Evaluation of pathogenicity on cereal and potato of Fusarium spp. isolated from field of Solør area

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Evaluation of pathogenicity on cereal and potato of *Fusarium spp.* isolated from fields of Solør area

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List of Acronyms

ANOVA- ANalysis Of VAriance

CZID - Czapeks Ipodrione Dichloran

DCPA - Dichloran Chloramphenicol Peptone Agar

FHB - Fusarium Head Blight

FDR- Fusarium Dry Rot

PDA- Potato Dextrose Agar

PPA- Peptone PCNB Agar

RbGU- Rose bengal Glycerine Urea medium

SE mean- Standard Error mean

SNA-Spezieller Nahrstoffarmer Agar

WA- Water Agar

1 Summary

Fusarium Dry Rot (*Fusarium spp.*) caused by fungi from the Fusarium genus is a problem of great importance in Norwegian potato production. This thesis presents results from a study on Fusarium population present in agricultural soil of a specific area in Norway and their pathogenicity on potato (*Solanum tuberosum*), Oat *(Avena sativa)* and Barley *(Hordeum vulgare*).

Soil sample were taken from Solør , an important potato production area . Recurrent postharvest losses attributed to Fusarium Dry Rot (FDR) have been observed despite the use of rotation between cereal and potato. The presence of particular Fusarium species able to sustain the rotation was hypothesized.

Eight soil samples from 8 fields of different crop history were plated following a modified Warcup plate technique and incubated for 7 days at room temperature. After identification, 45 isolates belonging to 8 species were recovered with predominance of *F.oxysporum, F.merismoides* and *F.avenaceum*. Isolate of *F.dimerum* and *F.venenatum* two rarely isolated species in Norway as well as *F.solani* and *F.sporotrichioides* were also recovered.

The pathogenicity of soil *Fusarium* species was evaluated. Two potato varieties of various resistances to Fusarium Dry Rot, *cv.* Asterix and *cv.* Mandel, were used in this experiment. Several pathogenicity tests were carried out and a modified method was established. Tubers were wounded, inoculated with a mycelium solution and incubated at 10°C for 4 weeks. *Cv.*Mandel tubers appeared to be more susceptible to *Fusarium spp* than *Cv.*Asterix. *F.avenaceum* and *F.sporotrichioides* were the two most aggressive species, causing significant increase of wound volume and the formation of cavities. Isolates of *F.oxysporum* and *F.merismoides* showed a rather weak pathogenicity as well as the other minor species recovered.

A seed germination assay was carried out for evaluation of isolated *Fusarium* pathogenicity on cereal. The oat variety Belinda and the barley variety Habil were used for this experiment. Inoculum solution was prepared following a bubble breeding methods. Seeds were germinated in a petri dish filled with filter paper imbibed of mycelium suspension. After the second day the number of germinated seeds were assessed daily for a 5 day period and compared to an uninoculated control. The size of cotyledon was measured in the fifth day. *F.avenaceum* and *F.sporotrichioides* had a significant effect on germination rate for both *cv.*Belinda and *cv.*Habil reducing significantly the number of germinated kernel in comparison to the control. *F.oxysporum* were weakly pathogenic measured as germination rate but seemed to affect cotyledon size at the fifth day. The other *Fusarium spp*. affected only weakly the germination rate and the cotyledon size.

Due to its pathogenicity on both potato and cereal as well as to be frequently isolated from fields *F.avenaceum* seems to be an important species responsible of symptom conservation over time in the field of Solør area.

2 Background

Dry rot of potato tuber due to *Fusarium spp.* cause important loss during storage in numerous area of the globe .Recent work performed in Great Britain (*Peters et al, 2008*) attributed this disease to four Fusarium species *: Fusarium solani var. coeruleum, F.avenaceum, F.culmorum, F.sambucinum*. Nevertheless, the incidence of each of these species vary greatly from one country to another (*Chehri et al, 2011; Seppanen, 1983; Tivoli et al, 1981*) depending on biotic (aggressivity of isolates, type of tuber wound) or abiotic (cultural practices, humidity, temperature) factors.

Despite its importance, few studies have been carried out on *Fusarium spp.* on potato in Norway. Surveys on the different species of *Fusarium* present in Norwegian soil (*Kommedhal et al, 1988*) as well as assessment of species present in soil adhering to potato tubers were previously carried out *(Bjor, 1978*; *Glorvigen ,1996; Jensen, unpublished data*) representing the core of publications on that subject for the country. Recent problem due to these pathogens led to further research on the subject and among other the realization of this work.

Solør is a district, part of Hedmark County, situated in South-East Norway. This area encompasses the valley between Elverum in the North and Kongsvinger in the South. This large flat area situated in a fluvial valley with deep silty sandy soil is particularly adapted for potato production.

Potato growing in a 4 years rotation with cereals (barley, oat) is common. This cultural practice is expected to decrease inoculum pressure in the field thanks to host variation between seasons. During the last years, this effect has been questioned. A conservation of *Fusarium* species common to potato and cereals causing dry rot and head blight for cereal respectively, have been proposed.

The hypothesis developed considers the presence of one or more *Fusarium* species able to infect both crops. This thesis present several experiment carried out to test this hypothesis.

- In the first part, reliable isolation methods and a survey of the different *Fusarium* species are presented.
- Secondly, the pathogenicity tests of the different species found on both potato and cereal are presented.
- Finally, the results found and possibilities of action against these pathogens are discussed.

The obtained results could be beneficial for further studies on this subject, to growers in their understanding of the disease epidemiology or for Fusarium Dry Rot resistances breeding program.

3 Litterature review

3.1 Taxonomy

Fungi of the *Fusarium* genus are true fungi belonging to the *Ascomycota* phylum and *Deuteromyce*s class. Past that first classification, *Fusarium* taxonomy can appear rather chaotic. Taxonomic history of *Fusarium* is long and complex starting in 1809 by the first diagnosis of the pathogen - realized by Link - and the primary character describe as a distinctive banana-shaped conidia. The first real classification was established by *Wollenweber & Reiking* (1935) and stands as the origin of all taxonomic work on the subject. Numerous changes occurred during the past 200 years and yet, ever changing modifications do not facilitate the understanding. However, nowadays, species determination based on genetical traits appears to be a reliable method for standardized classification.

One of the main confusion when dealing with *Fusarium* comes from the parallel classification of asexual (*Fusarium*) and sexual (*Giberella, Haematonectria, Albonectria*) stages with often two designations for the same pathogen (ex: *Fusarium graminearum =Giberella zeae*). Nevertheless, teleomorphic stage *(sexual)* is not commonly observed in the field and anamorphic *(asexual)* appellation is often preferred. In this report, *Leslie & Summerel* (2006) classification was used, organized according to morphological traits of the asexual stage of the fungus.

3.2 Fusarium morphology

Fusarium fungi are characterized by well developed and branched mycelium. Sexual reproduction is rarely observed and asexual spores structure (conidia) are commonly used for identification (*Leslie et al, 2006*). The mycelium of *Fusarium* produces 3 different types of structures: microconidia, macroconidia and chlamydospore varying in shape, size, and number from one species to another (*Figure 4.3*).

Microconidia are non septate to 2 septates organelles often formed along the mycelium on varying sized outgrowth named phialide and constitute another distinctive feature used for identification. Depending on the specie two types of phialide can be found. One branch corresponding to one cell called monophialides or several branches corresponding to one cell called polyphialides.

Macroconidia are large organelles bearing 3 to 8 septa with a characteristic heel on the basal cell. These conidias are often grouped in structure of various colors (orange, yellow, brown) called sporodochia. They are differentiated by their curvature as well as by the shape of their foot and basal cell. For survival certain species of *Fusarium* form thick walled structure called chlamydospores that can be differentiated by their grouping (in chain or isolated), their number and their rapidity of formation.

3.3 Ecology

3.3.1 Survival structure

Fusarium species all use infected host debris for carryover in soil where they survive saprophytically *(feeding on dead material)*.The most common overwintering structure are chlamydospores. Their formation is result of conidial germination, when this germination is aborted certain species have the ability to reinforce cell walls of the conidia or to form chlamysdospores within conidia *(Tivoli, 1983)*.

3.3.2 Temperature

Fusarium species responsible of dry rot have optimal temperature varying greatly between species. It was established the optimal temperature of 20-25°C for growth on artificial media and 10-20°C the optimal temperature for infection of tubers *(Stevenson et al, 2001)*. However, on tuber, 3 groups according to temperature optimum can be determined *(Tivoli et al, 1981)*:

- high,
- temperate,
- no optimum

As example; potato pathogenic *F.culmorum* develop symptom from 25-30°C but do not progress at 5-10 °C belonging to the first group. *Fusarium sambucinum* and *F.coeruleum* develop rot between 15-20° C and belong to the temperate group. However, *F.sambucinum* appears to be able to infect and develop in a very large spectrum from 5-30°C. *F.roseum* as well as *Fusarium* producing no significant damages belongs to the no optimum group.

3.3.3 Humidity

The soil humidity can have various effects on the conservation of Fusarium in soil. A high humidity can lead, for some species (*F.avenaceum*), to rapid lyses of conidia but for others (*F.sambucinum*) to an increase in the formation of resistant structures (chlamydospores) improving the carry over in soil *(Tivoli et al,1983)*.In a general manner, *Fusarium* species would survive better as spores and mycelium at low humidity. Study of influence of humidity on *Fusarium* during potato storage (*Clayton et al, 2001*) showed that in case of high humidity, *Fusarium* infection would be lowered. In the presence of water on tuber's surface, *Fusarium* spores may not be able to adhere and to infect wounded areas.

3.3.4 Organelles responsible for infection

It is not clear whether the organelles responsible of tubers infection are conidia or mycelium. It has been observed that *F. graminearum* on cereals had both mycelia and conidia responsible of infection (*Takegami & Sasai, ,1970).* Comparison of pathogenicity tests realized in this thesis might provide answers to this question concerning potato*.* In general, it appears that every *Fusarium* species has requirement regarding environmental factor varying from one species to another.

3.4 Symptoms

Symptoms of Fusarium Dry Rot often originate after wounds created by mechanical damages or desprouting during handling. Damages caused by other potato pathogens like - powdery scab (*Spongospora subterrenea*), late blight (*Phytophtora infestans*) - and pests injuries can also be cause of *Fusarium* infections. Surface lesions appear as sunken and wrinkled areas of skin with a light dark

discoloration surrounding the wound. In the case of heavy infection concentric rings can be observed with the infection point as epicenter. The presence of FDR can often be detected thanks to white, pink or orange colored mycelium proliferating on the surface of the tuber. This particularity allows to differentiate this pathogen from gangrene caused by *Boeremia spp.* having similar symptoms.

Internal lesions are characterized by necrotic areas with a sharp brown to black margin. In advanced case of infection, cavities are formed. They are often filled with yellow, white or orange mycelium and dry potato flesh. A light brown discoloration of the flesh often appears around the wound (*Figure 3.3*). In storage, dramatically fast decay is often due to secondary bacterial infection (soft rot, *Pectobacterium carotovorum*), Pythium spp. or pink rot. These pathogens liquefy the tuber flesh and are spread over adjacent tubers.

During the growing period, some species of *Fusarium* (*F.avenaceum, F.roseum, F.oxysporum*) are responsible of Fusarium wilt causing leaf discoloration and yellowing as well as a wrinkling of leaves. In case of heavy infection the pathogen can cause death of the entire plant but this phenomenon rarely occurs. (*Peter et al, 2007; Peters et al, 2008; Choiseul et al, 2011*)

3.5 Life cycle

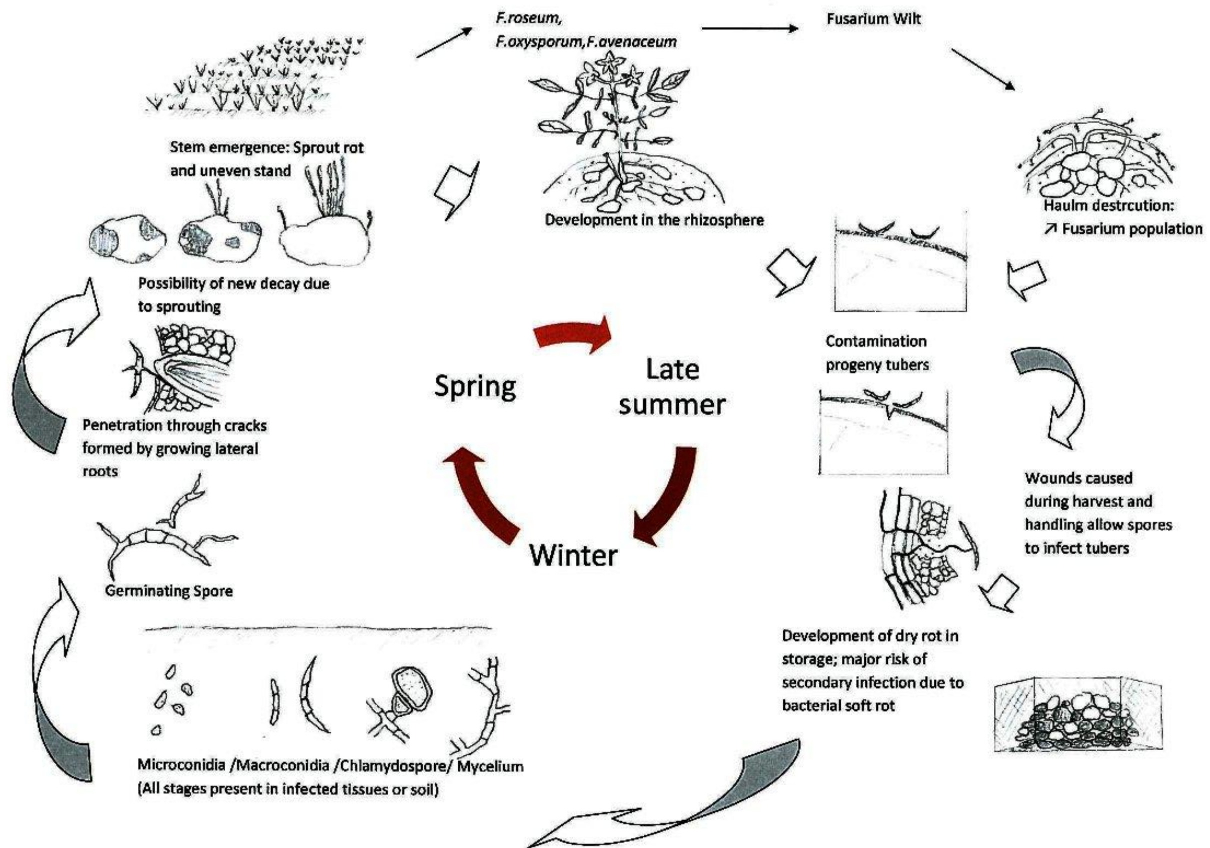


Figure 3-1 - Life cycle of Fusarium Dry Rot. J.Gomez

3.6 Effect of cultural practices on Fusarium Dry Rot

3.6.1 Planting

Planting of contaminated tubers (particularly by *F. coeruleum* and *F. sambucinum*) was found responsible of soil and progeny contamination *(Tivoli et al, 1986*). Contradictorily, other observations *(Leach, 1985*) showed that *Fusarium* infected mother tubers have a rather low influence on the increase of field inoculums affecting mainly daughter tuber. Nevertheless, transmission of the pathogens to tubers via contaminated soil remains the main cause of tuber infection. Field liming (pH increase) showed the ability to decrease disease severity *(Wale et al, 2008).*

3.6.2 Haulm destruction

It appears that this operation modified significantly the *Fusarium* flora with an often dramatic increase of *F. culmorum,F. roseum* and *F.avenaceum* . Comparison of propagule density before and after haulm destruction showed, in certain case, an increase of 25 % of the population for these species. (*Tivoli et al, 1986*). This phenomenon can be explained by an enhancement of the saprophytic activity of these particular *Fusarium* species especially at stem and roots level.

3.6.3 Harvesting

Harvest is the most critical stage for *Fusarium* contamination of the tubers. Infested soil, stem, roots and potato tubers are mixed together creating external contamination of the tubers. Harvesting and handling generate unhealed bruises and wounds in which pathogens can enter.

Different *Fusarium* species have specific mode of infection. For species such as *F. coeruleum* the transmission through wounds is decisive. For other species (F.sambucinum or *F.avenaceum*) infections by wound is possible but also via colonization of secondary roots and stems followed by a systemic spread to the tubers. In both case, tubers with important mechanical damage are more susceptible to infection than undamaged tubers *(Tivoli and al, 1986)*.

Another important aspect of *Fusarium* populations (*F.sambucinum, F.coeruleum, F.avenaceum*) behavior at harvest lies in their increase over time. It was observed (*Tivoli et al, 1986*) that late harvesting resulted in an increase of rotted tubers. It is therefore advised to avoid late harvest and particularly long laps of time between haulm destruction and harvest.

3.6.4 Crop rotation

Studies *by Folsom (1959) and Leach (1985*) showed that crop history does not affect quantities of Fusarium inoculum in soil. However, interesting patterns can be observed in their results (*Appendix IV*). Monocropping of potato appears to keep the level of *Fusarium* inoculums (*F.solani var coeruleum*) rather low when, comparatively; rotation of oat and potato showed high density of

Fusarium propagules in soil. These observations will be compared and discussed to results found in this study.

3.6.5 Storage

The contamination of tuber during storage is mainly due to propagules present in the dust of storage facilities (*Tivoli et al, 1982*). Air movement allows particles to deposit and fix on potato tubers. However, and as described previously, high humidity lower *Fusarium* infections (*Clayton et al, 2001*).First, by limiting the adherence of spores but also by the fact that surface moisture may act as lubricant resulting in less damage of the tuber skin. Nevertheless, high humidity (95-100% RH) favors many other pathogen especially bacteria (soft rots) and Pythium rot limiting a possible use of humidity increase as integrated management. Physiological age of stored potato appears to play a role in *Fusarium* infection. It has been established that young potato tubers have higher resistance to dry rot with obvious low levels of disease development in many cases (*Tivoli et al, 1986*).

