Survey of *Fusarium* species on yellow onion (*Allium cepa*) on Öland

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Abstract

It has been observed by both onion producers and a plant protection advisor on Öland (an island off the east coast of Sweden) that basal rot is the largest contributory factor to reduced onion quality and yield. Basal rot is mainly caused by species of *Fusarium* fungi. The aim of this study was to: *a*) investigate which species of *Fusarium* that can be found in onion produced on Öland, *b*) describe the symptoms caused by the different *Fusarium* fungi found and *c*) explore, through interviews with the onion producers on Öland, the mechanisms that may be involved in the observed increase in basal rot.

Onion bulbs (*Allium cepa*) were sampled on two occasions. In total 181 onions from 11 different fields were analysed. In addition, eight onion producers were interviewed. Tissue from the sampled onion bulbs were placed on water agar for fungal growth. The fungal isolates were sub-cultured, scraped and subsequently, DNA was extracted. The identification of the fungal species was performed with PCR amplification using species specific primers as well as amplification and sequencing of the ribosomal DNA segments of translation elongation factor (TEF) and internal transcribed spacer (ITS). The sequences were compared to reference species in order to identify any species of non-*Fusarium*, as well as *Fusarium* fungi present.

The result from the first sampling was that 63.3% of the onions were infected by *F. oxysporum*, 22.4% by *F. redolens* and 2.0% by *Fusarium.* sp., *F. oxysporum* and *Trametes* sp. co-occured in 2.0% of the onions and another 2.0% of the onions had a double infection of *F. oxysporum* and *F. culmorum.* The result from the second sampling showed that 18.9% of the onions were infected with *F. oxysporum*, 3.0% had a double infection of *F. oxysporum* and *Penicillium* sp., 0.8% had a double infection of *F. oxysporum* and *Sclerotium cepivorum* and 0.8% was infected with both *F. oxysporum* and bacteria.

The symptoms observed on the sampled onions in this study were basal rot starting at the basal plate and spreading up in the scales, resulting in discoloured and watery bulb tissue. This study observed that 35.7% of all onion bulbs determined to be infected with *F. oxysporum* were symptomless, both at the time of sampling and analysis.

This study suggests that the observed decline in onion quality and yield may be a result of shorter crop rotation periods, accumulation of chlamydospores in the soil and possibly the planting of infected bulb sets.

Sammanfattning

Både lökproducenter och en växtskyddskonsulent på Öland har iakttagit att basalröta är den största bidragande faktorn till försämrad lökkvalitet och minskad skörd. Basalröta orsakas framför av olika svamparter ur släktet *Fusarium*. Syftet med denna studie var att: *a*) undersöka vilka arter av *Fusarium* som kan hittas i löken på Öland, *b)* beskriva symptomen orsakade av de funna *Fusarium* arterna samt *c)* att genom intervjuer med ölandska lökodlare undersöka mekanismerna som kan vara involverade i den iakttagna ökningen av basal röta i lök.

Kepalök (*Allium cepa*) samlades in vid två tillfällen. Totalt analyserades 181 lökar från 11 olika fält och dessutom intervjuades åtta lökproducenter. En liten lökskiva från de insamlade lökarna placerades på vattenagar för att eventuell svamp skulle växa ut. Svampisolaten renodlades för att sedan skrapas för DNA extrahering. Identifieringen av svamparterna utfördes med PCR amplifiering med artspecifika primrar samt amplifiering och sekvensering av de ribosomala DNA segmentet av translation elongation factor (TEF) och internal transcribed spacer (ITS). Sekvenserna jämfördes med referensarter för att identifiera både *Fusarium* och andra svamparter.

Resultatet från första insamlingen visade att 63,3 % av löken var infekterad med *F. oxysporum*, 22,4 % med *F. redolens*, 2,0 % *Fusarium* sp., 2,0 % hade en dubbelinfektion med *F. oxysporum* och *F. culmorum*. I ytterligare 2,0 % av löken samexisterade *F. oxysporum* och *Trametes* sp. Resultatet från den andra insamlingen visade att 18,9 % av löken var infekterad med *F. oxysporum*, 3,0 % hade en dubbelinfektion med *F. oxysporum* och *Penicillium* sp., 0,8 % hade en dubbelinfektion med *F. oxysporum* och *Sclerotium cepivorum* samt att 0,8 % hade en dubbel infektion med både *F. oxysporum* och bakterier.

Symtomen på löken i denna studie var snarlika de som beskrivits i tidigare studier, det vill säga basalröta som börjar i basalplattan och som sprider sig upp i bladen vilket resulterar i missfärjad och vattnig lökvävnad. En intressant observation var att 35,7 % av lökarna i vilka *F. oxysporum* identifierats inte hade några symptom, varken vid insamlingen eller vid analys.

Studien pekar på att orsaken bakom den upplevda ökningen i försämrad lökkvalitet på Öland är resultatet av kortare växtföljd, ansamling av klamydosporer i jorden och eventuellt på grund av sådd med infekterad sättlök.

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Introduction

It has been observed by both onion producers and a plant protection advisor on Öland (an island off the east coast of Sweden) that basal rot is the largest contributory factor to reduced onion quality and yield. Approximately one fifth (~150 hectares) of the total area in Sweden used for onion production is situated on Öland (SCB; Stefan Lundgren, pers. comm.[*]). The use of locally produced food, rather than imported vegetables, is thought to contribute to reducing the impact on the climate (Naturvårdsverket). Therefore, it is important that the yield from onion producing fields on Öland is of good quality and high quantity.

Basal rot is mainly caused by species of *Fusarium* fungi. Therefore this study has undertaken to survey which *Fusarium* species that cause the observed increase in basal rot in onion bulbs on Öland.

