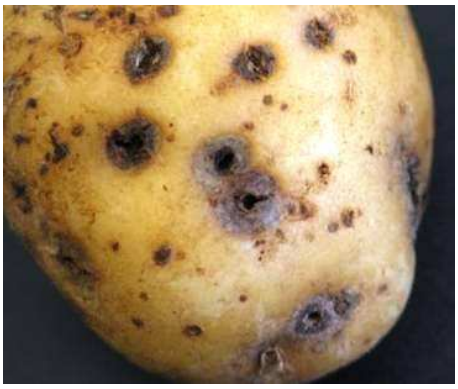


P. Shallow scab

Other pitted or raised blemishes

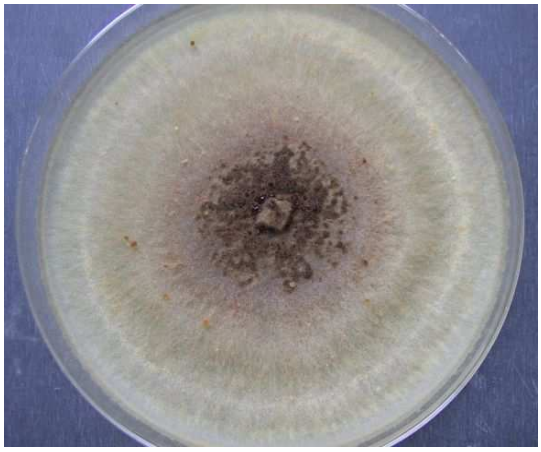
Q. Black scurf or sclerotia

R. Tuber cracks



S. Dry core

Figure 2.1 Classification of potato blemishes



Chapter 3

Diversity of microorganisms associated with atypical superficial blemishes of potato tubers and pathogenicity assessment

Chapter 3

Diversity of microorganisms associated with atypical superficial blemishes of potato tubers and pathogenicity assessment

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Abstract

Skin blemishes of potato (*Solanum tuberosum* L.) tubers can cause severe economical losses to the production. Some blemishes are due to known pathogens and others whose causes are unknown are called atypical blemishes. The present work aims at determining the origin of superficial atypical blemishes on a set of 204 tubers coming from 12 different French regions producing potato. The diversity of fungi and *Streptomyces* bacteria associated with blemishes was investigated by systematic isolation followed by identification of the strains by sequencing the internal transcribed spacer of the ribosomal DNA for fungi and by sequencing the 16S ribosomal DNA for bacteria. We found a high microbial diversity represented by 349 fungal isolates belonging to at least 47 different species and 21 bacterial strains of *Streptomyces* sp. The most represented fungi belonged to the genera *Fusarium*, *Rhizoctonia*, *Alternaria*, *Penicillium*, and *Clonostachys*. The pathogenicity of representative isolated strains was assessed in three bioassays; two bioassays based on single inoculations in previously sterilized potting mixture, and one bioassay based on both single and double inoculations under hydroponic conditions. We fulfilled the Koch's postulates for *Rhizoctonia solani* AG 3 causing sclerotia. For other fungal and bacterial strains, our results did not show any causality or relationship between a strain or a complex of strains and the occurrence of the blemishes. Moreover, the observation of irregular polygonal sunken corky lesions (polygonal lesions) - the most frequent atypical blemish - on non-inoculated tubers, suggested that the atypical blemishes could be as well a reaction of the plant to stressful environmental conditions.

Introduction

Potato (*Solanum tuberosum* L.) is the fourth main crop in the world after wheat, rice, and maize. Since the early 1990s, the potato sector is undergoing major changes worldwide. The global production increased by 20 % especially in developing countries which are changing their nutritional habits particularly in urban areas (Lutaladio and Castaidi, 2009). In most European countries, ware potatoes are now washed before selling. Washing the tubers reveals superficial blemishes and may reduce the commercial value of the commodity. Blemishes or superficial alterations affect only the tuber skin, without affecting the taste or the nutritional properties. However, they have a negative cosmetic effect on the tubers and destroy the integrity of the natural barrier of the skin. Thereby, they form an entry point for pathogenic microorganisms. Moreover, it has been shown that skin visual appearance is the most important factor influencing consumers' behaviour in fresh potato purchase. Economical data about such potential losses are difficult to estimate but all potato sectors, i.e. seed, ware, and processing are concerned.

Potato tubers can show a large range of superficial blemishes. These blemishes may result from a pathogen attack or from unfavourable environmental factors. When their causes are known and the Koch's postulates have been fulfilled, these blemishes are called typical blemishes. The typical blemishes of pathogenic origin are due to various diseases caused by fungi, bacteria, nematodes or viruses. Black dot caused by *Colletotrichum coccodes*, silver scurf (*Helminthosporium solani*), skin spot (*Polyscytalum pustulans*), black scurf (*Rhizoctonia solani*), and powdery scab (*Spongospora subterranea*) are well known fungal diseases (Radtke and Rieckmann, 1991; Stevenson *et al.*, 2001). The most widely spread bacterial disease of potato in the world is due to *Streptomyces* spp. causing common scab and netted scab and the most frequently observed symptom due to nematode is stubby-root nematode lesions caused by *Paratrichodorus* spp. and *Trichodorus* spp. nematodes. Potato Virus Y^{ntn}, Tobacco Rattle Virus (TRV), and Tobacco Necrosis Virus (TNV) are also known to cause superficial blemishes on potato tubers. Abiotic factors such as humidity, temperature, light, chemical products, nutrient deficiency or

mechanical damage cause enlarged lenticels, skin discoloration, tuber cracks or bruising. On the contrary, the blemishes for which the causal agent has not been clearly identified are called atypical blemishes.

In a previous study (Fiers, 2010), most of tuber blemishes were classified according to the type of symptom: sclerotia, enlarged lenticels, skinning, russeting, common scab, netted scab and atypical corky blemishes. The latter including corky cracks, corky spots or "rhizoscab", star-like corky lesions and blemishes commonly called "elephant hide", described as irregular polygonal sunken corky lesions. These will hereafter be called polygonal lesions. Atypical blemishes frequently observed in ware potato production are the atypical corky blemishes, especially polygonal lesions and corky spots. Black scurf or sclerotia are known to be the long term survival form of *R. solani* (Anderson, 1982; El Bakali and Martin, 2006) and common and netted scab have been unequivocally demonstrated to be caused by *Streptomyces* spp. (Lambert and Loria, 1989). These typical blemishes (sclerotia, common scab and netted scab) were integrated in this study as reference blemishes.

Production of all types of potato commodities aims at providing high quality tubers, either ware potatoes or seed tubers that need to meet the market demands related to the visual quality of the tubers. Atypical blemishes are then a predominant obstacle to the fulfilment of this quality requirement. Thus the determination of the causes of blemishes is needed. Assuming that atypical blemishes are of biological origin, two related hypotheses were considered: atypical blemishes are due to pathogenic microorganisms not yet identified or they are due to known pathogens producing atypical symptoms. Some atypical blemishes closely resemble netted scab caused by *Streptomyces* spp. but they are also occasionally attributed to *R. solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Franck) Donk) (Campion *et al.*, 2003). This is the reason why *R. solani* and *Streptomyces* were investigated as well. The objectives of this study were (1) to isolate potential pathogens from atypical skin blemishes, and (2) test whether the isolated microorganisms are able to re-create the atypical blemishes on progeny tubers and so doing, verifying Koch's postulates.

Materials and methods

Plant material

Potato tubers were collected in 2006 and 2007 in 12 different French departments representing production bases for seeds as well as for ware potatoes. In 2006 and 2007, samplings were made in 51 and 39 fields, respectively. From each field, 1 to 4 tubers representative of the overall diversity of blemishes were chosen for the study, resulting in a collection of 148 and 56 tubers sampled in 2006 and 2007, respectively. Though 42 different cultivars of potato were represented, the genetic background of *S. tuberosum* has been set aside deliberately in this study because the relationship between potato cultivar and soil-borne parasites are highly complex and still not fully understood. Blemishes were observed and classified into 10 groups (Table 3.1). Atypical corky blemishes are illustrated in Figure 3.1. The tubers were stored in paper bags at 4℃ during several weeks until the start of the experiment.

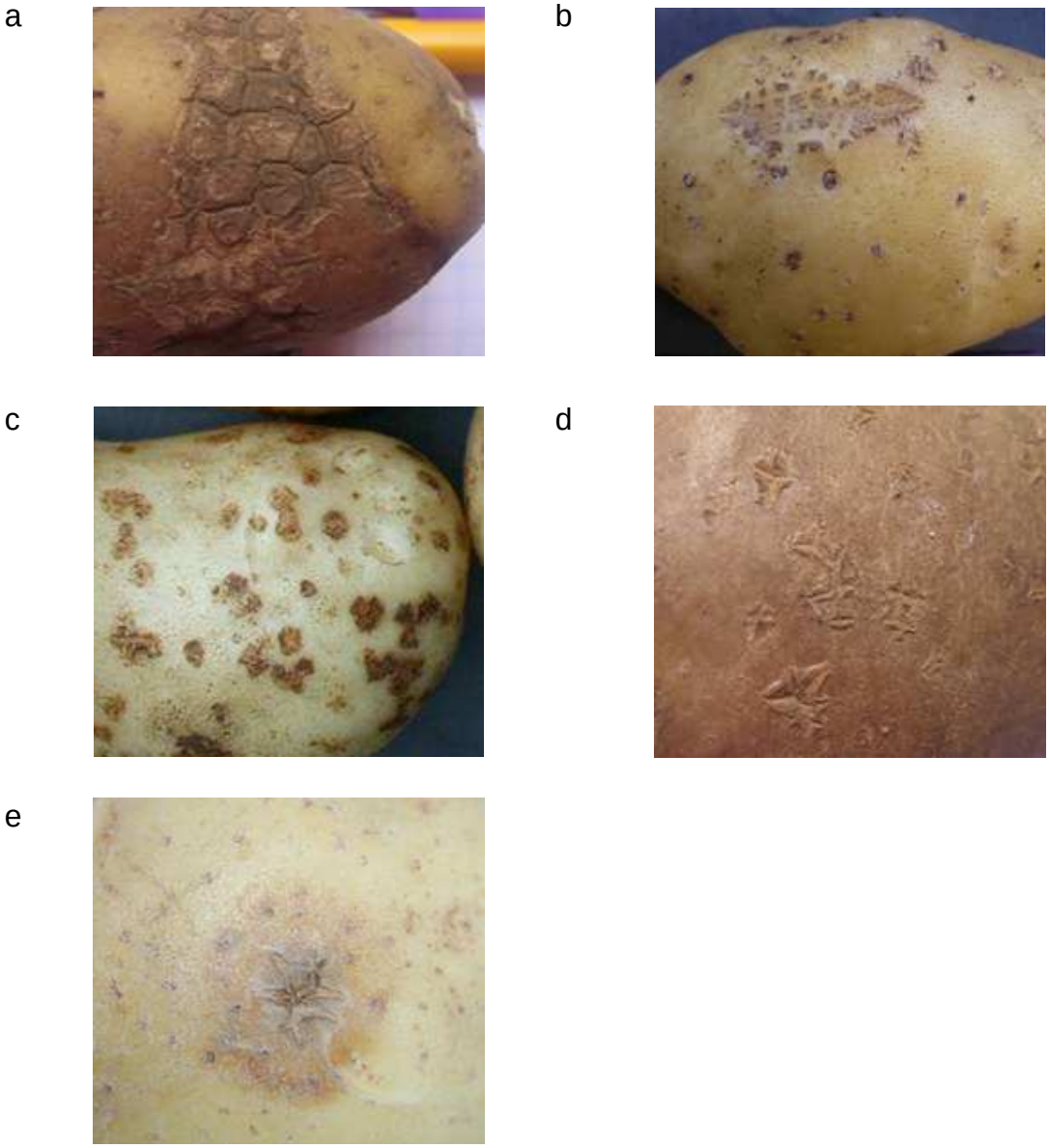

Figure 3.1 Pictures of atypical corky blemishes
(a) Irregular polygonal sunken corky blemishes or polygonal lesions; (b) Corky crack; (c) Corky spot; (d) Star-like corky lesions without halo; (e) Star-like corky lesions with halo

Isolation of fungi

Tubers were washed under running tap water and air dried. A picture of each affected tuber was taken. A 6 mm diameter and 5 mm deep piece was excised with a cork borer from the affected area of each tuber. The explants were surface sterilized in 1 % bleach for 15 s and rinsed three times in sterile water. Each tuber explant was dried on sterile paper, and plated on potato

dextrose agar (PDA). For the tubers collected in 2006, a second explant per tuber was taken and plated on water agar.

After 5 days of incubation at room temperature under natural light, fungal colonies developing from the plant material were identified by microscopic observations and purified at least twice by serial transfers on PDA. A total of 349 fungal isolates was recovered (Table 3.1) and stored both on PDA at room temperature and by cryopreservation at -80℃ in the collection "Microorganisms of Interest for Agriculture and Environment" (MIAE, INRA Dijon, France).

Isolation of *Streptomyces*

Streptomyces spp. were isolated from tubers collected in 2007 showing skinning (1 tuber), common scab (5 tubers), star-like corky lesions (1 tuber), polygonal lesions (16 tubers) and netted scab (4 tubers). Isolations were made according to the method described by Bouchek-Mechiche et al. (2000b). Tubers were washed under tap water, disinfected in ethanol from 1 min for very superficial blemishes to 5 min for deeper blemishes. They were rinsed in two consecutive baths of sterile water and air dried for at least 3 h. About 50 mg of affected skin was excised by scraping the tuber surface with a sterile scalpel and collected in a sterile mortar. One hundred µl of sterile water was aseptically added, and the mixture was homogenised with a pestle; then, about 400 µl of sterile water was added to get a smooth and homogenous mixture. After serial dilutions in sterile water, 200 µl of dilutions 10^{-3} and 10^{-5} for superficial blemishes and dilutions 10^{-4} and 10^{-6} for deep blemishes were deposited in a 9 cm Petri dish. Twenty ml of tyrosine, sodium caseinate, sodium nitrate (TCN) medium (1 g l^{-1} of L-tyrosine, 25 g l^{-1} of sodium caseinate, 10 g l^{-1} of sodium nitrate, 15 g l^{-1} of agar) maintained at 45℃ was added. Four replicates per dilution were made. Plates were incubated at 27℃ for 10 days. Each colony of *Streptomyces* was transferred to PDA and stored at 4℃. A total of 21 isolates of *Streptomyces* were collected from 27 tubers collected in 2007 (Table 3.1).

Molecular identification of fungal isolates

For DNA extraction, all the collected fungal isolates were cultivated in tubes on PDA slants. Two ml of potato dextrose broth (PDB) was poured into PDA tubes and vortexed to disperse the spores, and the spores-PDB mix was poured into Roux flasks containing 100 ml of PDB. For non-sporulating fungi, 6 explants of PDA were directly placed into Roux flasks. Flasks were incubated at room temperature without shaking for 2 to 3 days. The mycelium was harvested by filtration, frozen at -80℃ during 30 min, lyophilized and stored at -80℃.

The mycelium was ground in liquid nitrogen in a sterile mortar to obtain a mycelium powder. The DNA was extracted from 20 mg of mycelium powder using DNeasy plant mini kit (Qiagen, Courtaboeuf, France) according to the manufacturer's protocol. The DNA quantity and quality were checked by electrophoresis on a 0.8 % agarose gel, revealed with ethidium bromide and visualized by UV trans-illumination. The DNA concentrations calculated with the image analysis software Bio-Profil Bio1D++ (Windows Application V11.9, Copyright ©2004 Vilbert-Lourmat) were between 3.5 and 125 ng/µl.

For each fungal isolate, the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) was amplified by PCR with the primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) (White *et al.*, 1990; Gardes and Bruns, 1993). PCR amplifications were performed in a final volume of 50 µl by mixing 2 µl of DNA with 0.5 µM of each primer, 150 µM of dNTP, 6 U of *Taq* DNA polymerase (Q-Biogen, Evry, France) and PCR reaction buffer. Amplification was conducted in a mastercycler (Eppendorf, Hambourg, Germany) with an initial denaturation of 3 min at 94℃, followed by 35 cycles of 1 min at 94℃, 1 min at 50 ℃, 1 min at 72℃, and a final extension of 10 min at 72℃. Aliquots of PCR products were checked by electrophoresis on a 1 % agarose gel, revealed with ethidium bromide and visualized by UV trans-illumination.

The PCR products were sequenced by Beckman Coulters Genomics (Takeley, UK) using primers ITS1-F and ITS4. For each PCR product, sequences from both strands were assembled to produce a consensus sequence. Sequence identities were determined using BLAST analyses from the National Center for Biotechnology Information (NCBI) available on line.

Molecular identification of *Streptomyces* isolates

Streptomyces isolates stored at 4°C on PDA were cultivated in 25 ml of Luria Bertani (LB) media (10 g l^{-1} of bacto tryptone, 5 g l^{-1} of yeast extract, 10 g l^{-1} of NaCl; pH 7) for 6 days at 27°C. The DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's specifications. The DNA quantity and quality were checked by electrophoresis as above and the DNA concentrations calculated as above were between 4 and 650 ng/µl.
For each *Streptomyces* isolate, the 16S rDNA was amplified by PCR with the primers 27F (AGAGTTTGATCCTGGCTCAG) (Edwards *et al.*, 1989) and 1392R (ACGGGCGGTGTGTACA) (Braker *et al.*, 2001). PCR reactions were performed in a final volume of 50 µl by mixing 10 µl of DNA with 0.2 µM of primer 27F, 0.2 µM of 1392R, 200 µM of dNTP, 12 U of *Taq* DNA polymerase (Q-Biogen) and PCR reaction buffer. Amplifications were conducted in a mastercycler (Eppendorf) with an initial denaturation of 3 min at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 57°C, 1 min at 72°C, and a final extension of 5 min at 72°C. Aliquots of PCR products were checked by electrophoresis on a 1 % agarose gel as above.
The PCR products were sequenced using primers 27F and 1392R. For each PCR product, sequences from both strands were assembled to produce a consensus sequence. Sequences identities were determined using BLAST as above.

Pathogenicity tests

Strains representative of the most frequently isolated fungal and *Streptomyces* species were tested for their pathogenicity on potato tubers. In order to fulfil Koch's postulates for these strains, two different types of bioassays were set up: a pot-test with artificially infested soil and a test where potatoes were grown under hydroponic conditions.

Bioassays in soil

The bioassays were conducted in 2007 and 2008 in a greenhouse from April to September. Healthy potato tubers were grown in pots containing soil artificially infested with fungal or *Streptomyces* isolates. Thirty-three strains were tested in 2007 and 48 strains in 2008. Nine strains were tested twice (Table 3.2). In addition, four reference strains were tested: *Fusarium sambucinum* (TFSa, isolated in France 2007), *F. solani* var. *coeruleum* (T FSC1, isolated in France 2006) *R. solani* AG 3 (i4 / 9729-1, isolated in France 1999), and *R. solani* AG 2-1 (0799-001 2N WA, isolated in Morocco 2007).

Fungal inoculum was prepared on autoclaved millet seeds. Forty grams of seeds were mixed in jars with 32 ml of sterile deionised water and autoclaved for 45 min at 110℃ on two consecutive days and for 20 min at 120℃ the third day. The jars were stored at room temperature during 4 days before inoculation to allow the release of putative toxic compounds (mainly NH_3). Six plugs of 12 day-old fungal cultures on PDA were introduced and mixed with the millet seeds. The cultures were incubated at room temperature for 3 weeks with regular shaking.

For the *Streptomyces* inoculum, *Streptomyces* spp. were grown on 9 cm PDA plates. Prior to the inoculum setting, a specific substrate for the *Streptomyces* inoculum was prepared. One liter of vermiculite, mixed with 150 ml of deionized water was autoclaved for 1 h at 120℃, on two conse cutive days. 170 ml Say's media (sucrose 20 g l^{-1}; asparagine 1.2 g l^{-1}; K_2HPO_4 0.6 g l^{-1}; yeast extract 10 g l^{-1}) was added to the vermiculite and autoclaved for 30 min at 115℃. *Streptomyces* mat and spores were scraped from 7 to 10 day-old culture on oatmeal agar (oatmeal powder 50 g l^{-1}; agar 23.5 g l^{-1}) at 27 ℃ and ground in a sterile mortar with 8 ml sterile water. The resulting mixture was poured into the sterile vermiculite substratum and was incubated for 2 to 3 weeks at 27℃ with daily shaking (Wanner, 2004).

The potting mixture (one third of sand and two thirds of peat) used to grow the potatoes was steam-disinfected and stored at room temperature for 7 days to allow putative toxic compounds to be released. The fungal and *Streptomyces* inocula were mixed separately with about 6 l of disinfected potting mixture with a three-dimensional shaker (Turbula, System Schatz). The infested potting

mixture was placed in 10 l plastic pots (25 cm diameter, 30 cm high). The pots infested with *Streptomyces* were prepared two weeks before planting, covered with a plastic cover and kept at room temperature for about 15 days to allow the establishment and multiplication of the bacteria.

One seed tuber was planted in each pot. Cultivar Charlotte was chosen for soil assays because it is one of the cultivars that is mostly cited in the sample collections (2006 and 2007) and because it has a medium to high overall susceptibility to atypical superficial blemishes (FNPPPT and GNIS, 2007, Chatot, personnal data). Commercial certified seeds (Class A; 25-32) were used and visually examined for absence of any skin blemish. Plants were grown in a glasshouse at room temperature with minimal temperature of 10℃ and maximal temperature of 25℃ with a 16 h day length, for approximately 4 months, until natural maturity; additional light (200 W/m^2) was provided when needed. Plants were regularly watered, but soil moisture content was not monitored. Fertilizing watering was carried out every week with a fertilizer solution (N P K, 20 20 20). Plants were harvested in September. A non inoculated control and three replicate pots per treatment were set up.

At harvest, one gram of soil from each pot was spread on PDA in a Petri dish to check the survival and viability of the inoculated fungi and bacteria during the assay. After 4 days, the presence of the inoculated microorganisms was checked under a microscope.

Progeny tubers from the same plant were washed under running tap-water, air-dried, weighed and stored in a paper bag. The number of tubers per plant was recorded and the tubers larger than 3 cm long were scored individually according to the different classes of blemishes. The scoring scale edited by the French official service of control and certification (SOC) (GNIS and SOC, 2005) was adapted to black scurf, netted scab and common scab scales, each with 10 different levels of disease severity. Black scurf scale was: 0 = no lesion, 1 = 1 % of area covered by lesions; 3 = 4% of area covered by lesions; 5 = 9 % of area covered by lesions; 7 = 14 % of area covered by lesions; 9 = 35 % of area covered by lesions. Netted and common scab scales scored tuber as following: 0 = no lesion, 1 = 4 % of area covered by lesions; 3 = 6% of area covered by lesions; 5 = 30 % of area covered by lesions; 7 = 45 % of area covered by lesions; 9 = 60 % of area covered by lesions

The intensity of sclerotia was scored according to the *R. solani* scale, from level 1 (1 % of the tuber surface covered by the blemish) to level 9 (>35 % of the tuber surface covered by the blemish). Other blemishes were scored according to the common and netted scab scale, from level 1 (4 % of the tuber surface covered by the blemish) to level 9 (> 60% of the tuber surface covered by the blemish). The intensity was calculated by averaging the intensity of the reproduced blemish for the 3 replicates (6 when the strain was tested twice (Table 3.2). Fungi and *Streptomyces* were isolated from the progeny tubers following the same protocol as above.

Co-inoculation tests under hydroponic conditions

In the second type of test, potatoes were grown under hydroponic conditions in order to follow the development of the blemishes *in-situ*. A total of 22 strains of fungi and *Streptomyces* spp. were tested in single and co-inoculations, resulting in 49 different treatments (Table 3.3).

The experimental system (adapted from Gray, 1973) (Figure 3.2) consisted of a tray (40 cm in diameter and 10 cm in depth) with a 5 cm diameter hole in the centre, placed on a 5 l pot containing nutrient solution (N P K, 13 21 13 or N P K, 8 17 24, see below) (Algospeed Flo, Compo, France). A seed tuber was placed in the centre of the tray on a plastic screen of 1 cm mesh, maintaining the tuber in the tray, away from the water but allowing the roots to elongate into the nutrient solution. Certified seeds (Class A, 28-35) of the cultivar Bintje were used; this cultivar is known for its susceptibility to overall skin blemishes

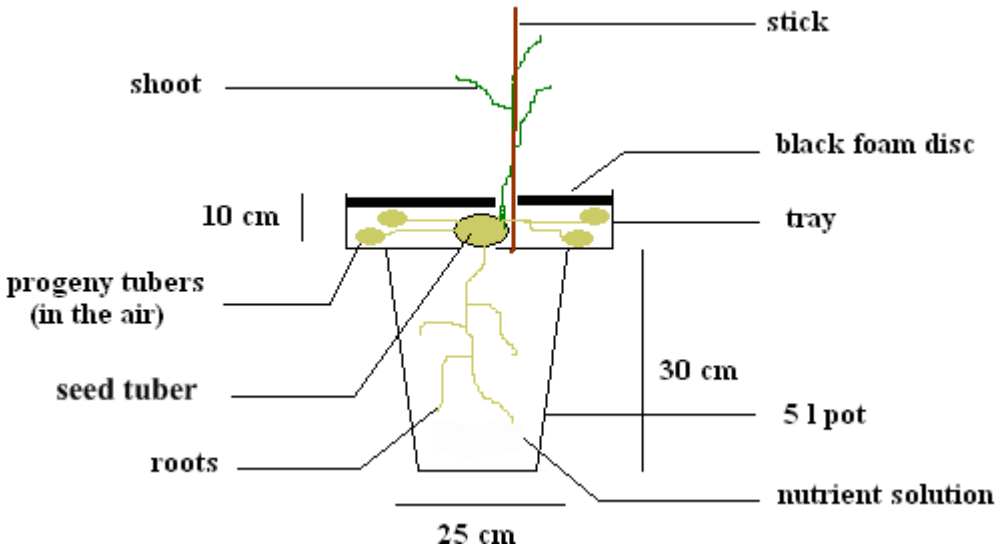

Figure 3.2 Scheme of the experimental set up of the assay under hydroponic conditions

A sterile mixture of 7 l of perlite and vermiculite (50/50 v/v) was placed in the tray as to completely cover the seed tuber; it was regularly moistened to initiate the development of roots and stolons. Stems were supported by a stick. All pots were set in a greenhouse; the day length was 12 h 30 and temperature was kept at 15℃ during the day and 10℃ during the nig ht. After 2 weeks, when sprouting was initiated the perlite-vermiculite mixture was removed and the tray was covered with a black foam disc to keep the newly formed tubers in the dark. During the course of the experiment, nutrient solution was aerated with an air pump (Hiblow, Airpump Takatsuki). The nutrient solution (N P K, 13 21 13) was used during the initiation of the stolons with a high content in phosphorus to favour root development. After 2 months it was changed to (N P K, 8 17 24), a solution with more potassium to strengthen tubers. The pots were emptied and filled again with fresh nutrient solution (pH ≈ 6 and conductivity ≈ 1500 µS) once a week.

Two and a half months after plantation, when progeny tubers reached a sufficient size (i.e. > 2 cm long), they were inoculated with one or two strains. Inoculations were performed using plugs of 10 day-old microbial cultures on PDA plates. Six visually healthy progeny tubers per plant were chosen. Three of them were wounded with a sterile toothpick and the three others were not. For each treatment, plugs of the inoculated strains were placed side by side on the tubers, on the wound for wounded tubers. For each treatment corresponding to single or double inoculations, three independent plants were inoculated. A

control treatment corresponding to three non-inoculated plants was also conducted. Plants were grown until natural senescence and harvested individually.

After inoculation, newly formed tubers were observed weekly during 7 weeks. The blemishes were scored when they appeared and all along their development.

At harvest, tubers from each plant were washed under running tap-water, air-dried and placed in a paper bag. The number of tubers per plant was recorded and the tubers were scored individually for atypical corky blemishes with the scoring scale from 1 to 9.