3.7 Epidemiology

The transmission to progeny tubers of different species of Fusarium Dry Rot has been studied in detail by Tivoli (*1983-1986*) in a study taking into account the role of the seed, soil and plant as well as the different cropping stage *(Figure 3.2)*.

1- Result found showed that seed tuber quality is determinant of soil and progeny infection by potato pathogenic Fusarium. Daughter tuber can be affected even when the density of inoculum is low.

2- The plant, at soil level, appears to enhance significantly *Fusarium* population density. The rhizospheric effect might be responsible of fungal dormancy breaking (via roots exudates signal) and favor the establishment of infectious inoculum in soil (favorable conditions of growth). For potato plant, the underground system (roots, runners, tubers) has various effects on transmission of *Fusarium* species. *F.coeruleum* , rarely isolated from plant debris, seems to be transmitted mainly by

soil when *F.avenaceum, F.culmorum, F.sambucinum* appear to use both plant and soil as infection pathways.*(Tivoli et al, 1986)*

3- During haulm destruction, the production of dead material associated with high humidity enhance the saprophytic abilities of Fusarium using plant as infection pathway.

4- During harvesting, soil and plant mixing allows the inoculum to infect tubers especially on wound created by mechanical damages. Level of contamination vary during the culture and are often high in the case of late harvesting.

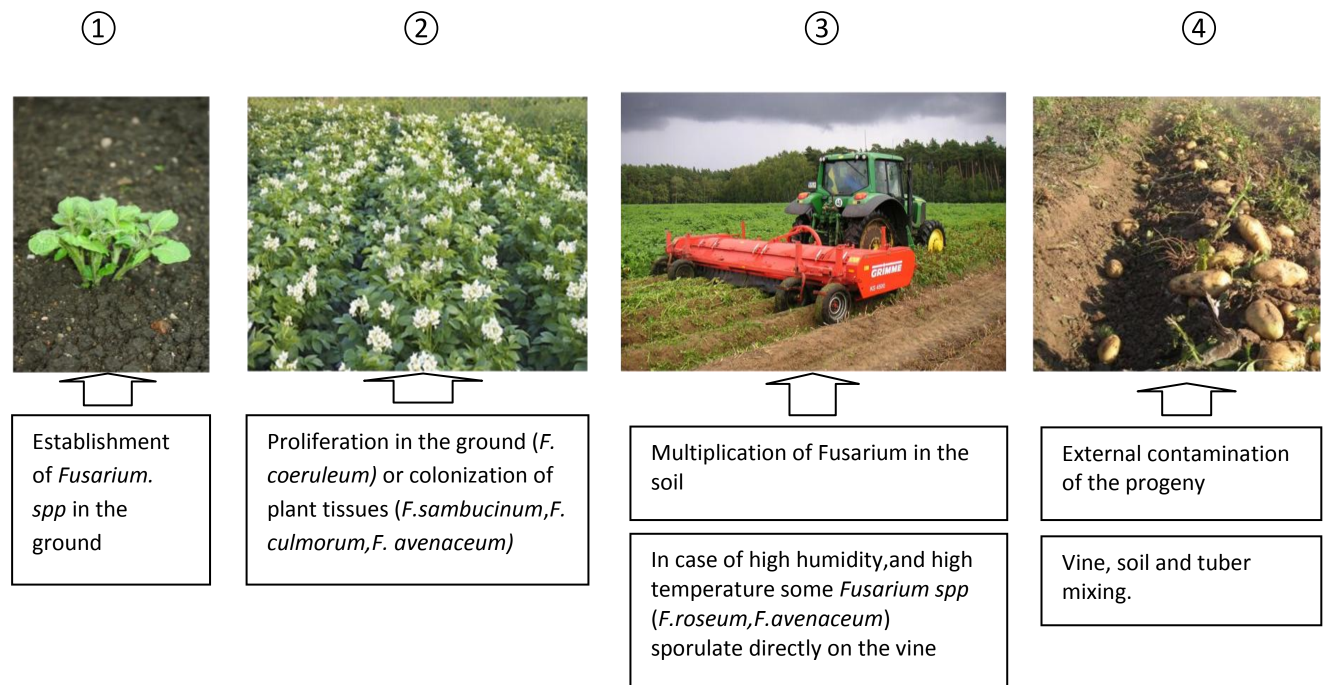


Figure 3-2 : Epidemiology of Fusarium Dry rot. Tivoli et al, 1986.

3.8 Mycotoxin production

Certain species of *Fusarium* causing Dry rot produce metabolites toxic for human and animals (*Abbas et al; 1987; El-Hassan.K.I., 2007,*). The reason are still unclear but condition related to stress factors - temperature variation, humidity or competition with other organisms is considered. Mycotoxins produced in potato tubers have been detected from tubers inoculated with *F.solani var.coeruleum*

that produced Trichothecene such as Deoxynivalenol (DON), Acetyl-DON, and HT-2 toxins (*El-Banna, 1984*). *F.sambucinum* was also reported producing several Trichotecenes and Sambutoxin (*Jin-Cheol et al, 1994*). So far, no case of human poisoning due to potato consumption has been mentioned in Norway (*Abbas et al, 1987*).

3.9 Management and control

3.9.1 Cultural practices

Numerous growers targeted publications give similar advices (*Peters, 2007; Wharton et al, 2007; Pringle, 2009*) concerning control and management of the disease.

During planting it is necessary to use clean if not disease free tuber. Even when certified, it is often relevant, prior planting, to assess seed quality sorting out tubers with as little Dry Rot as possible. Determination of the *Fusarium spp.* present during the previous years allows to establish adapted management, knowing that every species has its own specificity concerning infection pathways, and requirement for growth (humidity, temperature). At planting, warm tubers are favored as well as soil and temperature conditions promoting rapid sprout and growth.

Harvest under adapted condition constitutes also a decisive step in the integrated management of Dry Rot. Dry condition will be preferred, allowing low amount of dirt and clods on tuber. The crop is preferably harvested and handled when tuber pulp is warm and vines are dead. Prior to storage careful handling lead to minimized bruise and wound greatly lowering the impact of the disease.

Tuber storage in clean and disinfect facilities (warehouse, boxes, grading machine) lower risks of infection by dusts borne conidia of *Fusarium*. Proper curing condition (90-95% RH, 12-15°C) and a progressive decrease of temperature when wound healing is completed reduce the number of potential infection points. In addition to temperature regulation; a proper management of the ventilation in storage by recirculation of the airflow can be judicious. Uniform ventilation to keep the crop free from moisture is essential, especially in the hour following harvesting where condensation due to respiration is important and lead to rapid development of secondary bacterial infection.

Finally, appropriate use of fungicide (*Cf. Fungicides and biological control*) being aware of potential resistance can lead to a significant decrease in *Fusarium* inoculum in storage.

3.9.2 Fungicides and biological control

For years, Thiabendhazol (TBZ) has been the main post-harvest treatment used against potato Dry-rot, recent and numerous case of resistance (*Hide et al, 1992, Hanson et al, 1996, Satrapyasad, 1997*) have reappraised its efficiency and its use. Product based on *Imazalil* (Fungazil©, 100 SL. 150ml/ton) as active compound are now commonly applied having a broad spectrum of action on potato seed borne diseases. This aspect as well as the reduction of chemical compound authorized led to experiment on several other control methods. Several biological treatment trials have been carried out with promising result using the antagonistic bacteria *Pseudomonas fluorescens* (*Schisler and al, 1995, 2000*), or using mycorrizhae (*Niemira et al.1996*). Nonetheless, these solutions remain experimental. Commercial products Bio-Save 10 LP- 11LP© (*Pseudomonas syringae*) are commonly used in USA and Canada but not approved in Norway have shown good efficiency- single or associated to other commonly used fungicides (*Mughrabi, 2006*) - and could offer new opportunities for disease control.

3.10 Fusarium Head Blight of small grain

Organism from the Fusarium genus are responsible of Head Blight (Scab) of small grain (wheat, barley, oat, rye, triticale). Despite the fact that almost 17 agents are known to be responsible of that disease, most isolated organisms from grain in Norway are : *Fusarium culmorum, F.avenaceum, F.graminearum, F. poae* and *F. tricinctum* (*Kosiak and Torp, 1995; Bernhoft et al, 2010*). Usual symptoms of that disease are water-soaked brownish spots followed by salmon-pink to red color at the base of the spikelet and on the edge of the glumes. In advanced infection stages, grains shrink and become grey/brown with floury and discolored interior. The life cycle of FHB is rather similar to

Fusarium Dry Rot (*Figure 3.1*). Infected seeds but more commonly infected soils influenced by the presence of plant debris (cereal grains and stem nodes) are the primary source of inoculums (*Osborne and Stein, 2007*). Disease develops on seedling, stem and sporulate on heads to be dispersed by water splash, wind or microfauna. The pathogen overwinters as chlamydospores on plant debris and re-infect seedling under favorable conditions.

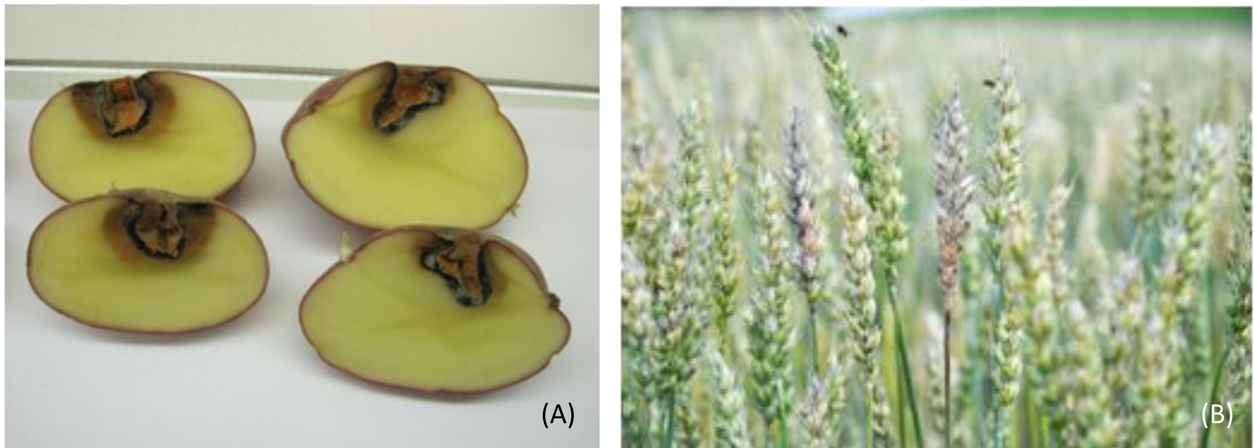


Figure 3-3: Symptoms (A) Symptom of Fusarium dry rot on tuber. P.Jensen, Bioforsk. (B) Symptom of Fusarium head blight on wheat. J.Razzaghian,Bioforsk.

4 Characterization of Fusarium species present in agricultural soil of Solør area.

4.1 Introduction

This chapter is dedicated to several experiments aiming to isolate *Fusarium* species out of soil sample from agricultural soil of Solør area. The first part of this chapter describes the characteristics of every sampled field and methods of sampling. The second part describes the pilot study first carried out to determine an adapted isolation technique, a proper growth medium and a standardized dilution factor. This preliminary study was conducted on a single soil sample and extended to the whole sample lot once the adapted method was established. The different methods and procedures are described hereafter. The final part of the chapter contains investigations on the different *Fusarium* species found as well as result from the pilot study.

4.2 Materials and methods

4.2.1 Method of sampling and field characteristics

In May 2011, 8 samples of soil were collected in 8 fields of the Solør area (*Figure 4.1, 4.2)*. Superficially worked (cultivator, rotovator) field were selected to secure that the upper soil layers had directly contact with the previous crop. The quality and quantity of plant debris were assessed *(Table 4.1)*.

Four samples were taken from fields with potato as previous crop and 4 samples were taken from fields with cereal as previous crop. Samples were collected with an Oakfield soil sampler extracting 10 centimeter of soil including crop debris. One hectare surface field was sampled by taking one sub-sample every 20 m (≈ 1kg soil/ field). Soil and plant debris samples were placed into paper bags, air dried for 48 h and stored at room temperature (25°C) until use.

Table 4-1 : Characteristics of sampled fields

Field	***Characteristics***
Potato 1	Few root and tuber debris, spring harrowing with animated tool (rotovator)
Potato 2	Moderate quantity of roots and tuber debris, spring harrowing with unanimated tool (cultivator)
Potato 3	Few roots and tuber debris, spring harrowing with animated tool (rotovator), *manure spraying 30 min before sampling*
Potato 4	Important quantity of roots and tuber debris, autumn harrowing with unanimated tool
Cereal 1	Important quantity of plant debris, no harrowing
Cereal 2	Moderate quantity of plant debris, no harrowing
Cereal 3	Moderate quantity of plant debris, no harrowing
Cereal 4	Low quantity of plant debris, no harrowing

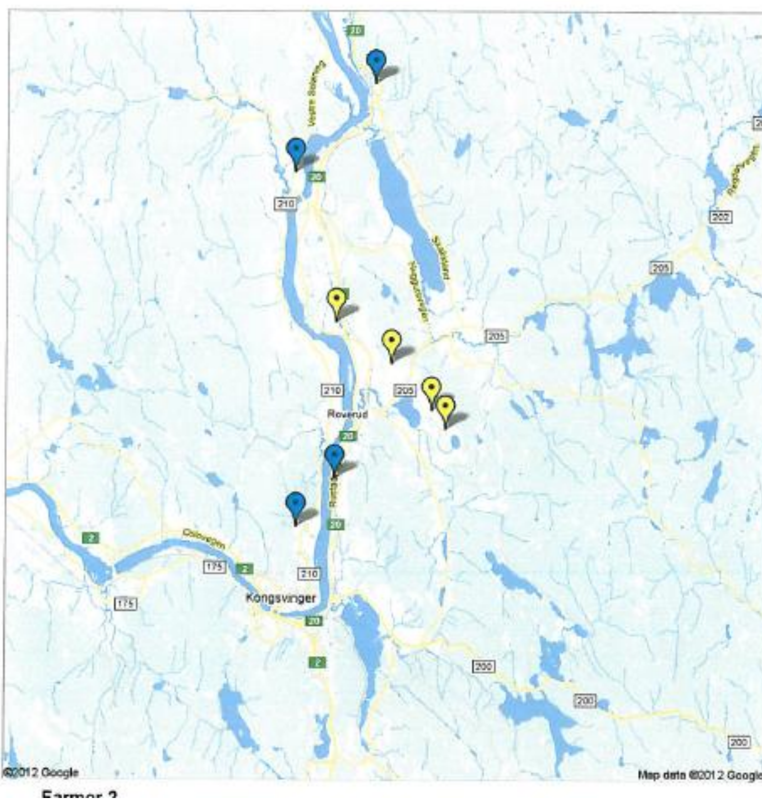


Figure 4-1: Field localisation

Figure 4-2: Sampled field pictures (A)Potato 1 (B)Cereal 1 (C) Potato 3 (D) Potato 4 (E) Potato 3 (F) Cereal 2 (G) Cereal 4

4.2.2 **Fungal isolation**

4.2.2.1 Modified Warcup soil plate technique

The method used was first described as *Modified Warcup soil plate technique* by *Maciejowska (1962).* Soil samples were filtered through a 2mm sieve to remove every residue (stones and plant debris). 300 ml of sterile water agar (0,1 %) were added to 30 g of soil and shacked vigorously for one minute. 1ml of this solution were transferred into a 99 ml of sterile water agar (0,1%) screw capped bottle giving a first dilution of 1:1000 (10^{-3}).

4.2.2.1.1 Method for pilot study

For the preliminary experiment 1 ml of solution was spread evenly on the surface of each of the 5 media selected (CZID, DCPA, PPA, PDA, RbGU). 5 plates per medium, per dilution factor and per soil sample were used. After homogenization, 1ml of the 10^{-3} dilution was transferred into 99ml of sterile water agar (0, 1%) obtaining a dilution of 1:100000 (10^{-5}). To obtain a 1:10000 (10^{-4}) dilution the method described by *Tuite* (1969) was used. 0, 1 ml of solution was pipetted out of the 10^{-3} bottle and transferred into a 99ml sterile water agar (0, 1%). Out of every dilution factor, 1ml of solution was pipetted and evenly spread on the adapted media with the use of a spatula.

Petri dishes were incubated for 7-10 days at room temperature under artificial white light to be assessed for colony counting.

4.2.3 **Tuber disk technique**

The method used as first been described by *Stanghellini & Kronland* (1985)*.*Tubers of *cv.* Asterix were washed under running tap water to remove adhering soil and surface disinfected (3% sodium hypochlorite, (d) H_20). Tubers were let to dry on paper towel for 5 minutes. Petri dishes (9 cm diameter) were filled with 24 g of sieved soil (2mm). Ten plugs were cut from tubers using a cork borer (*5 mm diameter, 10 mm thick*) halfway between rose and heel ends. Potato plugs were placed on the soil surface of every Petri dish and incubated for 24 h in the dark at room temperature (25 °C). After incubation, plugs were gently washed with tap water to remove adhering soil and growing

mycelium. Disks were surface-sterilized for 2 min in 70% ethanol, rinsed for 3 min in distilled water and left to dry on sterile paper towels in a laminar airflow cabinet.

Every plugs were cut into five sequential sections (approx. 2 mm thick), plated on a *Dichloran-chloramphenicol-peptone* agar (DCPA) and incubated under UV light bank for 7 days at room temperature (25°C).