The aim of this study was to:

- investigate which species of *Fusarium* that can be found in onions produced on Öland.
- describe the symptoms caused by the different *Fusarium* fungi found.
- explore, through interviews with the onion producers on Öland, the mechanisms that may be involved in the observed increase in basal rot.

Background

Onion and onion production

The yellow onion (*Allium cepa*, hereafter referred to as "onion") is a member of the lily family (*Liliaceae*) (Ögren, 1992). The onion is thought to originate from central Asia and today the production is spread to all temperate zones (Schwartz & Mohan, 1995; Fogelfors, 2001). It has been produced in Sweden since the middle ages and is currently grown on approximately 800 hectares. Öland accounts for approximately 150 hectares of the onion production in Sweden, although this area is declining (Stefan Lundgren, pers. comm.[*]). Onion and other *Allium* species have shown a positive effect on human health (Schwartz & Mohan, 1995; Keusgen, 2002). The intake of *Allium* species may lower the risk of gastrointestinal cancers and may help to prevent arteriosclerosis and other cardiovascular diseases. Onions are also important as a common ingredient in Swedish and international cooking due to their special taste and nutritional value.

Onions have a diploid genome and are biennials undertaking three development phases throughout the growing season (Fogelfors, 2001).The leaves are formed and grow during the first phase. The second phase begins when the number of daylight hours exceeds 14-16 hours per day, producing the bulb through modification of the stem, the inner leaves swell and form the scales. The third and final phase is the ripening of the bulb, when the outer leaves mature, turn yellow and start to wilt, eventually forming a hard peel around the bulb. The onions are mechanically pulled from the soil when 50-80% of the leaves have wilted. They are then left to dry in the field for about a week, if the weather is dry, before harvesting.

[*] Stefan Lundgren, Production manager, Kalmar Ölands Trädgårdsprodukter. Personal communication. 2010-12-17

Onions have fleshy leaves and fibrous roots, which form a small, shallow root system (Fogelfors, 2001). The roots are sensitive to oxygen deficiency and are also too shallow to reach deeper soil moisture; therefore the amount of water is vital for successful onion production (Ögren *et al.,* 2003). The average precipitation on Öland during the growing season, approximately April to August, is ~38 mm per month (Sveriges meteorologiska och hydrologiska institut, SMHI). Irrigation of the onion fields is therefore necessary, allowing the amount of water to be controlled in order to obtain the best conditions for growing onions (Ögren *et al.,* 2003). The roots can generate a symbiosis with mycorrhizae, which can stimulate the nutrient uptake from the soil. Onions also have a low demand for nitrogen, especially in the beginning and end of the growing season.

A site for successful onion production should be on warm soil as the soil will dry earlier in the spring, lengthening the onion growth period. For instance, the well-drained, humus rich, light sandy soil situated in the south and middle of Öland is favourable for onion production (Ögren *et al.,* 2003; Nilsson, 1983; 1987). A long growing season is preferential for onions to develop successfully. Öland has often the most sun hours per year in Sweden, making it a suitable place for growing onions (Ögren, 1992; Schwartz & Mohan, 1995; Fogelfors, 2001). Onions can be planted as seeds, small bulb sets or seedlings. The latter two are used in order to get a head start in the growing season and therefore an earlier harvest can be achieved. Weed control is also considered important, as onions have a low competition advantage. Crop rotation should allow at least four to six years between onion species to minimize accumulation of pathogens in the soil.

Species of *Fusarium* causing disease in onion

Four different species of *Fusarium* have been observed in onions (Table 1), although it is not fully understood to what extent *F. avenaceum* causes disease in onions, or if the onion is merely used as an alternative host.

Table 1. Four different *Fusarium* species found in onions.

Fusarium spp.	Disease	Reference
Fusarium oxysporum	Fusarium basal plate rot	Schwartz & Mohan, 1995
Fusarium redolens	Fusarium basal plate rot	Booth, 1971; Shinmura, 2002
Fusarium culmorum	Weakly virulent	Galván *et al.,* 2008
Fusarium avenaceum	Unknown	Galván *et al.,* 2008

Fusarium oxysporum

F. oxysporum is considered one of the world's most harmful pathogens (Correll, 1991; Schwartz & Mohan, 1995). It is a common soil borne pathogen, which has a high level of host specificity with over 120 different *formae specialis* (f.sp.). *F. oxysporum* f.sp. *cepae* causes serious disease in onions with recorded yield losses of more than 50% (Lacy & Roberts, 1982). Examples of *F. oxysporum* f.sp. are shown in Table 2.

Table 2. Different *formae specialis of F. oxysporum* and the disease they cause.

Formae specialis of *F. oxysporum*	Disease	Reference
F. oxysporum f.sp. *apii*	F. yellows of celery	Lori *et al.* 2008
F. oxysporum f.sp. *cepae*	Fusarium basal plate rot	Schwartz & Mohan 1995
F. oxysporum f.sp. *cubense*	Fusarium wilt of banana	Agrios 2005
F. oxysporum f.sp. *cucumerinum*	Fusarium wilt of cucumber	Jenkins & Wehner 1983
F. oxysporum f.sp. *lycopersici*	Fusarium wilt of tomato	Agrios 2005
F. oxysporum f.sp. *niveum*	Fusarium wilt of watermelon	Zhang *et al.* 2005

F. oxysporum f.sp. *cepae* occurs worldwide, causing fusarium basal rot in a number of *Allium* species in addition to onion, such as chive, garlic and shallot (Schwartz & Mohan, 1995).The pathogen is a deuteromycete and has no known teleomorphic (sexual) stage (Brayford, 1996). *F. oxysporum* f.sp. *cepae* produces mycelium as well as three types of asexual spores: microconidia, macroconidia and chlamydospores (Cramer, 2000). Microconidia are the most commonly produced spores and are 5-12 μm in length. They are typically without septate and their shape varies from oval to kidney shaped. Macroconidia have a characteristic falcate shape making them easily identifiable. In addition, they typically have three or four septa (Cramer, 2000; Agrios, 2005). Chlamydospores are produced in or on older mycelium, have one or two round cells and have thick cell walls, which defend the cells against degradation and antagonists. This type of spores helps *F. oxysporum* f.sp. *cepae* survive in the soil, in the absence of its host, for a very long time, usually indefinitely.