Fungal and *Streptomyces* isolations on progeny tubers were done following the same protocol as above.

Results

Among the samples collected in 2006 and 2007, the blemishes the most frequently observed were polygonal lesions, representing 22 % of the total blemishes, followed by sclerotia (16 %), enlarged lenticels (15 %), corky spots (13 %), common scab (8 %), corky cracks (8 %), star-like corky lesions (7 %), skinning (5 %), netted scab (4 %), and russeting (2 %). The atypical corky blemishes (polygonal lesions, corky spots, corky cracks and star-like corky lesions) all together represented 50 % of the observed blemishes.

Identification of fungi

Twenty six different fungal genera were found among the 349 isolates collected from blemishes and identified from their ITS sequence (Table 3.1). The most represented genera were *Fusarium* (80 strains), *Rhizoctonia* (68 strains), *Alternaria* (46 strains), *Penicillium* (33 strains), and *Clonostachys* (27 strains). Most of the isolates were identified at the species level, however, some isolates were only identified at the genus level, because either their ITS sequence had the same percentage of similarity as two or more fungal species or the obtained ITS sequence was too short. Finally, at least 45 different fungal

species were identified. In the case of *R. solani* isolates, the anastomosis groups (AG) were also determined based on ITS sequences (Table 3.1).

Table 3.1 Fungal and *Streptomyces* species isolated from the different blemishes

Fungal or *Streptomyces* species	Sclerotia	Polygonal lesions	Corky cracks	Corky spots	Star-like corky lesions	Enlarged lenticels	Skinning	Russeting	Common scab	Netted scab	Other	Total
Absidia glauca							1					1
Alternaria arborescens		1										1
A. citri					1							1
A. longissima		1		1						1		3
Alternaria sp.	3	17		4	4	4	3	1	3		2	41
Bjerkandera adusta		1				1	1					3
Bjerkandera sp.		3					1					4
Ceratobasidium sp.				1								1
Cercophora grandiuscula						1						1
Cladosporium cladosporioides					1		1					2
Cladosporium sp.	1	1			1	1			1			5
Clonostachys rosea	2	12			1	6	1		5			27
Colletotrichum coccodes	1	2	1			1			2			7
Colletotrichum sp.		2										2
Cylindrocarpon olidum		1										1
Epicoccum nigrum					1				4	1	1	7
Fusarium avenaceum					1					1		2
F. culmorum	1											1
F. equiseti				1	1				1			3
F. graminearum	1						1		1			3
F. oxysporum	4	20	3	3	4	2	2		6	1	1	46
F. redolens		2				1						3
F. sambucinum						1	1				1	3
F. sambucinum or *F. tumidum*											1	1
F. solani			2			2					1	5
F. venenatum			1		2		1	1				4
Fusarium sp.		3		2		1			1	1	1	9
Gliomastix murmorum		1										1
Microdochium bolleyi			1	1					1			3
Microdochium sp.									1			1
Mortiella elongata										1		1
Mucor circenelloides	1	4				2			1		1	9
M. fragilis	1											1
M. hiemalis	1	1		1			1					4
Mucor sp.					1							1
Neonectria radicicola		1				1					2	4
Neonectria sp.		1										1
Penicillium brasilianum	1		1			1						3
P. brevicompactum	1	3		1		12		1	2	1		21
P. freii					1							1
P. paneum										1		1
P. raistrickii						1						1
P. swiecickii		1										1
Penicillium sp.	1			1		2			1			5
Phoma exigua		1	2	1	2	1			1	2		10
Plectosphaerella cucumerina	1	4		2		5			1			13
Rhizoctonia solani AG 2-1	5								1			6
R. solani AG 3 - PT	29	9	1			6	2		7	1	1	56
R. solani AG 5				1		2		1				4
Rhizoctonia sp.				1	1							2
Rhizopus oryzae				1								1
Rhizopus sp.				1								1
Stereum rugosum							1					1
Trichocladium asperum										1		1
Trichoderma tomentosum										1		1
T. velutinum		1										1
T. viride		1										1
Trichoderma sp.							1	1				2
Ulocladium capsicum		1										1
Ulocladium sp.		1										1
Verticillium dahliae		1										1
Total fungi												349
Streptomyces scabiei									4			4
Streptomyces sp.		11							5	1		17
Total *Streptomyces*												21

Pathogenicity tests

Bioassays in soil

The inoculated strains were re-isolated from the soil in 39% of the cases. Fungi belonging to the *Rhizoctonia solani* species were more frequently re-isolated than the other species.

The results of the two bioassays conducted in soil are presented in table 3.2. Among the 62 fungal strains and the 8 *Streptomyces* strains inoculated, 30 fungi and 1 *Streptomyces* were re-isolated from the progeny tuber surfaces at the end of the tests. Several other fungi that were not initially inoculated in pots were also isolated such as *Trichoderma* spp., *Mucor* spp. and *Colletotrichum* spp. Among the inoculated fungi, *R. solani* was the species the most frequently re-isolated from tubers (16 strains out of 21 were recovered on the progeny tuber surface). Conversely, only one out of the 7 *Alternaria* strains tested and none of the 8 *Clonostachys* strains tested were recovered from the progeny tuber surface.

Table 3.2 Strains tested in the two bioassays conducted in infected soil

Species	Strains [(a)]	MIAE accession number[(b)]	Host of origin (potato cultivar)	Blemish of origin	Bioassays results		
					Observed blemishes		Detection of the inoculated species on the tubers[(d)]
					Identical to the original one (intensity[(c)])	Different from the original one	
Alternaria spp.	0629-002 J 1B PDA	MIAE00160	Juliette	Star-like corky lesion		Skinning, enlarged lenticels	-
	0629-002 M 2A PDA	MIAE00162	Marine	Polygonal lesions		Skinning, enlarged lenticels	+
	0629-041 1B PDA	MIAE00096	Daisy	Corky spot		Skinning, enlarged lenticels	-
	0629-045 3B WA	MIAE00099	Isabelle	Polygonal lesions	Polygonal lesions (1)	Skinning, enlarged lenticels	-
	0629-058 2 PDA	MIAE00182	Samba	Polygonal lesions	Polygonal lesions (1)		-
	0629-058 3A PDA *	MIAE00183	Samba	Polygonal lesions	Polygonal lesions (1)		-
	0629-059 1Aβ WA	MIAE00184	Fuego	Skinning	Skinning (2)	Enlarged lenticels	-
Cladosporium sp.	0629-022 2 PDA	MIAE00169	Chérie	Common scab		Polygonal lesions	-
Clonostachys rosea	0628-013 3 PDA	MIAE00156	Anoe	Enlarged lenticels	Enlarged lenticels (1)	Skinning, star-like corky lesions	-
	0629-022 1 WA	MIAE00210	Charlotte	Common scab		Skinning, enlarged lenticels	-
	0629-023 3A WA	MIAE00199	Charlotte	Common scab		Skinning, enlarged lenticels, polygonal lesions	-
	0629-030 1 WA	MIAE00175	Samba	Polygonal lesions		Skinning, enlarged lenticels	-
	0629-038 2 WA	MIAE00084	Samba	Polygonal lesions		Skinning, enlarged lenticels	-
	0629-038 4 PDA *	MIAE00088	Samba	Polygonal lesions	Polygonal lesions (1)		-
	0629-040 3C WA	MIAE00095	Samba	Polygonal lesions		Skinning, enlarged lenticels	-
	0629-056 1 PDA	MIAE00180	Spunta	Enlarged lenticels	Enlarged lenticels (1)	Skinning, sclerotia	-
Colletotrichum coccodes	0610-001 2A PDA	MIAE00075	Daifla	Corky crack		Skinning, polygonal lesions	+
Fusarium equiseti	0629-023 1B PDA	MIAE00079	Charlotte	Common scab		Polygonal lesions	+
F. oxysporum	0628-015 1A PDA	MIAE00157	Amandine	Corky spot		Skinning, enlarged lenticels	-
	0629-002 J 2Bβ PDA *	MIAE00163	Juliette	Star-like corky lesions		Skinning, enlarged lenticels	+
	0629-023 3B PDA	MIAE00172	Charlotte	Common scab		Skinning, enlarged lenticels	-
	0629-024 2 PDA	MIAE00173	Désirée	Common scab		Skinning, enlarged lenticels	-
	0629-040 1A PDA	MIAE00091	Samba	Polygonal lesions		Skinning, enlarged lenticels	-
	0629-040 2B WA	MIAE00214	Samba	Polygonal lesions	Polygonal lesions (1)	Skinning, enlarged lenticels	+
	0629-055 1 WA	MIAE00178	Pamela	Corky spot		Skinning, enlarged lenticels	-
	0629-058 1 PDA	MIAE00181	Samba	Star-like corky lesions		Skinning, enlarged lenticels	-
	0629-067 2 WA	MIAE00186	Samba	Polygonal lesions		Skinning, enlarged lenticels	+
F. sambucinum / *F. tumidum*	0628-012 3 PDA	MIAE00209	Charlotte	Dry rot		Skinning	+
F. sambucinum	T FSa **		unknown	Dry rot		Skinning, star-like corky lesions	+
F. solani var. *coeruleum*	T FSC 1 **		unknown	Dry rot		Polygonal lesion	-
F. solani	0610-001 2 WA	MIAE00074	Daifla	Corky crack		Skinning, enlarged lenticels, sclerotia	+
	0610-004 2 WA	MIAE00201	Nicola	Corky crack		Skinning, enlarged lenticels	+
F. venenatum	0610-001 1A PDA	MIAE00070	Daifla	Star-like corky lesions		Skinning, enlarged lenticels	-
	0610-004 1 PDA *	MIAE00076	Nicola	Corky crack		Skinning, polygonal lesions	-
Fusarium sp.	0629-021 2A PDA	MIAE00167	Atlas	Mould lesion		Skinning	+
Microdochium sp.	0629-024 2 WA	MIAE00174	Désirée	Common scab		Polygonal lesions , skinning, enlarged lenticels	-

Neonectria radicicola	0628-019 1A WA	MIAE00158	Anoe	Enlarged lenticels		Polygonal lesions	-
Penicillium brevicompactum	0628-006 3B PDA	MIAE00151	Amandine	Enlarged lenticels	Enlarged lenticels (1)	Skinning , polygonal lesions	-
	0628-012 1A WA	MIAE00153	Charlotte	Enlarged lenticels	Enlarged lenticels (1)	Skinning	+
	0628-013 2 WA	MIAE00215	Anoe	Enlarged lenticels	Enlarged lenticels (1)	Skinning , polygonal lesions	-
Penicillium sp.	0628-001 1B PDA	MIAE00149	Adriana	Enlarged lenticels		Skinning , polygonal lesions	+
Plectosphaerella cucumerina	0629-039 1 WA	MIAE00089	Amandine	Enlarged lenticels	Enlarged lenticels (2)	Polygonal lesions	+
Rhizoctonia solani AG2-1	0799-001 2N WA **	MIAE00189	Nicola	Corky crack		Skinning, enlarged lenticels	-
R. solani AG3	0602-001 1B PDA *	MIAE00072	Mixed	Polygonal lesions	Polygonal lesions (1)	Sclerotia, skinning	-
	0628-006 1A WA	MIAE00150	Amandine	Enlarged lenticels	Enlarged lenticels (2)	Skinning, polygonal lesions, sclerotia	+
	0628-006 3A PDA	MIAE00152	Amandine	Enlarged lenticels		Skinning, polygonal lesions, sclerotia	+
	0629-004 1A WA *	MIAE00164	Spunta	Sclerotia	Sclerotia (3)	Polygonal lesions	+
	0629-014 3 WA	MIAE00165	Chérie	Common scab		Skinning, enlarged lenticels, polygonal lesions, sclerotia	+
	0629-017 1 PDA	MIAE00166	Charlotte	Polygonal lesions	Polygonal lesions (2)	Sclerotia	+
	0629-023 1A PDA	MIAE00170	Charlotte	Common scab		Polygonal lesions, sclerotia	+
	0629-030 2 PDA	MIAE00176	Samba	Polygonal lesions	Polygonal lesions (1)	Sclerotia	+
	0629-030 3 PDA	MIAE00081	Samba	Polygonal lesions	Polygonal lesions (1)	Sclerotia	+
	0629-033 2B WA *	MIAE00082	Juliette	Skinning		Enlarged lenticels, sclerotia	+
	0629-036 1A WA	MIAE00083	Juliette	Skinning	Skinning (6)	Polygonal lesion, sclerotia	-
	0629-038 3A WA *	MIAE00087	Samba	Sclerotia	Sclerotia (2)	Skinning , polygonal lesions	+
	0629-039 2A WA	MIAE00090	Amandine	Enlarged lenticels	Enlarged lenticels (2)	Skinning, polygonal lesion, sclerotia	+
	0629-040 2A WA *	MIAE00092	Samba	Polygonal lesions	Polygonal lesions (1)	Sclerotia	+
	0629-049 2 WA	MIAE00006	Juliette	Polygonal lesions		Skinning, enlarged lenticels, sclerotia	+
	0629-055 3 WA *	MIAE00179	Pamela	Sclerotia	Sclerotia (2)	Polygonal lesions	+
	0629-059 3 PDA	MIAE00185	Fuego	Corky crack	Corky crack (2)	Skinning, sclerotia	+
	i 4 */**		unknown	Sclerotia	Sclerotia (2)	Skinning , polygonal lesions	+
R. solani AG5	0628-023 1B WA	MIAE00213	Chérie	Enlarged lenticels	Enlarged lenticels (1)	Skinning, polygonal lesions, sclerotia	-
S. scabiei	B M_ 0745-001 P		Bintje	Common scab		Skinning, enlarged lenticels	-
Streptomyces sp.	E M1_ 0747-002 P		Yona	Common scab		Skinning, enlarged lenticels	+
Streptomyces sp.	F M1_ 0729-049		Rosabelle	Netted scab		Skinning, enlarged lenticels	-
Streptomyces sp.	H P _0762-005		Bintje	Netted scab		Skinning, enlarged lenticels	-
Streptomyces sp.	L M_ 0756-093		May Flower	Netted scab		Skinning, enlarged lenticels, common scab	-
Streptomyces sp.	L P_ 0756-093		May Flower	Netted scab		Skinning, enlarged lenticels	-
Streptomyces sp.	M P1_ 0729-019		Urgenta	Common scab	Common scab (1)	Skinning, enlarged lenticels	-
Streptomyces sp.	V P2 _0762-007		Bintje	Netted scab		Skinning, enlarged lenticels	-
Non-inoculated control						Skinning, enlarged lenticels, polygonal lesions	

(a) The codification of the strains includes the year of isolation (two first digits), the geographical zone of isolation corresponding to the French departments (third and fourth digits) and the strain identification number (last digits and letters). Strains followed by an asterisk (*) were tested in the two different bioassays (2007; 2008). Strains followed by two asterisks (**) are reference strains, which were not sequenced in this study.
(b) Collection MIAE, Microorganisms of Interest for Agriculture and Environment (INRA Dijon, France).
(c) Intensities of blemishes were scored according to the official French scales of the tuber potato diseases (GNIS and FNPPPT). Sclerotia were scored from level 1 (1 % of the tuber surface covered by the blemish) to level 9 (>35 % of the tuber surface covered by the blemish) and the other blemishes were scored from level 1 (4 % of the tuber surface covered by the blemish) to level 9 (> 60% of the tuber surface is covered by the blemish).
(d) + means that the inoculated strain was isolated from the progeny tubers; - means that the inoculated strain was not isolated from the progeny tubers.

In all the treatments, all types of blemishes were observed with a variable intensity, but the original blemishes were rarely reproduced by the inoculated strains. There was no clear relationship between the type of blemish and the taxonomic status of the strains. However the strains of *R. solani* AG 3 were able to cause sclerotia (4 of the strains) as well as polygonal lesions (4 other strains) and one strain generated corky crack, on the progeny tubers. One of 9 *F. oxysporum* strains was able to cause polygonal lesions. One of the three strains of *P. brevicompactum,* two strains of *R. solani*, and the only strain of *P. cucumerina* caused enlarged lenticels. Among the strains of *Streptomyces* tested, none has produced the same blemish as the one from which it was isolated. Progeny tubers coming from the non-inoculated controls also showed blemishes: skinning (6 out of 6 tubers), enlarged lenticels (5 out of 6 tubers), and polygonal lesions (1 out of 6 tubers).

The intensity of reproduced blemishes varied from 1 to 6 with an average of 1.6, which means that in average, less than 6 % of tuber surfaces was covered by a given type of blemish. In general, blemishes intensities were low. Some tubers showed several different types of blemishes making it difficult to make a connection between the isolated strain and a specific blemish.

Co-inoculation tests under hydroponic conditions

Before inoculation, 43 % of the progeny tubers already displayed polygonal lesions with an average intensity of 3, meaning that 6 % of the tuber surface was covered by the blemish. The blemishes observed during the seven weeks following the inoculation, are listed in Table 3.3. Corky cracks, polygonal lesions, enlarged lenticels, skinning, and star-like corky blemishes were produced on progeny tubers grown under hydroponic conditions and inoculated with one or two selected strains. For each treatment, a variety of blemishes was observed without any relationship between the strain inoculated and blemishes. In none of the treatments, the inoculated fungus or *Streptomyces* were re-isolated from progeny tubers after harvest. However, numerous *Mucor* spp. and *Penicillium* spp. were isolated. The diversity of blemishes and of fungi isolated from the non-inoculated tubers was similar to the one from the inoculated tubers.

Table 3.3 Strains tested in the bioassay conducted with single and co-inoculations under hydroponic conditions

					Observed blemishes in the bioassay [(a)]							
						Double inoculations [(b)]						
Species	Strains	MIAE accession number	Host of origin (potato cultivar)	Blemish of origin	Single inoculations	*R. solani* AG 3 0629-023 1A PDA	*Fusarium oxysporum* 0629-023 3B PDA	*Alternaria* spp. 0629-048 1B PDA	*Penicillium brevicompactum* 0629-056 3A PDA	*Streptomyces* spp. A M1_ 0729-008	*Streptomyces* spp. L P_ 0756-093	*Plectosphaerella cucumerina* 0629-023 1A WA
Alternaria spp.	0629-045 2B PDA	MIAE00191	Isabelle	Polygonal lesions	C, I, SL	NT	NT	NT	NT	NT	NT	NT
	0629-048 1B PDA	MIAE00192	Spunta	Skinning	C, G, I, L, P, S, SL	C, G, I, P, S	C, I, L, P, SL	NT	NT	NT	NT	NT
	0629-056 2B PDA	MIAE00193	Spunta	Enlarged lenticels	L, P	NT	NT	NT	NT	NT	NT	NT
	0629-058 3B WA	MIAE00194	Samba	Polygonal lesions	L, S, SL	NT	NT	NT	NT	NT	NT	NT
Bjerkandera adusta	0629-048 1Bβ WA	MIAE00195	Spunta	Skinning	I, L, P, S	NT	NT	NT	NT	NT	NT	NT
Clonostachys rosea	0629-023 3A WA	MIAE00199	Charlotte	Common scab	C, I, L, P, S, SL	NT	NT	NT	NT	NT	NT	NT
	0629-048 1Bα WA	MIAE00196	Spunta	Skinning	C, I, P	G, I, L, P, S	C, L	I, L, P, S	C, G, I, L, P, S, SL	C, I, L, P, SL	C, I, L, S, SL	C, I, L
Fusarium equiseti	0629-023 1B PDA	MIAE00079	Charlotte	Common scab	C, I, L, P, rot	NT	NT	NT	NT	NT	NT	NT
F. oxysporum	0629-023 3B PDA	MIAE00172	Charlotte	Common scab	C, I, G, L, P, S	I, L, S	NT	NT	NT	NT	NT	NT
	0629-067 2A PDA	MIAE00197	Samba	Polygonal lesion	I, L, P	NT	NT	NT	NT	NT	NT	NT
F. solani	0628-006 1A PDA	MIAE00198	Amandine	Enlarged lenticels	C, L, P, rot, S	NT	NT	NT	NT	NT	NT	NT
Penicillium brevicompactum	0628-006 2A WA	MIAE00003	Amandine	Enlarged lenticels	C, I, L, P, S	NT	NT	NT	NT	NT	NT	NT
	0629-056 3A PDA	MIAE00200	Spunta	Enlarged lenticels	C, I, P, S	C, I, L, P, S	C, L, P, S, SL	I, L, P	NT	NT	NT	NT
	0729-008 PDA	MIAE00004	Naga	Polygonal lesion	C, I, P	NT	NT	NT	NT	NT	NT	NT
Plectosphaerella cucumerina	0629-023 1A WA	MIAE00202	Charlotte	Common scab	C, I, L, P, SL	C, I, L, P, S	I, L, P	C, G, I, L, P, S, SL	C, I	C, I, L, P, S	I, L	NT
	0680-006 2Bα WA	MIAE00203	Hybride A	Sclerotia	C, G, L, P, S, SL	NT	NT	NT	NT	NT	NT	NT
Rhizoctonia solani AG 2-1	0680-006 2Aβ WA	MIAE00208	Hybride A	Sclerotia	G, I, L, SL	NT	NT	NT	NT	NT	NT	NT
R. solani AG 3	0629-023 1A PDA	MIAE00170	Charlotte	Common scab	C, I, L, P, S	NT	NT	NT	NT	NT	NT	NT
Streptomyces scabiei	Y P1_0747-001 P	MIAE00204	Hybride B	Common scab	C, I, P, S	NT	NT	NT	NT	NT	NT	NT
Streptomyces spp.	A M1_0729-008	MIAE00205	Naga	Polygonal lesion	G, L, rot, S	C, I, L, P, S	C, I, L, P, S	L, P, S	C, I, L, P, rot, S, SL	NT	NT	NT
	L P_0756-093	MIAE00206	May Flower	Netted scab	C, G, I, L, P, S, SL	C, I, L, P, S, SL	C, G, P, S	G, I, L, P, S	C, G, I, L, P, S	NT	NT	NT
	X M_0747-001 L	MIAE00207	Hybride B	Netted scab	C, I, P, S	NT	NT	NT	NT	NT	NT	NT

(a) Observed blemishes are scored with the following abbreviations. C: corky cracks; G: greening; I: Polygonal lesion; L: enlarged lenticels; P: Tuber becoming pink; S: Skinning; SL: Star-like corky lesion
(b) NT, not tested.

Growing tubers under hydroponic conditions allowed the observation of formation and development of blemishes during tuber growth. It often appeared, on inoculated tubers as well as on non-inoculated tubers that wound and opened lenticel healing formed polygonal and star-like corky lesions. We also observed that polygonal lesions and corky cracks frequently resulted from the development of skinning and star-like corky lesions along with tuber growing period. Finally, crack formations were observed at the end of the growing period, when tubers tended to grow faster.

Discussion

The objective of this study was to investigate the microbial origin of potato skin tuber blemishes. We made a large collection of tubers showing different types of blemish, and isolated and identified microorganisms associated with these blemishes. At least 45 different species of fungi and 1 species of *Streptomyces* were isolated. *Fusarium*, *Rhizoctonia*, *Alternaria*, *Penicillium* and *Clonostachys* were the genera the most frequently isolated. *Fusarium*, *Rhizoctonia* and *Alternaria* are known to be present at the surface of potato tubers and in the rhizosphere of potato plants (Cwalina-Ambroziak, 2002; Pieta and Patkowska, 2003). They are known to be responsible for dry rots, black scurf and stem canker and early blight, respectively, on potato crop (Radtke and Rieckmann, 1991; Stevenson *et al.*, 2001). *Clonostachys*, especially *C. rosea* (formerly Gliocladium roseum, Schroers *et al.*, 1999) could be either a pathogenic agent causing dry rot (Theron, 1991) or a biological agent controlling several potato soil-borne diseases (Keinath *et al.*, 1991; Davide and Zorilla, 1995). *Penicillium* species were isolated from potato tubers and identified as saprophytic strains (Cwalina-Ambroziak, 2002). To our knowledge it is the first time that *P. brevicompactum,* the most common *Penicillium* species found in our study, was isolated from potato tubers. Thus, tubers are an ecological niche favourable for the development of a diversity of fungi and bacteria, both pathogens and saprophytes.

Though quite diverse, the microflora present at the surface of tubers displaying blemishes might have been underestimated by the isolation technique. Extracting the DNA directly from the tuber blemishes could have revealed an even larger spectrum of microorganisms. However, such a direct approach was not adapted to our purpose

since it would not have allowed us to isolate the microorganisms, characterize and test them individually in bioassays.

Only one sample of each blemish type, skinning and star-like corky lesion, was analysed for the isolation of *Streptomyces*. No *Streptomyces* were isolated from these blemishes. Further research needs to be done to know if *Streptomyces* does interfere with the formation of skinning and star-like corky lesions, or not.

A diversity of fungi was isolated in this study, direct observation using a microscope often allowed their identification at the genus level but further identification at the species level frequently required molecular tools. The ITS sequence and the 16S rDNA sequence are generally used as a species marker among fungi or *Streptomyces*, respectively. In our study, 75 % of the strains were identified at the species level by sequencing their ITS region or the 16S rDNA. Most of the remaining strains were not identified at the species level because of a too short sequence. However, this does not stand for the strains belonging to the genus *Alternaria.* Several of them could not be identified at the species level even with an almost complete ITS sequence because their ITS sequence matched two or more *Alternaria* species. In these cases, sequencing of the genes coding the elongation factor, the beta-tubulin, or the mitochondrial ribosomal large subunit (mtLSU) might have been more discriminative (Peever *et al.*, 2004). On the other hand, the variability of ITS sequences is much higher in *R. solani*, allowing the differentiation of AG within the species (Guillemaut *et al.*, 2003). In our study, three different AGs were identified.

The most important one was AG 3 - PT known to be the most frequent AG on potato (Kuninaga *et al.*, 2000). In addition, 6 strains of *R. solani* AG 2-1 and 4 strains of *R. solani* AG 5 were also isolated. *R. solani* AG 3 – PT and AG 2-1 were mostly isolated from sclerotia whereas *R. solani* AG 5 was isolated from enlarged lenticels and russeting. Similar results were found in potato crops in France and Great-Britain (Campion *et al.*, 2003; Woodhall *et al.*, 2007).

Concerning the identification of *Streptomyces*, identification at the species level has not been possible in all cases. A complementary identification method based on biochemical characteristics of streptomycetes could be used (Bouchek-Mechiche *et al.*, 1998). Otherwise, a specific PCR for each species of *Streptomyces* could allow a quicker and complementary detection. An amplification of the 16S rDNA, highly conserved, and amplification of the ribosomal intergenic spacer (RIS), more

discriminative, could allow a better identification of *Streptomyces* species (Lehtonen *et al.*, 2004; Park and Kilbane, 2006).