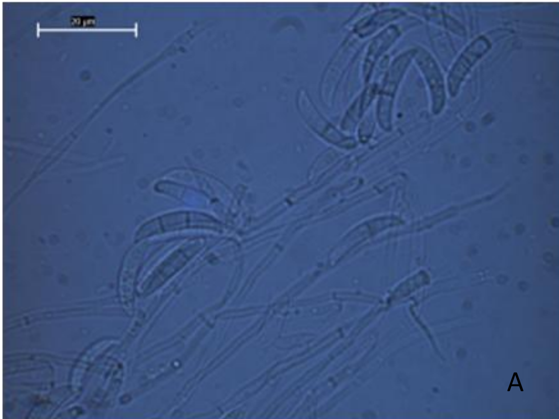
4.2.4 Single species isolation

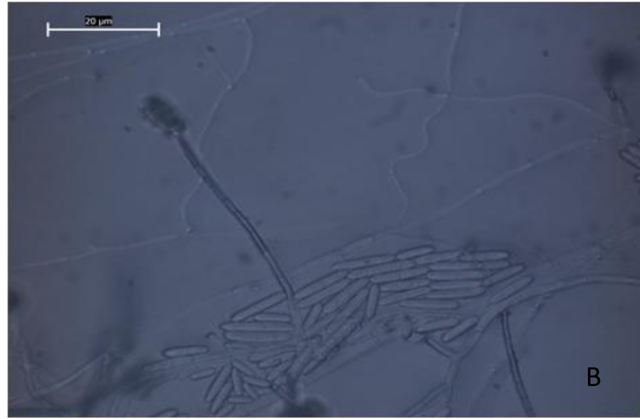
After identification of distinct *Fusarium* colonies using binocular under low magnification (x100) the colonies were sub-plated on PDA. Every colony from these plates was transferred on both PDA for growth measurement and SNA for determination of morphological traits. Plates were exposed for 3 days under UV light and observed for sporodochia formation.

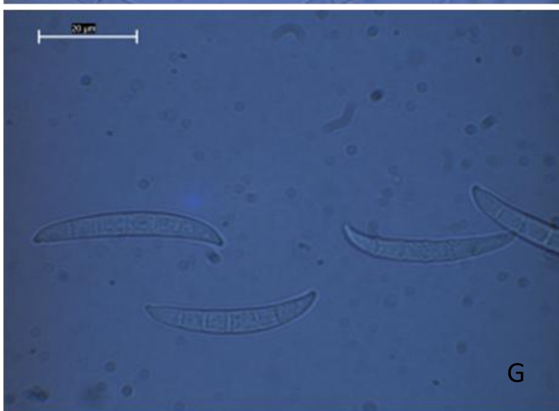
4.2.5 Identification

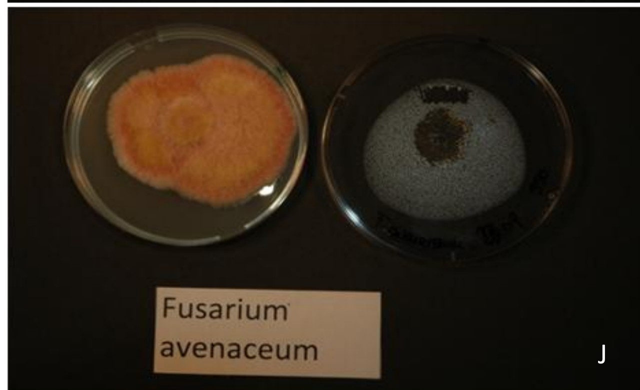
The Fusarium identification was performed using a light microscope (Leica DFC420) and with help of identification manuals (*Leslie et al, 2006; Burgess et al, 1988*). Single spore isolation was carried out for some of the identified isolates (*Appendix II*). Staining was performed for photographic purpose using iodine.

4.3 Results









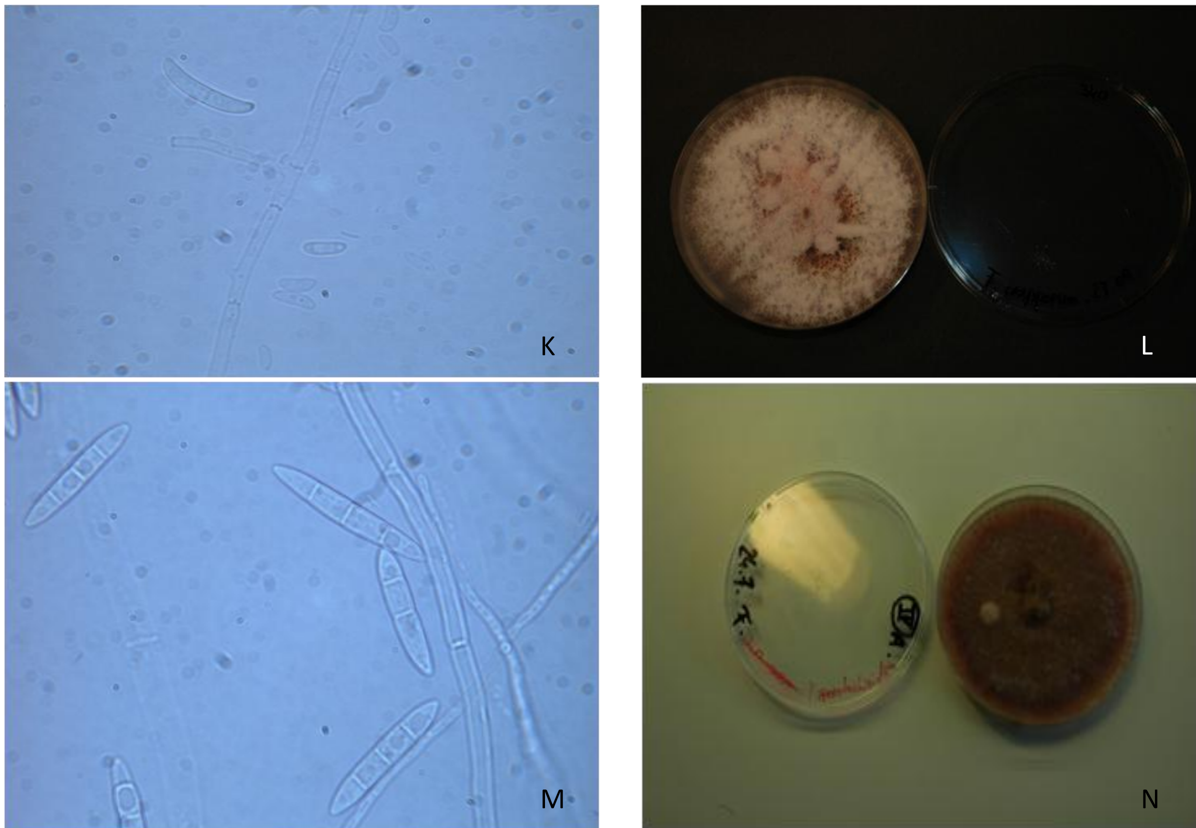


Figure 4-3: From left to right, binocular view, pathogen on PDA. Fusarium dimerum (x1000) (A), F.solani (x 400)(B), F. equiseti (x1000) (C,D),F.venenatum (x1000) (G,H), F.avenaceum (x1000) (I,J), F.oxysporum macro and mesoconidia (x200) (K,L), F.sporotrichioides (x1000) (M,N). J.Gomez.

4.3.1 Isolation technique and media assessment

Among the different method of isolation tested, the modified Warcup method allowed to treat a great number of sample with ease. Test of media using this method led to fungal and bacterial overgrowth on PDA for the different concentration of soil dilution (1:1-1:8) tested. Plating using RbGu and PPA as artificial media showed slow to no growth for all concentration (1:8-1:1). Plating with CZID led to low growth for low concentration (10^{-5}) increasing in an exponential way with the concentration gradient. Colonies isolated out of CZID mainly belonged to one species (*F.merismoides*). DCPA showed the same pattern of fungal development with a higher diversity of *Fusarium* species isolated (F.*avenaceum, F.oxysporum, F.merismoides*). The dilution factor of 10^{-3} allowed appropriate amount of fungal growth on every medium.

The potato plug experiment led to poor isolation of *Fusarium* due to extensive colonization of the plates by non pathogenic *Colletotrichum spp.* These plates were discarded and the result will not be discussed hereafter.

4.3.2 Characterization of Fusarium species from soil samples

Forty five isolates of *Fusarium spp.* were recovered from soil samples of various fields of Solør area *(Table 4.2)* were identified as *F. avenaceum* (7 isolates or 15,5%)*, F.dimerum* (1 isolate or 2,2 %)*, F.equiseti* (2 isolates or 4,4 %)*, F.merismoides* (17 isolates or 37,8%)*, F.oxysporum* (14 isolates or 31,1%)*, F.sporotrichioides (1 isolate or 2,2%), F.solani* (1 isolates or 2,2%) *and F.venenatum* (1 isolate or 2,2%).

An estimation of the number of Fusarium propagules present in the soil of each plot *(Figure 4.4)* was calculated thanks to the following formula (*Jeschke et al, 1990*):

$$\frac{\textit{No. of propagule}}{\textit{Gram of soil}} = \frac{\textit{Total No. of Fusarium isolates}}{\textit{No. of dilution Plates}} \times \textit{Dilution factor } (10^{-3})$$

Propagules of *F. merismoides* were present in 3 potato fields (mean =533,33 propagule.gr) and 3 cereal fields (mean= 600 propagule.gr) in high density. *F.oxysporum* was found in 2 potato fields and in high quantity in one cereal fields (mean =1400 prop.gr). *F.avenaceum* was isolated in 4 fields, 2 with potato (400 prop.gr) and 3 with cereal (300 prop.gr). The other *Fusarium.spp* (*F.equiseti, F.venetatum, F.dimerum* and *F.sporotrichioides*) except for *F.solani* isolated in cereal field, were only recovered from potato fields in low density (200 prop.gr).

Table 4-2: Isolated species of from different potato and cereal fields of Solør area.

Fusarium spp.	Field type								
	Potato 1	Potato 2	Potato 3	Potato 4	Corn 1	Corn 2	Corn 3	Corn 4	Total
F. avenaceum	0	2	0	2	1	1	1	0	7
F. dimerum	1	0	0	0	0	0	0	0	1
F. equiseti	1	0	1	0	0	0	0	0	2
F. merismoides	2	4	2	0	4	3	0	2	17
F. oxysporum	4	0	0	3	0	0	7	0	14
F.sporotrichioides	0	0	0	1	0	0	0	0	1
F. solani	0	0	0	0	0	0	0	1	1
F. venenatum	0	0	0	1	0	0	0	0	1

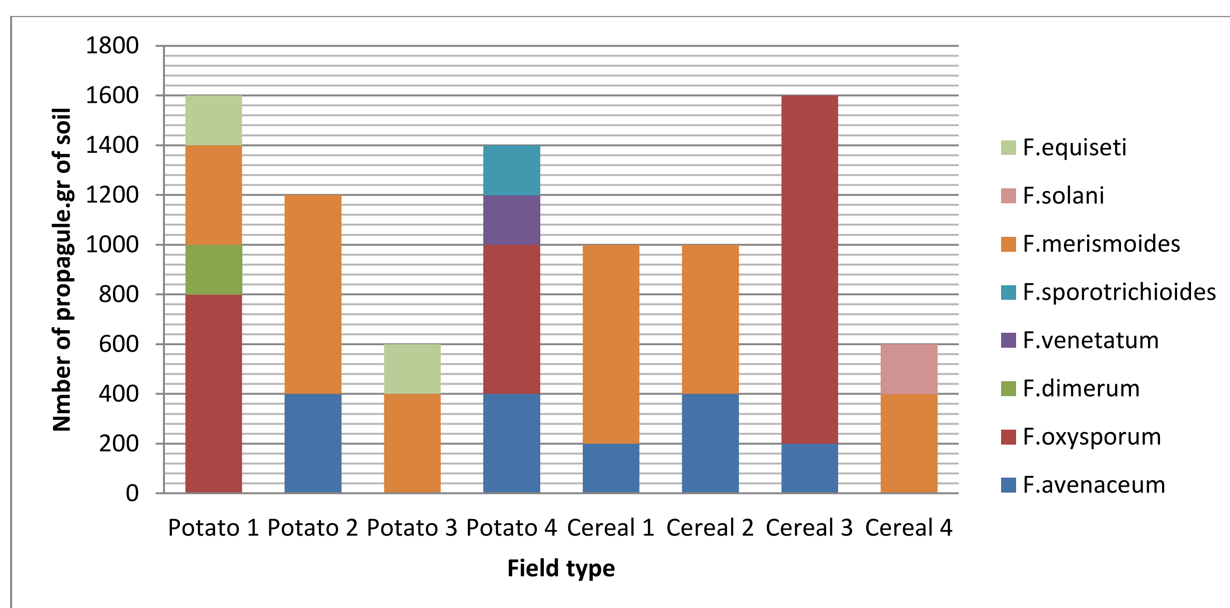


Figure 4-4 : Distribution and inoculums density of Fusarium spp. in the different fields

4.4 Discussion

4.4.1 Choice of growth media

Many type of media can be used for isolation of *Fusarium* from soil or plant debris. The main challenge is to find a medium that allows clear counting of *Fusarium* colonies with limited toxicity but still with a strong selectivity against any other fungi than *Fusarium*. This medium must also keep its ability for a long period of time to allow certain slow growing species to develop. *Table 4.3* summarizes advantages and weaknesses of every media tested. DCPA appears to be the most suitable media for Fusarium isolation from soil *(Andrews et al, 1986).*

4.4.2 Isolated species

F. merismoides (37, 8%) was the most commonly isolated species in this survey, *Fusarium oxysporum* (31, 1%) and *Fusarium avenaceum* (15, 5%) are the two second most isolated species.

F. oxysporum and F. *merismoides* present are common telluric fungi. Potato and cereal pathogenic *(Chehri et al, 2011)* as well as non-pathogenic Fusarium *oxysporum (Alabouvette et al, 1997*) strains have been reported from other areas of the globe. However *F. merismoides* has only been mentioned as a non-pathogenic soil fungus.

In this study, *F.avenaceum* was found in both cereal and potato fields. This species has been reported pathogenic on these two crops (*Sutton, 1982; Peters et al, 2008).* It has the ability to develop from infected tubers and survive on plant stems contrarily to other potato pathogenic species (*F.oxysporum, F.coeruleum)* that survive mainly in the soil.

This particularity could explain the presence of this species in two of the potato fields with the highest amount of residues. The same observation can be made for the cereal fields with the presence of *F. avenaceum* in three fields with the highest amount of residue and its absence in fields with low levels of plant debris.

F. venenatum, F. dimerum, Fusarium equiseti and Fusarium sporotrichioides was only found in potato fields. *F.venetatum* and *F.dimerum* are species rare to be isolated from Norwegian soils and have never been reported as pathogenic species on potato or cereals. *F. equiseti* and *F. sporotrichioides* have been mentioned as pathogens of both crops (*Theron et al, 1991; Xue et al, 2006; Peters et al, 2008*). However, their low amount weakens their position as potentially dominant pathogen of potato and cereals.

Fusarium solani was found in cereal fields. This species is known to be pathogenic on potato *(Morid et al, 2009)* but has never been mentioned has pathogenic on cereal.

Table 4-3 : Characteristic of growth media tested in the pilot study

Media	***Advantages***	***Disadvantages***
Potato Dextrose Agar (PDA)	No toxicity, no selectivity against any Fusarium specie, easy to prepare in quantity.	No selectivity massive growth of any type of fungi, impossibility of colony counting.
Rose Bengal-glycerin-urea (RbGU)	Clear colony counting	High toxicity, limited growth of fungi (high selectivity?)
Peptose PCNB agar (PPA)	Clear colony counting, abundant literature on use of this media for isolation from soil sample.	High toxicity, limited growth of fungi (high selectivity?)
Dichloran Chloramphenicol Peptone agar (DCPA)	Clear colony counting, good growth of fungi (appropriate selectivity)	
Czapek ipodrione dichloran agar (CZID)	Good growth of fungi (appropriate selectivity)	Low toxicity, few literature references on use of this media for isolation from soil

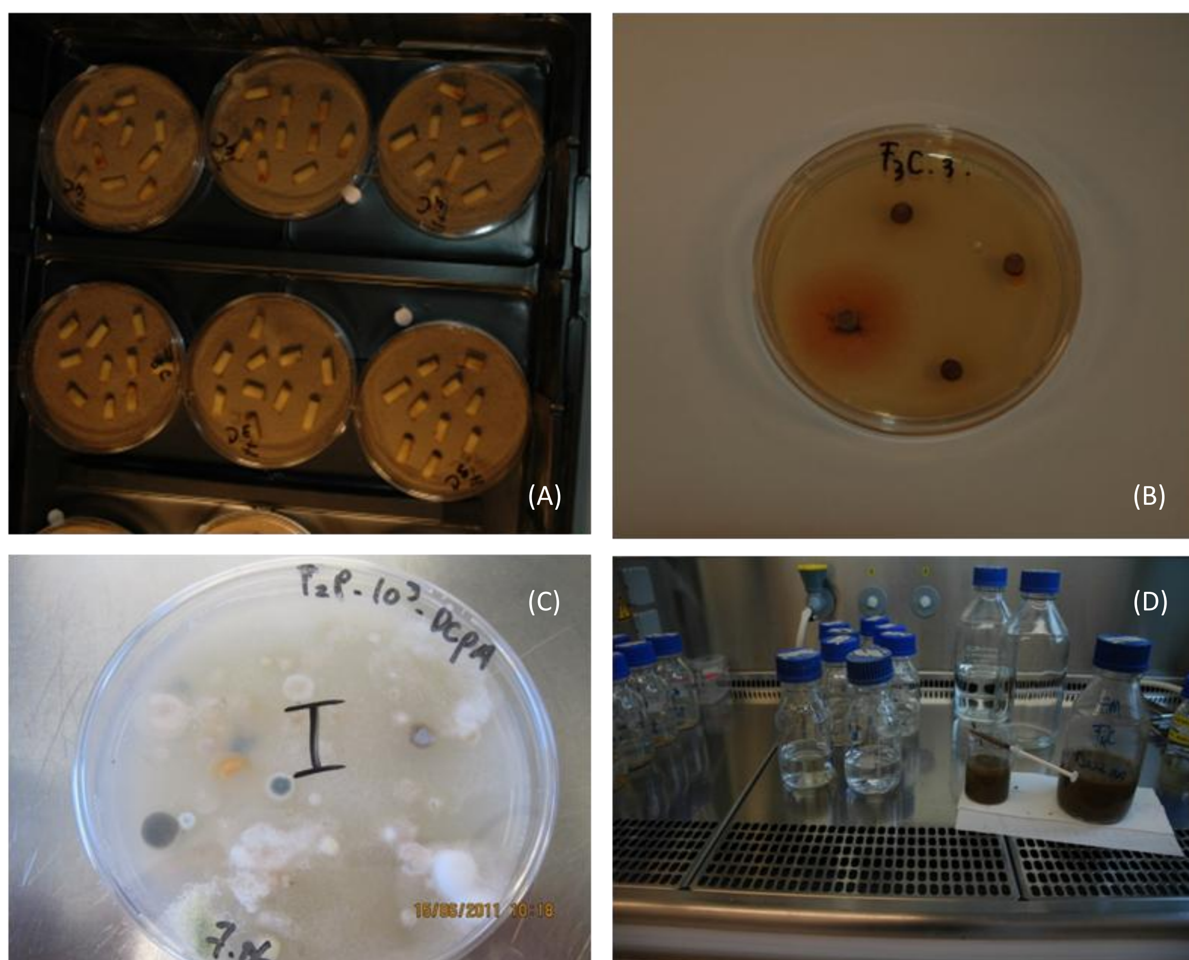


Figure 4-5 : Isolation techniques (A) Potato plug on sampled soil (B) Plated potato plug on DCPA (C) Plated soil solution on DCPA (D) Soil solution prior to plating. J.Gomez.