Fusarium species can survive either as mycelium or as spores on plant debris in the soil (Agrios, 2005). Although in colder climates, it is necessary for *Fusarium* species to produce chlamydospores to survive unfavourable periods, such as winter. The fungus can disperse with soil particles and plant debris, which can be transported by both water and farm equipment (Cramer, 2000). Longer transportation of the fungus can be by infected plant material or soil attached to them, for example bulb sets for sowing.

The infection of *F. oxysporum* f.sp. *cepae* commences when the onion comes into contact with the fungus, usually via the soil (Abawi & Lorbeer, 1971). The fungal spores germinate and produce mycelium, penetrating both damaged and healthy root tissue. The fungus then grows into the stem base plate and advances into the bases of the fleshy leaves. The fungus can also penetrate directly through the basal plate of the bulb or the bases of the fleshy leaves below the soil if the tissue is damaged or there is a high concentration of propagules in the soil. The mycelium of the fungus grows into the intercellular spaces or inside the cells of the roots and stem plate tissue as well as the vascular system, *i.e.* the xylem. The penetration of the xylem reduces the ability of the plant to transport water, causing the plant to wilt. Bulb injuries caused by the onion maggot, *Delia antique*, increase the incidence of disease (Everts *et al.*,1985; Schwartz & Mohan, 1995).

The initial symptoms of fusarium basal rot on the leaves of seedlings can be difficult to observe and plants can be killed before any other symptoms can be visually recognised (Cramer, 2000). Symptoms on seedlings include delayed emergence, seedling damping-off and stunted growth. The symptoms above ground of mature bulbs are chlorosis and the curving of all leaves (Schwartz & Mohan, 1995; Tahvonen, 1981; Cramer, 2000). The chlorosis progresses to necrosis from the tip of the leaves and downwards, eventually killing the plant. The rot will spread from roots through the stem plate and up the storage leaves and

may cause discolouration of the outside of the bulb. The affected tissue appears brown or reddish-brown and watery when the onion is cut in half. The stem plate is often the first part of the onion to show symptoms, usually as brown discolouration or occasionally white mycelium. When the entire stem plate is fully decayed it can easily be separated from the rest of the bulb. The roots typically rot, causing the plant to die. Some bulbs that are infected in the field may appear healthy and later develop rot in storage (Brayford, 1996).

The fungus develops when the soil temperature is between 15-32 °C (Schwartz & Mohan, 1995; Cramer, 2000). Studies showed that there is almost no disease when the soil temperature drops below 12°C and the temperature optimum for the development of the fungus is 25-32°C.

Several crop management procedures can be performed to prevent the spread and infection by fusarium basal rot, such as using resistant onion cultivars, crop rotation, solarisation, regular cleaning of equipment, dipping seedlings in fungicide and fumigation of the soil (Schwartz & Mohan, 1995; Cramer, 2000). Once the onion bulb or seed is planted in an infected field there is no known method to reduce the likelihood of infection by *F. oxysporum* f.sp. *cepae*. Storage of the harvested onions at less than 4ºC reduces losses, since the fungus develops very slowly at low temperatures.

Fusarium redolens

F. redolens is genetically very similar to *F. oxysporum* and was at one stage thought to be a variety of *F. oxysporum*, but studies have now shown that they are distinct from each other (Baayen *et al.*, 2001). *F. redolens* produces chlamydospores, short monophialides, microconidia and stout macroconidia (Shinmura, 2002). The symptoms resulting from a *F. redolens* infection are similar to those caused by *F. oxysporum*, such as wilting symptoms, damping-off in seedlings and sometimes a cortical rot. The only practical control measure according to Booth (1971) is to use resistant crop varieties. Shinmura (2002) showed that *F. redolens* can infect *A. cepa* and *A. fistulosum* (Welsh onion), where the recorded symptoms were leaf blight, plant stunting and root rot. The fungus is profited from a temperature around 25°C and a high concentration of salts in the soil increase the severity of the disease.

Fusarium culmorum

F. culmorum has proved to be weakly virulent on *Allium* species compared to *F. oxysporum* and *F. redolens*, which are highly pathogenic (Galván *et al.*, 2008). It is not known whether *F. culmorum* has a pathogenic effect on onions or if it merely uses them as an alternative host. Specific strains of *F. culmorum* are typically the causal organism of fusarium basal rot in garlic, although these strains are non-pathogenic to onions (Schwartz & Mohan, 1995). *F. culmorum* does not produce microconidia, however, macroconidia are produced abundantly and have distinguished septates that are stout and thick-walled (Figure 1) (Booth, 1971). The fungus also produces oval to spherical chlamydospores, which are formed on conidiosphores, either singularly, in chains or as a cluster (Booth, 1971; Schwartz & Mohan, 1995). Root extracts from onion and garlic have shown to prevent germination of other strains of *F. culmorum* which are pathogenic to for example wheat (Clerke, 1966).