The second part of the study was to assess the pathogenicity of the isolated strains. Two different types of bioassays were set up. In the first one, the soil was artificially infested by the microbial strains in order to mimic field conditions. In the second one, potato plants were grown under hydroponic conditions and inoculated with one or two strains of potential pathogens, in order to follow the development of the blemishes. Although millet seeds used as inoculum were fully colonized, inoculated strains were not always detected in the soil at the end of the assay. This could be because the soil volume tested was too small, or because other species isolated from soil such as *Trichoderma* or *Mucor* having a high growth rate, prevented the growth of the fungi of interest. Among the 72 fungal strains tested in soil, only 14 were able to reproduce the same blemishes they originated from; the other strains did not reproduce the blemish of origin. Among the 11 *Streptomyces* strains tested, none was able to reproduce the type of blemish it originated from. According to Koch's postulates, a given strain can be considered as responsible for a symptom when it reproduces the original symptom and when the identical strain can be isolated at least once from the diseased progeny tubers (Rapilly, 2001). The 14 potentially pathogenic strains belonged to the species *F. oxysporum*, *P. brevicompactum, P. cucumerina*, and *R. solani* AG 3 - PT. Skinning, enlarged lenticels and polygonal lesions seemed to be the basal blemishes observed under the conditions of the assay rather than a result of the inoculum. Even though *Fusarium* spp. and more especially *F. oxysporum* were frequently isolated from blemishes and particularly associated with polygonal lesions, it seems that this species is well adapted to live on tuber surface as an opportunist. Because, polygonal lesions were also observed on the non-inoculated controls, it is not possible, at this point, to infer the involvement of this species in the formation of polygonal lesions. Similarly, we cannot consider *P. cucumerina, P. brevicompactum* and *R. solani* AG 3 - PT as responsible for enlarged lenticels on potatoes. We know that a high level of humidity in the soil is favourable for the opening of lenticels as it was observed in the non-inoculated controls of the bioassays. Opened lenticels facilitate penetration of microorganisms. *P. cucumerina, P. brevicompactum* and *R. solani* AG 3 - PT could be well adapted species to this specific niche and might be considered as opportunistic colonizers of the lenticels. Moreover, isolations made from soil from the bioassays showed that some species were better adapted to

survive in the soil than others (data not shown). *R. solani* did not spread homogenously into the soil since it was much more often recovered from the tuber surface than from the soil. *Alternaria* spp. and *Clonostachys* spp. were recovered neither from the soil nor from the tuber surface. While *Clonostachys* is known to be good saprophyte (Theron, 1991), *Alternaria and R. solani* are not. *R. solani* behaves as a root inhabiting fungus rather than as a soil fungus (Garrett, 1970).

The involvement of *R. solani* AG 3 - PT in the formation of polygonal lesions was observed in 4 independent replicates: this result confirms what is currently observed in open field conditions but needs further investigation under controlled conditions and artificial inoculation. Similarly, the contribution of *R. solani* AG 3 to the formation of corky crack was verified with only one strain. On the contrary, the pathogenicity of *R. solani* AG 3 - PT causing sclerotia was clearly demonstrated in four independent replicates. The involvement of *R. solani* AG 3 in the occurrence of sclerotia has already been demonstrated several times (Anderson, 1982; Woodhall *et al.*, 2008).

Since the inoculation of single strains did not allow the reproduction of observed atypical blemishes, we assumed that a consortium of strains might be involved in the occurrence of blemishes. Co-inoculations, in the bioassay in hydroponic conditions, did not demonstrate clear fungal or *Streptomyces* contribution into the occurrence of skin blemishes. Blemishes like skinning or star-like corky lesions could be the early stage of other blemishes like polygonal lesions. We also found a connection between wound healing and the formation of polygonal lesions. Moreover, the appearance of severe polygonal lesions on the non-inoculated tubers under hydroponic conditions confirmed that this blemish could be skin response to other environmental factors. In the 60-70's and later in the 2000's, several publications suggested that polygonal lesions were due to an excess of humidity or organic matter (Hart, 1971; Sexton, 2003). In our experimental set up, the high moisture content due to hydroponic conditions is probably one of the causes of some of the blemishes. Polygonal lesions could be the result of a physiological reaction of the plant to stressful abiotic factors.

Although, we noticed that cultivar Samba was particularly frequently associated with polygonal lesions, the genetic background of the potato germplasm was deliberately not taken into account in the expression of tuber blemishes because too little reliable data are available on the host-parasite relationships. However, the *S. tuberosum* – *Streptomyces* spp. complex is one of few that gather practical data at the cultivar level when assessed under controlled conditions and artificial inoculum (Pasco *et al.*,

2005), although its expression can vary because it is highly dependant upon biotic and abiotic environmental factors.

This study showed the diversity of the microbial flora on the surface of potato tubers and demonstrated that, except for *R. solani* AG 3 - PT causing sclerotia, blemishes analyzed in this study could not be directly attributed to specific microorganisms. Evidences indicate that stress factors induce a differential response of the plant and the diversity of the microorganisms associated with the potato could be a natural situation. Cultivar susceptibility is also probably a key to blemish development and thus control. Complementary studies about the environmental conditions responsible for development of potato blemishes and the assessment of susceptible and resistant cultivars are needed.

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Chapter 4

Differential evolution of the structures of fungal and bacterial communities in the geocaulosphere of healthy and blemished potato tubers

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Differential evolution of the structures of fungal and bacterial communities in the geocaulosphere of healthy and blemished potato tubers

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Abstract

Appearance of atypical blemishes on potato tubers is unexplained. The involvement of soil borne microorganisms has so far never been demonstrated except for *Rhizoctonia solani* causing black scurf. To test if the microbial communities of the geocaulosphere of tubers are associated to the occurrence of blemishes, the bacterial and fungal community structures of the soil surrounding healthy and blemished tubers were analysed by terminal restriction fragment length polymorphism (T-RFLP) before and after haulm destruction.

The same T-RFLP procedure was used to characterize microorganisms previously isolated from blemished potatoes. Bacterial communities around healthy tubers evolved between the two sampling dates; they changed toward a single structure around blemished tubers. Fungal community structures around healthy and blemished tubers were different one from the others and at the two sampling dates what reveals the succession of different populations along with the sanitary status of the potato and with the season. However, although differential evolutions of the structures of microbial communities were shown to be related to the blemish occurrences, no causality link was depicted apart from the one supposed to be *R. solani* population that significantly increased around blemished tubers after haulm destruction.

Introduction

Potatoes (*Solanum tuberosum* L.) are cropped almost everywhere in the world and the production increases every year since several decades. In 2007, about 325 million tons were produced in the world. The quality of the tubers warranties good production sell. However, since fresh tubers are washed before selling in most of the developed countries, several kinds of blemishes became visible on the tuber surface. Some of these blemishes (polygonal lesions, corky cracks, corky spots, star-like lesions with or without halo, rugosities, skinning) are caused by factors that are still not well known (Campion *et al.*, 2003). In a previous study, it has been demonstrated that except for the fungus *Rhizoctonia solani* which causes sclerotia, the microorganisms present at the tuber surface were not directly implicated in the appearance of those blemishes. Conversely, a wide diversity of microorganisms is associated with potato surface (Fiers *et al.*, 2010). Edaphic factors such as soil pH, organic matter rate, water supply, or the presence of particular nutrients could be involved. In addition to abiotic factors, the microflora harboured by the soil surrounding the tuber (geocaulosphere) might influence the potato development and the occurrence of blemishes. The term 'geocaulosphere' built up with the same etymological bases as the term 'rhizosphere' refers to the soil of the nearby environment of tubers, under the influence of the subterranean stems. Microorganisms in the geocaulosphere could have an indirect impact on tubers and create favourable (i.e. stimulation of defence mechanisms) or unfavourable conditions (i.e. modification of soil pH or soil moisture) which might affect potato development and blemish occurrence. To assess this hypothesis, the bacterial and fungal community structures of the geocaulosphere of healthy, blemished and partially-healthy and blemished plants were compared at different periods of time by terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S and 18S ribosomal DNA (rDNA), respectively (Liu *et al.*, 1997). This method allows the characterization of the microbial community structures in the form of variable terminal restriction fragments (TRF). To go further and identify the microorganisms of the geocaulosphere potentially implicated in the occurrence of blemishes, the specific TRF of 56 microorganisms isolated from potatoes were determined using the same T-RFLP procedure.

Material and methods

Soil sampling

Soil sampling was done in 2008 in a potato field (cv. Juliette) located in Morbihan in Western France (48°05' N, 2°51' W) at t wo consecutive dates: T1 at the end of July, before haulm destruction and T2 at the end of August, after haulm destruction. The field was chosen because of recurrent problems of blemished tubers. A plot (15 m x 3 m) with 2 rows of potatoes (60 cm large) and 3 inter-rows (60 cm large) was delimited. At T1, 5 healthy plants and 5 plants whose progeny tubers were either healthy or blemished were chosen. At T2, 4 plants whose progeny tubers were either healthy or blemished and 4 blemished plants were chosen (Table 4.1). There were no completely healthy plants in the plot at T2. Three tubers were collected from healthy plants, 3 healthy tubers and 3 blemished tubers were collected from partially-healthy and blemished plants, and 6 tubers were collected from blemished plants. About 100 g of soil from the geocaulosphere of the collected tubers were sampled; the soil samples were placed in a plastic bag with the corresponding tuber. At each sampling date, 5 samples of soil from the inter-rows were collected, pooled together and divided into 3 homogenised samples. A total of 99 soil samples were collected and brought back to the lab where they were immediately prepared for subsequent analyses. Each soil sample was stored in 10 tubes preserved at -20°C until the experiment proceeded. Potato tubers were observed and the superficial blemishes were scored according to chart approved by the French Ministry of Agriculture (GNIS and SOC, 2005). A picture of each tuber was taken.

Table 4.1 Collected soil samples, corresponding number of plants and graphic representations.

	N° of plant	Sanitary status of potato plants	Sanitary status of progeny tubers	Number of samples	Symbols for graphic representation (PCA)
T1	C	Inter-row soil		3	△
	1	Half-healthy half-blemished	Healthy	3	○
	1		Blemished	3	●
	2	Half-healthy half-blemished	Healthy	3	○
	2		Blemished	3	●
	3	Half-healthy half-blemished	Healthy	3	○
	3		Blemished	3	●
	4	Half-healthy half-blemished	Healthy	3	○
	4		Blemished	3	●
	5	Half-healthy half-blemished	Healthy	3	○
	5		Blemished	3	●
	6	Healthy	Healthy	3	□
	7	Healthy	Healthy	3	□
	8	Healthy	Healthy	3	□
	9	Healthy	Healthy	3	□
	10	Healthy	Healthy	3	□
T2	C	Inter-row soil		3	△
	11	Half-healthy half-blemished	Healthy	3	○
	11		Blemished	3	●
	12	Half-healthy half-blemished	Healthy	4	○
	12		Blemished	2	●
	13	Half-healthy half-blemished	Healthy	3	○
	13		Blemished	3	●
	14	Half-healthy half-blemished	Healthy	3	○
	14		Blemished	3	●
	15	Blemished	Blemished	6	■
	16	Blemished	Blemished	6	■
	17	Blemished	Blemished	6	■
	18	Blemished	Blemished	6	■

Bacterial and fungal community structures

Nucleic acids were extracted from 1 g of soil of the 99 soil samples as described previously (Edel-Hermann *et al.*, 2004). Briefly, a physical disruption (bead-beater) and a chemical extractant (sodium dodecyl sulfate) at 70℃ were used. The crude nucleic acid extracts were purified twice using a polyvinylpolypyrrolidone spin column to remove coextracted humic acids and once using a Geneclean Turbot

kit (Q-BIOgene, Illkirch, France). Purified DNA extracts were quantified by electrophoresis in agarose gels using dilutions of calf thymus DNA and stored at -20 ℃.

The genetic structure of microbial communities in the different soil samples was investigated using T-RFLP of 16S and 18S ribosomal RNA (rRNA) genes for bacteria and fungi, respectively (Edel-Hermann *et al.*, 2004; Perez-Piqueres *et al.*, 2006). Bacterial 16S rDNA was amplified by PCR using the primer 27F (AGAGTTTGATCCTGGCTCAG) (Edwards *et al.*, 1989) labelled with the fluorescent dye D3 and the primer 1392R (ACGGGCGGTGTGTACA) (Braker *et al.*, 2001). Fungal 18S rDNA was amplified by PCR using the primer nu-SSU-0817-5′ (TTAGCATGGAATAATRRAATAGGA) labelled with the fluorescent dye D3 and the primer nu-SSU-1536-3′ (ATTGCAATGCYCTATCCCCA) (Borneman and Hartin, 2000). The labelled and unlabelled primers were synthesized by Sigma-Aldrich and Eurofins MWG Operon (Ebersberg, Germany), respectively. PCR amplification of 16S rDNA was carried out in 25 µL reactions including: *Taq* polymerase buffer, 200 µM dNTP, 0.2 µM of each primer, 2 U *Taq* polymerase, 20 ng DNA, and H_2O. All PCR reagents were obtained from Q-BIOgene. Amplifications were conducted in a Mastercycler (Eppendorf, Hamburg, Germany) using the following reaction conditions: initial denaturation at 94℃ for 3 min followed by 30 cycles of 1 min at 94℃, 1 min at 57℃ and 1 min at 72℃ and a final e longation for 10 min at 72℃. PCR amplification of 18S rDNA was carried out using identical reagent conditions except the primers at a concentration of 0.25 µM each. The PCR conditions for 18S rDNA included an initial denaturation at 94℃ for 3 min followed by 35 cycles of 1 min at 94℃, 1 min at 56℃ and 1 min at 72℃ and a fina l elongation for 10 min at 72℃. Bacterial and fungal PCR reactions were checked by electrophoresis on a 1 % and 2 % agarose gel, respectively. PCR products were purified using the MinElute PCR purification kit (Qiagen, Courtaboeuf, France). Purified PCR products were quantified as above and 120 ng of purified PCR products were digested with 12 U of restriction enzyme in a final volume of 100 µl for 3 h at 37 ℃. The restriction enzymes *Hae*III (Promega) and *Msp*I (Q-BIOgene) were used to digest 16S and 18S PCR products, respectively.

Fluorescently-labelled terminal restriction fragments (TRF) were separated and detected using a capillary electrophoresis sequencer CEQ^{TM} 2000XL (Beckman Coulter, Fullerton, CA, USA). Analyses were run with the Frag-4-30s-70min method,

including a denaturation of 2 min at 90°C, an injection at 2000 V during 30 s and a separation at 4 800 V during 70 min. The sizes of the TRF were determined by comparison with Size Standard-600 (Beckman Coulter). Fungal and bacterial community structures were characterised by the size and fluorescence intensity of the TRF. For each PCR product, the T-RFLP analysis was performed in triplicate. Mean values for the relative intensity of peaks found in at least two of the three analyses were considered for further statistical analyses of microbial community structure. Moreover, the fragments between 60 to 640 bp corresponding to the size range of the standard were considered. The comparison of the TRF sizes between samples was automated by assigning them to discrete categories using the program Lis with an interval of 1.25 bp (Mougel *et al.*, 2002). The communities characterized by the sizes of the TRF and their intensity measured by the height of the peaks were compared by principal component analysis (PCA) using the ADE-4 software (Thioulouse *et al.*, 1997). This ordination method summarizes multivariate data to a few variables or dimensions and provides an arrangement of the communities in a two-dimensional diagram based on their scores on the two first dimensions. The significance of the resulting structures was checked using Monte Carlo tests with 1 000 random permutations of the data.

Typing of microbial strains by T-RFLP

In order to identify microorganisms corresponding to characteristic TRF in the geocaulosphere, diverse fungi previously isolated from potato tubers were analysed using the same procedure of T-RFLP analysis as above. As *Streptomyces* bacteria are frequently associated to potatoes (Bouchek-Mechiche *et al.*, 1998), their characteristic TRF was also determined in order to identify them in bacterial community structures. Three strains of *Streptomyces* (*S. europeaiscabiei*, *S. mirabilis*, *S. scabiei*) and 53 fungal strains belonging to 27 different genera and 56 species were used (Fiers *et al.*, 2010). The strains were stored in the collection "Microorganisms of Interest for Agriculture and Environment" (MIAE, INRA Dijon, France). Strains of *Streptomyces* stored at 4°C on potato dextrose agar (PDA) were cultivated in 25 mL of Luria Bertani media (10 g L^{-1} of bacto tryptone, 5 g L^{-1} of yeast extract, 10 g L^{-1} of NaCl; pH 7) for 6 days at 27°C. The DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's

specifications. The DNA quantity and quality were checked by electrophoresis as above and the DNA concentrations calculated as above were between 6 and 650 ng μL^{-1}.

The fungal strains were cultivated in tubes on a PDA slope. Two mL of potato dextrose broth (PDB) were poured into each tube. Each tube was then vortexed to disperse the spores, and the mixture was poured into Roux flasks containing 100 mL of PDB. For fungi which do not sporulate (i.e. *R. solani*), 6 explants of PDA were directly placed into Roux flasks. Flasks were incubated at room temperature without shaking for 2 to 3 days. The mycelium was harvested by filtration, frozen at -80℃ during 30 min, freeze dried and stored at -80℃. Th e mycelium was ground to powder with liquid nitrogen using cold sterile mortar and pestle. The DNA was extracted from 20 mg of mycelium powder using the DNeasy plant mini kit (Qiagen). The DNA quantity and quality were checked by electrophoresis on a 0.8 % agarose gel, revealed with ethydium bromide and visualized by UV transillumination. The DNA concentrations were between 3.5 and 125 ng μL^{-1}. The T-RFLP analysis of each strain was performed as above.

Results

Before haulm destruction (T1), 61 % of the tubers had blemishes. At this date, blemishes were light; they affected about 1 % of the tuber surface. The most frequently observed blemishes were superficial polygonal lesions (39 %), star-like lesions with (5 %) or without (9 %) halo and netted scab (7%). Other blemishes represented 1 %. After haulm destruction (T2), 86 % of the tubers were blemished, 52 % presented black scurf on at least 9 % of the tuber surface, 14 % were blemished by star-like lesions with halo and 13 % by star-like lesions without halo. Seven percents of the tubers presented netted scab (Figure 4.1).

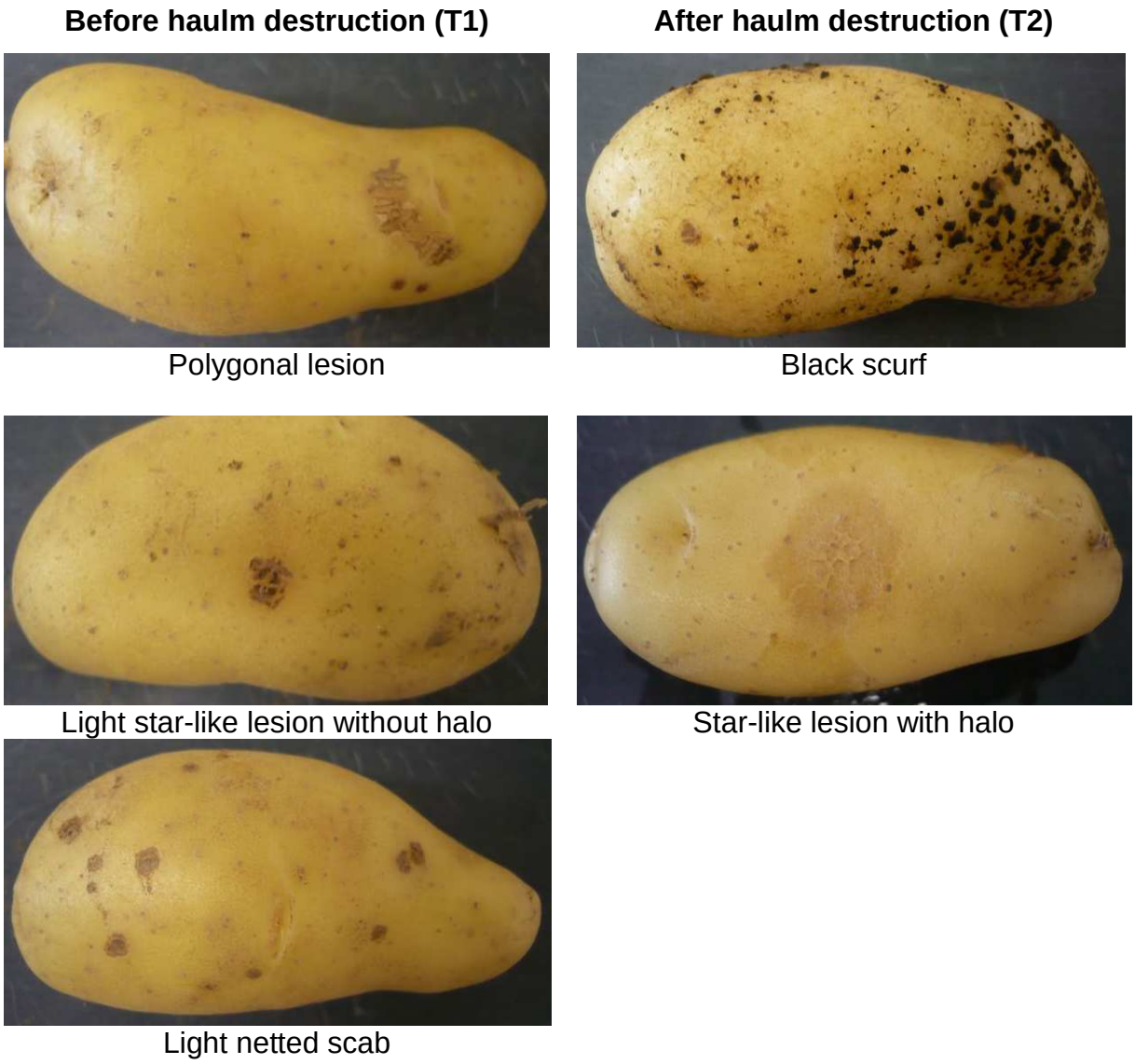

Figure 4.1 Pictures of some blemishes observed before (T1) and after (T2) haulm destruction on potato tubers.

T-RFLP analyses provided complex profiles for bacteria and fungi at each sampling time. The mean number of TRF per soil sample was 90 for the bacteria and 37 for the fungi. PCA showed that inter-row soils were always different from samples from geocaulospheres especially at T2 and they were more variable at T1 than at T2. There were as much differences between tubers from the same plant as between tubers from different plants (Figure 4.2). At T1, the permutation tests revealed that the bacterial community structures were not different whatever the sanitary state of the tubers (Figure 4.2a). However, at T2 the structures of the bacterial community differed significantly according to the sanitary status of the tubers (Figure 4.2c).

Concerning fungi, significant differences were observed at T1 between communities of geocaulospheres harbouring healthy potatoes from healthy plants and potatoes from partially-healthy and blemished plants (Figure 4.2b). At T2, differences were observed between the communities of geocaulospheres harbouring blemished potatoes from blemished plants and potatoes from partially-healthy and blemished plants (Figure 4.2d). The structures of bacterial communities, respectively fungal communities in soil samples around healthy and blemished tubers from partially-healthy and blemished plants were always similar. However, they were different from the structures of the community of soil samples around healthy tubers from healthy plants for fungi and from the community structures of soil around blemished tubers from blemished plants, for bacteria and fungi.

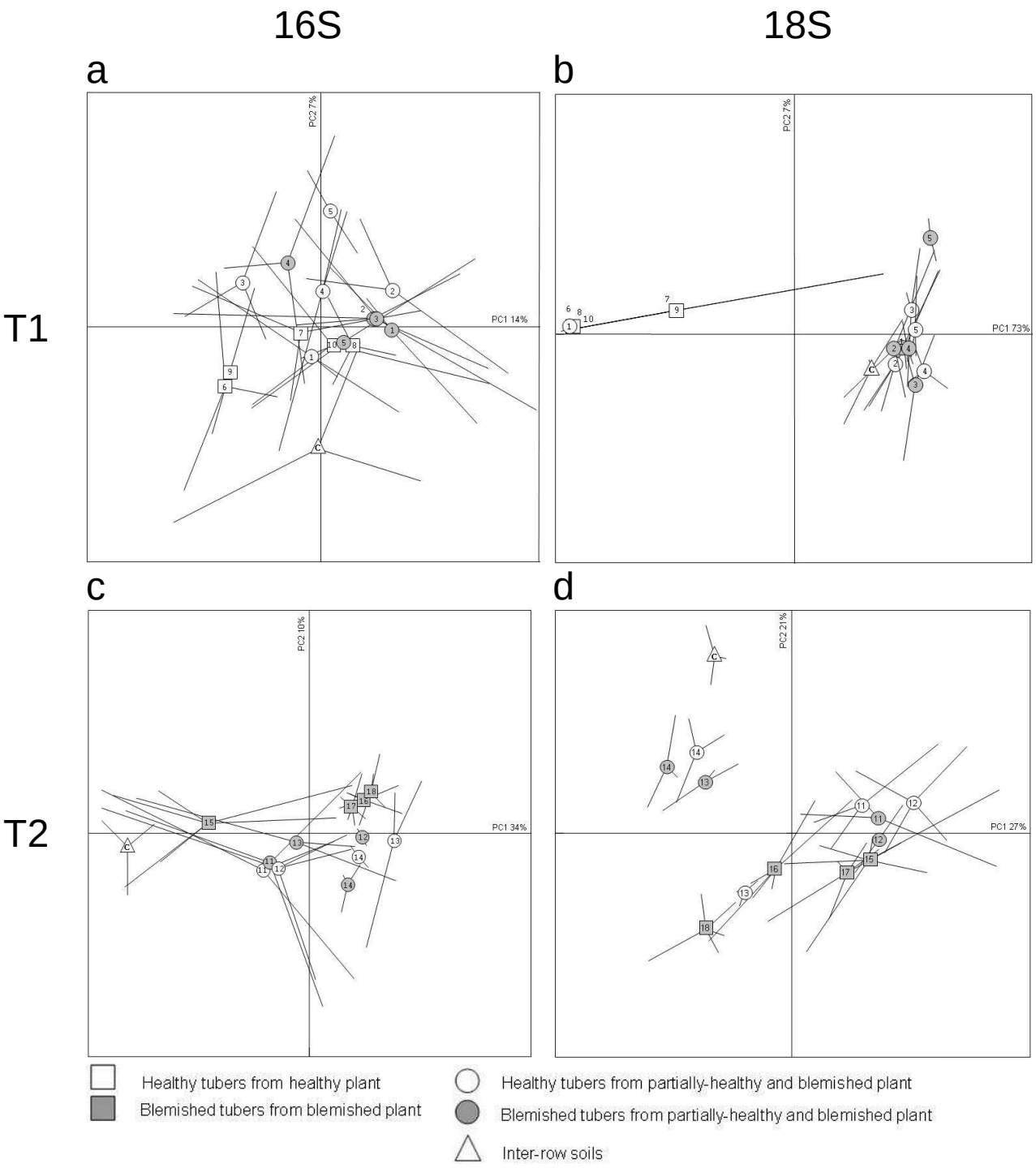

Figure 4.2 Principal component analysis of bacterial 16S (a and c) and fungal 18S (b and d) terminal restriction fragment length polymorphism data sets before, T1 (a and b) and after, T2 (c and d) haulm destruction of the potato plants.
Analyses were performed on soil from the geocaulosphere of healthy plants (3 tubers, white squares), blemished plants (6 tubers, grey squares) or partially -healthy and blemished plants (3 healthy tubers, white circles and 3 blemished tubers, grey circles). Each plant was represented by a single number. Analyses of uncultivated soil were performed on three independent samples of inter-row soil as control (triangles). Each soil set is represented by its gravity centre. The branches show the divergence of the tubers from the same plant from the respective gravity centres.

A global analysis showed significant discrimination in bacterial community structures between the two periods of sampling, T1 and T2 (Figure 4.3a). However, the permutation test did not show significant difference between the community structures around blemished tubers from blemished plants at T2 and around blemished tubers from partially-blemished plants at T1. Among the peaks representing the bacterial TRF, five of them were high in intensity in all the treatments. One peak was intense only at T1, another was observed in all treatments except inter-row soils. Some peaks we only observed in inter-row soils.

The fungal global analysis revealed a marked discrimination between the two samplings T1 and T2 (Figure 4.3b). For all treatments, three fungal TRF were among the peaks of highest intensity. The TRF of ~ 583 bp was intense (5.5 % of the relative intensity in mean) in all the treatments except in inter-row soil at T2. In addition to the three main peaks, seven dominant peaks were observed in soils sampled at T1 and in soils sampled at T2, but they were different for the two sampling dates. One of the seven major TRF at T2, a peak around 565 bp, was particularly intense in fungal community structures around blemished tubers from blemished plants at T1 and T2.