5 Pathogenicity test on potato tuber

5.1 Introduction

The following chapter describes the various pathogenicity tests of different *Fusarium spp.* isolates from soil samples carried out on two potato cultivar (*cv.*Asterix and *cv.*Mandel) with known different resistance to potato Dry Rot. On a scale from 1 to 9 - where 9 is very resistant and 1 is very susceptible – *cv.Mandel* is scored as 1 and *cv.Asterix* is scored as 6 (*Møllerhagen et al, 2010*). The optimal period to carry out resistance tests is from early November until late December to avoid sprouted potatoes. This period was also used for the pathogenicity test.

5.2 Materials and methods

5.2.1 Standard method

The following method is usually performed as test for the potato breeding program in Norway to establish data on resistance to Dry rot (*Kirsten Topp, Graminor*).

This protocol was also used in our test. From SNA, *Fusarium* isolates were transferred to PDA and grown for a 4 weeks period in the dark at room temperature (25°C). *Fusarium* solutions were prepared by transferring PDA plates into a Heerlen Meyer (2 liters) filled with distilled water (1l for 24 plates). The solution were homogenized with a mixer *(Braun, 300 W)* and poured into cap-sealed 500 ml bottles.

Fusarium solutions were prepared the day before testing and put in cold storage (9-10 ° C) during the night. Prior to the experiment potato tubers were washed with tap water to remove soil particles and placed in cold storage (4°C) until 2 day before the experiment started and transferred at 9-10 degrees store before use. Tubers of equal size and without damage were selected. 17 isolates belonging to 8 *Fusarium* varieties (*F.avenaceum, F.dimerum, F.equiseti,F.merismoides, F.oxysporum, F.solani,F.sporotrichioides , F.venenatum)* were used. The pathogenicity of these *Fusarium* was tested

on 5 tubers per isolate with 3 repetitions for a total number of 15 tubers per isolate for each potato variety.

Fusarium solution (inoculum) was poured into a plastic box (Hennig Olsen ice box, *2 l)*. Tubers were wounded using a nail board (consisting of four, 4mm diameter, 6 mm depth pins forming a square of 20 mm sides). Tubers were rolled and pressed towards these pins within the box filled with the inoculum. Wounded but uninoculated control was prepared. Tubers were placed in a plastic tray (20 tubers per tray) with wounds facing upwards. A filter paper covering tubers was imbibed with 3ml of distilled water to create humidity in the crates. After inoculation trays were covered with a transparent plastic bag and sealed with tape. Boxes were rapidly transferred to a cold store (9-10 ° C) room and left for incubation during a 4 weeks. Prior to assessment; trays were moved to room temperature for 1 week. A preliminary check for possible soft rot was performed after the first week of experiment.

5.2.2 Modified method

After a first unsuccessful trial with the original method *(Figure 5.1)* and significant loss of plant material due to secondary infection, several parameters in the method were changed. The humidity level within boxes was too high favoring fast bacterial development (Soft rot contamination). Tuber were, therefore, cover with filter paper only for a week, resulting in a decreased humidity and a climate closer to conventional storage. The second parameter changed was the amount of inoculums used. In the previous experiment, complete deeping of tuber within the Fusarium solution led, primary, to a rough estimate of the inoculums quantity applied over each tuber, and, generate an important risk of various pathogen transmission from one tuber to another when using the same solution for a whole lot. A defined quantity of inoculums (*0, 75 ml*) measured with a calibrated spoon (*Picture 5.1*) allowing a precise application with no risk of disease transmission was used.

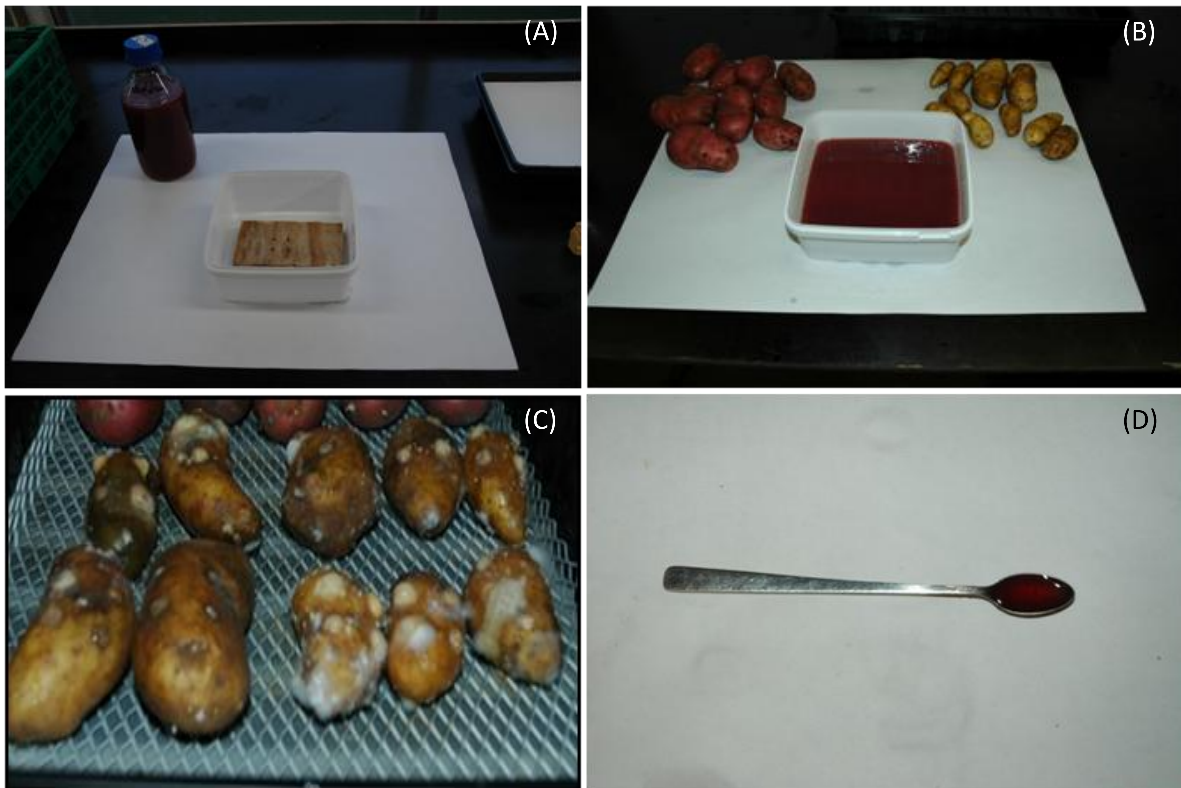


Figure 5-1: (A) Nail board,plastic box and fungal solution (Standard method), (B) Spore solution, cv.Mandel (white)and cv. Asterix (pink) to be wounded/inoculated(C) Soft rot infected tuber following Standard method (D) Calibrated spoon. J.Gomez.

5.2.3 Assessment

Tubers infected by soft rot were discarded. Every tuber was cut transversally through the wounds. The depth (mm) and width (mm) of the wound were measured and compared to an untreated control. In addition possible presence and color of mycelium and presence or absence of cavity were noted. A final calculation corresponding to the total volume of the wound was established. The rot was assumed to be conical, therefore the volume of every rot point (hole) was calculated using the equation:

$$\textbf{Volume} = \pi r^2 (h/3)$$

Where **r** is ½ the width of rot and **h** the depth of rot.

5.2.4 Data analysis

Statistical analyses were conducted using Minitab (15-16). A general linear Model were used performing analysis of variance (ANOVA) test for width, depth, volume and cavity (1- cavity; 0- no cavity) variable (y= Isolate + Variety). A correlation analysis were carried out between isolate and variety (y= Isolate nr *Variety)

5.3 Results

Wound size variation results showed significant differences between variety and between isolates. Significant differences were observed between variety for depth (P value <0,001) and width (P value< 0,001) as well as between volume (P<0,001).

Plot of wound volume mean *(Figure 5.2)* allows to quantify these differences and allows comparison with the uninoculated control. For 'Mandel', one isolate of *F.avenaceum* (*550*- Mean =147, 9 mm; SE mean= 12, 2 mm) had an important effect on wound volume as well as one isolate of *F.sporotrichioides* (*44*- Mean= 90,7 mm; SE mean=12,2). Differences in wound size were smaller with still 3 remarkable differences of two isolates of *F.avenaceum* (*360* - Mean=61, 1 mm; SE mean=12,2; *43*- Mean=54,9 mm; SE mean= 12,8) and one isolate of *F.oxysporum* (*41*- 54,6 mm; 12,2). The other isolates although statistically significant had small difference with the control (45, 8 mm; 12, 2).

Fusarium isolate had a larger effect on *cv.* Mandel than on *cv.* Asterix (Total mean volume = 51, 2 mm < 73,51mm). On *cv.Asterix*, one isolate of *F.sporotrichioides* had an important effect on wound volume (*44*- Mean= 60, 75; SE mean =1,36). The two isolates of *F. avenaceum* have also affected wound volume (*360*- Mean=55,62mm; SE mean =1,36; *550*-Mean =49,25; SE mean= 1,36). Results for the other isolates showed no differences with the uninoculated control.

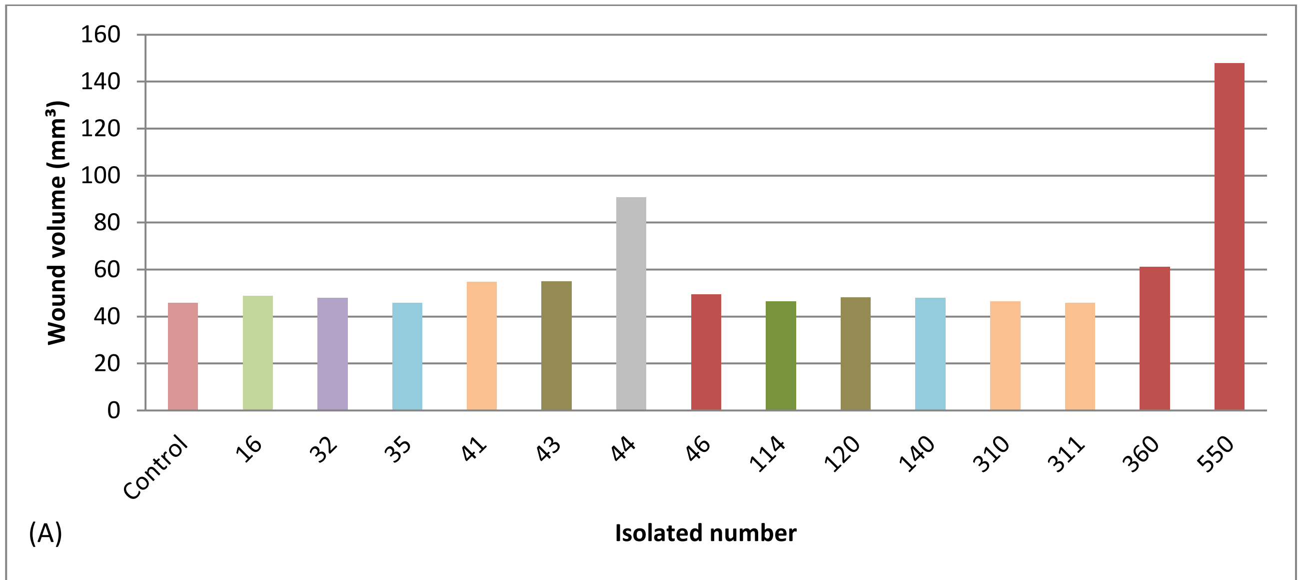
Significant differences in cavity formation were observed between varieties (P value=0,019) and between isolates (P value <0,001). On the total number of Mandel tubers found with cavity 14 % were inoculated with *F.avenaceum*, 25% with *F.merismoides*, 34% with *F.sporotrichioides* and 14%

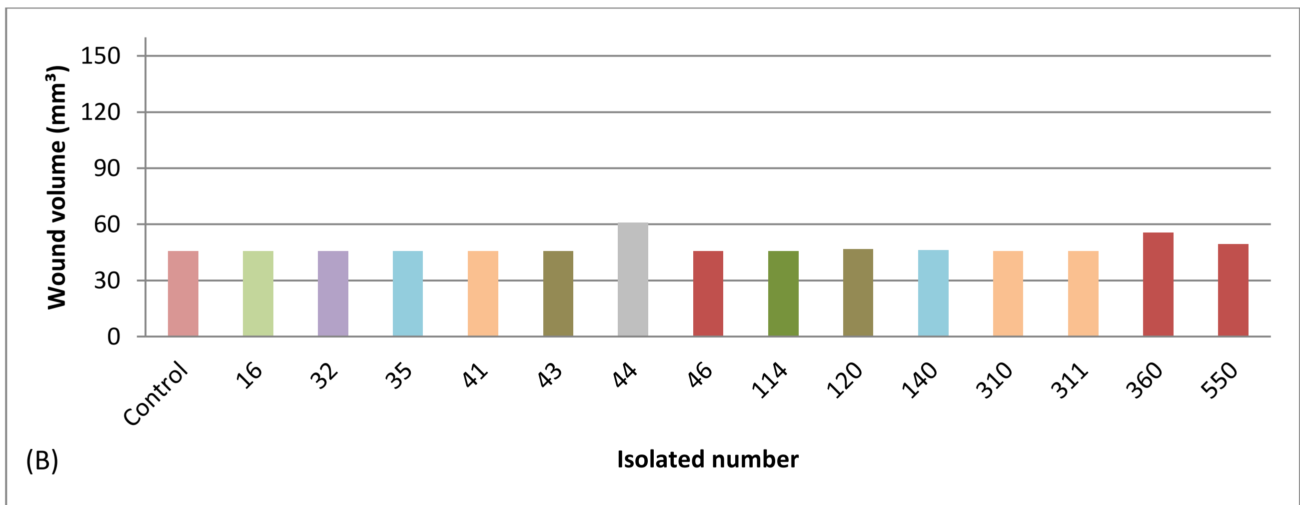
with F.venenatum. On the total number of 'Asterix' tubers found with cavity, 40% were inoculated with *F.avenaceum,* 12 % with *F.merismoides*, 8% with *F.solani* and 40% with *F.sporotrichioides*.

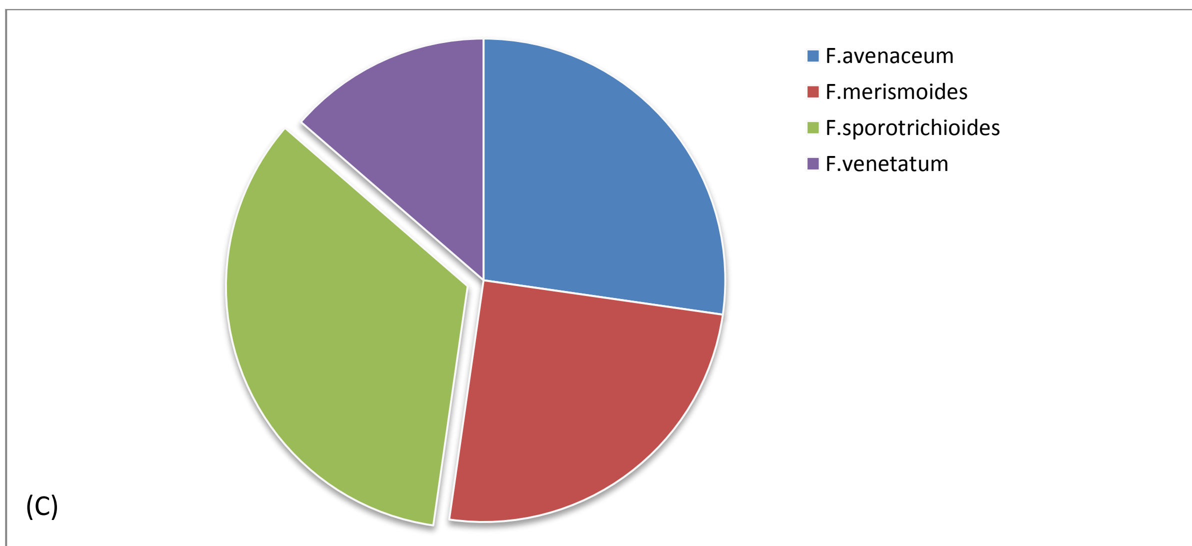
The last visual element evaluated was the presence/ absence as well as the color of the mycelium. Numerous tubers inoculated with *Fusarium avenaceum* had a dark brown coloration of the wound margin with, in certain case, a light to dark brown discoloration of the flesh at the surrounding *(Figure 5-3*). These symptoms were often accompanied by circular, white mycelium filled cavity. The surface of tubers was often covered by white fungal hyphae. Isolates of *F. sporotrichioides* produced, in many cases, mycelium on the surface of the tuber of pink and white color and formed characteristic dry cavities on both of the potato cultivar tested. *F.merismoides* isolates had a rather quick development of their mycelium over the wound, however (and despite the fact that small cavities were formed under the skin layers) fungal infection did not carry inward the tuber. *F.solani* isolate had the same pattern of development with important mycelium production - particularly during the first week - but poor flesh damages. Isolates of *F.equiseti, F. dimerum* and *F. oxysporum* formed white mycelium in the surrounding of the cut with no damage to tuber flesh. Observations of low damages to tuber flesh were often linked with the presence of a dry light brown crust delimiting clearly the wound from the inside fresh flesh result of tuber healing.