Fusarium avenaceum

F. avenaceum (teleomorph *Gibberella avenacea*) is known to cause root rot in for instance field peas, wheat and maize, but has also been found in onion in tropical regions such as Uruguay (Booth, 1971; Galván *et al.*, 2008; Feng *et al.*, 2010). It is not yet known whether *F. avenaceum* causes basal rot in onion or if the onion is merely used as alternative host (Galván *et al.*, 2008).

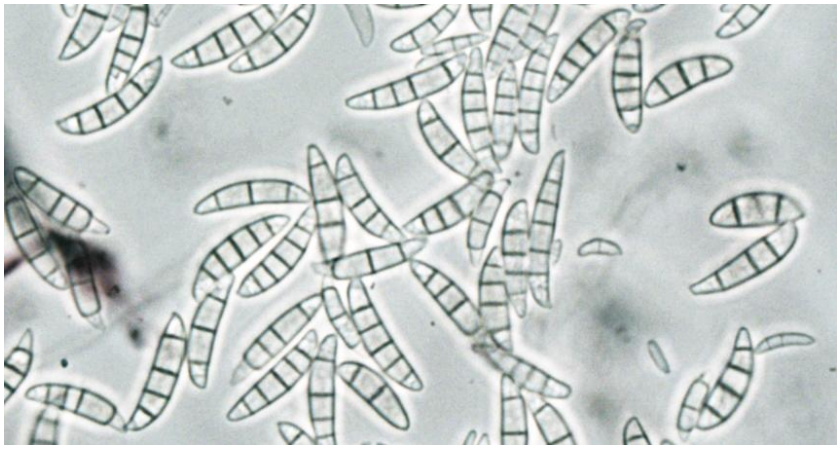

Figure 1. *Macroconidia of* F. culmorum *isolated from an onion bulb. The average size of the large conidia is 31.5 µm. (photo Sara Lager).*

Species specific primers as well as more general primers, such as the translation elongation factor (TEF) and internal transcribed spacer (ITS) primers, can be used to identify the different species of *Fusarium* as well as other fungal species that may infect onions (Geiser *et al.,* 2004). A TEF-primer is used for amplification and sequencing of the TEF-region, allowing the detection and identification of a broad range of *Fusarium*. The TEF-primer amplifies ~700 bp of the TEF-region. The subsequent sequencing of this highly informative region allows the different species of *Fusarium* fungi to be distinguished from each other. The ITS-primer, used for amplification and sequencing of the ITS region, is considered to be a more general primer in comparison with the TEF-primer (O'Donnell *et al.,* 1998a).

Ocular inspection of the morphology of the fungus is an alternative method used to identify *Fusarium* species. Although a fully reliable result using this method is hard to achieve since the morphology of the species is very similar. In addition, the shape and colour of fungal isolates can change depending on the environment in which they grow (Booth, 1971).

Material and Methods

Sampling procedure

Eight onion producers were selected because of their location, as they were all situated on the island of Öland off the east coast of Sweden (Figure 2). Eleven fields, managed by the eight producers, were subsequently selected for sampling. More than one field per producer was selected if the producer employed alternative management techniques. For instance, one producer used different crop rotation periods on two fields and onions were therefore sampled from both fields (Skärlöv N and Skärlöv S). All onion producers planted seed onions except one producer who used onion bulb sets as well as planting seeds, hence three fields (two bulb set fields: Hammarby N and Hammarby S and one seed field: Bjärlinge) managed by this producer were selected for sampling. The 11 onion fields were sampled on two different occasions. The samplings were considered to be biased on both occasions, since approximately half of the onions were sampled because they showed various disease symptoms. The onions from the first sampling were used for isolating fungi and extraction of DNA from the isolated fungi, whereas the onions from the second sampling were used for growing fungal isolates and extracting DNA from the isolated fungi as well as for extracting DNA directly from the onion tissue.

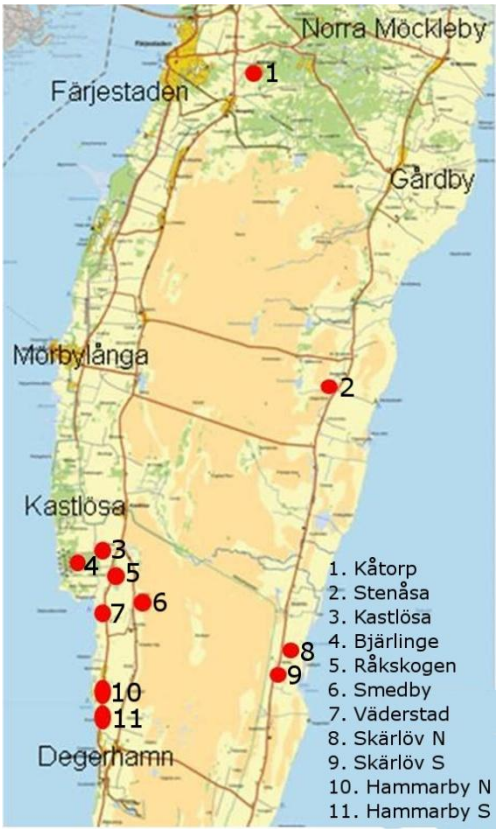

Figure 2. *Location of the 11 sampled onion fields on Öland (Mörbylånga kommun and modified by Sara Lager).*

The first sampling, of 20-40 onions from each field, was made on the 2nd of July 2010. The onions were sampled from an area of approximately half a hectare per field. Both onions with wilted or discoloured leaves, as well as, onions with healthy leaves were selected in the laboratory, in order to obtain the full range of visible symptoms, as well as determine the presence of any latent infection. The onions were then selected by the condition of the roots or other observable characters in the laboratory and were divided into either of the groups: smut, allium white rot, healthy roots (but with slightly discoloured basal plate), rotten roots and no symptoms. The "smut" and "allium white rot" -categories were chosen since the onion showed symptoms of these diseases (Schwartz & Mohan, 1995). The symptoms of smut (pathogen *Urocystis cepulae*) and allium white rot (pathogen *Sclerotium cepivorum*) can be seen in Figures 3a and 3b.