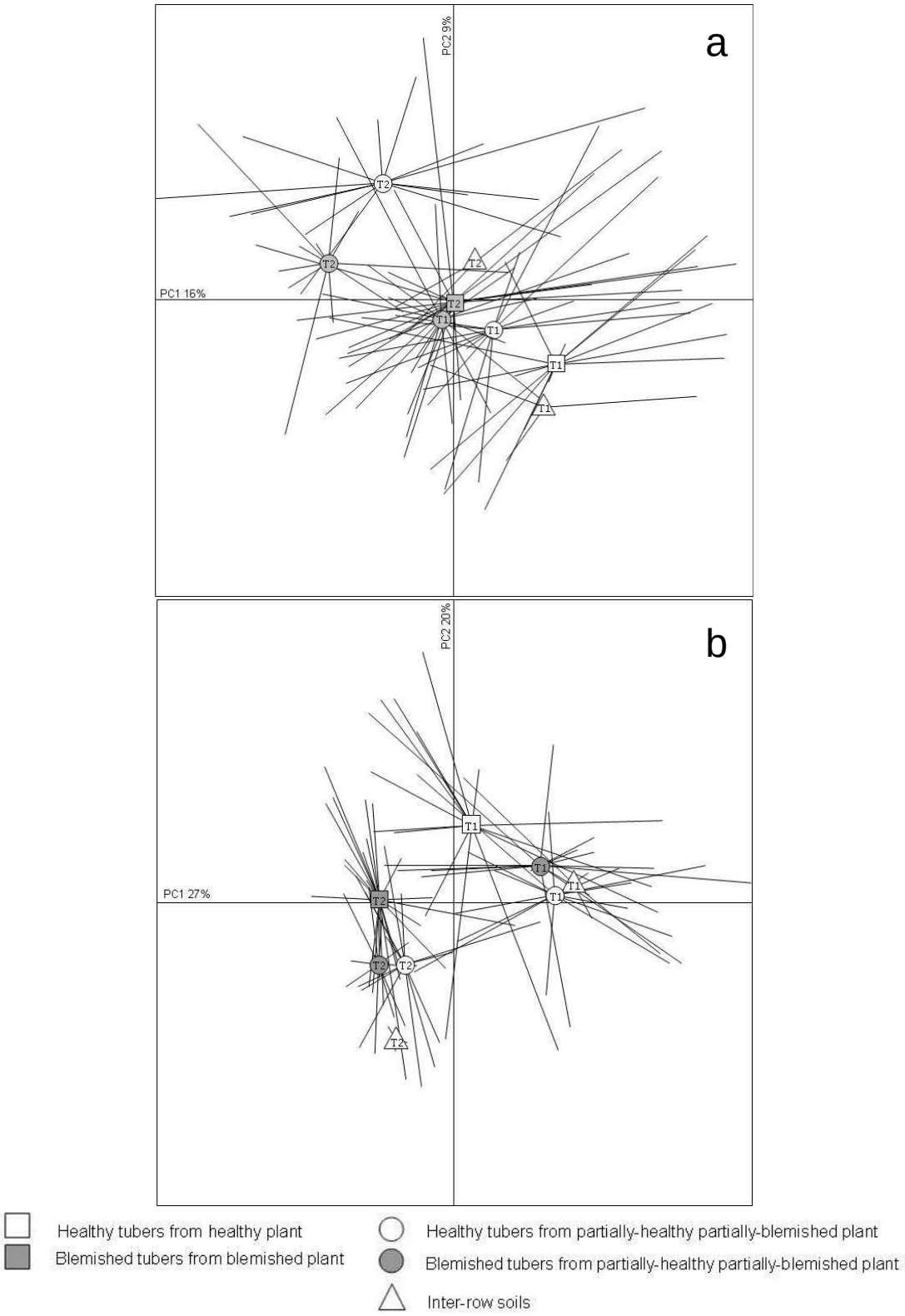

Figure 4.3 Principal component analysis of 16S (a) and 18S (b) terminal restriction fragment length polymorphism data sets before (T1) and after (T2) haulm destruction of the potato plants.
Analyses were performed on soil from the geocaulosphere of healthy plants (3 tubers, white squares), blemished plants (6 tubers, grey squares) or partially -healthy partially -blemished plants (3 healthy tubers, white circles and 3 blemished tubers, grey circles). Analyses of uncultivated soil were performed on three independent samples of inter-row soil as control (triangles). Each soil set is represented by its gravity centre. The branches show the divergence of the tubers from the same plant from the respective gravity centres.

The T-RFLP analysis of reference strains revealed that the three species of *Streptomyces* analyzed had about the same length of TRF with an average of 222.88 bp. In soil community structures, peaks around 222 or 223 bp were not detected at T1 and only slightly detected at T2. Concerning fungi, the species from the same genus have about the same TRF length except for *Trichoderma* (Table 4.2). The peaks associated with *Rhizopus orzyae*, *Verticillium dahliae*, *Absidia glauca* and *Fusidium griseum* were never detected in soil communities. Among strains for which the TRF length was determined, none was detected in soil samples from healthy plants. The TRF corresponding to *Macrophomina phaseolina* (561.34 bp), *Colletotrichum* sp. (580.06 bp) and *Cylindrocarpon olidum* (580.10 bp) were detected in complex profiles of soil samples from partially-healthy and blemished plants only at T1. The four genera *Alternaria*, *Ulocladium*, *Phoma* and *Epicoccum* have about the same TRF length (583.04 to 583.40 bp). TRF of similar length were detected in T-RFLP profiles from partially-healthy and blemished plants at T1, all the treatment at T2, as well as in soil samples from inter-row at T1 and T2. The TRF of *Penicillium* sp. (558.56 bp), *Rhizoctonia solani* (565.19 bp), *Trichoderma* sp. (580.86 bp) and *Cladosporium cladosporioides* (580.94 bp) were detected in complex T-RFLP profiles of soil samples from partially-healthy and blemished plants and from blemished plants at T1 and T2 but neither in geocaulosphere of healthy plants nor in inter-row soil. The same situation was observed for the TRF of *Mortiella elongata* but only at T2. *Bjerkandera adusta* had a TRF of 564.59 bp. Such length was only detected in inter-row soils at T2.

Table 4.2 Terminal restriction fragment (TRF) lengths of fungal strains.

TRF length (bp) [a]	Species [b]
155.14 ± 0.04	*Rhizopus orzyae*
306.81 ± 0.04	*Mortiella elongata*
524.76 ± 0.32	*Verticillium dahliae*
556.94 ± 0.27	*Absidia glauca*
558.36 ± 0.18	*Fusidium griseum*
558.56 ± 0.20	*Penicillium* spp. (9)
561.34 ± 0.37	*Macrophomina phaseolina*
564.59 ± 0.08	*Bjerkandera adusta*
565.19 ± 0.31	*Rhizoctonia solani* (3)
580.06 ± 0.37	*Colletotrichum* sp.
580.10 ± 0.19	*Cylindrocarpon olidum*
580.86 ± 0.10	*Trichoderma* spp. (*T. velutinum*; *Trichoderma* spp.)
580.94 ± 0.25	*Cladosporium cladosporioides*
583.04 ± 0.14	*Alternaria* spp. (4)
583.13 ± 0.28	*Ulocladium capsicum*
583.22 ± 0.21	*Phoma exigua*
583.40 ± 0.12	*Epicoccum nigrum*
> 640	*Cercophora grandiuscula*, *Clonostachys rosea*, *Fusarium* spp., *Gliomastix murmorum*, *Microdochium bolleyi*, *Mucor* spp., *Neonectria radicicola*, *Plectosphaerella cucumerina*, *Stereum rugosum*, *Trichocladium asperum*, *Trichoderma tomentosum*

[a] values are mean of three replicates
TRF > 640 bp are out of the range of detection
[b] Numbers in brackets indicate the number of strains analyzed when it was more than 1.

Discussion

The main objective of the present study was to investigate whether the microbial community structure in the geocaulosphere of potato tubers depends on the occurrence of blemishes on the tubers. In addition, although the same TRF may correspond to different microorganisms, the analysis of a collection of strains with the same T-RFLP procedure can provide hypotheses concerning the microorganisms responsible for the variations observed in community structures. The occurrence of blemished tubers observed before and after haulm destruction increased by 41 % in 27 days and all the sclerotia appeared after haulm destruction.

The community structures of inter-row soils were different at T1 and T2. The slightest variability in inter-row soil sampled at T2 can be explained by climatic conditions or by a drought event at the end of the growing season. Inter-row soils were also generally distinct from the soil samples from geocaulosphere. This was confirmed by

the fact that some TRF were specifically associated with inter-row soil samples. Thus, non cultivated soils had different microbial community structures than soils where potatoes were grown. This implies that potato plants have an effect on the structure of the communities of their geocaulosphere, probably through phenological and physiological changes. The geocaulosphere is an evolving and alive environment that can be compared with the rhizosphere in which exudates are produced and have an effect on their environment.

Our study showed that the structures of microbial communities were homogeneous in the geocaulosphere of partially healthy and partially blemished plants. The microbial communities of healthy plants had different structures compared with the microbial communities of partially healthy and blemished plants, which were also different from the microbial community structures of blemished plants. Therefore, the microbial communities evolved according to the sanitary status of the plant.

The data showed significant differences between bacterial community structures before and after haulm destruction, especially among healthy tubers. In the case of healthy plants, the structure of the bacterial communities was different at T1 and T2, while in the case of blemished plants, the structure of the bacterial communities in the geocaulosphere of blemished tubers was similar at T1 and T2. The bacterial consortium was quite different for healthy tubers from healthy plant at T1 and for healthy tubers from partially-healthy plants at T2. Conversely, it was relatively homogenous and constant around blemished tubers from half blemished plant at T1 and blemished tubers from blemished plant at T2. The intermediate states, healthy tubers from partially-healthy plant at T1 and blemished tubers from partially-blemished plant at T2, had in-between bacterial community structures. There was a progressive evolution from two different structures of the bacterial communities at T1 and T2 around healthy tubers toward a common structure around all blemished tubers. For fungi, the variations of community structures induced by time were higher than the variations induced by the health state of plants. The two distinct structures at T1 and T2 showed that there was a succession of the fungal communities in potato geocaulosphere along with time. Considering the analysis of the most intense peaks for each community, the differences of structures between T1 and T2 were much more marked for fungi than for bacteria. We observed that the main fungal TRF were very different at T1 and T2, whereas the most intense bacterial TRF were the same at T1 and T2. The distinction between T1 and T2 is not only due to the time delay

between the two sampling dates. Although the weather report for 2008 showed that July and August had about the same climatic conditions in France, the physiological variations of plants and the impact of chemical products applied for haulm destruction on the microbial community structures should also be considered.

Data about the TRF lengths of reference strains could have allowed a more precise analysis of the complex soil profiles. Although some TRF provided information about the occurrence of putative fungal species in the geocaulosphere, the majority of the peaks in complex T-RFLP profiles could not have been associated with a particular species or genera. Although the T-RFLP analyses were performed on a representative sample of microorganisms living on the surface of the potato tubers (Fiers *et al.*, 2010), only few microorganisms could be allocated to the main peaks observed for each profile. Moreover, our analysis could not determine the TRF lengths of some species because they were longer than 640 bp, the maximal detectable length with the size standard that was used. TRF data remain rare and databases providing information on the TRF lengths of various microorganism species could be an interesting tool allowing a more precise analysis of complex T-RFLP profiles. Several papers report works on databases development, but sources of error remain possible and existing databases are still poor concerning environmental microorganisms (Matsumoto *et al.*, 2005; Dickie and FitzJohn, 2007). In our results, it appeared that all the determined TRF were not present in complex T-RFLP profiles. TRF corresponding to TRF of *R. orzyae*, *V. dahliae*, *A. glauca* and *F. griseum* were not detected in any of the soil samples. Those fungi were absent from the geocaulosphere of the studied potatoes. The strains analyzed, isolated from blemished tubers were never detected in the geocaulosphere of healthy tubers. This shows that those strains were specifically associated to the geocaulospheres of blemished tubers. It also happened that several genera had the same TRF length. This was the case for the ~ 583 bp TRF which matched at least with four different genera: *Alternaria*, *Ulocladium*, *Phoma* and *Epicoccum*, and for the ~ 580 bp TRF which matched with *Colletotrichum* and *Cylindrocarpon* genera. Among the most intense peaks observed in soil community structures, some could correspond to some of the strains that were analysed. For example, the TRF of ~ 565 bp represented 5 % in average of the total intensity of each complex profile. This length corresponds to the mean TRF of *R. solani*. This fungal species or a fungus with the same TRF length was highly present in the geocaulosphere of blemished potato

tubers after haulm destruction (T2). This coincided with the period of time that black scurf was first observed on blemished tubers in the experimental field. As we know that black scurf is caused by *R. solani* forming sclerotia, at the tuber surface (El Bakali and Martin, 2006), we can confirm that this particularly intense TRF of ~ 565 bp corresponded to *R. solani*. This is in agreement with the previous results indicating that sclerotia are formed after haulm destruction (Otrysko *et al.*, 1988). Likewise, TRF of ~ 581 bp could correspond to *Trichoderma* sp. This TRF represented 10.7 % of the total intensity of the T-RFLP profile of inter-row soil at T2. Another peak representing 10 % of the total intensity of the profile of blemished tubers at T2 was 583 bp long. This TRF could be associated to *Alternaria, Ulocladium*, *Phoma* or *Epicoccum*. *Trichoderma* spp., *Alternaria* spp., *Ulocladium* spp., *Phoma* spp. and *Epicoccum* spp. are not pathogenic for potatoes; however, they are good saprophytes and can easily develop in soil. *Alternaria* spp. were particularly associated with blemished tubers (Fiers *et al.*, 2010), probably because it takes advantage of the niche created by the blemish formation. The bacterial TRF of ~ 222 bp was detected in some inter-row soil samples. This TRF length can be associated to the TRF of *Streptomyces*, a potentially pathogenic bacterium of potato causing netted scab, observed on some of the analyzed tubers.

As just demonstrated, the fungal community structures of the geocaulosphere of potato tuber evolve by succession of different fungal community structures in time. The apparition of a *R. solani* population around blemished tubers after haulm destruction is the only case for which a causality link between the presence of this fungus and the occurrence of specific blemishes can be proposed (Fiers *et al.*, 2010). For the other microorganisms, it is so far difficult to conclude whether the modification of the structure of their communities has provided conditions for, or was a consequence of the occurrence of blemishes, but this study clearly demonstrates a direct relationship between these simultaneous events.

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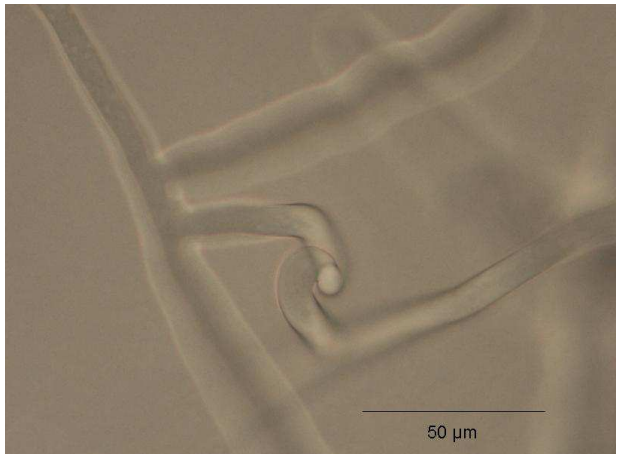

Chapter 5

Genetic diversity of *Rhizoctonia solani* associated with potato tubers in France

Chapter 5

Genetic diversity of Rhizoctonia solani associated with potato tubers in France

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Abstract

The soil borne fungus *Rhizoctonia solani* is a pathogen for many plants and causes severe damages in crops all around the world. Strains of *R. solani* from the anastomosis group (AG) 3 attack potatoes that lead to great yield losses and to the downgrading of the production. The study of the genetic diversity of the strains of *R. solani* in France allows determining the structure of the populations and establishing adapted control strategies against this pathogen. The diversity of 73 French strains isolated from tubers grown in the mains potato seed production areas and 31 strains isolated in 9 other countries was assessed by phylogenetic analyses of i) the internal transcribed spacer sequences (ITS1 and ITS2)) of ribosomal RNA (rRNA), ii) a part of the gene *tef-1α* and iii) the total DNA fingerprints of each strain established by amplified fragment length polymorphism (AFLP). The determination of the AGs of *R. solani* based on the sequencing of the ITS region showed 3 different AGs among our collection (60 AG 3 PT, 8 AG 2-1 and 5 AG 5). Grouping of the strains belonging to the same AG was confirmed by the sequencing of the gene *tef-1α* used for the first time to study the genetic diversity of *R. solani*. About 42 % of the ITS sequences and 73 % of the gene *tef-1α* sequences contained polymorphic sites where several nucleotides are possible, suggesting that the cells of *R. solani* strains contain several copies of ITS and gene *tef-1α* within the same nucleus or between different nuclei. Phylogenetic trees showed a greater genetic diversity within AGs in *tef-1α* sequences than in ITS sequences. The AFLP analyses showed an even greater diversity among the strains demonstrating that the French strains of *R. solani* isolated from potatoes were not a clonal population. Moreover, there was no relationship between the geographical origins of the strains or the variety from which they were isolated and their genetic diversity.

Introduction

The fungus *Rhizoctonia solani* (teleomoph *Thanatephorus cucumeris*, Kühn, 1858) is a widely spread plant pathogen which cause severe damages on numerous plant species. *R. solani* includes many related but genetically different subspecific groups. The hyphae of the closely related strains can fuse and, hence, form an anastomosis group (AG), whereas the distantly related strains are unable to anastomose (Parmeter, 1970; Kuninaga and Yokosawa, 1984; Carling *et al.*, 2002). The known strains of *R. solani* can be classified into at least 13 AGs, but the classification is not strictly fixed as some 'bridging strains' are able to anastomose with strains of at least two AGs (Parmeter, 1970; Carling *et al.*, 2002; Sharon *et al.*, 2008). Each AG is either host specific or with a wide host range (Ogoshi, 1987; Carling *et al.*, 2002). For example, AG 2 is associated with diverse host plants but AG 8 is more specifically associated with cereals. AG 3 is divided into two genetically different subgroups: AG 3 PT which is associated with potatoes, and AG 3 TB, associated with tobacco (Kuninaga *et al.*, 2000; Woodhall *et al.*, 2008). *R. solani* AG 3 PT reduces tuber quality by producing sclerotia (black scurf) on progeny potato tubers. The pathogen can also infect underground organs (stems, stolons and roots) which affect crop yield (tuber size and number) (El Bakali and Martin, 2006). Beside those typical symptoms, *R. solani* is associated with several types of blemishes on potato tubers (Fiers *et al.*, 2010). Other AGs of *R. solani* are sometimes considered as potential pathogens of potato in France and in the United Kingdom, although the Koch's postulates have still not been assessed (Campion *et al.*, 2003; Woodhall *et al.*, 2008).

The AG differentiation is traditionally carried out by pairing unknown isolates with reference strains and by identification of the hyphal anastomosis reaction (Carling, 1996; Guillemaut *et al.*, 2003). However, this method is time consuming, needs experienced eyes and does not show the diversity among AGs. Sequencing of the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) is now a more convenient and rapid method to differentiate *R. solani* AG. However this region is less variable for detection of differences between isolates of the same AG (Kuninaga *et al.*, 2000; Woodhall *et al.*, 2007; Lehtonen *et al.*, 2008b). The gene *tef-1α* encoding the translation elongation factor 1α and more variable than the ITS region has already proved its usefulness to reveal genetic variability within fungal genera,

including *Fusarium* (Geiser *et al.*, 2004) and *Trichoderma* (Anees *et al.*, 2010b). This genetic marker could be useful to reflect polymorphism both between and within AG of *R. solani*. In addition to targeted DNA markers, strategies based on amplified fragment length polymorphism (AFLP) allow a large number of DNA fragments to be screened in order to assess intraspecific variability to differentiate closely related isolates and reveal clonal lineages (McDonald, 1997).

The aim of this study was to characterize the genetic diversity of isolates of *R. solani* collected from the different potato-growing areas in France. The genetic variability between and within AG was evaluated using ITS and *tef-1α* sequencing, together with AFLP analysis.

Material and methods

A collection of 73 isolates of *R. solani* was collected from potato tubers of several varieties and from 9 different French departments producing potato in 2006 and 2007 (TABLE 18). The potato tubers were affected by various superficial blemishes (Fiers *et al.*, 2010). In addition, 31 isolates of *R. solani* from other countries were analyzed: 6 from Finland, 1 from Germany, 1 from Japan, 4 from Morocco, 1 from the Netherlands, 2 from Poland, 1 from Spain, 12 from Switzerland, and 3 from the United Kingdom (Table 5.1).

DNA of all the fungal isolates was extracted from freeze dried powder of mycelium using the DNeasy plant mini kit according to the protocol previously described (Fiers *et al.*, 2010).

Table 5.1 Isolates of *Rhizoctonia solani* analyzed in this study

Anastomosis group	Strains	MIAE accession number [a]	Geographical origin		Host of origin (potato cultivar)	ITS sequence type [b]	*tef-1α* sequence type [b]	Two-locus type
2-1	0722-092 2 B PDA	MIAE00269	France	Côtes d'Armor	Juliette	m1	m1	1
2-1	0629-011 1 WA	MIAE00231	France	Finistère	Rosanna	p1	m2	2
2-1	0680-006 2 Aβ WA	MIAE00208	France	Somme	Hybride	m2	p1	3
2-1	0680-006 1 PDA	MIAE00262	France	Somme	Hybride	m2	p1	3
2-1	0680-006 1 WA	MIAE00263	France	Somme	Hybride	m2	p1	3
2-1	0680-006 2 A PDA	MIAE00264	France	Somme	Hybride	m2	p1	3
2-1	0680-006 2 Aα WA	MIAE00265	France	Somme	Hybride	m2	p1	3
2-1	0680-006 2 Bβ WA	MIAE00266	France	Somme	Hybride	m2	p1	3
2-1	173-4	MIAE00348	Finland		Unknown	Nd	m1	
2-1	R114	MIAE00349	Finland		Unknown	Nd	p2	
2-1	R25	MIAE00357	Finland		Unknown	m1	p3	4
2-1	0799-001 2 N WA	MIAE00189	Morocco		Unknown	p2	p4	5
2-1	Y25	MIAE00350	United Kingdom		Unknown	m2	m3	6
3	0602-001 1 B PDA	MIAE00072	France	Aisne	Unknown	p3	p5	7
3	0722-001 1 B PDA	MIAE00267	France	Côtes d'Armor	Charlotte	m3	m4	8
3	0722-001 2 A PDA	MIAE00268	France	Côtes d'Armor	Charlotte	m4	p6	9
3	0628-006 1 A WA	MIAE00150	France	Eure-et-Loir	Amandine	m5	p7	10
3	0628-006 3 A WA	MIAE00152	France	Eure-et-Loir	Amandine	p4	Nd	
3	0628-006 3 A PDA	MIAE00219	France	Eure-et-Loir	Amandine	p4	Nd	
3	0728-012 PDA	MIAE00270	France	Eure-et-Loir	Amandine	p5	p8	11
3	0728-091 A PDA	MIAE00271	France	Eure-et-Loir	Ditta	p3	Nd	
3	0629-049 2 WA	MIAE00006	France	Finistère	Juliette	p6	p9	12
3	0629-033 2 B WA	MIAE00082	France	Finistère	Juliette	m6	m4	13
3	0629-036 1 A WA	MIAE00083	France	Finistère	Juliette	m4	p10	14
3	0629-038 3 A WA	MIAE00087	France	Finistère	Samba	m7	p9	15
3	0629-039 2 A WA	MIAE00090	France	Finistère	Amandine	p7	Nd	
3	0629-040 2 A WA	MIAE00092	France	Finistère	Samba	p8	p9	16
3	0629-040 2 A PDA	MIAE00093	France	Finistère	Samba	p8	p9	16
3	0629-004 1A WA	MIAE00164	France	Finistère	Spunta	m8	p11	17
3	0629-014 3 WA	MIAE00165	France	Finistère	Chérie	p9	p9	18
3	0629-023 1 A PDA	MIAE00170	France	Finistère	Charlotte	m6	p12	19
3	0629-030 2 PDA	MIAE00176	France	Finistère	Samba	m5	Nd	
3	0629-055 3 WA	MIAE00179	France	Finistère	Pamela	p10	p11	20
3	0629-059 3 PDA	MIAE00185	France	Finistère	Hybride	m7	p13	21
3	0629-004 1 B WA	MIAE00222	France	Finistère	Spunta	p4	m5	22
3	0629-004 3 WA	MIAE00224	France	Finistère	Spunta	p9	m5	23
3	0629-004 4 WA	MIAE00225	France	Finistère	Spunta	p10	p14	24
3	0629-005 1 A PDA	MIAE00226	France	Finistère	Spunta	m5	m4	25
3	0629-005 1 WA	MIAE00227	France	Finistère	Spunta	m5	m4	25
3	0629-005 3 B PDA	MIAE00228	France	Finistère	Spunta	p11	p15	26
3	0629-005 3 WA	MIAE00229	France	Finistère	Spunta	p12	p15	27
3	0629-014 1 WA	MIAE00232	France	Finistère	Chérie	m5	p9	28
3	0629-014 2 WA	MIAE00233	France	Finistère	Chérie	p5	p9	29
3	0629-017 4 WA	MIAE00235	France	Finistère	Charlotte	p8	p16	30
3	0629-021 1 A WA	MIAE00236	France	Finistère	Atlas	m4	m4	31
3	0629-023 1 B WA	MIAE00237	France	Finistère	Charlotte	m6	m4	13
3	0629-023 2 B WA	MIAE00238	France	Finistère	Charlotte	m6	m4	13
3	0629-030 2 A WA	MIAE00239	France	Finistère	Samba	m5	p9	28
3	0629-038 1 A PDA	MIAE00240	France	Finistère	Samba	p4	m5	22
3	0629-038 1 WA	MIAE00241	France	Finistère	Samba	p4	m5	22
3	0629-038 3 A PDA	MIAE00242	France	Finistère	Samba	m7	p17	32
3	0629-038 3 B WA	MIAE00243	France	Finistère	Samba	m7	p9	15
3	0629-049 1 A PDA	MIAE00248	France	Finistère	Juliette	p13	p18	33
3	0629-049 1 Bα PDA	MIAE00249	France	Finistère	Juliette	m5	p19	34
3	0629-051 1 A WA	MIAE00250	France	Finistère	Marine	p8	p20	35
3	0629-055 2 PDA	MIAE00251	France	Finistère	Pamela	m5	p14	36

3	0629-055 2 WA	MIAE00252	France	Finistère	Pamela	m5	p14	36
3	0629-059 2 B PDA	MIAE00253	France	Finistère	Hybride	m5	p21	37
3	0729-015 PDA	MIAE00272	France	Finistère	Urgenta	m9	m4	38
3	0729-032 C PDA	MIAE00273	France	Finistère	Bintje	m8	Nd	
3	0729-093 1 PDA	MIAE00274	France	Finistère	Nicola	p13	p9	39
3	0729-093 2 PDA	MIAE00275	France	Finistère	Nicola	p14	p9	40
3	0745-001 1 B PDA	MIAE00276	France	Loiret	Bintje	p15	Nd	
3	0745-001 2 A PDA	MIAE00277	France	Loiret	Bintje	p16	m4	41
3	0656-003 2 WA	MIAE00255	France	Morbihan	Kennebec	p17	p22	42
3	0656-004 1 A WA	MIAE00256	France	Morbihan	Spunta	p8	p23	43
3	0656-004 2 B WA	MIAE00257	France	Morbihan	Spunta	m5	Nd	
3	0656-004 2 PDA	MIAE00258	France	Morbihan	Spunta	p8	p24	44
3	0656-006 2 N A WA	MIAE00259	France	Morbihan	Charlotte	m4	p9	45
3	0656-006 2 O PDA	MIAE00260	France	Morbihan	Charlotte	m4	p9	45
3	0656-006 2 O WA	MIAE00261	France	Morbihan	Charlotte	m4	p25	46
3	0756-091 A PDA	MIAE00280	France	Morbihan	Nicola	p18	Nd	
3	0762-002 3 PDA	MIAE00281	France	Pas-de-Calais	Hermes	m5	p26	47
3	R11	MIAE00356	Finland		Unknown	p19	m5	48
3	R98	MIAE00359	Finland		Unknown	p20	Nd	
3	CBS 363.82	MIAE00352	Germany		Unknown	p7	p9	49
3	i1	MIAE00351	Japan		Unknown	p21	m6	50
3	0799-001 2 N PDA	MIAE00217	Morocco		Unknown	p7	p9	49
3	0799-001 3 N A PDA	MIAE00283	Morocco		Unknown	Nd	p9	
3	0799-001 3 N WA	MIAE00284	Morocco		Unknown	p22	p25	51
3	CBS 163.83	MIAE00374	The Netherlands		Unknown	p9	p9	52
3	P1	MIAE00354	Poland		Unknown	m9	m7	53
3	P2	MIAE00355	Poland		Unknown	m9	m7	53
3	S1 1	MIAE00361	Switzerland		Bellini	m4	Nd	
3	S1 2	MIAE00362	Switzerland		Bellini	m4	Nd	
3	S1 3	MIAE00363	Switzerland		Bellini	m4	m5	54
3	S2 1	MIAE00364	Switzerland		Magnum	p23	Nd	
3	S2 2	MIAE00365	Switzerland		Magnum	Nd	p9	
3	S3 2	MIAE00366	Switzerland		Charlotte	p24	Nd	
3	S3 3	MIAE00367	Switzerland		Charlotte	p25	p27	55
3	S4 1	MIAE00368	Switzerland		Gourmandine	m4	p9	45
3	S5 1	MIAE00369	Switzerland		Hybride	p26	p28	56
3	S5 2	MIAE00370	Switzerland		Hybride	p3	Nd	
3	S6 2	MIAE00371	Switzerland		Ludmilla	p27	Nd	
3	S7 2	MIAE00372	Switzerland		Naviga	p28	m5	57
3	CBS 117241	MIAE00373	Spain		Unknown	p12	p9	39
3	HA	MIAE00353	United Kingdom		Unknown	m4	p9	45
3	Rs08	MIAE00360	United Kingdom		Unknown	m6	Nd	
5	0628-023 1 B PDA	MIAE00159	France	Eure-et-Loir	Chérie	m10	p29	58
5	0628-023 1 B WA	MIAE00213	France	Eure-et-Loir	Chérie	m10	p29	58
5	0628-023 1A PDA	MIAE00221	France	Eure-et-Loir	Chérie	m10	p29	58
5	0747-001 1 A PDA	MIAE00278	France	Lot-et-Garonne	Hybride	p29	Nd	
5	0747-002 2 A PDA	MIAE00279	France	Lot-et-Garonne	Samba	m10	p30	59
5	R96	MIAE00375	Finland		Unknown	m10	p29	58

[a] Collection MIAE, Microorganisms of Interest for Agriculture and Environment (INRA Dijon, France, http://www2.dijon.inra.fr/umrmse/).
[b] m and p followed by a number designate the different monomorphic and polymorphic sequence types, respectively. Nd indicates not determined.