Isolate nº	***Fusarium species***
16	*F.dimerum*
32	*F.equiseti*
35	*F.merismoides*
140	*F.merismoides*
44	*F.sporotrichioides*
46	*F.venenatum*
114	*F.solani*
41	*F.oxysporum*
310	*F.oxysporum*
311	*F.oxysporum*
43	*F.avenaceum*
120	*F.avenaceum*
360	*F.avenaceum*
550	*F.avenaceum*

Table 5-1 : Isolate number and Fusarium species


Wound volume (mm³)
160
140
120
100
80
60
40
20
0
Control
16
32
35
41
43
44
46
114
120
140
310
311
360
550
(A)
Isolated number


Wound volume (mm³)
150
120
90
60
30
0
Control
16
32
35
41
43
44
46
114
120
140
310
311
360
550
(B)
Isolated number


F.avenaceum
F.merismoides
F.sporotrichioides
F.venetatum
(C)

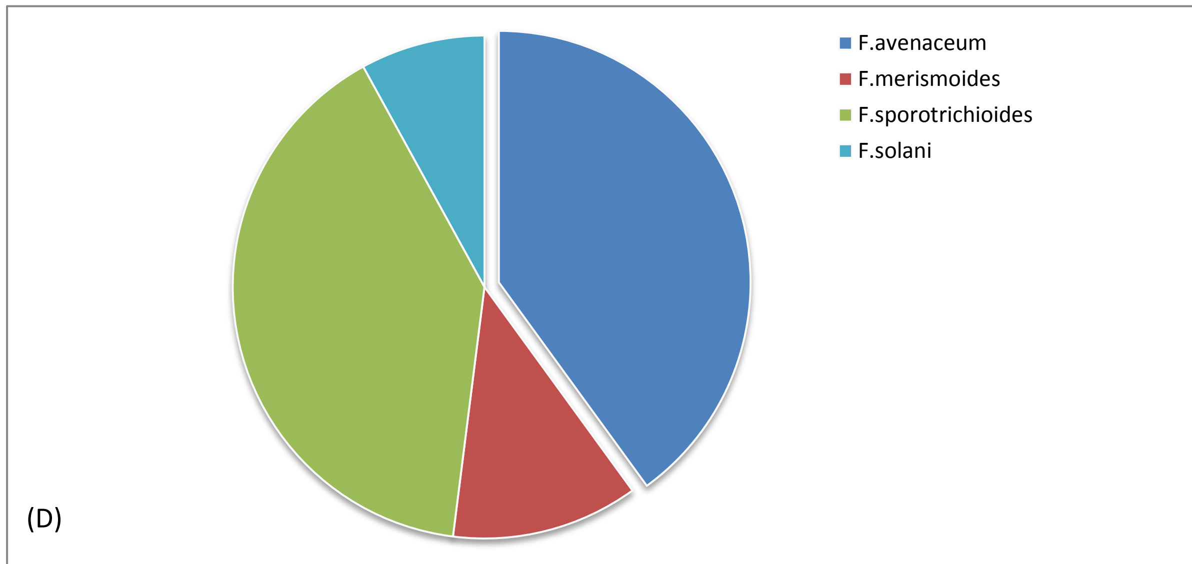


Figure 5-2: Plotted result from potato tuber pathogenicity experiment (A) Wound size (mm^3) vs isolate for cv. Mandel. (B) Wound size (mm^3) vs isolate for cv. Asterix. (C) Number of cavity vs isolate for cv. Mandel. (D) Number of cavity vs isolate for cv. Asterix.

5.4 Discussion

In the overall, damages were more severe on Mandel variety than on Asterix variety. The variety Mandel is known (*Møllerhagen et al, 2010*) to be very susceptible to dry rot having a low resistance index. Results found tend to confirm these findings.

F.avenaceum had a significant effect on wound size. For both 'Mandel' and 'Asterix' an increase in wound volume was observed (until 10 times the volume of the uninoculated control for some tuber of 'Mandel'). The pathogenicity of this specie was established in previous experiment (*Choiseul, 2007; Peters, 2008*) for other countries. In Norway, Jensen (unpublished data) also found this specie aggressive after isolation from soil adhering to tuber. A remarkable feature can be observed on the result concerning this species. It appears that the different isolates of *F.avenaceum*, despite a certain pathogenicity for all of them, have various degree of aggressiveness. Thus, isolate 550 recovered from potato field seems to be particularly pathogenic on 'Mandel' when isolate 360 recovered from cereal is more pathogenic on 'Asterix'. This particularity shows, first, that isolates recovered from

cereal fields can be pathogenic on potato but also that within the same crop type, species of *Fusarium* can have various degree of pathogenicity.

F. sporotrichioides was the second most aggressive species; this species was previously tested on potato but found weakly pathogenic (*Peters, 2008*). The effect on wound size and the formation of cavity for both inoculated 'Mandel' and 'Asterix' tuber shows that this specie is pathogenic to potato in Norway.

Isolates of *F.merismoides*, despite the occasional production of surface cavities, showed very low damages to the tuber inner flesh. Due to evaluation of a high number of isolates for this specie (4 isolates tested) belonging to both crops, *F.merismoides* can be considered as weakly pathogenic.

F. dimerum, F.equiseti, F. oxysporum, F.solani and *F.venenatum* had low to no effect on wound size. In view of results found, isolates of these species can be considered as non- pathogenic. This argument is confirmed by the presence of a dry light brown crust delimiting clearly the wound from the inside fresh flesh result of tuber healing on most of the tuber inoculated. However, except for *F.oxysporum* (4 isolates), the number of isolates tested for these species is low (1 isolate), further studies with higher amount could confirm those observations.

Two species can therefore be considered as pathogenic, *F.avenaceum* and *F.sporotrichioides*. However, it is difficult, so far, to confirm the strong pathogenicity of any of this two species. Sizes of cavity, as well as damage of the tuber flesh remain limited if compared to analog experiment *(Peters et al, 2004; 2008)*. This lack of pathogenicity among the different species of *Fusarium* can be explained by different factors. First, the size of the wound (3 mm x 4 mm) might not be sufficient enough to allow fungal infection. Many of the tests previously used, *(Choiseul et al, 2007; Peters et al, 2008)* used larger wound size (5-8 mm x 4mm). Second possibility, the media used for fungal propagation was not adapted.PDA is a rich nutrients media, for that reason it favor rapid development of fungal mycelium, but inhibit the production of conidia. As described previously, it is

unclear whether mycelia or conidia are responsible of Dry Rot primary infection, therefore the use of mycelium alone might not be relevant to obtain a proper infection.

Final possibility, species recovered are weakly pathogenic and the pathogen affecting potato in Solør area is mainly present in plant debris rather than in soil.

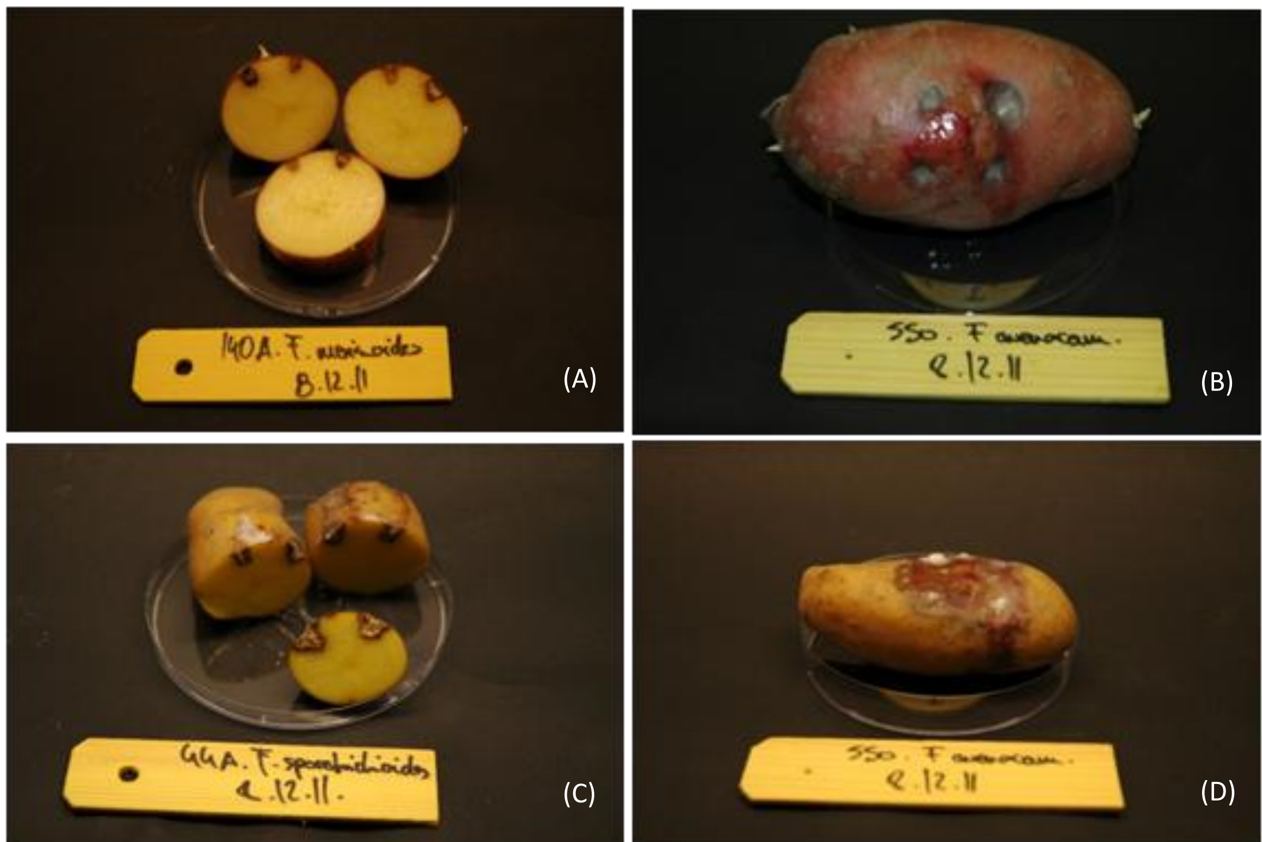


Figure 5-3: Wounded and inoculated tuber with (A) Fusarium merismoides, (B) F.avenaceum, (C) F.sporotrichioides, (D) F.avenaceum. J.Gomez.

6 Evaluation of pathogenicity of inoculums type on potato tuber

6.1 Introduction

This chapter present the work realized following analysis results of the previous pathogenicity experiment. As explained in the discussion of the preceding chapter, incidence of the most pathogenic *Fusarium spp*. did not seem sufficient to establish one species as strong pathogen. Several possibilities were hypothesized, among them, a change in inoculums type. The previous protocol was based on the use of PDA as growth media, allowing a good and fast development of fungal mycelium but limiting the production of macroconidium structure. In the following experiment, the use of SNA allowed the production of sporodochia containing macroconidia producing mycelium. The size of wounds remained unchanged but the amount of conidia applied to every wound was precisely measured. Results found will allow, first, to confirm the pathogenicity of previous *Fusarium spp*. Secondly, the use of two isolates known to be highly pathogenic on potato (*F.sambucinum, F.coeruleum*[1]) might lead to a clear comparison of pathogenicity between species. Finally, results from the two various inoculation types will allow to estimate whether conidia or mycelium were responsible of tuber infection. Isolates of *Fusarium avenaceum, F.coeruleum, F.dimerum, F.oxysporum,F.sporotrichioides* and *F.solani* were used in this experiment. In regards results found in the previous chapter, only the most aggressive isolate of each species where selected.

6.2 Material and method

The method used was first described by *Peters & Lees (2004).*Tuber were hand washed, surface sterilized with 0, 5% chlorine then wounded using a nail board (4 pins (6 mm x 2 mm) forming a 20mm side square). A 20µl drop of conidial suspension (approx 5.0 x 10^4 conidia/ml) was pipetted

[1] *Isolate collected from soil adhering to tuber from various area of Norway. P.Jensen.*

into each wound. 1 ml of every spore solution was pipetted onto a plate of water agar for viability testing (spore germination assessment).

Tubers were left to dry for ½ hour at room temperature (25 °C). Uninoculated but wounded tubers were used as controls. Wounded tubers were placed in clean, labeled, paper bags and incubated at 10 °C. After 4 weeks incubation, each tuber was cut across the wound and the width and depth of rotted tissue was measured. Mycelium and cavity presence was also assessed. As previously realized the volume of the wound was calculated using the formula of a cone volume *(Cf. page 37)*.

6.2.1 Data analysis

Statistical analyses were conducted Minitab (15-16). A general linear Model was used as analysis of variance (ANOVA) test for width, depth, volume and cavity (1- cavity; 0- no cavity) variable (y= Isolate + Variety).

For result comparison between the two pathogenicity experiment a 2 sample T-Test was used as comparison test for width, dept and volume. Graphic comparison was realized for cavity formation results.

6.3 Results

Wound size variation results showed significant differences between variety and between isolates. Significant differences were observed on both variety between depth (P value <0,001) and width (P value< 0,001) as well as between volume (P<0,001).

Plot of volume mean *(Figure 6.1)* allows quantification of these differences and allows comparison with the uninoculated control. For 'Asterix', *F.sporotrichioides* (Mean=49,2 mm. SE= 1, 05) had the strongest effect on wound size as well as as *F.coeruleum* (Mean =46,9mm. SE=1, 05) and *F.avenaceum* (44, 9 mm. SE= 1, 05) that showed the same pattern of volume increase.

F.dimerum, F.oxysporum, F.solani did not affect wound size.

For Mandel variety, *F.coeruleum* had a strong effect on wound size (Mean= 200,5 mm- SE= 3, 5). *F.sporotrichioides* (50,9 mm-3, 59) and *F.avenaceum* (46,8 mm-3, 5) had a lower but significant effect on wound size increases. *F.dimerum, F.oxysporum* and *F.solani* did not affect wound size.

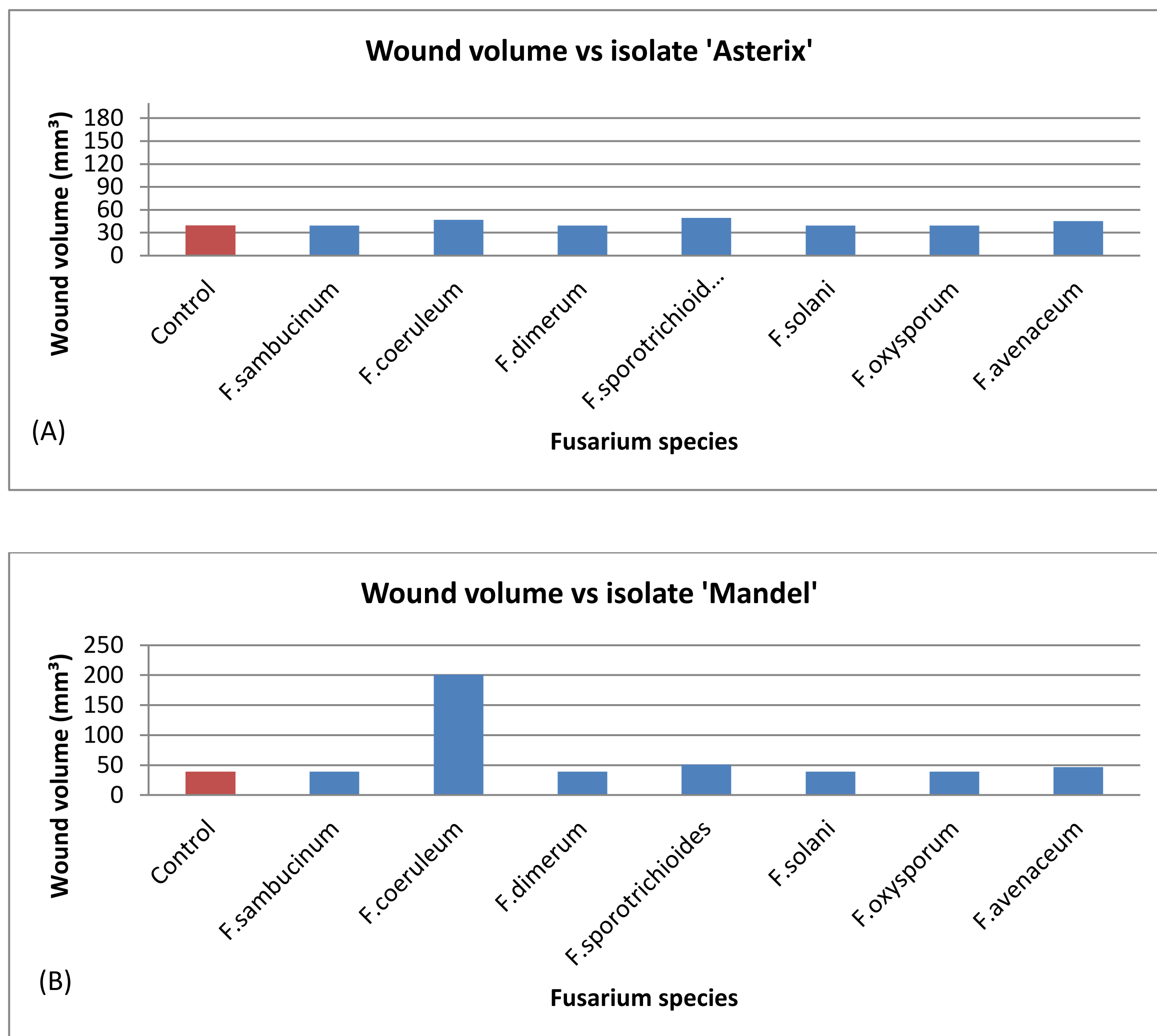


Figure 6-1: Result from the pathogenicity experiment (A) Wound volume vs isolate for Asterix variety (B) Wound volume vs isolate for Mandel variety