The second sampling took place on the 31st of August 2010 and onions were sampled from an area of half a hectare per onion field. From each field, between 20 and 110 rotten and healthy looking onions were collected. In the laboratory, six healthy looking and six rotten onions per field, where possible, were selected to include a broad range of visible symptoms, in order to determine if there was any latent infection in the healthy looking onions. In total, 181 onions were collected over the two sampling occasions and these were all subsequently analysed by this study.

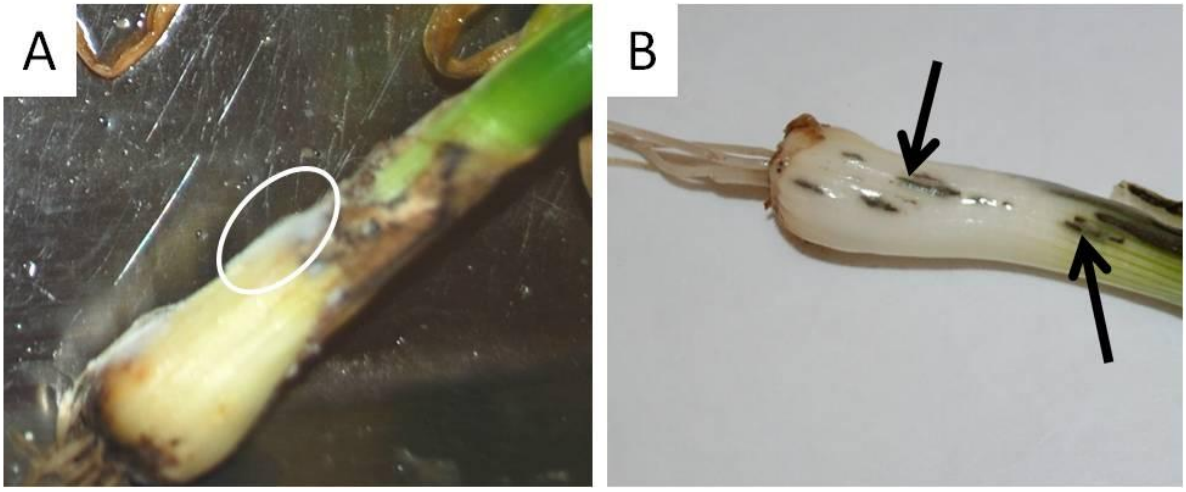

Figure 3. *Symptoms of A) allium white rot* i.e. *white fluffy mycelial growth on stem plate and bulb and B) smut i.e. dark striped lesions on the outer surface (Photo Sara Lager).*

Sample treatment

All the sampled onions from the first sampling were cleaned and divided in halves and between five to ten of the onions were selected to grow fungal isolates. The isolation was as follows: the cut surface was rinsed and sterilised with 70% ethanol. Thereafter, a slice of onion of approximately one cm^2 including basal plate tissue, as well as bulb tissue, was put on water agar (Figure 4a). The slices from the onions with visible symptoms were cut from the border region of the lesion, including rotten as well as healthy onion tissue (Figure 4b). The mycelia grown from the bulb tissue was sub-cultured on water agar and stored in a fridge for approximately two months. Some of the fungal isolates were contaminated during storage and were therefore disposed. Mycelium from the fungal isolates was transferred from the water agar to half-strength PDA (potato dextrose agar). Some isolates seemed to include more than one fungal species and in those cases, the different fungi were transferred to separate plates of PDA. The mycelium was scraped from the agar plates when they had grown on the PDA and placed in two ml microcentrifuge tubes with five glass beads (3 mm Ø) for DNA extraction. The plates and spores from the isolates were photographed in stereomicroscope (appendix 1, figures I-V).

The selected onions from the second sampling were divided in halves. One half was photographed and the other half was rinsed and peeled. The rinsed half was cleaned with a sterile cut across the bottom part of the onion and the slice was subsequently placed on water agar for fungal growth. The fungal isolates were isolated, subcultured, scraped and put in microcentrifuge tubes as described for the first sampling, although no storage was needed with these isolates. In addition, a second slice was cut from the onions from the second sampling and were put directly in two ml microcentrifuge tubes with five glass beads (3 mm Ø) and frozen. Both slices included tissue as described above. Each tissue slice had a fresh weight of about 0.08 g.