For each fungal isolate, the ITS region of the rDNA and part of the *tef-1α* gene were amplified by PCR. ITS PCR was performed with the primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) (White *et al.*, 1990; Gardes and Bruns, 1993) in a final volume of 50 µL by mixing 2 µl of DNA with 0.5 µM of each primer, 150 µM of dNTP, 6 U of *Taq* DNA polymerase (Q-Biogen, Evry, France) and PCR reaction buffer. PCR amplifications of *tef-1α* were performed with primers EF1–645F (TCG TCG TYA TCG GMC ACG TCG A) and EF1–1190R (TAC CAG TGA TCA TGT TCT TGA TGA) (Andersen *et al.*, 2009) in a final volume of 25 µL by mixing 1 µL DNA with 0.068 µM of each primer, 1.5 mM $MgCl_2$, 150 µM dNTP, 1 U *Taq* DNA polymerase (Q-Biogen, Evry, France) and PCR reaction buffer. Amplifications were conducted in a mastercycler (Eppendorf, Hambourg, Germany). ITS amplification consisted in an initial denaturation of 3 min at 94 C, followed by 35 cycles of 1 min at 94 C, 1 min at 50 C, 1 min at 72 C, and a final extension of 10 min at 72 C. Amplification of *tef-1α* was carried out with an initial denaturation of 5 min at 94 C, followed by 40 cycles of 30 s at 94 C, 30 s at 52 C, and 80 s at 72 C and a final extension of 7 min at 72 C. Aliquots of PCR products were checked by electrophoresis on a 1 % agarose gel, revealed with ethydium bromide and visualized by UV trans-illumination.

ITS and *tef-1α* PCR products were sequenced by Beckman Coulters Genomics (Takeley, UK) using primers ITS1-F and ITS4 and EF1-645F and EF1-1190R, respectively. For each PCR product, sequences from both strands were assembled to produce a consensus sequence using the software SeqMan (DNASTAR Lasergene, GATC Biotech SARL, Marseille, France). The identities of the sequences were determined using BLAST analyses from the National Center for Biotechnology Information (NCBI) available on line. For each DNA region, sequences were aligned using ClustalX (Thompson *et al.*, 1997). The multiple sequence alignments were carried out with PHYLO-WIN (Galtier *et al.*, 1996) using the Kimura's two parameters distance model (Kimura, 1980) and the neighbor-joining method (Saitou and Nei, 1987). The topology of the resulting tree was tested by bootstrapping with 1000 re-samplings of the data. Phylogenetic trees were drawn using the NJPLOT program (Perrière and Gouy, 1996).

The digestion and ligation of 125 ng of extracted DNA were carried out according to the manufacturer's specifications of the AFLP Core Reagent kit (Invitrogen) (Vos *et al.*, 1995). The ligation products were pre-amplified by PCR. PCR amplifications were performed in a final volume of 25.5 µL by mixing 2.5 µL of DNA with 0.3 µM of each primer E-0 (GACTGCGTACCAATTC) and M-0 (GATGAGTCCTGAGTAA), 212 µM of dNTP, 2.5 U of *Taq* DNA polymerase (Q-Biogen, Evry, France) and PCR reaction buffer. Amplification was conducted in a thermal cycler GeneAmp PCR system 9600 (Perkin Elmer Applied Biosystems, Foster City, Calif.) with 20 cycles of 30 s at 94 C, 1 min at 56 C, and 1 min at 72 C. A 1:50 dilution was performed with 1.5 µL of pre-amplified product diluted in 73.5 µL TE buffer. Selective amplification was performed in a final volume of 20 µL by mixing 5 µL of DNA with 0.4 µM of E-AA 5′-IRD800 fluorescent primer (GACTGCGTACCAATTCAA) (MWG), 0.30 µM of M-C primer (GATGAGTCCTGAGTAAC), 202.5 µM of dNTP, 0.5 U of *Taq* DNA polymerase (Q-Biogen) and PCR reaction buffer. PCR was conducted in a thermal cycler GeneAmp PCR system 9600 with a first cycle at 94 C during 30 s, 65 C during 30 s, and 72 C during 1 min. The 12 following cycles were performed by reducing the annealing temperature of 0.7 C at each cycle. Then, 23 cycles were performed at 94 C during 30 s, 56 C during 30 s, and 72 C during 1 min. Aliquots of PCR products were checked by electrophoresis on a 1 % agarose gel, revealed with ethydium bromide and visualized by UV trans-illumination. Separation of the amplified fragments was performed on a 41 cm polyacrylamide gel (Li-Cor, Germany) with 6.5 % acrylamide KB (Li-Cor), during 10h30 at 1 500 V for a resolution of the fragments from 50 to 700 bp. A size standard (50-700 bp sizing standard, Li-Cor) and a strain of reference for which the profile is known, were added on each gel in at least five wells. A picture of each gel was taken. For each strain, the analysis was performed in duplicates from independent DNA extracts.

Li-Cor gel pictures were analyzed with ONE-Dscan software (Scanalytics BD Biosciences-Bioimaging, version 2.05), measuring the band sizes of the 115 most intense bands between 100 and 500 bp for each profile. Categories grouping fragments, whose weight differed by less than 1.5 bp, were created with LisAFLP program (Mougel *et al.*, 2002). The LecPCR application (ADE-4 v. 2001 Thioulouse *et al.*, 1997) was then applied to the data to transform the fragment weights matrix into a binary matrix. The binary matrix indicated the absence (0) or presence (1) of

each fragment for each profile. Finally, only fragments present in the two repeats performed for each strain were included in the analysis. Genetic relationships between each profile was estimated using the Nei and Li similarity index (Nei and Li, 1979) and bootstrap values corresponding to the appearance frequency of branches in 1000 data permutations was calculated with Treecon software (Vandepeer and Dewachter, 1994 version 1.3b). The similarity matrix was represented by a dendrogram with the UPGMA (unweighted pair grouping method with arithmetic mean) algorithm (Sneath and Sokal, 1973).

Results

PCR amplification of the ITS region with the primers ITS1-F and ITS 4 gave a single product of approximately 700 bp for each isolate. The ITS1, 5.8S and the ITS2 regions were sequenced for 100 isolates (Table 5.1). For all isolates, BLAST analyses allowed to determine the corresponding AG on the basis of 99 to 100 % sequence similarity with corresponding sequences. Among the 73 French isolates, 60 were identified as *R. solani* AG 3 PT, the remaining were AG 2-1 (8 isolates) and AG 5 (5 isolates). A total of 64 polymorphic sites was identify in ITS1 but only 24 polymorphic sites in ITS2. Among the large number of AG 3 PT isolates analyzed, 10 and 3 variable sites were observed in ITS1 and ITS2, respectively (Figure 5.1). Heterogeneity within isolates was observed for 48 out of the 100 isolates. This heterogeneity was observed as two overlapping peaks at the same position in the electrophoregram, indicating that multiple ITS types might exist within isolates. Among the AG 3 PT sequences, this heterogeneity within isolates was observed at all the 13 variable sites identified within the AG. Conversely, only 2 and 4 sites were identified to be polymorphic within isolates among AG 2-1 and AG 5 sequences; however the number of sequences was also lower with 11 and 6 isolates from AG 2-1 and AG 5, respectively.

All the sequences were assigned to a type number from m1 to m10 for the 10 monomorphic sequences observed and p1 to p29 for the 29 sequences with polymorphic sites, coded as m and p followed by a number designate the different monomorphic and polymorphic sequence types, respectively (Figure 5.1).

Comparisons of all sequences allowed identifying 39 ITS types. Four types were identified within AG 2-1 (type m1, m2, p1, p2), 33 within AG 3 PT (type m3 – m9 and type p3 – p28) and 2 within AG 5 (type m10 and p29) (Table 5.1).

The 52 sequences without any polymorphic site were compared in a phylogenetic tree (Figure 5.2). The three AG were genetically different as they appeared on three distinct branches of the tree with significant bootstrap values above 99 %. Isolates belonging to AG 5 were more similar to isolates from AG 2-1 than to isolates from AG 3 PT (Figure 5.2). The tree of ITS sequences showed 7 different ITS types among AG 3 PT. Type m3 comprised one isolate (MIAE00267) from France (Côtes d'Armor) and isolated from cultivar Charlotte. Type m5 included 12 isolates from France (Finistère, Eure-et-Loir, Pas-de-Calais). They were isolated from cultivar Spunta, Samba, Pamela, Chérie, Amandine, Hermes and Juliette. ITS type m4 comprised 11 isolates from France (Finistère, Morbihan, Côtes d'Armor), Switzerland and United Kingdom. They were isolated from cultivars Charlotte, Juliette, Atlas, Gourmandine, and Bellini. Type m7 included 4 isolates all from France (Finistère) isolated from cultivar Samba or a hybrid. The ITS types m6, m8 and m9 were grouped in the same branch, distantly related from the other types in AG 3 PT. Type m6 comprised 5 isolates from France (Finistère) and United Kingdom isolated from cultivars Charlotte and Juliette. Type m8 comprised 2 isolates from France (Finistère) isolated from cultivars Spunta and Bintje. Type m9 comprised 3 isolates from Poland and France (Finistère) isolated from Urgenta and unknown cultivars.

Two different ITS types were observed among AG 2-1. Type m2 included 7 isolates from France (Somme) and United Kingdom. They were isolated from a hybrid cultivar and an unknown cultivar. Type m1 included 2 isolates from Finland and France (Côtes d'Armor) and they were isolated from an unknown cultivar and cultivar Juliette. Finally, monomorphic isolates from AG 5 were grouped in only one ITS type (m10). They were 5 isolates from France (Eure-et-Loir, Lot-et-Garonne) and Finland isolated from cultivars Chérie, Samba, a hybrid and an unknown cultivar.

(a)

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                      10        20        30        40        50        60        70        80        90       100
             ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AG 2-1  m1   CCCATTCATTTGGGCATGTGCACACCTTTCTCTTTCATCCCACACACACCTGTGAACCTGTGAGACAGATGGGGAATTTACTTGTTGCTTTTTGGAATAC
        m2   .T....T.....................C.............................................................C...........
AG 3    m3   T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        m4   T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        m5   T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        m6   T.T...T....A...............C.............................T..........T...........T.....AT.A....T....A
        m7   T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        m8   T.T...T....A...............C.............................T..........T...........T.....AT.A....T....A
        m9   T.T...T....A...............C.............................T..........T...........T.....AT.A....T....A
AG 5    m10  .T...GA...................A..........-...............C..T......G..............T.GA...T.....AAG.ACA
AG 2-1  p1   ......T....A...............C............T......................T...............................
        p2   .T....T.....................C.............................................................C...........
AG 3    p3   T.T...T....A...............CY............................T..........T...........T.....AT.W....T....A
        p4   T.T...T....A...............C.............................T..........T...........T.....AT.A....T....A
        p5   T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p6   T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p7   T.T...T....A...............CY............................T..........T...........T.....AT.W....T....A
        p8   T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p9   T.T...T....A...............CY............................T..........T...........T.....AT.A....T....A
        p10  T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p11  T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p12  T.T...T....A...............CC............................T..........T...........T.....AT.W....T....A
        p13  T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p14  T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p15  T.T...T....A...............C.............................T..........T...........T.....AT.A....T....A
        p16  T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p17  T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p18  T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p19  T.T...T....A...............CY............................T..........T...........T.....AT.A....T....A
        p20  T.T...T....A...............CC............................T..........T...........T.....AT.W....T....A
        p21  T.T...T....A...............CY............................T..........T...........T.....AT.W....T....A
        p22  T.T...T....A...............C.............................T..........T...........T.....AT.A....T....A
        p23  T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p24  T.T...T....A...............CC............................T..........T...........T.....AT.W....T....A
        p25  T.T...T....A...............CC............................T..........T...........T.....AT......T....A
        p26  T.T...T....A...............CY............................T..........T...........T.....AT.W....T....A
        p27  T.T...T....A...............CY............................T..........T...........T.....AT.W....T....A
        p28  T.T...T....A...............CY............................T..........T...........T.....AT.W....T....A
AG 5    p29  .T...GA...................A..........-...............C..T......G..............T.GA...T.....AAG.ACA

                     110       120       130       140       150       160       170       180       190
             ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AG 2-1  m1   AAAGGCAATAGGTTATTGGACCCT-CTGTCTACTCAATTAATATAAACTCAATTTATTTTAAAACGAATGTAATGGATGTAACACATCTC
        m2   .......................-..............................A........T.........................
AG 3    m3   ..T.AT....AA.C....A.....T............C.C.......A...............TG-......................
        m4   ..T.AT....A..C....A.....T............C.C.......A...............TG-......................
        m5   ..T.AT....AA.C....A.....T............C.C.......A...............TG-......................
        m6   ..TAAT....A..C.C..A.....T............C.C......................TG-......................
        m7   ..T.AT....A..C....A.....T............C.C.......A........CA....TG-......................
        m8   ..TAAT....A..C.C..A.....T............C.C......................TG-......................
        m9   ..TAAT....A..C.C..A.....T............C.C.....C................TG-......................
AG 5    m10  .TT..TGC.G.--------.....-............-.-...................AG...T..................G.....A
AG 2-1  p1   .......................-......................C..............CT.......................
        p2   .......................-......................Y.....A........T........................
AG 3    p3   ..TRAT....A..C.Y..A.....T............C.C.......M...............TG-......................
        p4   ..TAAT....A..C.C..A.....T............C.C......................TG-......................
        p5   ..T.AT....AR.C....A.....T............C.C.......A...............TG-......................
        p6   ..T.AT....A..C....A.....T............C.C.......A........WY....TG-......................
        p7   ..TRAT....A..C....A.....T............C.C.......M...............TG-......................
        p8   ..T.AT....AA.C....A.....T............C.C.......A...............TG-......................
        p9   ..TRAT....A..C.Y..A.....T............C.C.......M...............TG-......................
        p10  ..T.AT....AA.C....A.....T............C.C.......A...............TG-......................
        p11  ..T.AT....AA.C....A.....T............C.C.....M.A..............TG-......................
        p12  ..T.AT....AA.C....A.....T............C.C.....M.A..............TG-......................
        p13  ..T.AT....AR.C....A.....T............C.C.......A...............TG-......................
        p14  ..T.AT....AR.C....A.....T............C.C.......A........YW....TG-......................
        p15  ..TAAT....A..C.C..A.....T............C.C.....M................TG-......................
        p16  ..T.AT....A..C....A.....T............C.C.......A........YW....TG-......................
        p17  ..T.AT....AA.C....A.....T............C.C.......A........YW....TG-......................
        p18  ..T.AT....AA.C....A.....T............C.C.......A........YW....TG-......................
        p19  ..TRAT....AR.C.Y..A.....T............C.C.......M...............TG-......................
        p20  ..T.AT....A..C....A.....T............C.C.......A........W.....TG-......................
        p21  ..TRAT....A..C.Y..A.....T............C.C.....M.M..............TG-......................
        p22  ..TRAT....A..C.C..A.....T............C.C.......M...............TG-......................
        p23  ..T.AT....AR.C....A.....T............C.C.......A........YW....TG-......................
        p24  ..T.AT....A..C....A.....T............C.C.......A...............TG-......................
        p25  ..T.AT....A..C....A.....T............C.C.....M.A..............TG-......................
        p26  ..TRAT....A..C.Y..A.....T............C.C.......M...............TG-......................
        p27  ..TRAT....A..C.Y..A.....T............C.C.......M........YW....TG-......................
        p28  ..TRAT....AR.C.Y..A.....T............C.C.....C.M..............TG-......................
AG 5    p29  .TT..TGC.G.--------.....-............-..CATA..CYCA.T..AT..AG...TG-................G.....A
```

(b)

```
                        10        20        30        40        50        60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AG 2-1  m1      TTCTTCAAAGTAAATCTTTTG-TTAACTCAATTGGTTTCACTTTGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTTCATTAGCTGGAT
        m2      ......................-....T....C.......................................................G...........
AG 3    m3      A........A.C........TG...........A....G.TC.........................................GT...........
        m4      A........A.C........TG...........A....G.T..........................................GT...........
        m5      A........A.C........TG...........A....G.T..........................................GT...........
        m6      A........A.C........TG...........A....G.T................A.........................GT...........
        m7      A........A.C........TG...........A....G.T..........................................GT...........
        m8      A........A.C........TG...........A....G.T..........................................GT...........
        m9      A........A.C........TG...........A....G.T..........................................GT...........
AG 5    m10     A...................-G...............CT.....................-......................C..............
AG 2-1  p1      .......Y............-G....T..............................T.............................G...........
        p2      ....................-G....T....C.......................................................G...........
AG 3    p3      A........A.C........TG...........A....G.T................W.........................GT...........
        p4      A........A.C........TG...........A....G.T................W.........................GT...........
        p5      A........A.S........TG...........A....G.T..........................................GT...........
        p6      A........A.C........TG...........A....G.T..........................................GT...........
        p7      A........A.C........TG...........A....G.T................W.........................GT...........
        p8      A........A.S........TG...........A....G.T..........................................GT...........
        p9      A........A.C........TG...........A....G.T................A.........................GT...........
        p10     A........A.C........TG...........A....G.TY.........................................GT...........
        p11     A........A.C........TG...........A....G.T..........................................GT...........
        p12     A........A.C........TG...........A....G.T..........................................GT...........
        p13     A........A.C........TG...........A....G.T..........................................GT...........
        p14     A........A.C........TG...........A....G.TY.........................................GT...........
        p15     A........A.C........TG...........A....G.T..........................................GT...........
        p16     A........A.C........TG...........A....G.T..........................................GT...........
        p17     A........A.C........TG...........A....G.TY.........................................GT...........
        p18     A........A.C........TG...........A....G.T..........................................GT...........
        p19     A........A.C........TG...........A....G.T................A.........................GT...........
        p20     A........A.C........TG...........A....G.T..........................................GT...........
        p21     A........A.C........TG...........A....G.T..........................................GT...........
        p22     A........A.C........TG...........A....G.T................A.........................GT...........
        p23     A........A.C........TG...........A....G.T..........................................GT...........
        p24     A........A.C........TG...........A....G.T................W.........................GT...........
        p25     A........A.C........TG...........A....G.T..........................................GT...........
        p26     A........A.C........TG...........A....G.T................A.........................GT...........
        p27     A........A.C........TG...........A....G.T................W.........................GT...........
        p28     A........A.C........TG...........A....G.T..........................................GT...........
AG 5    p29     A...................-G...............CY.....................-......................Y..............

                       110       120       130       140       150       160       170       180       190
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
AG 2-1  m1      CTCAGTGTTATGCTTGGTTCCACTCGGCGTGATAAATTATCTATCGCTGAGGACACTGTAAAAGGTGGCCAAGGTAAATGCAGATGAACCGCTTCTAA
        m2      ......................................................................................................
AG 3    m3      .......................A......................................A.....................................
        m4      .......................A......................................A.....................................
        m5      .......................A......................................A.....................................
        m6      .......................A......................................A.....................................
        m7      .......................A......................................A.....................................
        m8      .......................A......................................A.....................................
        m9      .......................A......................................A.....................................
AG 5    m10     .......................A........G.......................C...........G.......................
AG 2-1  p1      ......................................................................................................
        p2      ......................................................................................................
AG 3    p3      .......................A......................................A.....................................
        p4      .......................A......................................A.....................................
        p5      .......................A......................................A.....................................
        p6      .......................A......................................A.....................................
        p7      .......................A......................................A.....................................
        p8      .......................A......................................A.....................................
        p9      .......................A......................................A.....................................
        p10     .......................A......................................A.....................................
        p11     .......................A......................................A.....................................
        p12     .......................A......................................A.....................................
        p13     .......................A......................................A.....................................
        p14     .......................A......................................A.....................................
        p15     .......................A......................................A.....................................
        p16     .......................A......................................A.....................................
        p17     .......................A......................................A.....................................
        p18     .......................A......................................A.....................................
        p19     .......................A......................................A.....................................
        p20     .......................A......................................A.....................................
        p21     .......................A......................................A.....................................
        p22     .......................A......................................A.....................................
        p23     .......................A......................................A.....................................
        p24     .......................A......................................A.....................................
        p25     .......................A......................................A.....................................
        p26     .......................A......................................A.....................................
        p27     .......................A......................................A.....................................
        p28     .......................A......................................A.....................................
AG 5    p29     .......................A........G.......................C...........G.....................R
```

Figure 5.1 Sequence alignment of part of the ITS1 (a) and ITS2 (b) regions among isolates of *Rhizoctonia solani* collected from potatoes.
Y indicates C and T, W indicates A and T, M indicates A and C and R indicates A and G. Polymorphic bases indicated in grey are common to those found by Justesen et al (2003) in the ITS1 region.

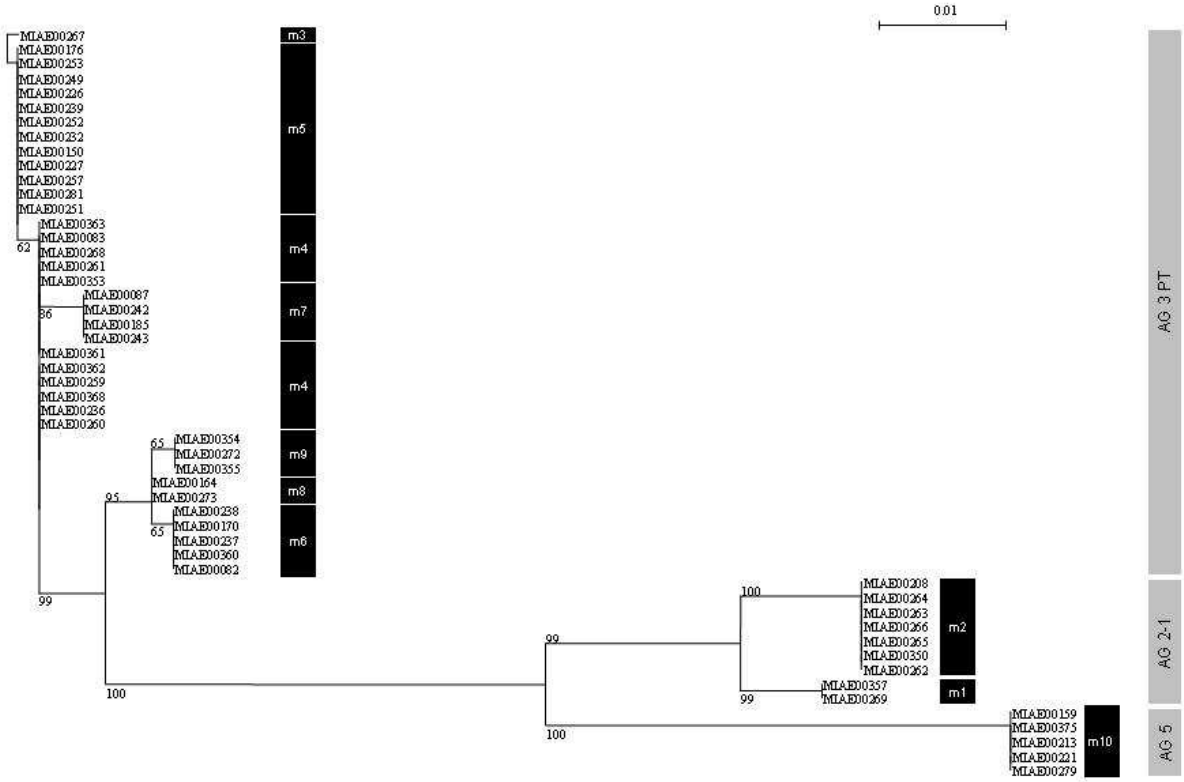

Figure 5.2 Neighbour-joining tree (Kimura two-parameter distance) of 52 monomorphic ITS sequences of *Rhizoctonia solani* isolated from potato tubers.
Bootstrap values (≥ 60 %) are shown near the equivalent branches.

The PCR amplification of the *tef-1α* gene with the primers EF1-645F and EF1-1190R gave a single product of approximately 400 bp. The *tef-1α* gene was sequenced for 86 isolates (Table 5.1). In BLAST analyses, all sequences matched a sequence of the same AG of *R. solani* than the AG identified through ITS sequence, with a similarity of 93 to 100 %. Among the 86 sequenced isolates 13 belonged to AG 2-1, 68 to AG 3 PT and 5 to AG 5.

A total of 60 variable sites were identified among *tef-1α* sequences (Figure 5.3). Among the 68 sequences of AG 3 PT, 20 variable sites were depicted. Among AG 2-1 and AG 5 isolates, 42 and 8 variable sites were observed, respectively. Like in the ITS sequences, some heterogeneity within isolates was observed for 64 isolates out of the 86, such as polymorphic sites, which revealed polymorphism within the *tef-1α* gene in the same individual. This heterogeneity was observed at 17, 14 and 5 variable sites observed within AG 3 PT, AG 2-1 and AG 5 sequences, respectively

(Figure 5.3). According to the variations in the *tef-1α* sequences, 37 different elongation factor types were identified: 7 within AG 2-1 (types m1 – m3 and types p1-p4), 28 within AG 3 PT (type m4 – m7 and type p5 – p28) and 2 within AG 5 (type p29 and p30) (Table 5.1).

A phylogenetic tree was built up on the basis of the 23 *tef-1α* sequences without any polymorphic site, together with one sequence of AG 5 (MIAE00279) including only one polymorphic site. Indeed all the sequences of *R. solani* AG 5 analyzed comprised at least one polymorphic site (Figure 5.4). The phylogenetic tree showed 4 different elongation factor types within AG 3 PT. Type m6 comprised one isolate (MIAE00351) from Japan; type m5 comprised 7 isolates from France (Finistère), Switzerland and Finland isolated from cultivar Spunta, Samba, Naviga and Bellini. Type m4 comprised 9 isolates from different department of France (Côtes d'Armor, Finistère and Loiret) isolated from cultivars Atlas, Bintje, Charlotte, Juliette, Spunta and Urgenta. Finally type m7 included 2 isolates from Poland isolated from unknown cultivars. All the strains belonging to the type m7 in *tef-1α* analysis corresponded to type m9 in ITS analysis; both being distantly related to other AG 3 PT sequences (Figures 5.2 and 5.4). Among AG 2-1 sequences, three elongation factor types m1, m2 and m3 were identified.

Combined data from ITS sequences and *tef-1α* sequences revealed a total of 59 two-locus sequence types among the 82 isolates for which both loci were analyzed (Table 5.1). Among the 59 types, only 10 two-locus sequence types were monomorphic for both loci.