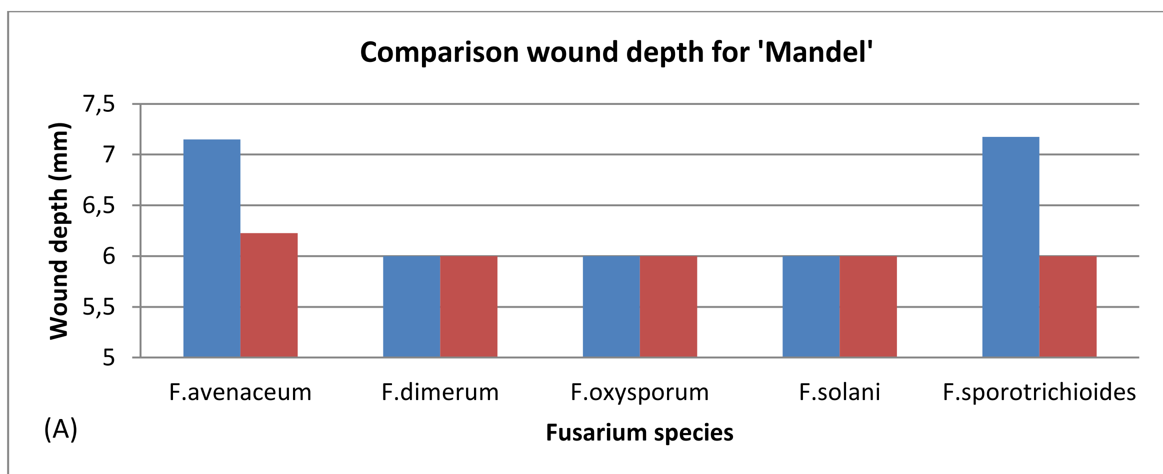
Result from the two pathogenicity experiments using isolates of *F.avenaceum, F.dimerum, F.oxysporum, F.solani* and *F.sporotrichioides* are compared hereafter.

Here was no significant differences (P value >0, 05) between wound volume for *F.avenaceum, F.dimerum, F.oxysporum* and *F.solani* neither on 'Mandel' nor 'Asterix'. Only *F.sporotrichioides*

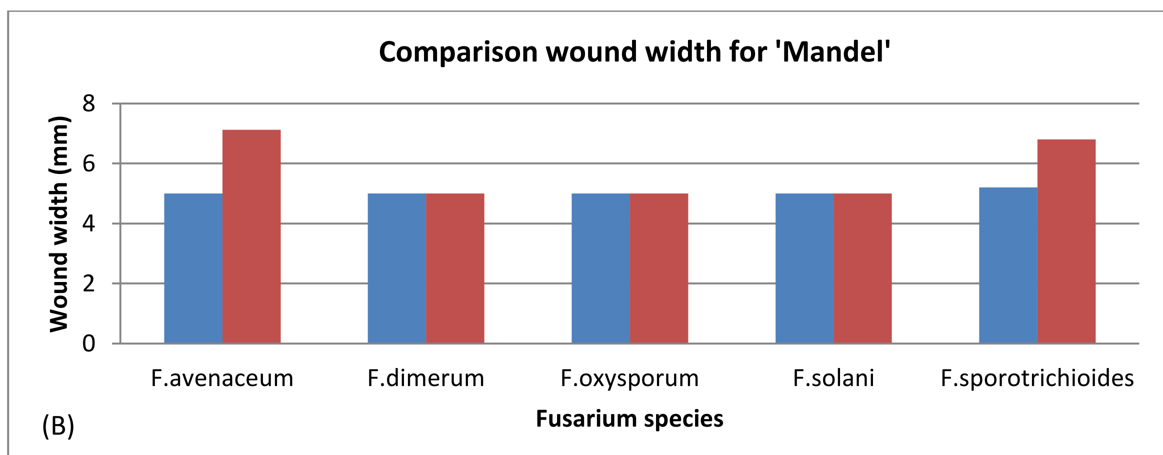
differed significantly in volume than other species regarding wound (P value< 0,001) in 'Mandel' but not in 'Asterix' (P value=0,327).

'Mandel' and 'Asterix' wound depth and width showed no significant differences between *F.dimerum, F.oxysporum* and *F.solani*. Significant differences between experiments were observed for *F. avenaceum* and *F.sporotrichioides* (P value <0,001), No significant difference was found on 'Asterix' wound width for *F.avenaceum (*P value >0, 001) between the 2 experiment.

Plotted result show that spore based pathogenicity experiment (blue) had greater effect on wound depth than mycelium based trial (red) for *F.avenaceum* and *F.sporotrichioides*. However, mycelium inoculated tuber had a larger wound width than spore inoculated tuber for *F.avenaceum* and *F.sporotrichioides* for both 'Asterix' and 'Mandel' variety.



(A)



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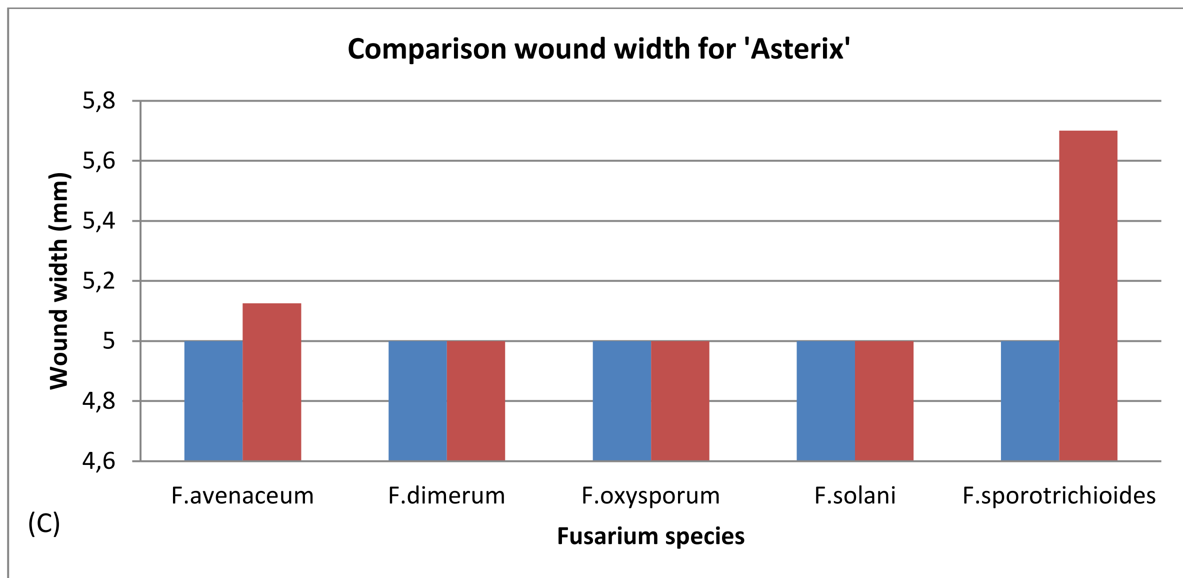
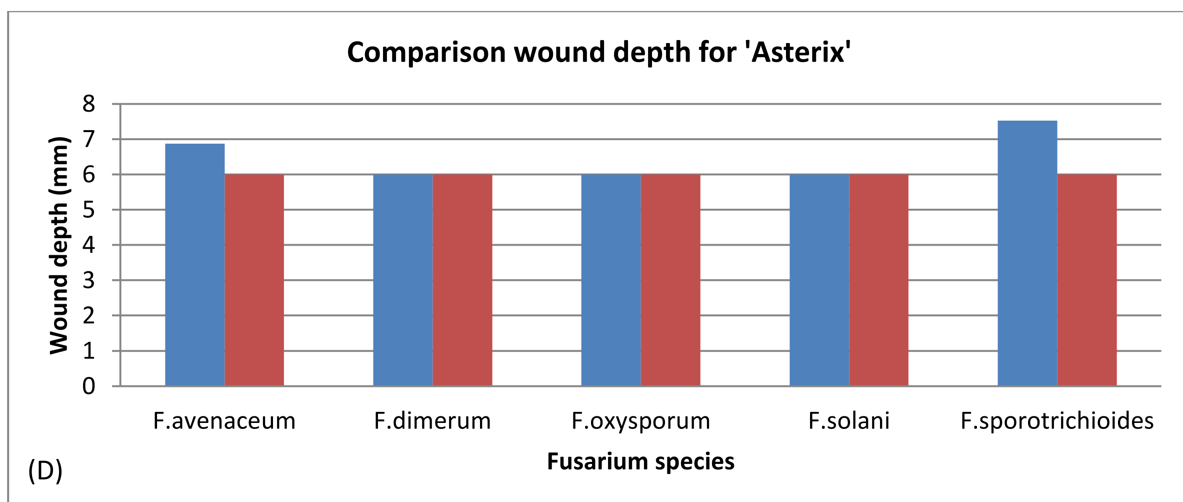




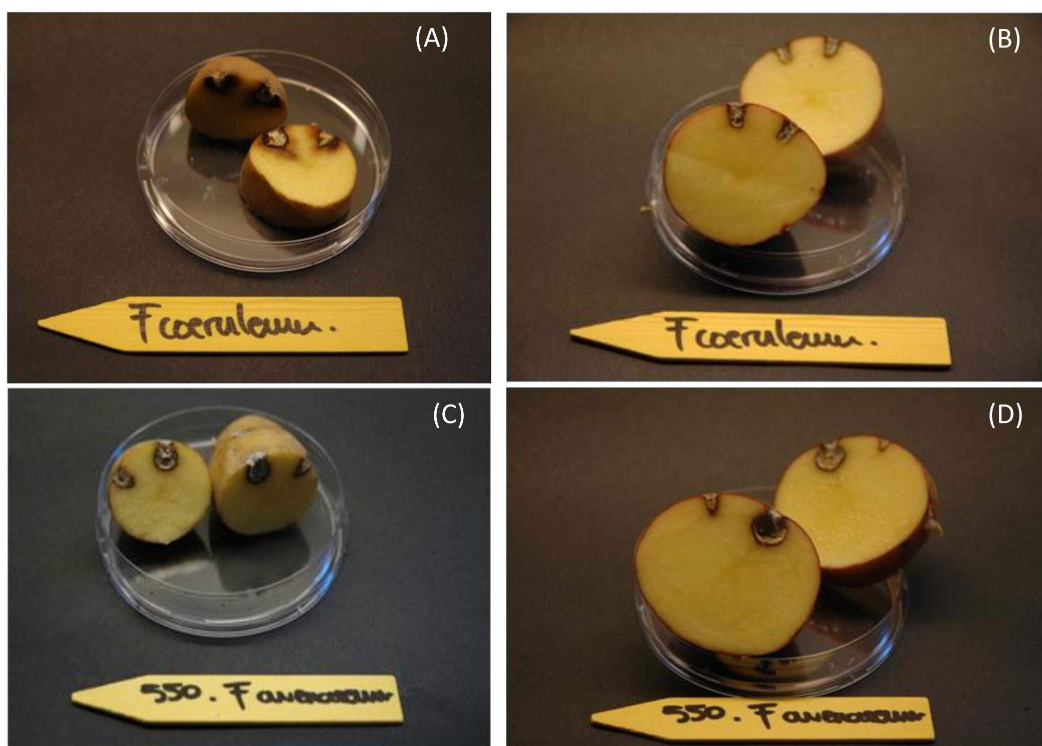
Figur 6-2: Comparison of result between mycelium (red) and spore (blue) pathogenicity test: (A) Wound depth vs isolate for Mandel variety (B) Wound width vs isolate for Mandel variety (C) Wound width vs isolate for Mandel variety (D) Wound depth vs isolate for Asterix variety

6.4 Discussion

Spore based pathogenicity experiment established the pathogenicity of three Fusarium species, *F.avenaceum* , *F.coeruleum* and *F.sporotrichioides*. *F.coeruleum* was known to be particularly aggressive on potato and caused important damage on Mandel variety *(Figure 6.3)*. Nevertheless, 'Mandel' is very susceptible to Dry Rot. 'Asterix', being more resistant showed lower damages. This experiment confirmed the pathogenicity of two of the species previously found as aggressive, *F.avenaceum* and *F.sporotrichioides,* and the low pathogenicity of the other *Fusarium*. When

compared to *Fusarium coeruleum, F.avenaceum* and *F.sporotrichioides* had a rather weak effect on wound size for 'Mandel', despite that *F.avenaceum* formed characteristic round, white mycelium filled, cavities in important quantity *(Figure 6.3)*.When comparing wound sizes on 'Asterix' ; *F.sporotrichioides* and *F.avenaceum* had similar to higher effect on tuber wound size than *F.coeruleum* confirming a real pathogenicity on potato tuber.

It appears that conidias and mycelium affect tuber wound size similarly. Nevertheless, the pattern of infection is different. Spores inoculation affect mainly wound depth while mycelium inoculations affect width. This can be due to the fact that spore inoculation is mainly targeted into the wound when mycelium inoculation is based on a deposit on the surface of the tuber and into the wound. Damage affecting the inside flesh rather than the surface would be likely to be prioritize in the evaluation of pathogenicity as well as a precise amount of inoculum applied to the wound . If following these criteria, inoculation with spores based inoculum producing fresh mycelium would be preferred.



Figur 6-3 : Inoculated tubers with (A) F.coeruleum on 'Mandel' -important damage- (B) F.coeruleum on 'Asterix' (C) F.avenaceum on 'Mandel' - formation of round cavities - (D) F.avenaceum on 'Asterix' - formation of round cavity-

7 Seed germination assay on oat and barley kernel infected by *Fusarium spp.*

7.1 Introduction

This chapter focuses on pathogenicity test carried out on cereals. Oats and Barley varieties were used in this study being the more commonly cultivated corn in the area of sampling. Were chosen, *cv.*Belinda for Oat and *cv.*Habil for Barley for their medium resistance (neither weak nor strong) to FHB (Fusarium Head Blight).This resistance were established after assay realized on harvest 2007-2009 in Barley and 2007-2010 in Oats in several field of Ås area (Akershus, South Norway) against *F. graminearum (unpublished data, Helge Skinnes)*. The objective of this experiment is to determine whether pathogenic *Fusarium* species of potato also affect cereal. Most aggressive isolates of *F.avenaceum, F.dimerum, F.oxysporum, F.solani, F.sporotrichioides* and *F.venenatum* were tested in this experiment.

7.2 Materials and methods

Inoculums were prepared using the bubble breeding method developped by *A Mesterhazy (1985).* Isolate of *F.dimerum, F.avenaceum, F.equiseti, F.oxysporum, F. sporotrichioides, F.merismoides, F.venenatum, F.solani,* extracted from soil samples of Solør area were used. The inoculum was prepared by the bubble breeding method (*Mesterhazy, 1985*) which provides 1-2 L of uniform inoculums for artificial inoculation. Liquid Czapek-Dox medium was prepared in 1 L Heerlen-Meyer and autoclaved. Inoculum was ready after 6 days at room temperature (25°C) under natural light. As mycelium might infect as well as conidia and knowing that this type of inoculums produces different amounts of both structure, pathogenicity were tested at different concentration. Seed were exposed to the original concentration, at 1:1, 1:4, and in the case of very high pathogenicity at 1:8 dilutions of the inoculums in Czapek Dox media.

Prior inoculation, inoculums were thoroughly mixed (*Ultra-Turrax. 20000 tr.min*) for 1 minute. A double layer of filter paper imbibed with 10 ml of the inoculum suspension of diverse concentration was placed in a 9 cm diameter Petri dish. 25 seeds of each variety were sown, embryo upward and placed at room temperature (25°C) in the dark.

7.2.1 Assessment

The number of germinated seeds was counted on the 2nd day, and the number of healthy seedling was then scored daily for a period of 5 days. Their mean related to the uninoculated control gave the relative germination rate. In addition, measurements of cotyledon length were taken the last day of the experiment.

7.2.2 Data analysis

Statistical analyses were conducted with Minitab (15-16). A General Linear Model were used as analysis of variance (ANOVA) test for the number of germinated seed (y= Isolated + Variety).

7.3 Results

7.3.1 Seed germination

Seed germination results *(Figure 7.2)* showed significant differences between species (P value< 0,001) and between isolates, for 'Belinda' (P value<0,001) and for 'Habil' (P value<0,001).

For 'Belinda' variety the isolate of *F.avenaceum* has strongly affected seed germination (mean = 2, 2 Germinated Seeds). In comparison, and despite an obvious effect on seed emergence, *F.oxysporum* (mean= 6, 2 GS), *F.dimerum* (mean = 6, 8 GS) and *F.sporotrichioides* (mean =6,6 GS) were rather weak pathogens. *F.venenatum* (mean= 7, 8) and *F.solani* (10, 5) did not affect seed germination, resulting in the same to higher germination rate than the inoculated control (mean= 7, 9 GS).