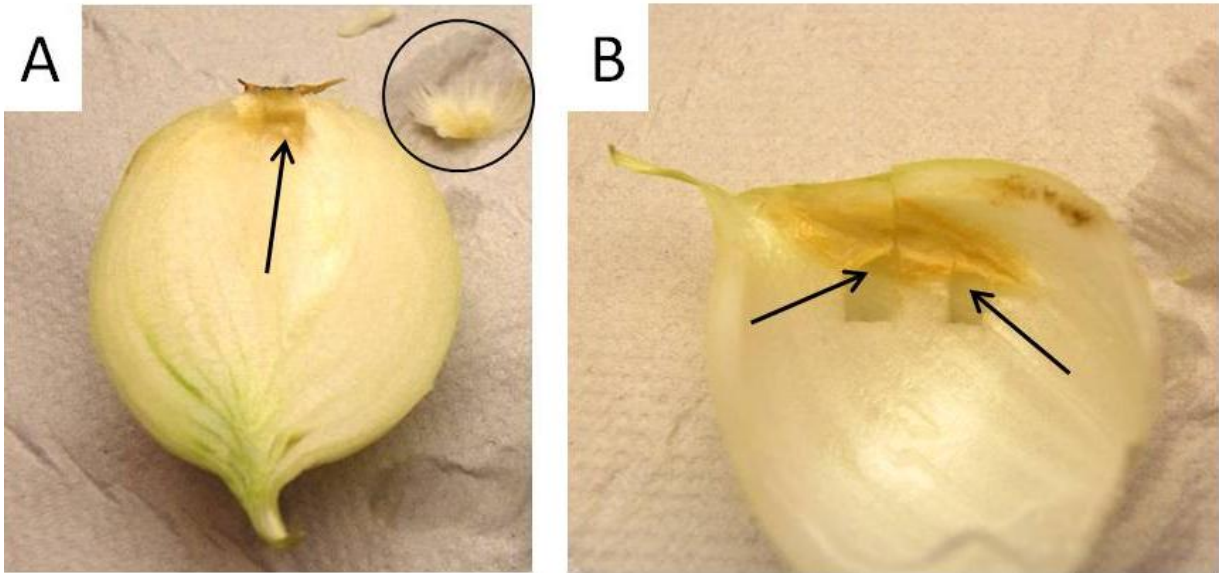

Figure 4. *A) A symptomless onion, having been cut in half, showing the location of the slice (arrow) used for culture. The slice, containing both basal plate and onion bulb tissue, is shown in the inset image. B) An onion showing symptoms of disease after being cut in half. A slice for culture was cut from the border region of the symptom to include both rotten and healthy tissues (arrows). (Photo Sara Lager).*

DNA extraction

DNA was extracted from both onion tissue and fungal isolates. The samples with onion tissue or mycelium were freeze-dried overnight and thereafter shaken at 5500 rpm for 30 seconds (Procellys, Bertin Technologies). Next, 800 µl CTAB was added to the samples, which were shaken again and incubated in a heat block at 65°C for an hour. The samples were then centrifuged at 13000 rpm for 5 minutes. The fluid was transferred to 1.5 ml tubes and 500 µl of chloroform was added. Next, the samples were centrifuged at 13000 rpm for 7 minutes. The chloroform step was conducted twice for a few samples, as some components did not dissolve in the chloroform during the first treatment. The top layer was transferred to new 1.5 ml tubes containing 700 µl isopropanol. The tubes were turned over a few times and the samples were incubated in room temperature for one hour. Next, they were centrifuged at 13000 rpm during 5 minutes. Afterwards, the fluid was poured out and the samples were rinsed with 200 µl of 70% ethanol and the fluid was once more poured out. Thereafter, the samples were left to dry for one hour and then dissolved in 70 µl ddH_2O and stored in a fridge at 4°C.

The concentration of DNA was measured with a spektrophotometric method. DNA absorbs light with a wave length of 260-280 nm and the amount of DNA is equivalent to the absorbed light (NanoDrop, Spectrophotometer, Thermo Scientific). The samples from the cultures, with a concentration above 100 ng μl^{-1} were diluted 1:20 and the samples with a concentration below 100 ng DNAμl^{-1} were diluted 1:10 and thereafter frozen. The DNA from the onion tissue was diluted first as for the fungal isolates and then diluted to about 0.5 ng DNA μl^{-1} to a final volume of 50µl.

Identification of fungal species

Species specific primers were used to determine which of the three *Fusarium* species known to infect onion that had infected the onions in the current study. The species specific primers were used for amplification of DNA from the fungal isolates as well as from the onion tissue. TEF and ITS primers were also used for amplification of DNA but only from the fungal isolates. Reference isolates for the species were used as a positive control to confirm that the matches were correct. PCR mixtures for the primers were blended according to the recipe in appendix 2, table I. All the work with the DNA samples and the PCR mixtures was conducted on ice. The PCR conditions are described in appendix 2 and the sequences of the different primers are presented in table 3.

Table 3. *Sequences and product size of the primers used for detecting* Fusarium *species.*

Pathogen/ region	Primer name	Sequence	Size (bp)	Reference
F. culmorum	Fc01-F Fc01-R	ATGGTGAACTCGTCGTGGC CCCTTCTTACGCCAATCTCG	570	Nicholson *et al*., 1998
F. oxysporum	Clox1-F Clox2-R	CAGCAAAGCATCAGACCACTATAACTC CTTGTCAGTAACTGGACGTTGGTACT	534	Mulè *et al*., 2003
F. avenaceum	JIA-F JIA R	GCTAATTCTTAACTTACTAGGGGCC CTGTAATAGGTTATTTACATGGGCG	220	Turner *et al*., 1998
ITS	ITS-1F ITS4	CTTGGTCATTTAGAGGAAGTAA TCCTCCGCTTATTGATATGC	~900	Gardes & Bruns, 1993
TEF	ef1-F ef2-R	ATGGGTAAGGAAGACAAGAC GGAGGTACCAGTGATCATGTT	~700	O'Donnell *et al*., 1998c

Electrophoresis was used to confirm positive amplification of the PCR products. The PCR products were applied in wells of an agarose gel (1 %) stained with 0.01% SYBR® safe DNA gel stain. In the first and last well a product size ladder was applied to confirm the size of the PCR products. Larger DNA products in the PCR mixture do not travel as far in the gel as the smaller ones when an electric field is applied. The travelled distance should be the same for the PCR products from the samples and from the reference isolates to ensure a match.