```
                        10        20        30        40        50        60        70        80        90       100
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AG 2-1  m1       CATTGAAAAGTTCGAGAAGGAAGCTGCTGAGCTCGGCAAGGGCTCCTTCAAGTATGGTGCGTACGCATATACTACTCGATCGA--CCGCCACTGATTAGT
        m2       ...C.......................................................G.............T.G....CCGT--...........
        m3       ...C...............................A........................G...............G....--TT.TT..........
AG 3 PT m4       G.......................................T...................G......C...G....C....GTTT........C....
        m5       G.......................................T...................G......C...G....CC...GTTT..T.....C....
        m6       G.......................................T...................G......C...G....C....GTTT..T.....C....
        m7       G.......................................T...................G.........G....C....GT.T..T.....C....
AG 2-1  p1       ...C...............................A........................G...............G....--TT.TT......Y...
        p2       .......................................................R...................--...............
        p3       G.......................................T...................G......C...G....C....GTTT........C....
        p4       ...C...............................A........................G..Y............G....--TT.TT.......R..
AG 3 PT p5       G.......................................T...................G......C...G....C....GTTT..T.....C....
        p6       G.......................................T...................G......Y...G....Y....GTYT..Y.....C....
        p7       G.......................................T...................G......Y...G....CY...GTYT..T.....C....
        p8       G.......................................T...................G......C...G....CY...GTTT..T.....C....
        p9       G.......................................T...................G......C...G....CY...GTTT..Y.....C....
        p10      G.......................................T...................G......C...G....C....GTTT........C....
        p11      G.......................................T...................G......C...G.Y..CC...GTTT..T.....C....
        p12      G.......................................T...................G......C...G....C....GTTT........C....
        p13      G.......................................T...................G......C...G....YY...GTTT..Y.....C....
        p14      G.......................................T...................G......C...G....C....GTTT..Y.....C....
        p15      G.......................................T...................G......C...G....CY...GTTT..T.....C....
        p16      G.......................................T...................G......Y...G....C....GTTT..Y.....C....
        p17      G.......................................T...................G......C...G....CY...GTTT..Y.....C....
        p18      G.......................................T...................G......Y...G.Y..C....GTYT..T.....C....
        p19      G.......................................T...................G......C...G.Y..C....GTYT..T.....C....
        p20      G.......................................T...................G......C...G....C....GTTT..T.....C....
        p21      G.......................................T...................G......C...G....C....GTTT..Y.....C....
        p22      G....-..................................T...................G......C...G....C....GTTT..Y.....C....
        p23      G.......................................T...................G......Y...G....C....GTYT..Y.....C....
        p24      G.......................................T...................G......C...G....C....GTYT..Y.....C....
        p25      G.......................................T...................G......C...G....CY...GTTT........C....
        p26      G.......................................T...................G......C...G....Y....GTTT........C....
        p27      G.......................................T...................G......C...G....C....GTTT........C....
        p28      G.......................................T...................G......C...G....CC...GTTT..T.....C....
AG 5    p29      ........................................T................Y..G......C.T.G...TCYR--GY..........C....
        p30      ........................................T...................G......C.T.G...--Y.C.GC..........C....
```

```
                       110       120       130       140       150       160       170       180       190       200
                 ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AG 2-1  m1       ATGCTACAGCGTGGGTGCTCGACAAACTCAAGGCTGAGCGTGAGCGTGGTATCACCATCGATATCGCGCTCTGGAAGTTCGAGACCCCCAAGTACATGGT
        m2       .C.T...................................................................T...............................
        m3       ..........C.............................................................................................
AG 3 PT m4       .G......................G................A..............T.......................................
        m5       GG......................G................A..............T.......................................
        m6       GG......................G.............................T........A..T......................T......
        m7       .G.............C..T.....G.........................T..T.....T..A...........................................
AG 2-1  p1       ..........S.....................Y.............................................Y.............
        p2       .........................................................................................................
        p3       .G.............S........G................R...........Y..T.....Y..R..............................
        p4       .........................................................................................................
AG 3 PT p5       .G......................G.............................T........A.......................T......
        p6       .G.............S..Y.....G.........................Y..T.....Y..A.......................W......
        p7       RG.............S..Y.....G................R...........Y..T.....Y...............................
        p8       GG......................G................R..............T........R..Y......................W......
        p9       RG......................G................A..............T.......................................
        p10      .G......................G................R..............T.......................................
        p11      GG......................G................A..............T.......................................
        p12      .G................Y.....G................A..............T.......................................
        p13      RG......................G................R..............T........R.......................W......
        p14      RG......................G................R..............T........R..Y......................W......
        p15      RG......................G................R..............T........R.......................W......
        p16      .G................Y.....G................R...........Y..T.....Y..R..............................
        p17      GG......................G................A..............T.......................................
        p18      RG.............S..Y.....G.........................Y..T.....Y..R..............................
        p19      GG.............S..Y.....G.............................T.....Y..R..............................
        p20      GG......................G.............................T........A..T......................T......
        p21      RG......................G................R..............T........R..Y......................W......
        p22      .G......................G................R..............T........R.......................W......
        p23      .G.............S..Y.....G................R...........Y..T.....Y..R..............................
        p24      .G................Y.....G................R...........Y..T.....Y...............................
        p25      RG......................G................A..............T.......................................
        p26      .G......................G................R..............T........R.......................W......
        p27      .G......................G................A..............T........R..Y......................W......
        p28      GG................Y.....G................R..............T.......................................
AG 5    p29      ....C...................G.......................................................T..............
        p30      ....C...................G.......................................................T..............
```

```
                    210       220       230
                 ....|....|....|....|....|....|
AG 2-1  m1       CACTGTAAGTATTGGCATATATGCTCAACA
        m2       ...............C..........C..
        m3       T..C............C.............
AG 3 PT m4       ...C......CC.T..T-........G..G
        m5       ...C......CC.T.-C........G..G
        m6       ...C......CC.T.-C........G...
        m7       ...C......CC.T..T-........G..G
AG 2-1  p1       Y..Y............C.............
        p2       ..............................
        p3       ...C......CC.T.-C........G..G
        p4       ...C............C.............
AG 3 PT p5       ...C......CC.T.-C........G..R
        p6       ...C......CC.T.-C........G..R
        p7       ...C......CC.T.-C........G..G
        p8       ...C......CC.T.-C........G..R
        p9       ...C......CC.T.-C........G..G
        p10      ...C......CC.T.-C........G..G
```