Germination of *cv.* Belinda (*Oat*) was more affected by *Fusarium spp.* than germination of *cv.* Habil (Barley)

For ‘Habil’ the isolate of *F.avenaceum* had notably affected seed germination (mean= 10,33 Germinated Seeds) as did *F.sporotrichioides* (mean=11,2 GS). Two other species, although having a lower effect, blocked seed germination; *F.oxysporum* (mean= 12, 7 GS) and *F.venenatum* (mean= 13, 7 GS). *F.solani* (16, 3 GS) and *F.dimerum* (14, 6 GS) had no to very low effect on seed germination when compared to the uninoculated control.

7.3.2 Cotyledon size

Cotyledon size showed significant differences between isolates in ‘Belinda’ (P<0,001) and in ‘Habil’ (P<0,001) and also between the two varieties (P value<0.001).

On ‘Belinda’, *F.venenatum* affected the cotyledon growth most (mean=2,2mm). *F.oxysporum* (mean=2,5mm), *F.avenaceum* (2, 7 mm) and *F.sporotrichioides* also limited cotyledon size. However, *F.dimerum* and *F.solani* did not have effect on cotyledon length when compared to an uninoculated control.

For ‘Habil’, *F.oxysporum* caused the strongest limitation with cotyledon growth (mean= 3,4 mm). Comparatively, *F.avenaceum* (4,4mm) and *F.venenatum* (4, 7 mm), despite visible effect on cotyledon size, have affected growth weakly. *F.solani* and *F.dimerum* had no to very low effect when compared to an uninoculated control.

When comparing graphs of germination rate and cotyledon size, it appears that there are differences between *Fusarium spp.* some affect cotyledon growth more than germination and vice versa.

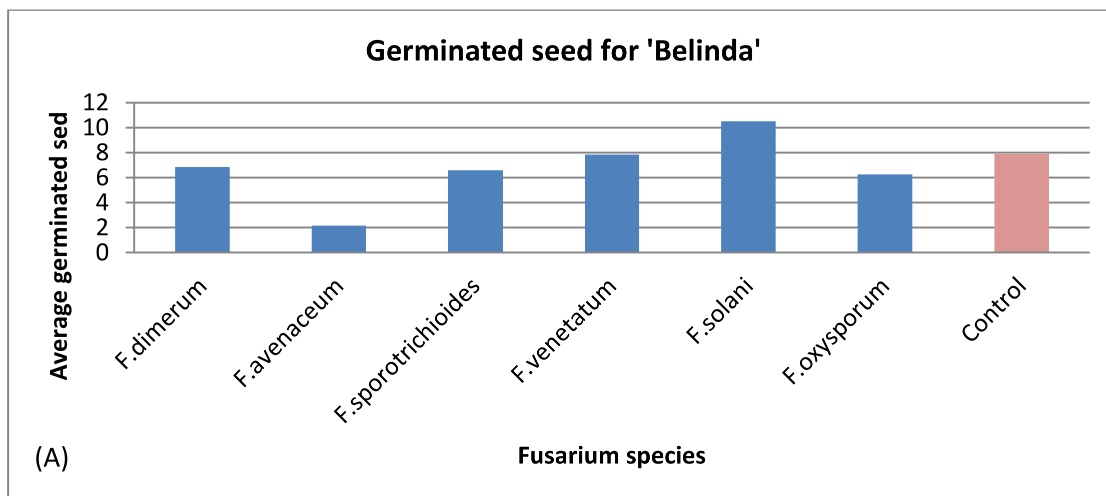
7.3.3 Visual characteristics

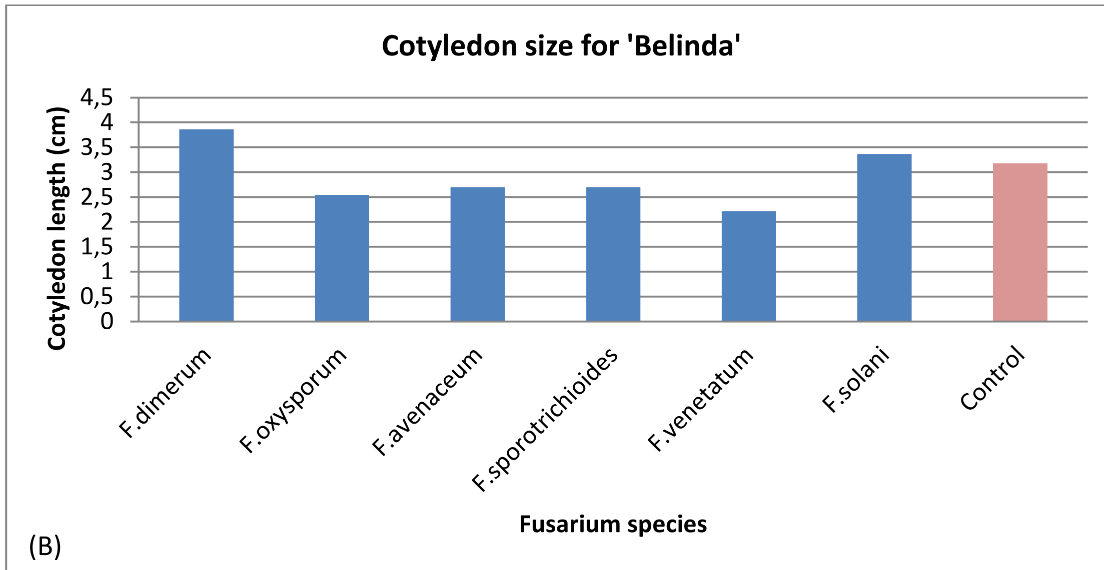
Color and presence of mycelium showed also interesting features, *F.avenaceum* had the particularity to form thick white mycelium on the kernel itself often starting by the two bottom end. This colonization appeared to block significantly seed germination. After cotyledon emergence symptom, were observed on the base of the germ with brown necrosis often followed by the decay of the young plant. *F.sporotrichioides* created an important quantity of white colored mycelium on plate

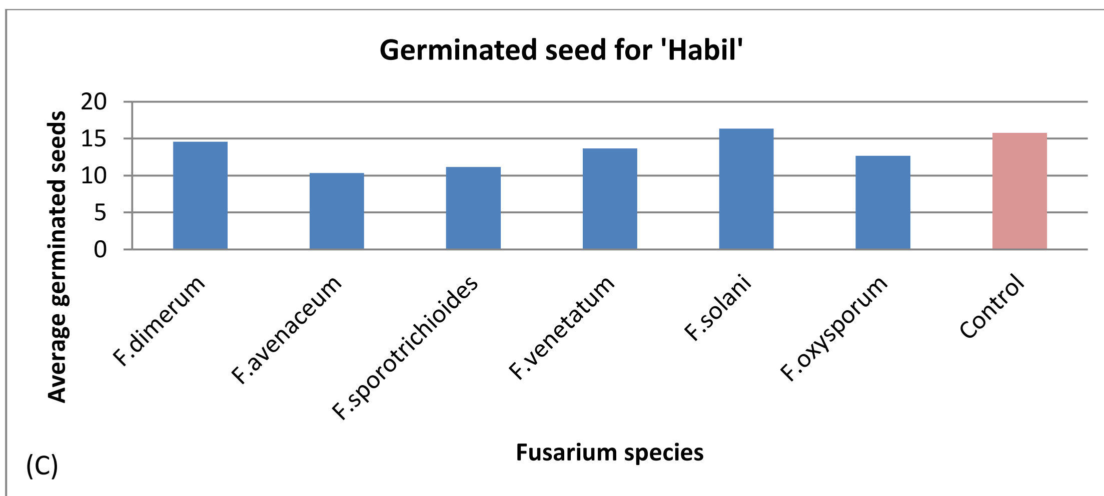
surface with the particularity of pink discoloration localized at the surrounding of each kernel after the first two days spreading to the entire plate the following days. On certain plate, the emergence of roots was not completed and supposed to be the result of fungal pathogenicity.

F.venenatum developing abundant white tainted pink mycelium covering the entire plate. This coverage might also be the reason of low growth rate more than specific pathogenicity

F.oxysporum formed white cotonish mycelium specifically located on grain, covering them completely. A purple taint was observed on the remaining space of the plate. This coverage had important effect on roots and cotyledon growth with the appearance, in numerous cases, of brown necrosis leading to quick decay of the germ. Control showed differences that need to be quoted, Habil showed a good germination rate with a small infection of the kernel during the last day. However, 'Belinda' had a rather low germination rate due to important infection of the grain leading to quick rot of cotyledons.







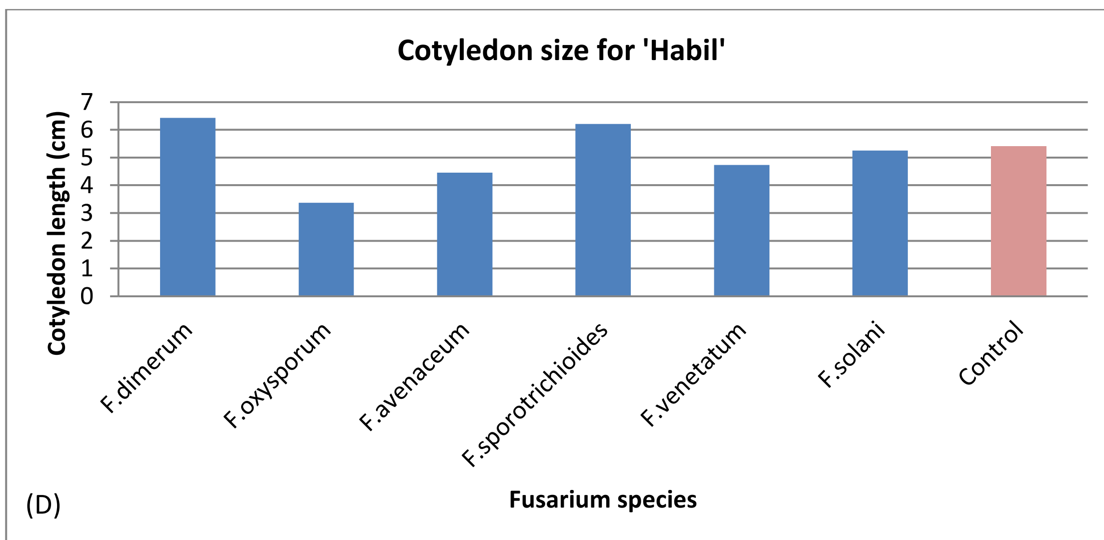


Figure 7-1 : Plotted result of seed germination assay. (A) Germinated seed vs isolate for 'Belinda'. (B) Cotyledon length vs isolate for 'Belinda' (C) Germinated seed vs isolate for 'Habil' (D) Cotyledon length vs isolate for 'Habil'

7.4 Discussion

F.avenaceum has highly affected both 'Belinda' and 'Habil' seed germination. Previous studies *(Salas et al, 1999; Xue et al, 2006)* have reported the ability of this specie to be responsible of FHB creating damages on the plant and seeds. Despite a strong effect on seed germination the pathogen affected weakly cotyledon size.

F.sporotrichioides moderately limited seed germination; this specie has been reported as pathogenic on small grain *(Salas et al. 1999).* In this experiment the isolate used did not affect cotyledon size.

F.venenatum had a significant effect on seed germination especially on Belinda variety. No report on the pathogenicity of this species on grain was found. In regards to these results, this specie could be considered as pathogenic on cereal seeds, however, only one isolate were tested. Further test on a larger number would authenticate the present findings. Isolate of this specie had a low effect on cotyledon growth.

Despite an important effect on germinated plant (cotyledon size) with foot rot symptom *(Parry et al, 1995)*, *F.oxysporum* seemed not to affect significantly germination.

F.dimerum and *F.solani* had no significant effect on seed germination or cotyledon size despite the formation and development of mycelium on plate surface and kernel for both pathogens. No reports have been found observing the pathogenicity of on e of this *Fusarium* specie.

Several observations can be highlighted in this experiment. Control seed, mainly for 'Belinda', appeared to be contaminated resulting in important rot, this might have affected result on seed germination. Secondly, many species that seemed to affect germination *(F.avenaceum, F.sporotrichioides)* did not affect further growth of the plant (cotyledon size), this phenomenon can be explained by the fact that infection of head and leaves occur via conidium or ascospores *(Parry et al, 1995)* probably poorly produced here.

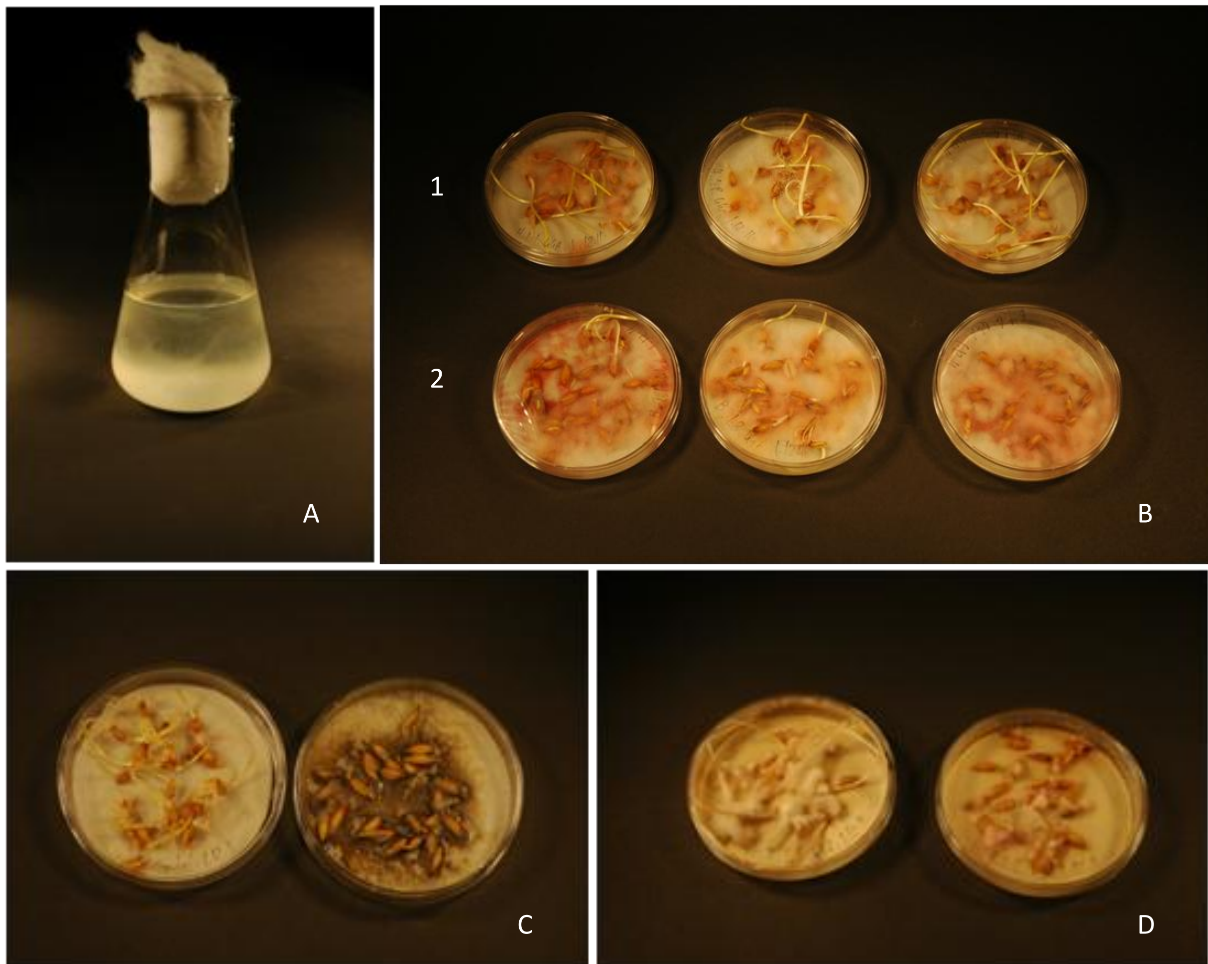


Figure 7-2 : Pathogenicity test on cereal (A) Bubble breeding method, mycelia growth in a Czapek Dox media,(B) Plate infected with F.sporotrichioides, 'Belinda' (2),'Habil' (1), (C) Control, 'Belinda' (right) and 'Habil '(left), (D) Plate infected with F.avenaceum, 'Belinda' (right) and 'Habil '(Left)

8 Comprehensive discussion

Inoculation methods

Two different types of inoculums corresponding to two inoculation methods were assessed in this work. The use of mycelium as main inoculum allowed a fast inoculation by spreading the solution over tuber surface. However, this method has the main weakness to wet the tuber skin for a long period (*ca.* 1 week) and favor the development of skin diseases. This type of contamination and the resulting symptoms can easily be mistaken with Dry Rot pathogenic effect. The enlargement of collar resulting from this infection can truncate measurement of wound width and thus, calculation of wound volume. Last but not least, the difficulties in measuring the amount of fungal pathogenic structure applied to the wound lead to a problem of comparison of pathogenicity between *Fusarium* species having various speed of growth on PDA.

The use of conidial suspension as main inoculum is a slower process – duration of sporodochia formation, spore counting, localized inoculation (pipetting) - but allows a precise measurement of inoculum quantity applied on every wound. A standardized amount (5.0×10^4 *sp.ml*) leads to accurate assessments of isolate pathogenicity and an evaluation of inoculum viability *(spore germination test*). In addition, use of localized inoculation decreases significantly the risk of collar infection by skin diseases.

In numerous publications on the subject *(Tivoli et al, 1986; Satyaprasad et al, 1999; Peters et al, 2004, 2008; Choiseul et al, 2007),* it is rarely clarified whether the presence of spore or mycelium is taking into account and their influence on Fusarium Dry Rot pathogenic abilities. In regards of results found concerning the incidence of spore and mycelium on wound size; spore based inoculation would be preferred being more accurate and resulting in more damages on tubers inner flesh.