Forty-five µl of the TEF and ITS PCR products were cleaned with 81 µl magnetic marbles (Agencourt AMPure) and processed according to the manufacturer's manual. The samples were set to dry at 37°C over night and were then sent to Macrogen Inc. in Seoul, South Korea (http://dna.macrogen.com) for sequencing.

The sequences were assembled in contigs with 95-98% accuracy in the program SeqMan Genome Assembler (DNAStar Inc.). Thereafter the sequences were matched with reference sequences in the National Centre for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov) and the fungi could be identified using the BLASTN algorithm of at least 99% matching.

Interview with onion producers

Two telephone interviews with each of the eight onion producers were conducted. The purpose of the first interview was to receive information about the onion production as well as information about which onion diseases they encountered in the onion fields. The additional

interview was conducted after harvest in order to receive information about how good the onion quality was compared to previous year's production.

The questions asked during the interviews were:

Have there been any problems with the onion quality? Rot or other. Yes/No
If yes, in which way?
Have the symptoms emerged in the field or in storage?
For how long have onions been produced on this farm? /When were onions produced here for the first time?
How often have onions recurred in the crop rotation?
Has the crop rotation changed the last 10 years? (Does onion recur more often now then 10 years ago?)
Are bulb sets used for planting or have they been used earlier?
Additional interview: How was the quality of the onions harvested 2010?

Result

Sample treatment

The number of fungal isolates from the first sampling was limited to between 0-10 onions per field due to contamination during storage (Table 4). Nine onions with symptoms were used for analysis from the second sampling from the location "Råkskogen" as three of the sampled onions first appeared to be healthy, but turned out to have symptoms when they were cut open. The second sampling took place late in the growing season and therefore had some fields already been harvested or pulled. Discarded onions left on the field after harvest were sampled in the harvested fields.

Table 4. *The number of onions sampled and subsequently used for analysis from the first sampling as well as the number of onions showing symptoms and the state of the onions in the field during the second sampling.*

	First sampling		Second sampling		
Location	sampled	used	sampled	symptoms	State in field
Kåtorp	5	3	12	0	Still growing
Stenåsa	5	3	12	1	Pulled
Kastlösa	5	5	12	0	Pulled
Bjärlinge	5	5	12	5	Harvested
Skärlöv N	5	0	12	0	Still growing
Skärlöv S	5	3	12	1	Still growing
Väderstad	5	4	12	6	Pulled
Råkskogen	7	5	12	9	Still growing
Smedby	5	2	12	5	Pulled
Hammarby N	9	9	12	6	Harvested
Hammarby S	10	10	12	6	Harvested

Identification of fungal species

The *Fusarium* species found in this study were *F. oxysporum*, *F. culmorum* and *F. redolens*. The *F. avenaceum* primer was excluded from this study since no PCR products were amplified when it was used with the reference isolates. The isolates with *F. oxysporum* had differences in morphology, especially in colour, which can be seen in appendix 1 figure III.

PCR products from some DNA samples were not amplified by the other species specific primers. The TEF region was amplified for the DNA samples from the fungal isolates that did not give any amplification with the species specific primers. In addition were the ITS-region sequenced for the samples that did not give any amplification in the TEF-region. Some samples which were amplified with the species specific primers were also amplified using the TEF and ITS primers to confirm the result. This can be seen as "A+C" in tables 5a and 5b. Each onion from the second sampling was analysed twice as the onion tissue as well as the fungal isolates were used. The result from this double analysis can be seen in table 5b as "A+B". The fourth method used was ocular inspection, which showed that several onions had a double infection (presented as "A+D" and "B+D" in table 5b). DNA from onion tissue was not extracted from the onions from the first sampling, nor was the method of ocular inspection used for the onions sampled during the first sampling. Therefore are the methods "B", "D", "A+B", "A+D" and "B+D" excluded from table 5a.

The result from all the different methods combined from the first sampling was that 63.3% of the onions were infected by *F. oxysporum*, 22.4% with *F. redolens*, 4.1% with *Penicillium* sp., 2.0% with *Fusarium.* sp. and bacteria respectively. *F. oxysporum* and *Trametes* sp co-occured in two percent of the onions and another two percent of the onions had a double infection of *F. oxysporum* and *F. culmorum.* The last two percent of the onion samples were excluded due to inadequate DNA sample or contaminated fungal isolates due to mites. These figure are based on table 5a.

The result from all the different methods combined from the second sampling showed that 18.9% of the onions were infected with *F. oxysporum*. About 4.6% of the onions had a double infection, which was distributed as follows: 3.0% had a double infection of *F. oxysporum* and *Penicillium* sp., 0.8% had a double infection of *F. oxysporum* and *Sclerotium cepivorum* and 0.8% was infected with both *F. oxysporum* and bacteria. The total percentage of onions with a *F. oxysporum* infection was 23.5%.

Among the rest of the onions from the second sampling 5.3% were infected with *Bionectria ochroleuca*, 0.8% with *Alternaria* sp., 25.0% with *Penicillium* sp., 3.0% with *Sclerotium cepivorum*, 3.0% with *T. atroviride* and 26.5% with bacteria. Contamination of isolates due to mites and inadequate DNA resulted in that 12.1% of the samples were excluded. The identity of one fungus from one onion (0.8%) could not be clarified using neither of the methods. These figures are based on table 5b.