```
        p11  ...C......CC.T.-C.........G..G
        p12  ...C......CC.T.-C.........G..G
        p13  ...C......CC.T..T-........G..R
        p14  ...C......CC.T.-C.........G..R
        p15  ...C......CC.T..T-........G..G
        p16  ...C......CC.T.-C.........G..G
        p17  ...C......CC.T..T-........G..G
        p18  ...C......CC.T.YT-........G..G
        p19  ...C......CC.T.YT-........G..G
        p20  ...C......CC.T.-C.........G..R
        p21  ...C......CC.T.-C.........G..G
        p22  ...C......CC.T.-C.........G..G
        p23  ...C......CC.T.-C.........G..G
        p24  ...C......CC.T.-C.........G..G
        p25  ...C......CC.T..T-........G..G
        p26  ...C......CC.T.-C.........G..G
        p27  ...C......CC.T.-C.........G..R
        p28  ...C......CC.T..T-........G..G
AG 5    p29  ...C......CCCAT.T-........G.TC
        p30  ...C...R..CCCAT.T-........G.TC
```

Figure 5.3 Sequence alignment of part of the tef-1α gene among isolates of *Rhizoctonia solani* collected from potatoes.
Y indicates C and T, W indicates A and T, M indicates A and C and R indicates A and G.

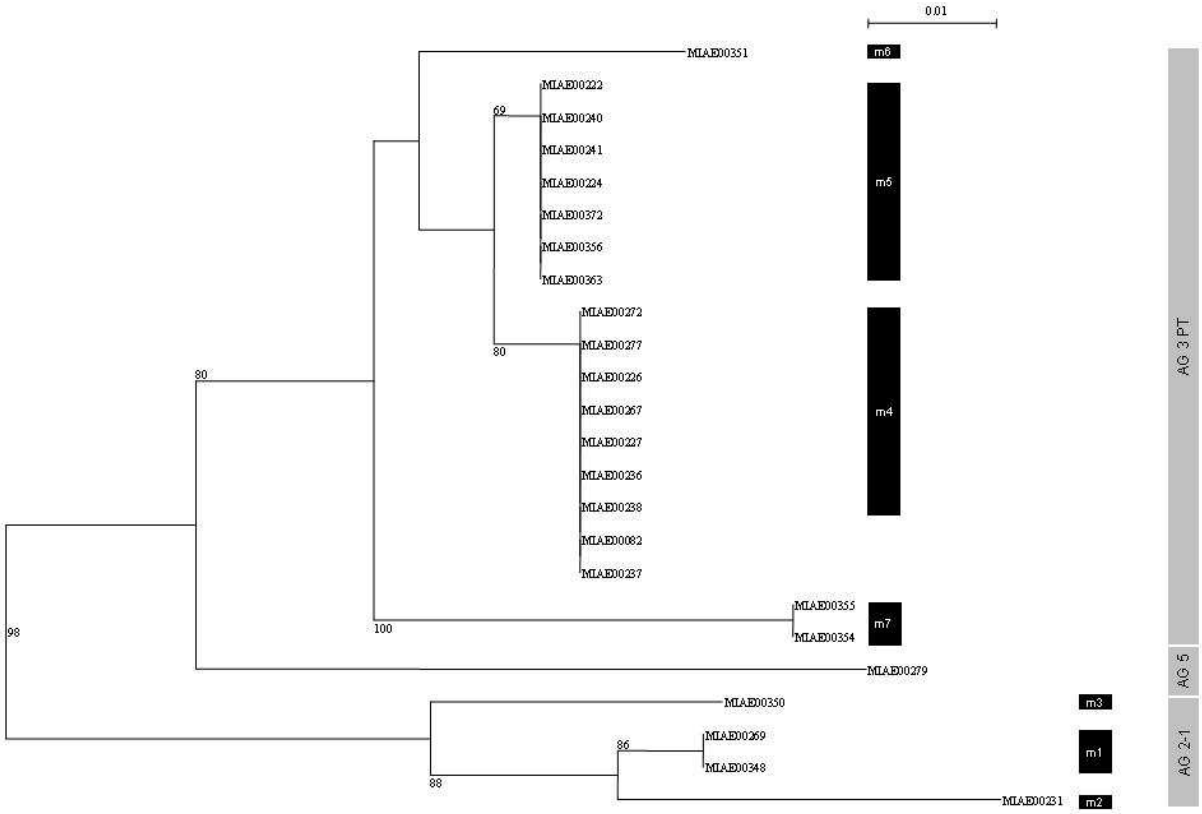

Figure 5.4 Neighbour-joining tree (Kimura two-parameter distance) of 23 tef-1α sequences of *Rhizoctonia solani* isolated from potato tubers from France and other countries.
Strain MIAE00279 belonging to AG 5 includes one polymorphic site. Bootstrap values (≥ 60 %) are shown near the equivalent branches

Eighty-nine isolates were analyzed by the AFLP method. The number of bands analyzed (from 100 to 500 bp) was 60 – 102 per profile, with an average of 78 bands, for a total of 254 useful markers. The phylogenetic tree of AFLP profiles showed three groups supported by significant bootstrap values above 96 %, corresponding to the three AGs (Figure 5.5). Regarding AFLP profiles, the AG 5 isolates were more related to the AG 3 PT isolates than to the AG 2-1 isolates, as it was shown on the tree of *tef-1α* sequences. A significant diversity was observed within each AG with so many AFLP profiles as isolates, but no particular clusters could be identified within AGs. Among AG 3 PT isolates, the French isolates were spread all over the tree. At a smaller scale, isolates originating from the same French Department (Finistère) were also found all along the tree, showing no direct relation of the diversity observed with the geographic origin.

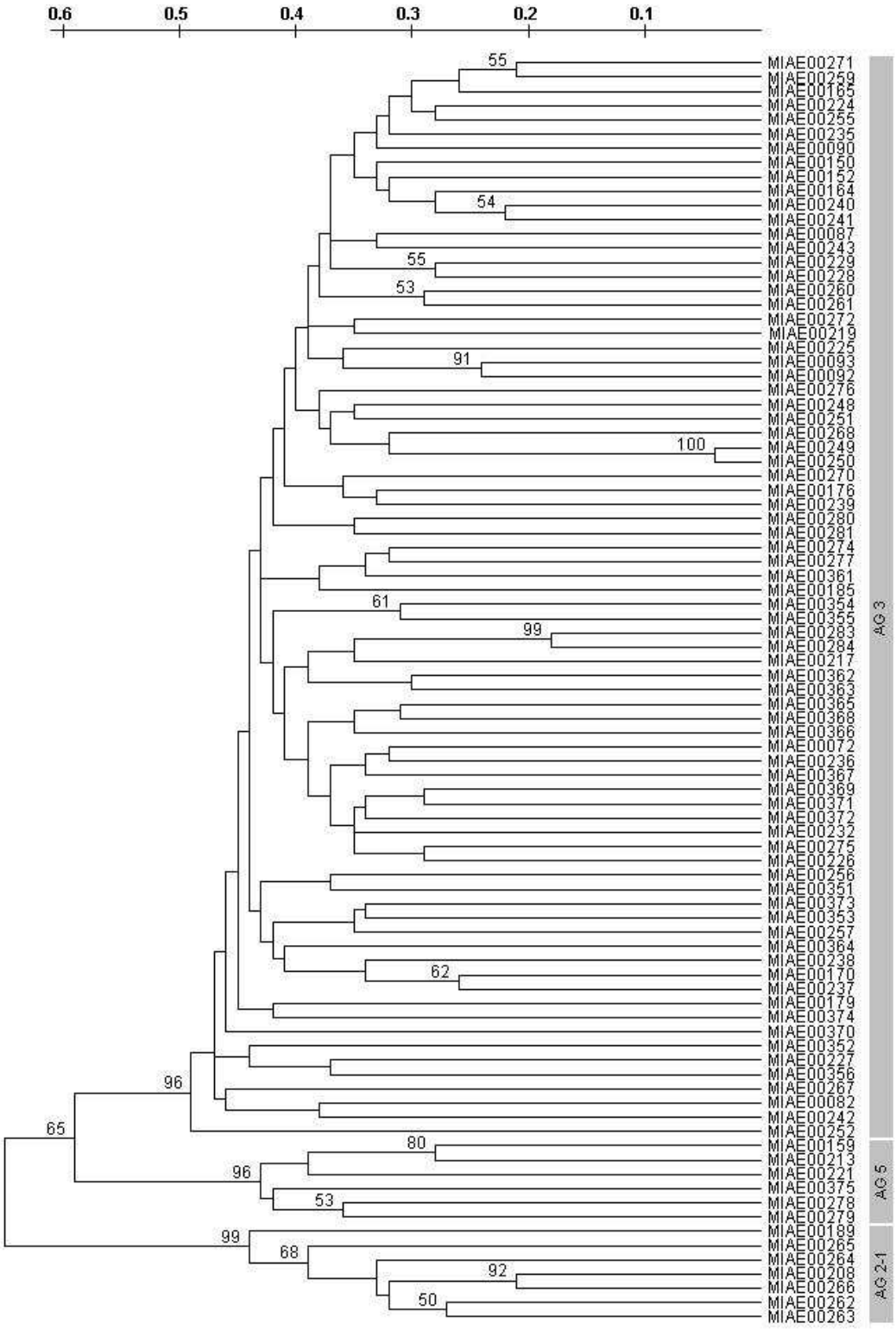

Figure 5.5 Phylogenetic relationships among 89 strains of *Rhizoctonia solani* inferred from AFLP data using a UPGMA analysis of Nei – Li distances.
Bootstrap values (≥ 50%) are shown above the equivalent branches

Discussion

In this study, 104 isolates of *R. solani*, all sampled from blemished potato tubers, were characterized by sequencing of the ITS region and part of the *tef-1α* gene and by AFLP fingerprinting. The 73 French isolates were representative of all area producing potatoes in France; they were compared with 31 isolates originating from other countries. AG 3 PT was found to be predominant, including 82 % of the French isolates. The remaining isolates belonged to AG 2-1 and AG 5. *R. solani* isolates belonging to AG 3 are frequently isolated from potato tubers and are known to be pathogens for this crop (Kuninaga *et al.*, 2000), but AG 2-1 and AG 5 are more rarely isolated from blemished tubers and their pathogenicity has still not been demonstrated (Campion *et al.*, 2003; Woodhall *et al.*, 2008; Fiers *et al.*, 2010).

Several distinct types of the ITS region and of the *tef-1α* gene were identified among the *R. solani* isolates and surprisingly, in the majority of isolates the coexistence of multiple types was observed. Polymorphism in the ITS region and *tef-1α* gene within isolates can be due to the existence of different ribosomal DNA units within the same nucleus or different sequences in different nuclei. This hypothesis could be confirmed after cloning (Justesen *et al.*, 2003). This heterogeneity results from mutations that would be fixed. Several ITS sequence types within the same individual were also identified for other fungi such as *Sclerotium* spp. (Almeida *et al.*, 2001), *Ascochyta* spp. (Fatehi and Bridge, 1998) and *Botrytis* spp. (Yohalem *et al.*, 2003). Among *R. solani* populations, ITS polymorphism was studied within AG 2-1 and AG 3 (Justesen *et al.*, 2003; Pannecoucque and Hofte, 2009). The same 10 within-isolates polymorphic sites previously identified by Justesen et al (2003) among ITS1 sequences of *R. solani* AG 3 were also detected in our study in addition to others sites. The finding of two different ITS types or elongation factor types within the same isolate and the possible association with two different nuclear types is consistent with the heterokaryotic nature of *R. solani* which has been confirmed by other DNA marker methods that revealed heterozygosity in isolates of *R. solani* AG 3 (Ceresini *et al.*, 2007).

Both loci, ITS region and *tef-1α* gene, showed a considerable level of variability among *R. solani* isolates. Concerning the ITS region, we found that ITS1 sequences were much more variable than ITS2 sequences, with 24 % and 11 % of variable sites

in ITS1 and ITS2, respectively. Our results are in agreement with the ITS variability recently described by Nilsson et al (2008) among *R. solani*. The second locus used, *tef-1α* gene, was found even more polymorphic, with 27 % of variable sites among all three detected AGs of *R. solani*, and 9 % of variable sites among AG 3 PT isolates. However, all this variability could not be illustrated in trees, since dimorphic sites were excluded from the phylogenetic analysis.

The different sequence types obtained for one DNA locus did not match exactly the sequence types obtained for the other locus. However, in both ITS and *tef-1α* trees, the AG 3 PT branch that diverged from other AG 3 PT sequences corresponded to the same isolates MIAE00354 and MIAE00355.

The analysis of ITS sequences is a wide spread method for specific identifications of fungi and our results indicated that the gene *tef-1α* showed a greater diversity among AGs of *R. solani* than the ITS region. This shows the complementarity of sequences from two or more loci in multilocus sequence typing (MLST) approaches. Such MLST strategy becomes widely used to resolve phylogenetic relationships among fungi such as *Fusarium* (Nitschke *et al.*, 2009; O'Donnell *et al.*, 2009) or *Trichoderma* (Kullnig-Gradinger *et al.*, 2002; Anees *et al.*, 2010b). These recent studies have shown the interest of including *tef-1α* gene in such studies. However, the use of this gene to resolve genetic diversity within *R. solani* has not been previously published and only 33 sequences of *tef-1α* gene of *R. solani* were available in Genbank before this study. We used primers EF1-645F and EF1-1190R originally described for *Alternaria* spp. (Andersen *et al.*, 2009). We adapted PCR conditions to amplify part of the *tef-1α* gene from *R. solani*. Multigene approaches may be useful for more precise molecular identifications of species or AGs, when a unique locus such as ITS does not always provide clear information (Anees *et al.*, 2010b).

ITS and *tef-1α* types did not show any relationship with the geographical origin of the isolates or the cultivar of origin. Moreover, AFLP data did not show particular structure among the isolates belonging to the same AG. Our findings support the hypothesis that *R. solani* populations are constantly always evolving following different genetic events. The absence of sporulation of *R. solani* may limit the dissemination of clonal isolates and may prevent the structuring of the populations. On the other hand, the diversity of the *R. solani* populations could be enriched by the spread of the fungal genes taking place within the field and at larger extent, between

French departments and between several countries separated by several hundred kilometers. As tubers are exchanged on the international market, seed born inoculum could be the predominant cause of the long distance dispersal of the fungus. Indeed, potato tubers are vegetatively multiplied what increases the risk of fungal transmission through infected seed. Therefore, these newly introduced genomes increase the diversity of the population by bringing in and exchanging genes with the endemic isolates.

Despite the large diversity at the intraspecific level within *R. solani*, no evident relationship between cultivars and associated populations of *R. solani* could have been depicted. Since there is no difference of pathogenicity between isolates of *R. solani* (Fiers *et al.*, 2010), it seems that the severity of the disease depends mainly on environmental conditions and perhaps on the behavior of the cultivar. This aspect of the plant – pathogen interaction was not previously studied in depth.

Our study is the first evidence of genetic variability among *R. solani* associated with blemished potato tubers in France. However, the genetic diversity of *R. solani* populations isolated from blemished tubers is neither dependant on the geographical origin of the isolates or on the host cultivar. The lack of population structure suggests a constant evolution within *R. solani*. Such evolution should be promoted by frequent genetic events, genetic mixing and anthropogenic activities. Further researches are needed to determine the phenotypical characteristics of the isolates in order to set up adapted control strategies against *R. solani*.

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Chapter 6

Effect of environmental conditions and cultural practices on the occurrence of blemishes on potato tubers

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Effect of environmental conditions and cultural practices on the occurrence of blemishes on potato tubers

Abstract

Potato production can be downgraded because of blemishes on the surface of the tubers that causes severe economical losses. These blemishes are either from known origin, they are called typical blemishes, either from unknown origin, they are called atypical blemishes. Environmental factors can enhance or reduce blemishes. This study dealt with the abiotic factors that could impact the occurrence of the blemishes.

A survey was conducted with 45 potato seed producers in France. Physico-chemical traits of the soil, cultural practices and climatic conditions were evaluated in relation with the occurrence of the blemishes. Chi square tests ($\alpha = 0.05$) established positive correlations between soil pH belonging to the 6-7 class and atypical blemishes and between a dehaulming - harvesting delay longer than 40 days and potato scabs. The number of sclerotia was also greater when the rotation was shorter than 5 years and when the amount of water received by the crop during the growing season was lower than 350 mm. In addition, a statistical study about the varietal susceptibility toward superficial blemishes was carried out. A collection of 204 blemished tubers from 55 different varieties was analysed by factorial correspondence analysis. Comparisons with the binomial law allowed showing particular susceptibility of Amandine variety to enlarged lenticels and Spunta variety to atypical blemishes, for two consecutive years.

Our results showed that superficial blemish occurrences depended on abiotic factors and that some potato varieties are particularly susceptible to some blemishes. Abiotic factors may also impact biotic ones which enhances blemish formation. Integrated management of soil characteristics, cultural practices and the choice of the varieties are of major importance to control blemishes on the surface of potato tubers and to warranty the good cosmetic quality of the production.

Introduction

Potato tuber quality is a predominant aspect of the potato production but can be altered by numerous damages essentially due to pathogenic attacks. Indeed, about 40 different diseases caused by fungi, bacteria, nematodes or viruses may damage potato crop all around the world. Quality of potato tubers may also be changed by environmental conditions due to natural phenomena - high soil temperature is responsible for heat necrosis in vascular ring tissues (Stevenson *et al.*, 2001) - or anthropogenic activities – mechanic harvesting and grading induce damages on tubers (Peters, 1996). Knowledge about the causes of the different kinds of tuber quality problems is generally proportional to the economical importance of the damages.

Potato blemishes are superficial alterations of the tubers that alter only the upper layers of the periderm (Fiers *et al.*, submitted). Very few studies are available on the subject because blemishes became only recently an important economical issue (Campion *et al.*, 2003; Woodhall *et al.*, 2008). A few years ago potatoes were not washed before being sold so, the superficial blemishes were hidden by the soil adhering to the tubers and thus, they were not considered as a problem. In the 80's, the habits of consumers began to change and tubers were washed to present "clean" products on the store shelves. Since then, blemished tubers were downgraded and the economical losses became more and more important. Although blemishes were observed since a long time (Peters, 1966; Hart, 1971), researches to determine their causes only began in the 2000's. Several hypotheses were proposed about the origin of the blemishes. The soil-borne fungus *Rhizoctonia solani* was supposed to be predominantly involved in the occurrence of some superficial blemishes but not all of them (Campion *et al.*, 2003; Woodhall *et al.*, 2008). Environmental stresses such as high soil temperature, proximity of decaying organic matter, excessive moisture etc. were also assumed to be responsible for the visual alterations of the tubers (Stevenson *et al.*, 2001; Sexton, 2003; Selman *et al.*, 2008) but a clear demonstration of the respective involvement of biotic and non biotic factors is still lacking. In the previous chapters, we tested the hypothesis of a pathogenic origin and we demonstrated that indeed, *R. solani* causes black scurf or sclerotia attachment to the tubers but none of the 283 other soil-borne microorganisms associated to the

various blemishes found on potato tubers were responsible for the appearance of those blemishes (Fiers *et al.*, 2010).

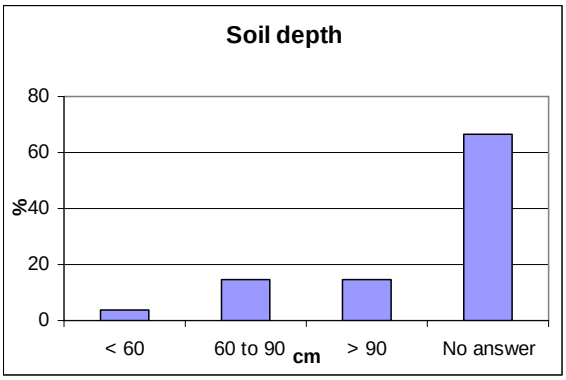
The second hypothesis about non biotic factors will be addressed in this part through a specific focus on the environmental conditions or cultural practices that can cause the occurrence of potato blemishes. Testing individually every factor of climate, soil characteristics and crop management plan would have been very tedious and not really representative of the field conditions. Thus, in order to be as close as possible to the real crop conditions, we decided to realize a survey with farmers to determine favourable and unfavourable environmental parameters to the development of tuber blemishes. Moreover, we performed statistical analyses to depict any putative relationship between the potato cultivar and the occurrence of potato blemishes. Indeed, it has been shown that the same biotic or/and abiotic factor can induce blemishes of various aspects and that potato cultivars differ in their susceptibility to the blemishes (Lutomirska, 2007; Wanner and Haynes, 2009).

Material and methods

The survey was conducted in the main French potato production basins in the North and the West of France with 45 potato producers chosen according to the amount of blemished tubers they harvested in 2007. A semi structured questionnaire was send to the farmers. They were asked to give data on their cultural practices and the soil characteristics of the field. The survey takes into account soil characteristics such as soil depth, pH value, cation exchange capacity (CEC) value, organic matter rate, texture, and structure (Figure 6.1). Soil structure was considered as “good” when the soil was aerated, fragmented with a good filtering coefficient. A “bad” soil structure was characterized by a compact and asphyxiated soil. Cultural practices were evaluated by comparing delays between the dates of planting and dehaulming and between the date of dehaulming and harvesting, rotation length, preceding crops, irrigation and drainage during the potato crop. Meteorological data were also collected. The amounts of water received by the crop, i.e. precipitations + irrigation, and the average temperature during all the growing season were taken into account.

For each parameter, answers were distributed in 2 to 4 categories that were related to the occurrence of blemishes with a Chi square (Khi^2) test ($\alpha = 0.05$).

In addition, blemished potato tubers were collected in 2006 and 2007 in the same French areas and in Switzerland, Italy (Sicilia), and Morocco. All the surveyed producers provided samples collected in 2007. Each sample was collected from a single field. In 2006 and 2007, a collection of 92 and 122 samples of tubers presenting blemishes from 29 and 45 different cultivars respectively was set up. A picture of each tuber was taken; blemishes were classified in 4 classes (sclerotia, atypical corky blemishes, potato scabs and enlarged lenticels) and they were scored according to the French notation scale edited by the French official service of control and certification (SOC) (GNIS and SOC, 2005). For each year, a test of conformity with the binomial law was performed ($\alpha = 0.05$) in order to test the conformity of the occurrence of the blemishes with the theoretical probability. A significant high frequency of occurrence of a given blemish on a cultivar would reveal a specific sensibility of the cultivar to that blemish. The dependence between the lines and the columns of the contingency tables was tested with a Chi square test. The relationships between the potato cultivars and the blemishes were represented by factorial correspondence analysis (FCA). The statistical tests were performed with the XLStat Pro 7.5 software (Addinsoft, version 7.5).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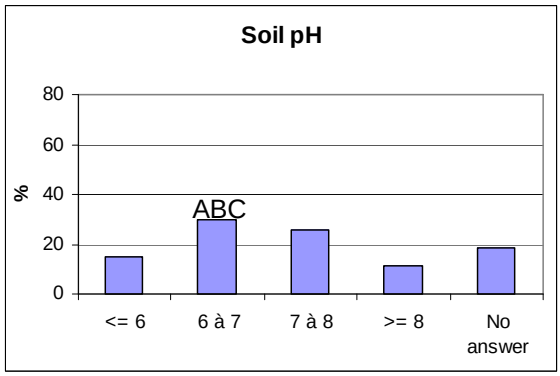
80
60
%40
20
0
< 60
60 to 90
cm
> 90
No answer


Soil pH
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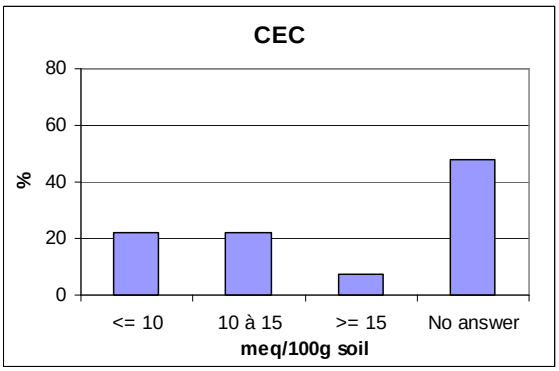
60
%
40
20
0
ABC
<= 6
6 à 7
7 à 8
>= 8
No answer


CEC
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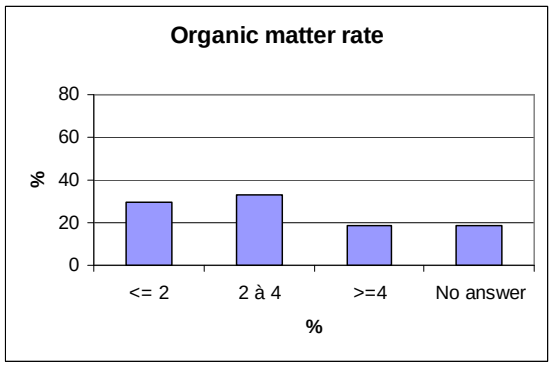
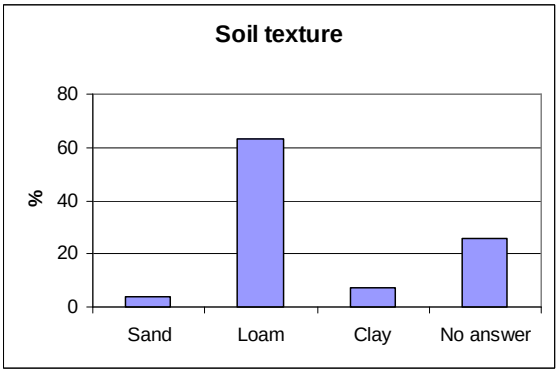
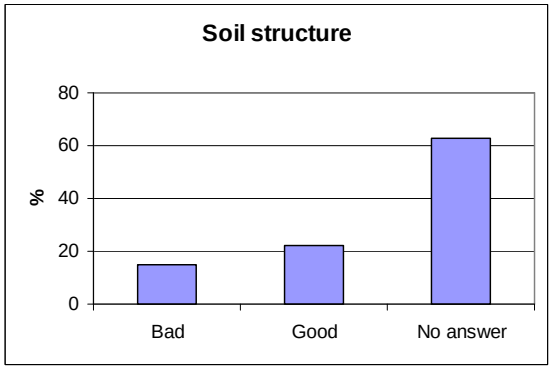
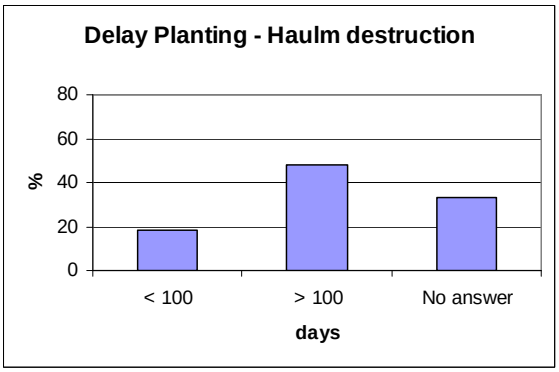
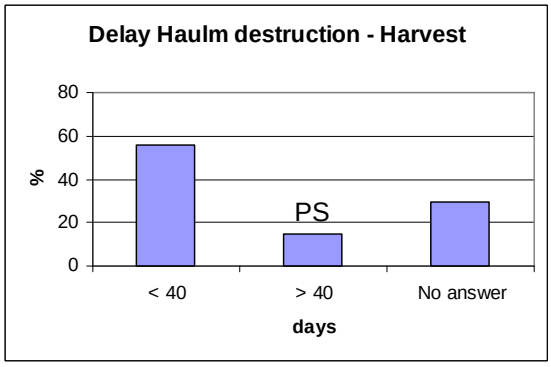
60
%
40
20
0
<= 10
10 à 15
>= 15
No answer
meq/100g soil


Organic matter rate
80
60
%
40
20
0
<= 2
2 à 4
>=4
No answer
%


Soil texture
80
60
%
40
20
0
Sand
Loam
Clay
No answer


Soil structure
80
60
%
40
20
0
Bad
Good
No answer


Delay Planting - Haulm destruction
80
60
%
40
20
0
< 100
> 100
No answer
days


Delay Haulm destruction - Harvest
80
60
%
40
20
0
PS
< 40
> 40
No answer
days

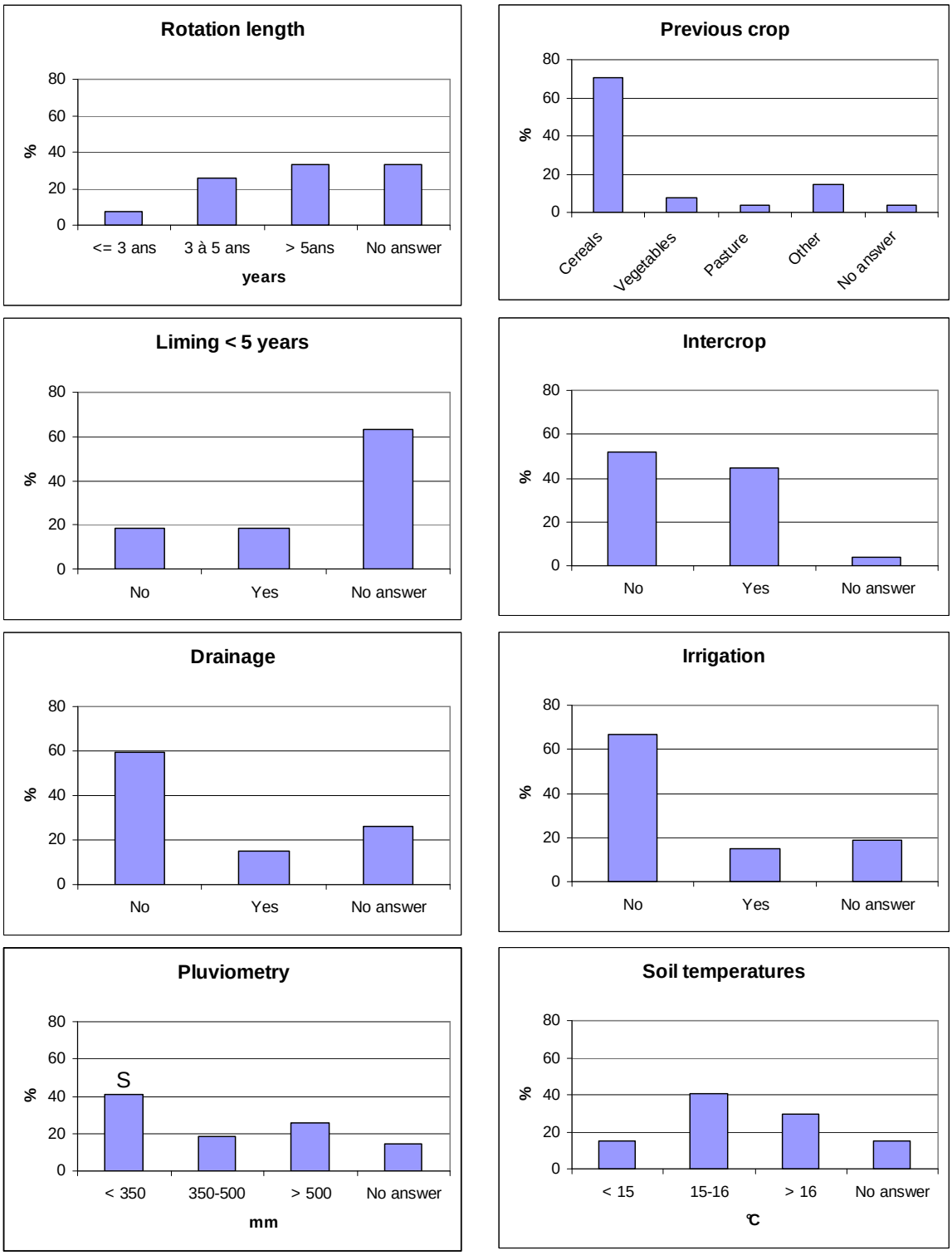


Figure 6.1 Percentage of answers for each category of surveyed parameters.
Letters indicate that values of the given parameter favoured significatively (α = 0.05) the occurrence of the indicated blemish (ACB = atypical corky blemishes; PS = potato scabs; S = sclerotia).

Results

Among the collected tubers, 38 % presented atypical corky blemishes in 2006 and 40 % in 2007, 34 % of the tubers had sclerotia both in 2006 and 2007, 15 % had potato scabs symptoms in 2006 and 10 % in 2007, whereas 13 % presented enlarged lenticels in 2006 and 16 % in 2007.

More than 53 % of the farmers answered the survey. The average rate of answers to every question was 67.7 %. Despite, the good answering rate of the survey, the total number of fulfilled questionnaires was too weak to reach a pertinent statistical signification. Nevertheless, interesting tendencies could be shown.

Average surveyed fields had a soil depth between 60 and 90 cm, a pH value between 6 and 7, the CEC value was under 15 meq / 100 g of soil and the organic matter rate was between 2 and 4 %. Soils were generally loamy, with a good structure. Delay between planting and dehaulming was commonly longer than 100 days and delay between dehaulming and harvesting was shorter than 40 days. There were as many rotations longer than 5 years than rotations shorter than 5 years and cereals were the most frequent crops included in the rotation. In general, no intercrop was used and there were neither drainage nor irrigation (Figure 6.1).

Results showed significant effects of pH and delay between dehaulming and harvesting ($\alpha = 0.05$). A pH value between 6 and 7 increased the occurrence of atypical corky blemishes and a period longer than 40 days between dehaulming and harvesting favoured the development of potato scabs symptoms (Figure 6.1). Results nearby the significant threshold indicated that a rotation length between 3 and 5 years increased the risk of sclerotia occurrence whereas a rotation longer than 5 years decreased the risk. About 70 % of the farmers grew cereals before potatoes in the rotation without any positive or negative effect on the occurrence of the blemishes. Climatic conditions, especially pluviometry below 350 mm had also an impact on blemishes by enhancing the occurrence of sclerotia all along the growing season.

Concerning the cultivars-blemishes relationships, data of 2006 and 2007 were not treated together, because the two sampling years have had very different climatic conditions in France. In 2006, the temperatures were exceptionally high (+ 1 or + 2℃

higher than seasonal averages) and the pluviometry was normal. In 2007, the cropping season was fresh and very humid. The majority of the sample comes from the North West of France where the precipitations in 2007 were 2 to 3 times higher than the seasonal average (Météo France). The Chi-square test showed that the lines and the columns were significantly independent ($\alpha = 0.05$) for the two years. The FCA represented 88.59 % of the total variability in 2006 (F1: 50.10 % and F2: 38.49 %, Figure 6.2) and 87.14 % of the variability in 2007 (F1: 49.97 % and F2: 37.17 %, Figure 6.3).

In 2006, the FCA showed that the cultivars Adriana, Chérie and Désirée were distinct from the other cultivars. Moreover, the cultivar Chérie was significantly associated with potato scabs (coefficient = 0.001). The tests of conformity with the binomial law indicated that Amandine and Anoe were displaying significantly more enlarged lenticels than the other cultivars, with coefficient values of 0.020 and 0.031 respectively ($\alpha = 0.05$). The FCA showed also that cultivars Spunta was significantly more susceptible to atypical corky blemishes than the other cultivars with a coefficient of 0.039. Other cultivars seem not to be particularly susceptible to a specific blemish (Figure 6.2).

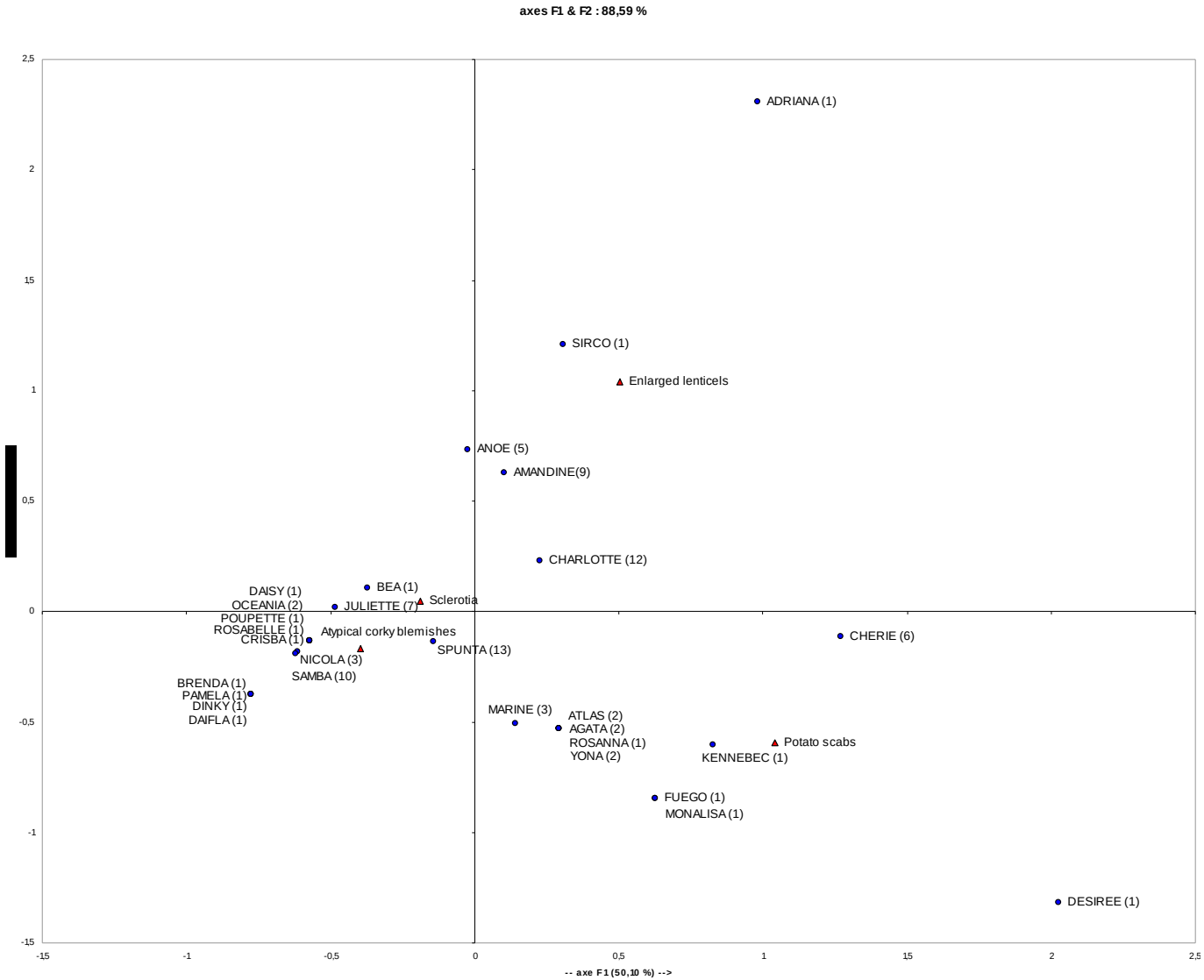


Figure 6.2 Correspondence analyses of blemishes x potato cultivars in 2006
Numbers between brackets represent the number of scored samples per related cultivar.

In 2007, the FCA showed again the close relationship between the cultivar Amandine and enlarged lenticels as confirmed by the test of conformity with the binomial law which had a coefficient value of 6.883×10^{-5} ($\alpha = 0.05$). As in 2006, cultivar Spunta was associated with atypical corky blemishes, as showed by the almost significant value of the conformity test (0.065). Potato scabs distinguished also from the other blemishes on the FCA. They seemed to affect more specially the cultivars Urgenta, Emeraude and Viola. However, the number of analysed tubers is too weak so that the test of conformity is significant. Nevertheless, the test showed that the cultivars Bintje and Désirée are significantly affected by potato scabs with coefficient values of 5.801×10^{-4} and 0.048 respectively. Otherwise, no particular relationship appears between the others cultivars and blemishes in 2007 (Figure 6.3).

Some genetically related cultivars appeared to be susceptible to the same blemishes. Nicola, Juliette, Charlotte and Amandine, which have a common genetic background presented similar susceptibility to atypical corky blemishes and sclerotia on the 2 AFC. In particular, Amandine issues from a crossing between Charlotte and another cultivar; this relationship between Amandine and Charlotte was emphasized through the FCA which revealed for the two consecutive years the common susceptibility of those two cultivars to enlarged lenticels and sclerotia. Likewise Désirée, Rosanna, Kennebec and Atlas which have also genetic links presented a particular susceptibility to potato scabs in 2006.

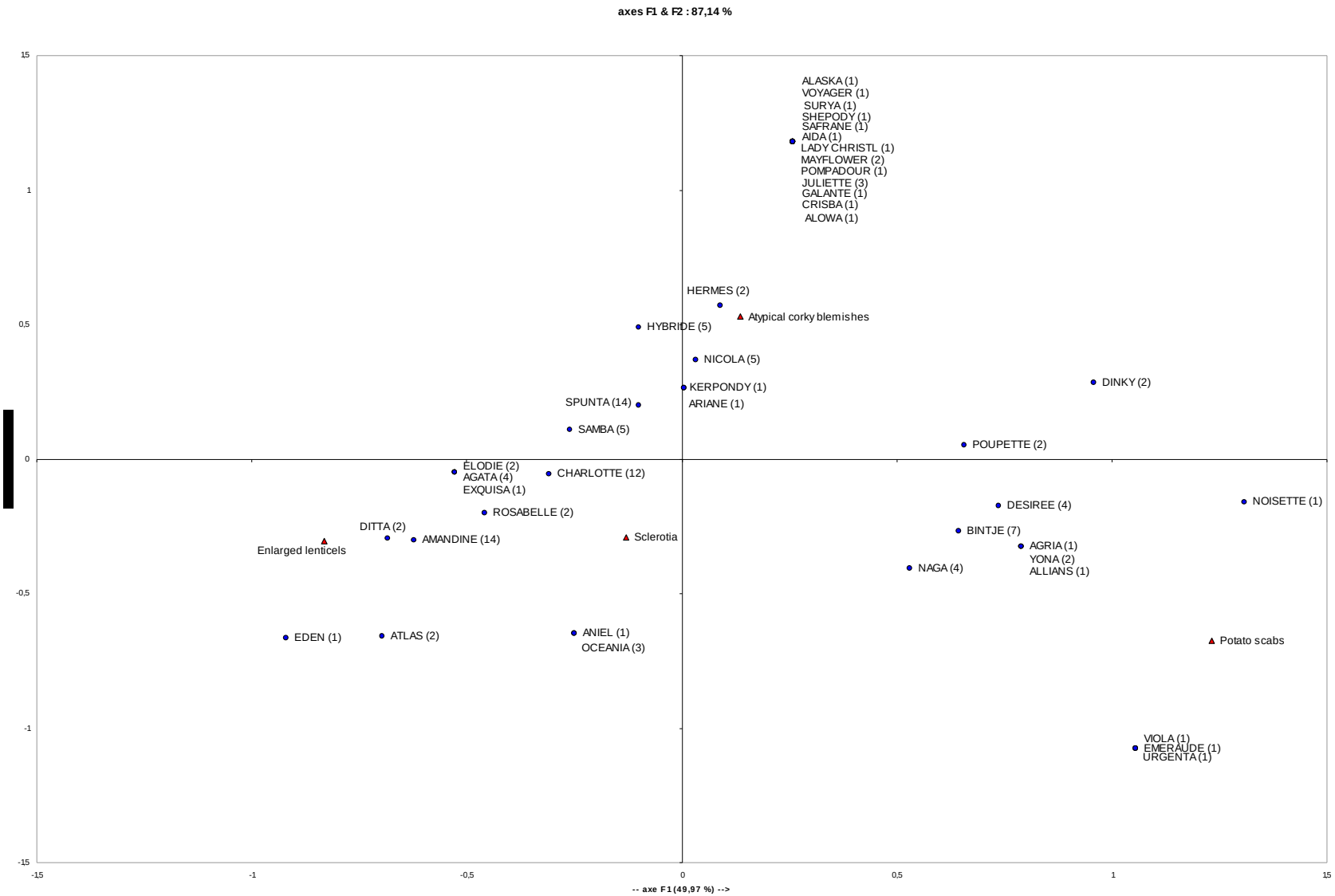


Figure 6.3 Correspondence analyses of blemishes x potato cultivars in 2007
Numbers between brackets represent the number of scored samples per related cultivar.

Discussion

In Brittany, our major sampling place, the soils are a bit acidic with an average of 6.3 compared with the range of values (from 5 to > 8) displayed by agricultural soils in France (Arrouays *et al.*, 2010). Almost 30 % of the surveyed fields belonged to the category of pH between 6 and 7, and those pH values seemed to be favourable to the occurrence of atypical corky blemishes. Nevertheless, pH is a complex factor that affects several other parameters. It determines the availability of soil nutrients and elements contents that can impact the potato sanitary status (Stengel *et al.*, 2009). Indeed a deficiency or an excess of one of the macronutrients (nitrogen, phosphorus, potassium) or micronutrients (iron, manganese, aluminium, etc.) is a source of stress for the plant and it can result into blemish occurrence on tubers (Zorn, 1995; Sarkar *et al.*, 2004). The element rates of each surveyed soils should be taken into account to determine the real role played by pH in the appearance of blemishes.

On the other hand, we showed that a period of time between dehaulming and harvesting longer than 40 days was harmful for potato as the severity of potato scabs developing on tubers increased. This confirms the results obtained previously indicating that the quality of ware potatoes can be controlled by adjusting planting and harvesting dates (Sikka and Singh, 1976). Minimum disease severity is generally recorded when tubers are directly harvested along with foliage. However, with delay in date of haulm cut, there is gradual and significant increase in tuber yield. As long as tubers are attached to the green plants, the photosynthates keep on accumulating in tubers, i.e. the biological sink. Once the green top is removed the tubers in soil attached to stolons and roots, are likely to be more liable to various infections including that of *Streptomyces* spp. and *R. solani* (Sangeeta and Pundhir, 2009). Time interval between dehaulming and harvesting has to be long enough to allow for the convenient growth of the tubers, but also short enough to avoid the formation of potato scab. The dehaulming – harvesting delay has to be calculated according to the pressure exerted by *Streptomyces* spp. inoculum on the crop. Concerning the rotation length, it is known since a long time that long rotations allow the decrease or the eradication of pathogen inoculums (Peters *et al.*, 2003). The longer the time between two potato crops, the less the concentration of *R. solani* inoculum, the less

severe black scurf will be. Rotation length is an important parameter to take into account as well as the rotation composition. Cereals in the rotation did not increase nor decrease the appearance of the blemishes. Consequently this crop can be integrated into the rotation without risk.

Our results did not show any effects of high soil temperature, heterogeneous distribution of the organic matter in soil and excessive moisture on the occurrence of blemishes, as it has been previously suggested (Stevenson *et al.*, 2001; Sexton, 2003; Selman *et al.*, 2008). However, we showed that excessive moisture decreased the occurrence of sclerotia. In literature, papers on this subject are rather contradictory. Some authors claimed that black scurf is favoured by high soil moisture (Lakra, 1995; El Bakali and Martin, 2006), others showed that irrigation decreased black scurf (Hide *et al.*, 1994), or that there was no influence of rainfall on sclerotia occurrence (Lutomirska, 2007). Nevertheless, rainfall and irrigation are not systematically correlated with high soil moisture content. Indeed, well drained soils avoid overcoming the water holding capacity of the fields even after strong rains or too important irrigation.

Since, the number of answered questionnaires was low, this study can not provide irrefutable information or concrete advises on favourable or unfavourable environmental traits and cultural practices for the management of healthy potato crop. Nevertheless, our results are good indicators to highlight the points that need to be watched in order to avoid blemish occurrence. Some factors are favourable to some blemishes and unfavourable to others. Therefore, a large scale survey should be undergone among the producers with some more specific and precise questions issuing from the present survey such as soil pH, soil texture, organic matter (soil content and dispersion in the soil), delay around haulm destruction, rotation length and previous crops, moisture (irrigation, pluviometry and drainage) and soil temperatures.

The choice of the potato cultivar is a major cultural practice that allows adapting the crop to the market expectations, to the environmental conditions and to the sanitary risks. This work aimed at established relationships if any between potato cultivars and superficial blemishes. We could have studied the relationships between the cultivars and the geographical origins of the samples or between the blemishes and

the geographical origins, but it would have been biased since some cultivars are more frequently cropped in some areas because of the climate and the way the potatoes will be used (seeds, ware, or processing).

Despite the different climatic conditions in the two years, the 2 AFC showed that the susceptibility of the cultivars to the blemishes was rather constant in 2006 and 2007. This confirms previous results claiming that the relative susceptibility of a cultivar to a given blemish remains approximately the same whatever the environmental conditions. For example, a susceptible cultivar will always have more severe blemishes or symptoms than a resistant cultivar whatever the climatic conditions (INRA and Agrocampus, 2006; 2007; 2008).

Some cultivars were represented by only one sample whereas other cultivars were represented by at most 28 samples. Indeed, some cultivars are more frequently used than others. The results concerning cultivars represented by one or two samples can not be considered as representative of the cultivar's behaviour.

Our results also showed that the cultivars with a common genetic background are liable to the same blemishes, what is in agreement with previous studies (Bozec *et al.*, 2005).