Fusarium species and epidemiology

Among the 8 species recovered, *F.avenaceum* appeared to be the best candidate for contamination of both cereal and potato crops*. F.sporotrichioides*, despite its pathogenicity on both cereal and potato has been feebly recovered (1 isolate). The inoculums level of this specie appears too low to be responsible of symptom preservation over time on the entire Solør area. Nevertheless, *F.sporotrichioides* is commonly described as specie associated with FHB of small grain, its presence in a potato field and now a confirmed multi-crop pathogenicity, may lead to an increase in occurence for the coming years. Further sampling may confirm this hypothesis. *F.oxysporum* and *F.merismoides* were found in great amount but showed rather weak pathogenicity on cereal and potato. Their abundant quantity can be explained by their natural occurrence as soil fungi in Norwegian soil *(Kommedhal et al, 1988)*. *F.solani, F.equiseti* and *F.dimerum* found in low quantity appeared to be weak pathogen and are likely to have different plant hosts than oat, barley or potato.

Correlation between results found in the different parts of the present study show interesting features between isolated species - their density and pathogenicity - and the quantity of debris present in the field at the moment of sampling. *F.avenaceum* was isolated in two of the potato field with the highest amount of residue (tubers, stem and roots). Knowing its saprophytic abilities, survival and dispersal from plant debris a schematic of its survival in the field was depicted *(Figure 8.1)*.

① Primary infection of a field (*Cereal in Figure 8.1*) by *F.avenaceum* can be caused by air- water borne conidia *(Parry et al, 1995)*, by planted infected seeds lot as well as inter field contamination via tillage tools. Fungal spores establish and develop on the crop resulting in seedling blight, foot rot or Head Blight. Conidia form on cereal ears (under high humidity), and, by the mean of water splash, human or micro/ macro fauna activity, these structure spread, leading to extensive field infection.

② During harsh period *F.avenaceum* produces chlamydospore (overwintering structures) after conidial germination or, in case of high humidity, by the thickening of spore walls (*Hargreaves et al,*

1977). The conservation of these structures is greatly eased by the presence of crop debris furnishing a continuous supply in organic matter.

In the spring, chlamydospores germinate under warm and moist condition *(Parry et al, 1995)*, and constitute the second step in crop contamination *(Potato in figure 8.1)*. Another source of inoculum coming from infected seed tuber can also be considered *(Tivoli et al, 1983)*.

③ *F.avenaceum*, thanks to perturbation occurring during planting (mixing of plant debris, soil and potato seed), water splash or faunal activity settle on the potato crop. The pathogen develops, in a first time, within the rhizosphere to progress on the plant collar and stem. Sporulation on the stem *(Tivoli et al, 1983*) will allow easy dispersal and infection of surrounding plants.

④ At the end of the growing season, haulm destruction constitute a first favorable step in fungal propagation by a supply of dead material enhancing saprophytic activity. Mixing of vine and tuber at harvest constitute the main cause for spore deposit onto tubers. In the field, left over roots, stems and tubers will bear chlamydospore formation and favor overwintering of the pathogen.

To conclude, the association of pathogenicity on cereal and potato as well as a conservation eased by residue can be reason of symptom conservation and inoculums build up over time. For further verification of this hypothesis, it could be relevant to evaluate the tuber disease level of potato fields with high amount of residue in comparison with several other having low amount of plant debris. An assessment of *Fusarium* wilt – often caused by *F.avenaceum*- in the field could be another way to verify this assumption.

Possibilities of control

Conclusion drawn from results and discussions allows to define a certain number of action to control the development of *Fusarium* Dry Rot on Potato.

Despite the fact that it was observed in other studies that the amount of debris present in the field did not affect the quantity of inoculums in soil (*Folsom, 1959; Leach, 1985*), observations done in this work (although only four fields were sampled for each crop type) tend to show the opposite. Indeed,

higher quantity of debris in fields led to a larger and more diverse density of pathogenic *Fusarium* (strong or weak isolates). The reduction of the amount of residue in the field before winter showed to reduce infection of the following crop, by deep ploughing , or residue burning *(Dill Macky et al, 2000; Champeil et al, 2004; Salas et al, 2005)*.

Some isolates of *F.avenaceum* were recently found moderately resistant to Amazalil (Fungazil ©) in Britain *(Peters et al, 2004)*. However, this product remains the only and best preventive option against FDR. The application of this product following harvesting when tubers are the most vulnerable constitutes a crucial step in disease control.

The conservation of *F.avenaceum* over time from cereal to potato reappraises the efficiency of crop rotation. The use of cereal (wheat, barley and oat) all susceptible to FHB appears to favor reappearance of symptoms. The introduction of crop from other families would be one of the solutions to break the disease cycle. One of the alternative crop could be Spring turnip rape *(Brassica rapa ssp oleifera, Vårrybs; cv. Kulta, 187 kg.da)* belonging to the *Brassicaceae* family and used by some growers of the area. Despite a low yield, crop of this family allows to decrease significantly the amount of *Fusarium* pathogenic population *(Larkin et al, 2007)* and provide beneficial green manure. The period of 4 year minimum between two consecutive potato crops must also be respected having influences on decrease of *Fusarium* population *(Peters et al, 2004)*.

Results of compared pathogenicity of spores and mycelium showed that conidia alone have an important in-depth effect and a great ability to infect potato wound forming numerous strongly damaging cavities. This observation is relevant especially in the case of management of the disease in storage. It was shown *(Tivoli et al, 1982)* that dust borne conidium are the main cause of tuber infection by FDR coming from mixing of stems, tuber and soil at harvest *(Tivoli et al, 1986)*. Disinfection of storage prior to harvest as well as a limited time between haulm destruction and picking appears to be determining factors in integrated disease management.

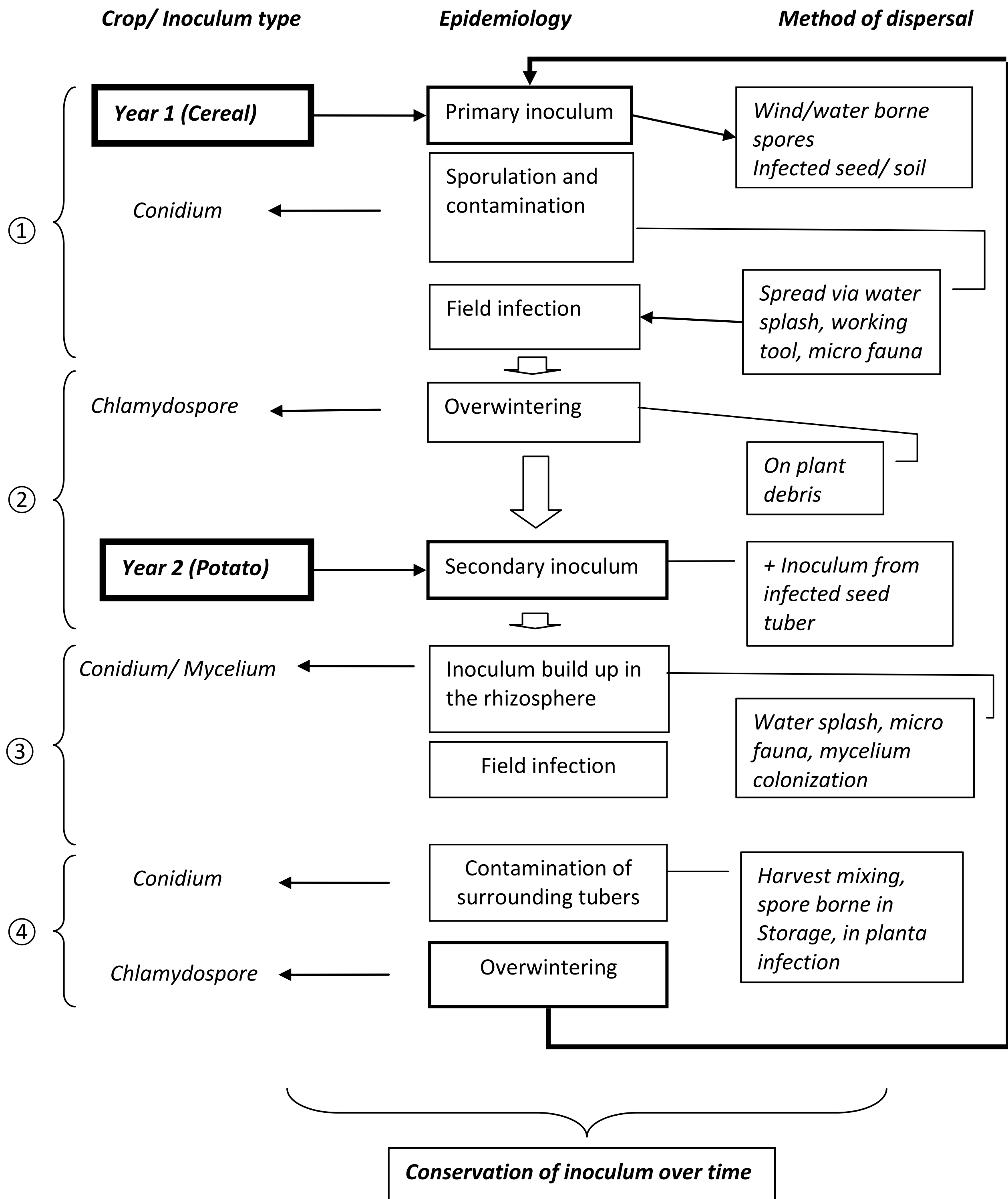


Figure 8-1 : Hypothesized schematic of F.avenaceum epidemiology in one of Solør field

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10 Annexes

10.1 Appendix I: Composition of growth media used

Potato Dextrose Agar (PDA): 20 g dextrose 20g agar 1L (d)H_20	***Peptose PCNB agar (PPA)-(Leslie et al, 2006):*** 15g Peptone 1g KH_2PO_4 0,5g Mg $SO_4.7H_2$ 750 mg PCNB 20g Agar 1L (d)H_2O
Rose Bengal-glycerin-urea (RbGU)-(Leslie et al,2006): 10g Glycerol 1g Urea 0,5g L Alanine 1g PCNB 0,5g Rose Bengal 15g Agar 1L (d)H_2O	***Czapek ipodrione dichloran agar (CZID)- (Leslie et al,2006):*** 35 g Czapek dox broth 8g Agar 1ml Dichloran 1ml Chloramphenicol 1ml Trace metal solution 1L (d)H_20 10ml Chlortetracycline 1ml Iprodrione suspension
Dichloran Chloramphenicol Peptone agar (DCPA)-(Stuart et al, 1986): 15g Peptone 1g KH_2PO_4 0,5g Mg $SO_4.7H_2$ 0,2g Chloramphenicol 2µg/ml ethanol Dichloran 20 g Agar 1L (d)H_2O	***Czapek-Dox media- (Tuite, 1969):*** 2 g Na NO_3 0,5 g KCl 0,5 g Mg SO_4 $7H_2O$ 0,01 g Fe SO_4 $7H_2O$ 0, 35 g K_2 HPO_4 30 g Sucrose 1L (d)H_2O

10.2 Appendix II: Preparation of single spore isolate

1. Culture of *Fusarium spp.* were transferred on clean media (SNA) and incubated for 7 days

2. After incubation, spore solutions were prepared at various concentrations (1:1; 1:4; 1:8)

3. 1ml of every solution was placed on water agar (0, 5%), 3 plates for every concentration gradient were used.

4. Plate were emptied from water in excess and left standing at 45 degree inclination under air flow cabinet to allow remaining water to flow.

5. Plates were left at room temperature and natural light for 12 hours

6. Plates were assessed for germination and single germinated spore was extracted and transferred on WA.

7. Plates stood 3 days at room temperature prior growth assessment and final transfer on SNA.

10.3 Appendix III

TABLE 1. — *Soils, cropping sequences and populations of* Fusarium solani *'Coeruleum' in Central and Northern Maine potato soils—1977.*

Soil Location	Crops previous to 1977: 1976 1975 1974 1973	*Fusarium solani* 'Coeruleum' prop/g dry soil
Aroostook Co.		
FK1[1]	Oats: Potatoes: Oats: Potatoes	28
FK2	Grass: Oats: Potatoes	3
FK3	Potatoes: Oats	50
FK4	Grass for over 10 years	9
FK5	Potatoes for over 10 years	58
TH1	Potatoes: Oats	34
TH2	Potatoes: Millet	16
TH3	Potatoes: Potatoes	3
TH3A[2]	Potatoes: Potatoes	2
TH4	Oats: Potatoes	100
TH4A[2]	Oats: Potatoes	51
TH5	Millet: Potatoes	25
Central Maine		
EC1	Grass for over 10 years	84
EC2	Potatoes: Corn	68
EC3	Corn: Potatoes	121
EC5	Potatoes: Oats	118
EC6	Oats: Potatoes	88
EC7	Oats: Potatoes: Corn	63
EC8	Potatoes: Oats	73
NEW1	Grass for 14 years	309
NEW1A[2]	Grass for 14 years	143
NEW1A[2]	Grass for 14 years	153

[1]FK = Fort Kent; TH = Fort Fairfield; EC = East Corinth; and NEW = Newport.
[2]Subsamples.

Statistical data

Mycelium inoculation

Mean and Standard Error (SE) Mean from ANOVA- Mandel Variety- Isolate vs wound size.

Isolate nr	Mean	SE Mean
Control	45,82	12,181
16	48,77	12,181
32	47,87	8,837
35	45,81	12,181
41	54,64	12,181
43	54,9	12,84
44	90,71	12,181
46	49,34	12,181
114	46,36	12,84
120	48,11	12,181
140	47,83	12,181
310	46,31	12,181
311	45,81	12,181
360	61,06	12,181
550	147,92	12,181

Mean and Standard Error (SE) Mean from ANOVA –Asterix variety- Isolate vs wound size

Isolate nr	Mean	SE Mean
Control	45,81	1,3631
16	45,81	1,3631
32	45,81	0,9638
35	45,81	1,3631
41	45,81	1,3631
43	45,81	1,3631
44	60,75	1,3631
46	45,81	1,3631
114	45,81	1,3631
120	46,82	1,3631
140	46,32	1,3631
310	45,81	1,3631
311	45,81	1,4368
360	55,62	1,3631
550	49,25	1,3631

Spore inoculation

Mean and Standard Error (SE) Mean from ANOVA –Asterix variety- Isolate vs wound size

Isolate	Volume (mm3)		Depth----		Width (mmm)	
nr	Mean	SE Mean	Mean	SE Mean	Mean	SE Mean
Control	39,27	1,05614	6	0,1112	5	0,03397
F.sambucinum	39,27	1,05614	6	0,1112	5	0,03397
F.coeruleum	46,947	1,05614	6,525	0,1112	5,2	0,03397
F.dimerum	39,27	1,05614	6	0,1112	5	0,03397
F.sporotrichioides	49,251	1,05614	7,525	0,1112	5	0,03397
F.solani	39,27	1,05614	6	0,1112	5	0,03397
F.oxysporum	39,27	1,05614	6	0,1112	5	0,03397
F.avenaceum	44,997	1,05614	6,875	0,1112	5	0,03397

Mean and Standard Error (SE) Mean from ANOVA- Mandel Variety- Isolate vs wound size.

Isolate	Volume (mmm3)		Depth----		Width (mmm)	
nr	Mean	SE Mean	Mean	SE Mean	Mean	SE Mean
Control	39,27	3,51512	6	0,14623	5	0,05801
F.sambucinum	39,27	3,51512	6	0,14623	5	0,05801
F.coeruleum	200,564	3,51512	9,5	0,14623	8,9	0,05801
F.dimerum	39,27	3,51512	6	0,14623	5	0,05801
F.sporotrichioides	50,992	3,51512	7,175	0,14623	5,2	0,05801
F.solani	39,27	3,51512	6	0,14623	5	0,05801
F.oxysporum	39,27	3,51512	6	0,14623	5	0,05801
F.avenaceum	46,797	3,51512	7,15	0,14623	5	0,05801

Mean and Standard Error Mean from ANOVA – Belinda variety- Germinated seed (A) and cotyledon size (B) vs isolate

Isolate	Mean	SE Mean
F.dimerum	6,833	0,8918
F.avenaceum	2,167	0,8918
F.sporotrichioides	6,583	0,8918
F.venetatum	7,833	0,8918
F.solani	10,5	0,8918
F.oxysporum	6,25	0,8918
Control	7,867	0,8918
Concentration		
0,125	8,167	0,7281
0,25	6,389	0,7281
0,5	6,278	0,7281
1	5,944	0,7281

(A)

Isolate	Mean	SE Mean
F.dimerum	3,862	0,1622
F.oxysporum	2,541	0,2295
F.avenaceum	2,7	0,3567
F.sporotrichioides	2,696	0,2471
F.venetatum	2,218	0,2119
F.solani	3,364	0,1907
Control	3,182	0,3726

(B)

Mean and Standard Error Mean from ANOVA – Habil variety- Germinated seed (A) and cotyledon size (B) vs isolate

Isolate	Mean	SE Mean
F.dimerum	14,58	0,9607
F.avenaceum	10,33	0,9607
F.sporotrichioides	11,17	0,9607
F.venetatum	13,67	0,9607
F.solani	16,33	0,9607
F.oxysporum	12,67	0,9607
Control	15,8	0,9607
Concentration		
0,125	13	0,7844
0,25	15,28	0,7844
0,5	13,83	0,7844
1	10,39	0,7844

(A)

Isolate	Mean	SE Mean
F.dimerum	6,427	0,1218
F.oxysporum	3,373	0,1977
F.avenaceum	4,457	0,2149
F.sporotrichioides	6,205	0,2226
F.venetatum	4,737	0,2036
F.solani	5,248	0,184
Control	5,405	0,327

(B)