New potato cultivar assessment takes into account several crop and tuber characteristics, especially disease resistance of the cultivars. However the aggressiveness of each pathogen is expressed according to the response of the cultivar and the environmental conditions. Resistance assessments frequently reveal that common scab is the only tested disease among the superficial blemishes affecting the potato tuber. Indeed, *Solanum tuberosum* – *Streptomyces* spp. relationship is one of the few potato parasitic complexes that gather available data at the cultivar level, although the behaviour of the potato phenotypes against *Streptomyces* spp. still remains environment-dependant (Pasco *et al.*, 2005; Anonymous, 2009). The description and the awareness about atypical superficial blemishes vary according to countries and remain frequently unclear. That is one of the reasons why official assessments for registration of new potato cultivars do not integrate cultivar behaviour to atypical blemishes into the evaluation criteria yet, even if some official assessments were recently validated in France about pitted vs. netted scab (Pasco *et al.*, 2005) and in Switzerland, about tuber deformations due to *R. solani* (Reust and Torche, 2008).

In the previous chapters, we concluded that no causality links could be established between the microorganisms associated to blemishes and the occurrence of the blemishes. In this chapter, we are inclined to think that some non biotic factors including climate and agricultural practices are involved in the appearance of the blemishes and also that susceptible cultivars could exacerbate this appearance. Therefore, it should be possible in the future to overcome the problem by innovative management of the production although it is still very difficult to adopt a course of action avoiding all the problems.

General discussion

General discussion

In the global current context of massive demographic increase, potatoes are desirable to feed the global populations and to contribute to their economical and social development. Obviously, the issues and constrains of potato production are not the same according to the country. Developing countries aim at supplying enough staple potato production to meet increasing food demands, whereas developed countries have to provide good quality products matching with the consumers' expectations. Among other factors, the economical development of fresh potato producers in developed countries depends on the visual quality of the tubers. Indeed, the losses due to the downgrading or rejecting of blemished tubers hamper the proper evolution of the fresh potato industry. Consumers are more and more demanding concerning food quality. The perception of fresh fruits and vegetables has changed since populations became more urban and are less familiar with the production systems. Consumers who were more familiar with the geographical origin of fruit and vegetable production mentioned non-sensory attributes – mode of production, variety - more often than those with less contact, who, in contrary mentioned sensory attributes – aspect, odour, touch - (Peneau, 2005). Concerning fresh potatoes, blemished tubers are not well perceived by the consumers, even if they do not affect the nutritional quality of the product. In the long term, efforts should be made to make the consumers aware of the quality of fresh potatoes despite some visual blemishes, but at the present time solutions to avoid the occurrence of blemishes are needed. The determination of their causes is a crucial point that has to be clarified in order to optimize its control. A global review of the knowledge about soil-borne potato diseases was proposed in **chapter 1** on this thesis. Data about the conditions of appearance and development of diseases are essential to understand the mechanisms underlying host - pathogen interactions and to set up efficient methods to control diseases. The first chapter of this work can be considered as a decision support system to avoid damages on potato crop since it resumes all the favourable and unfavourable conditions affecting the occurrence and the development of the soil-borne diseases of potato. Nevertheless, very little information

were collected in the literature on the blemishes despite the evident need of knowledge on this subject. Consequently, the demand of extension people and farmers for further researches aiming at solving this problem was foreseeable.

Ce qui se conçoit bien s'énonce clairement et les mots pour le dire arrivent aisement - What is well conceived is expressed clearly, and the words to say it come easily- wrote Nicolas Boileau-Despréaux, a famous French poet and writer in 1674 (Boileau-Despréaux 1674). This sentence sums up the first objective of this work that was to propose clear and consensual terminology and classification of the different blemishes that can be observed on tuber surface (**chapter 2**). Some atypical potato blemishes, such as polygonal lesions, were observed since a long time and were named "elephant hide", especially in USA. The first paper we found in the literature about this issue dates from 1966 (Peters, 1966). Other authors approached briefly the subject in the 70's (Hart, 1971). They mentioned some observations and suggested few probable causes but no actual study was carried out. In the 2000's, few papers dealing with blemishes were recorded. Two of them studied more in depth the implication of *Rhizoctonia solani* in the occurrence of the atypical blemishes (Campion *et al.*, 2003; Woodhall *et al.*, 2008). Nevertheless, the overview of those rare previous studies showed the heterogeneity and confusion in the different names used for the blemishes. Seeing the importance of the problem for the potato community, it was necessary to examine the matter in more detail, with experts from the seed, ware and processed potato sector, researchers and breeders, and the first thing to do was to harmonise the nomenclature and the classification of the blemishes in order to avoid misunderstandings and favour collaborative researches between different laboratories. Several choices aiming at keeping the consensual names and changing those which could introduce confusion had to be made. They imply that the potato community changes its denomination habits and adopts the new nomenclature in order to work on common and clear foundations at a global scale.

The second objective of this study was to identify the origin of the blemishes altering potato surfaces. Tubers were collected for two consecutive years, 2006 and 2007. The climatic conditions were very different during the two growing periods, spring and summer were hot and dry in 2006 and they were cold and wet in 2007.

Those variations allowed collecting very diverse types of blemishes during the two years.

Two causes were possible to explain the appearance of the blemishes. Either they were due to one or several pathogens or they resulted from a reaction of the plant to unfavourable environmental conditions (Agrios, 2005). Our first goal was to make an exhaustive isolation and identification of the microorganisms living at the surface of the tubers (**chapter 3**). However, the scope of the study constrained us to target some microorganisms. A large diversity of fungi was identified but the method we used allowed us isolating only cultivable fungi. Concerning bacteria, we targeted *Streptomyces*, known to be implicated in several potato diseases especially netted scab that causes blemishes, very similar to the atypical blemishes. The pathogenicity of the *Streptomyces* isolates and the fungi the most frequently isolated from the surface of the blemished tubers was tested in bioassays with infested soil. This classical technique aimed at favouring the development of a specific microorganism in the soil and at fulfilling the Koch's postulates. Koch's postulates fulfilment is the only known method to prove rigorously the pathogenicity of a microorganism (Rapilly, 2001). The fulfilment of the four postulates was not easy, especially as the bioassays were conducted in a non sterile environment. Aerial microorganisms could develop in the soil and establish interactions with the tested microorganism or the tuber. Some microbial interactions were tested by inoculating tubers with combinations of two microorganisms. In all those tests, blemishes were produced showing that we succeeded in creating favourable conditions to blemish formation, but apart from *R. solani*, there was no relationship between an inoculated microorganism and the occurrence of the blemishes. We enlarged the domain of investigations from the surface of the tuber to the surrounding geocaulosphere and from the artificial conditions of the greenhouse to the natural conditions of a field in which superficial blemishes were previously regularly observed (**chapter 4**). This approach based on the analysis of the microbial structure in the soil close to the altered and the non altered potato was used to overcome the practical limitation of the approach based on isolation. Such an approach was already successfully used to identify the fungal populations of *Trichoderma* spp. involved in the dynamics of infectious patches caused by *R. solani* in sugar beet crop (Anees *et al.*, 2010a). Unfortunately, no noticeable peak (TRF) which could have indicated the relative abundance of a microbial group to identify in the geocaulosphere of blemished tubers compared to

the one of healthy tubers was recorded, apart, once again, the TRF corresponding to the *R. solani* group. Although modifications of the structures of the fungal and bacterial communities were observed at the different sampling dates and in the vicinity of blemished tubers, we could not conclude that these modifications were responsible for the appearance of the blemishes or if, at the contrary, the blemished potato had specific exudates that could change the population ratios among the microbial communities. Anyway, this non-*a priori* approach gave the same results as the Pasteurian one based on the fulfilment of Koch postulates. No causality between the presence of microorganisms and the appearance of atypical blemishes was found apart in the case of *R. solani*. We concluded that *R. solani* was actually responsible for sclerotia but the other blemishes were not of pathogenic origin.

We therefore focused on this pathogen to know more about its diversity, although it is not really involved in atypical blemishes but it is in quite typical and already described blemishes called sclerotia. *R. solani* is a very common soil-borne pathogen with a large diversity of host plants and causing various diseases under diverse environmental conditions. Julius Kühn observed this fungus for the first time on potato in 1858. *R. solani* can cause seed decay, damping off, root rot or stem canker on bean, cotton, cabbage, sugar beet, alfalfa, tomato, potato etc (Parmeter, 1970). In fact, there is a relative host specificity of the different anastomosis groups (AG) of *R. solani*. On potato, we isolated especially AG 3, AG 2-1 and AG 5. AG 3 and more precisely the subgroup associated with potato, AG 3 PT is known to cause black scurf and stem canker on potato tubers (Kuninaga *et al.*, 2000). AG 2-1 and AG 5 were isolated at lesser extent than AG 3 but they are supposed to be implicated in the appearance of atypical blemishes. Nevertheless, Koch's postulates were not fulfilled for these two AGs neither in **chapter 3** nor in other studies (Campion *et al.*, 2003; Woodhall *et al.*, 2008). It is very difficult to eradicate *R. solani* once the pathogen is in the field. The fungus may cause up to 30 % of losses in the potato production (Banville, 1989). Moreover, chemical treatments against plant diseases are more and more limited because of the current regulations for the protection of the environment all over the world. Many chemicals previously used are now forbidden. New control techniques are studied; they include unfavourable cultural practices for the pathogen (chapter 6), sustainable chemical treatments and biological control (Wilson *et al.*, 2008). **Chapter 6** of this thesis mentioned that abundant irrigation and

rotation longer than 5 years could decrease the soil inoculum of *R. solani*. On the other hand, microbial cocktails, growth hormones and several other stimulators of plant defence response are tested (Mahmoud, 2007). Those new control methods are currently tested by Végénov-Bretagne Biotechnologie Végétale (BBV) to assess their efficacy against potato blemishes. The application of such control methods implies a deep knowledge of the epidemiology and the ecology of the pathogen. Even if *R. solani* has been studied for more than 150 years, uncertainties remain concerning its ecology, especially classification and taxonomy. *R. solani* is a multinucleate fungus. This can introduce many variations in its genome. In **chapter 5**, the study of the phylogenetic relationships between the French strains of *R. solani* compared with strains from other countries showed that there was no geographical structure of the *R. solani* population and no relationship between the host cultivars and the isolates of *R. solani*. This suggests that frequent genetic events and genetic mixing take place at a large scale all over the world, without any structure of the population could have been drawn, probably because of the limited dissemination of *R. solani*. The spread of genomes probably occurs because of the global exchange market of seed tubers inducing numerous international transportations of potatoes potentially contaminated by mycelium or sclerotia of *R. solani* (Justesen *et al.*, 2003).

The second possible cause of the blemishes was related to stressful environmental conditions. Potato tubers are subterranean stems. They develop in a complex environment which is the soil and which harbours both biotic and abiotic components. The survey we did was quite limited and we discussed, in **chapter 6**, that this was a restraint preventing us from general and definitive conclusion. Anyway, we saw that the physico-chemical traits of the soil such as pH and water availability interfere with the plant health status and impact the occurrence of atypical corky blemishes and sclerotia. This leads us to the conclusion that this hypothesis was more likely to bring us with the identification of the causes of the appearance of blemishes than the microbial one, although they are not exclusive mutually. However it is clear and we mentioned it, that a large scale survey should be conducted to provide us with more precise information about the list and the relative importance of the abiotic factors which seemed to be involved in the appearance of blemishes. Large scale means both geographic areas, climatic conditions and agricultural practices but also a large number of parameters surrounding the plant itself including

aerial part, geocaulosphere and rhizosphere of the potato. Biotic factors should also be included as parameters to measure as they interact with abiotic factors and thus might have an indirect role on the occurrence or development of the atypical blemishes. The rhizosphere is a favourable area for microbial development. Carbon, nitrogen, phosphorus, potassium and other micronutriments are released by the plants in the rhizosphere and they are a source of energy for the microorganisms. In return, microorganisms contribute to the plant nutrition by altering minerals and through symbiotic associations such as mycorhization or formation of nodosities (Stengel *et al.*, 2009). Exudates released by the plants modify the microenvironment of the rhizosphere. The soil pH and the chemical balances change increasing the availability of nutrients for the plant. Moreover, those major modifications change the structure of the microbial communities in the rhizosphere. As evocated in **chapter 4**, the structures of the microbial communities of the potato geocaulosphere evolve in time and according to the phenology of the plant. Similar mechanisms could take place in rhizosphere and geocaulosphere. Indeed, we showed that the microbial communities and especially the bacterial communities were different in the geocaulosphere of healthy plants according the period of time, but they evolve toward an identical structure in the geocaulosphere of blemished tubers. This showed that there are many mutual interactions between plants and soil-borne microorganisms in which the human can interfere in order to improve the agronomic systems.

We showed also in our study that the cultivar has to be chosen according the future use of the progeny tubers and also according to the sanitary risks incurred in a given field: amount of pathogenic inoculum, climatic conditions, soil characteristics, etc. Choice of unadapted cultivar will increase the sanitary risks. If the weather conditions are also unfavourable, it could be hard to control the risk even with good cultural practices and adapted control methods. Most of European potato cultivars belong to *Solanum tuberosum* species but about 10 other *Solanum* species are cultivated in South America and 200 wild species are registered (FAO, 2008). Disease and insect resistance of natural populations, or traditional cultivars developed by Andean farmers, is an important legacy. Genetic selection of potatoes for disease resistance did probably not begin before the end of the late blight epidemic in Ireland between 1845 and 1849. Since then, continuous progresses are made to breed new cultivars resistant to the new diseases and with good intrinsic qualities. Therefore, the

susceptibility of the potato plant, i.e. the cultivar, has to be included in the set of biotic and abiotic data to be considered to evaluate the risk of blemish appearance. Some efforts were already done to propose models aiming at evaluating the risk of production losses (Savary *et al.*, 2006). Similar efforts should be done using statistical analysis to identify indicators and bioindicators of the beneficial or deleterious environment of the potato during crop production but more precisely during the period during which blemishes appear (Janvier *et al.*, 2007). Such approach should stimulate the build up of decision support systems to prevent blemishes.

This thesis tackled topical issues at several scales. The project was initially consecutive to repeated requests of technical staff in the fresh-washed potato production to limit the annual non negligible amount of fresh potatoes that were down-graded due to poor visual quality. Because the tuber is also the plant for planting, ie the seed, then a strong demand for high visual quality seeds has challenged seed potato growers to produce very high quality seeds; with the hope that this measure would solve the tuber blemish issue in the following growing season. Seed potato producers in Brittany, together with fresh potato growers have then launched this project in order to find out solutions while respecting the current environmental regulations. This problem concerns also the global agriculture which needs to produce abundant and qualitative food products and has to protect the planet at the same time. Those preoccupations meet the first and the seventh millennium development goals of the United Nations Organisation: End poverty and hunger and ensure environmental sustainability (ONU, 2000). Measures introduced to reach those goals depend largely on our capacity to manage an alternative agriculture with beneficial socioeconomic and environmental impacts. This is the aim of agroecology. Agroecology provides the scientific basis to address the production by a biodiverse agroecosystem able to sponsor its own functioning (Altieri, 2002). Agroecology initially dealt primarily with crop production and protection aspects, in recent decades new dimensions such as environmental, social, economic, ethical and development issues are becoming relevant (Wezel *et al.*, 2009). Thus, by studying the causes of the occurrence of potato tuber blemishes at a microscopic scale, this project dealt with quite larger scales that aim at developing global

agriculture, improving the socio-economical conditions for farmers and finding sustainable techniques for crop management.

Future prospects

Based on the conclusions we drawn back from our study, and also, as an obvious corollary, on the analysis of the literature we did, I would suggest first a short term practical perspective about a common terminology we should have about potato blemishes.

But I think also that the approaches we used, although they provided a lot of information and partial responses to the original question, were not totally satisfying to the growers as we did not give a clear and definitive answer towards the causality of blemishes, we just provided with promising tracks. That is why I would propose some middle term perspectives about the i) plant-pathogen interactions and plant defence reactions, ii) the definition of indicators of the soil health to built up decision support systems for the growers and iii) an integrated pest management study directly related to the previous proposed perspectives.

Practical perspectives

New nomenclature and classification were suggested in order to harmonize further works on potato blemishes. It has been written in English for a scientific journal which will be available for a limited number of potato scientists and researchers. The value of such nomenclature is to be spread at all levels in the potato industry and among extension staff. Translations in several languages of this nomenclature and publication in technical reviews could allow farmers, technicians as well as engineers or researchers to exchange about blemishes with the same words and the same meanings. This nomenclature could be used in certification shemes to allow the identification and the precise description of tuber blemishes that are still rarely taken into account in the criteria of certification of the tubers.

Research perspectives

i) Plant-pathogen interactions and plant defence reactions

In addition to the microbial mechanisms and the environmental conditions we evoked during this study and that may lead to the reduction of the pathogenic attacks, plants interact with their environment, especially the soil environment through the rhizosphere activity. In the case of potato, the soil surrounding tuber is called geocaulosphere and can be compared to the rhizosphere. The impact of the geocaulosphere on the soil charcteristics and the microbial communities is not well known. We suggest that plant rhizosphere and potato geocaulosphere could be compared according to the release of exudates and their impact on the surrounding microbial communities. Regarding the lack of knowledge about this question it would be interesting to study the similarities and differences between rhizosphere and geocaulosphere in order to better understand the interaction between plants, especially potato, soil and microorganisms. Plants are able to defend themselves from pathogenic or environmental stresses by setting up several different mechanisms. The first defence system is mechanical; it includes physical barriers avoiding the entrance and spread of the pathogen in the plant. The second mechanism induces biochemical reactions that produce substances either toxic for the pathogens or inhibiting their growth and that modify the plant physiology. The physiological mechanisms of the blemish formation have never been studied but it is known that after an attack, plant cells produce suberin that forms a cork layer on the cell walls (Pollard *et al.*, 2008). The accumulation of suberin on the upper layers of the tuber periderm could lead to the formation of superficial corky structures such as those observed on blemished tubers. Even in the same host, defence reactions are different depending on the age of the plant, the cultivar or the weather conditions (Agrios, 2005). The physiological mechanisms of the plant leading to blemish formation are not well understood. They could be related to a hypersensitive response of the plant and all the physiological changes it induces. Are the plant defence process the same in response to a pathogenic or an environmental stress? What are the differences? Are some beneficial microorganisms able to potentialize the plant defence reactions? What are the genes implicated? What are the physiological functioning leading to the formation of the blemishes themselves?

Those are some questions that need to be answered in the next years in order to understand the mechanisms underlying the tuber blemish formation. Concerning *R. solani*, few studies report that this fungus produces phytotoxins leading to plant infections (Gvozdeva *et al.*, 2006; Brooks, 2007) but plant - pathogen interactions need to be clarified.

ii) Definition of indicators of the soil health to built up decision aid supports for the growers

Soil health and soil quality are closed concepts that allow soil functions to be related to specific purpose i.e. to maintain an appropriate productivity and good quality of the products while simultaneously reducing the effects on the environment and contributing to human health. However, the former takes into account more clearly plant health and therefore the phytosanitary aspect of the soil than soil quality (Janvier *et al.*, 2007). Soil health assessment includes the quantification of indicators of soil quality. Such indicators may be derived from specific soil parameters obtained from different disciplines of soil science, but descriptive indicators which are inherently qualitative can also be used in assessing soil health and soil quality (Schjonning *et al.*, 2004). Soil quality indicators condense an enormous complexity in the soil and therefore could be used as management tools to positively influence soil health. Nevertheless, they have to be chosen to be usefully used and not too complex to handle by policy makers or land managers (Doran and Zeiss, 2000). In our study, we showed the potential implication of soil characteristics and cultural practices in the occurrence of the blemishes thanks to the statistical analyses of a survey conducted with some farmers from the West of France. But we concluded that more indicators would be useful to identify the beneficial and deleterious components preventing from or leading to blemishes so that agricultural practices could promote the beneficial components. Therefore, we propose to conduct a survey at a larger scale than the one we previously used to collect a larger amount of both specific and descriptive parameters. Such a survey could be conducted among the population of French producers of seed potato whose number is estimated around 915 people (FNPPPT and GNIS, 2007). A representative sample of this population should be determined to provide information on both geographic areas, climatic conditions and agricultural practices but also a large number of parameters surrounding the plant

itself including aerial part, geocaulosphere and rhizosphere of the potato. It should provide with detailed information about the list and the relative importance of the abiotic factors which seemed to be involved in the appearance of blemishes. Biotic factors should also be included as parameters to measure as they interact with abiotic factors and thus might have an indirect role on the occurrence or development of the atypical blemishes. Appropriate multivariate statistical analyses combined with mathematical model based on the data set will identify the most appropriate indicators to be handled by the growers, i.e. a decision support system to manage the soil health to prevent blemishes and to conduct healthy potato crops.

iii) Integrated pest management to control soil-borne pathogens of potatoes

The soil microflora is composed of very diverse species that can be pathogenic or non pathogenic for the plant. The severity of a disease is regulated by the non pathogenic flora. Their ways of action are either active or passive. The antagonistic microorganisms reduce actively the pathogenic populations by parasitism or by antibiosis, whereas other species can compete passively with the pathogens and deprive them from essential nutrients or ecological niche (Alabouvette *et al.*, 1996; Stengel *et al.*, 2009). New environmental friendly control methods are inspired by those natural mechanisms. They consist in inoculating antagonistic populations in diseased fields or in favouring the development and the activity of the beneficial populations by sustainable cultural practices. The occurrence of sclerotia due to *R. solani* could be controlled by field inoculation of efficient antagonists such as *Trichoderma* spp. (Wilson *et al.*, 2008). Cultural practices showing a reduction of the occurrence of some tuber blemishes in **chapter 6** could also be a part of the sustainable management systems leading to the production of unblemished potatoes. Storage conditions were briefly evocated in this work. Indeed, important damages may occur if tubers are stored in bad conditions (Cwalina-Ambroziak, 2002). There was no study on the development of atypical blemishes during storage but generally fast drying of tubers after harvesting permits to avoid the development of several diseases (Scheid, 2006). Unfortunately, cultural management practices alone are not currently fully efficient to control diseases, causing the potato industry to become over-reliant on the use of agrochemicals for effective management. Current research efforts are directed at the identification and incorporation of genetic

resistance into cultivars with acceptable agronomical characteristics to provide more effective disease management (Gudmestad *et al.*, 2007).

The appropriate analyses of the databases built up during the previous proposal of perspective supported by the built up of mathematical models would be in perfect adequacy with the philosophy developed within the ENDURE network. Development of integrated pest management could be a sustainable solution to produce potatoes that will respond to the preoccupations of all the potato industry that aim at producing a fresh mass consumer product with high qualitative and quantitative yields, good presentation and environment friendly modes of production (Figure 7.1).

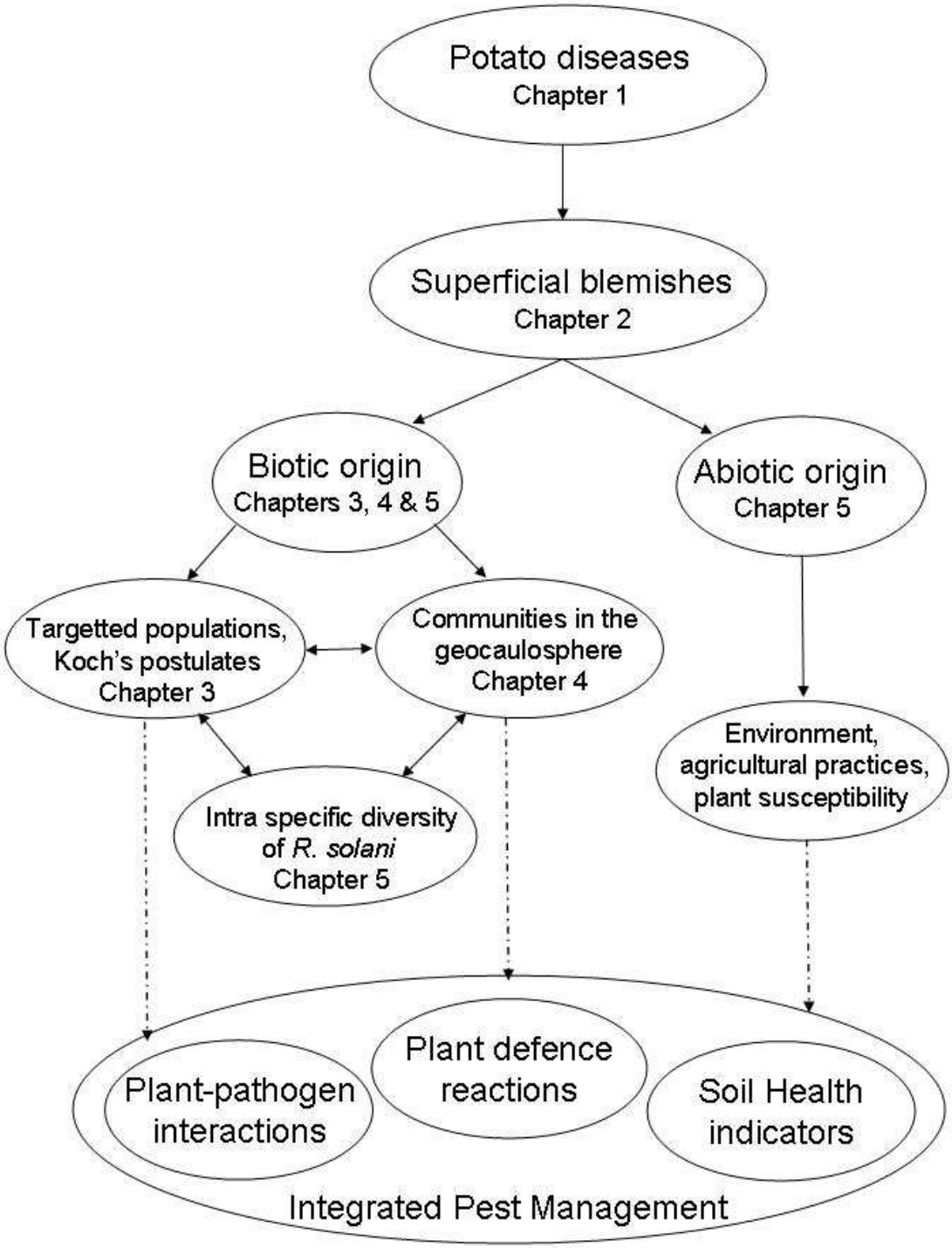


Figure 7.1 Diagram of the research perspectives issuing from the thesis

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Curriculum vitae

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Career

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Publications

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Fiers M., Chatot C., Edel-Hermann V., Le Hingrat Y., Konate A.Y., Gautheron N., Guillery E., Alabouvette C. and Steinberg C. (2010). Diversity of microorganisms associated with atypical superficial blemishes of potato tubers and pathogenicity assessment. European Journal of Plant Pathology, In press.

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