Table 5a. *Fungal species identified in onions from the first sampling, divided into sampling location and method or combination of methods. N shows the number of onions analysed from each location. An example from the table:* F. oxysporum *and* Trametes *sp. co-occured in one onion from the first sampling which was sampled from a field in Hammarby Norra and this co-occurrence was determined by using species specific primers on DNA from the isolates as well as from the onion tissue.*

		Methods				
Location	Infection	A	C	D	A+C	Total
Kåtorp N=3	*F. redolens*		2			2
	Fusarium sp.		1			1
Stenåsa N=3	*Penicillium* sp.			2		2
	F. redolens		1			1
Kastlösa N=5	*F. redolens*		5			5
Bjärlinge N=5	*F. oxysporum*	4				4
	F. redolens		1			1
Skärlöv S N=3	*F. redolens*		2			2
	excluded sample*			1		1
Väderstad N=4	*F. oxysporum*	4				4
Råkskogen N=5	*F. oxysporum*	4				4
	F. oxysporum/ F. culmorum				1	1
Smedby N=2	*F. oxysporum*	2				2
Hammarby Norra N=9	*F. oxysporum*	7			1	8
	F. oxysporum/ Trametes sp.				1	1
Hammarby Södra N=10	*F. oxysporum*	7			2	9
	bacteria			1		1
Total						49

A = Species specific primer; DNA from isolates, C = ITS and TEF primers, D = ocular inspection, "A+C" = result of method A and C combined.

*excluded sample includes inadequate DNA samples, pure cultivation samples destroyed by mites as well as samples where the fungi simply did not grow.

Table 5b. *Fungal species identified in onions from the second sampling and information from the interviews about the production system.*

Location	Year onion production started	Onion in crop rotation (years)	Years since bulb onions used	Infection	Methods								
					A	B	C	D	A+B	A+C	A+D	B+D	Total
Kåtorp	1995	~5	5	*F. oxysporum*	1					1			2
				Penicillium sp.				3					3
				Bionectria ochroleuca			1						1
				Bacteria				4					4
				excluded sample*				2					2
Stenåsa	2010	Never	Not used	*Bionectria ochroleuca*			2						2
				Penicillium sp.				1					1
				Bacteria				8					8
				excluded sample*				1					1
Kastlösa	2004	Never	Not used	*Penicillium* sp.				9					9
				Bacteria				1					1
				excluded sample*				2					2
Bjärlinge	1970th	5-6	0 (still planting)	*F. oxysporum*			1			1			2
				Sclerotium cepivorum			1						1
				Penicillium sp.				4					4
				Bacteria				3					3
				excluded sample*				2					2
Skärlöv N	2009	Never	Not used	*Bionectria ochroleuca*			2						2
				Penicillium canescens			1						1
				Bacteria				6					6
				excluded sample*				3					3
Skärlöv S	2009	Never	Not used	*F. oxysporum*						1			1
				Bionectria ochroleuca			2						2
				Penicillium sp.				5					5
				Bacteria				3					3
				excluded sample*			1						1

(Table 5a continues on the next page)

Location	Year onion production started	Onion in crop rotation (years)	Years since bulb onions used	Infection	Methods								
					A	B	C	D	A+B	A+C	A+D	B+D	Total
Väderstad	1930th	~5	2	*F. oxysporum*						1			1
				Sclerotium cepivorum			2						2
				Trichoderma atroviride			3						3
				Alternaria sp.			1						1
				Penicillium sp.				4					4
				excluded sample*			1						1
Råkskogen	1975	4-5	7	*F. oxysporum*	2					1			3
				F. oxysporum/ Sclerotium cepivorum			1						1
				F. oxysporum/ Bacteria								1	1
				Trichoderma atroviride			1						1
				Sclerotium cepivorum				1					1
				Penicillium sp.				1					1
				Bacteria				5					4
Smedby	1975	6-10	2	*F. oxysporum*	5				2				7
				Penicillium sp.				1					1
				Bacteria				3					3
				excluded sample*				1					1
Hammarby N	1970th	5-6	0 (still planting)	*F. oxysporum*	2								2
				F. oxysporum/ *Penicillium sp.*							1		1
				Penicillium sp.				2					2
				Unknown**			1						1
				Bacteria				3					3
				excluded sample*			2	1					3
Hammarby S	1970th	5-6	0 (still planting)	*F. oxysporum*	6				1				7
				F. oxysporum/ *Penicillium sp.*							1	2	3
				Penicillium sp.				2					2
Total													132

A = Species specific primer; DNA from isolates, B = Species specific primers; DNA from onion tissue, C = ITS and TEF primers, D = ocular inspection, "A+B" = result of method A and B combined, "A+C" = result of method A and C combined, "A+D" = result of method A and D combined and "B+D" = result of method B and D combined.

*excluded sample includes inadequate DNA samples, pure cultivation samples destroyed by mites as well as samples where the fungi simply did not grow. ** Unknown cause of the infection.

Ocular inspection and symptom description

The isolate samples which resembled *Penicillium* sp. fungi were not scraped from the PDA but confirmed as *Penicillium* sp. with a stereomicroscope according to the key of Barnett and Hunter (1998). One sample of *Sclerotium cepivorum* was also confirmed with the stereomicroscope and compared with the isolate containing *Sclerotium cepivorum* which had been identified with the PCR and sequencing method. Ocular inspection was also used for identification of bacteria, mite contamination or saprophytes.

The observed symptom from the onions from the first sampling is presented in table 6. Neither of the onions infected with *F. redolens*, *Fusarium* sp., *Penicillium* sp. nor the onion where *F. oxysporum* and *Trametes* sp. co-occured, showed any symptoms at all. Of the onions infected with *F. oxysporum* 61.3% showed symptoms of some kind and 38.7% were symptomless.

Table 6. *Number of onions with the different symptoms and the identified fungal species present in the onion. The category "healthy roots" included onions with a slightly discoloured basal plate.*

	smut	allium white rot	healthy roots	rotten roots	Symptomless
F. oxysporum	3	2	7	7	12
F. culmorum/ F. oxysporum	1				
F. oxysporum/ Trametes sp.					1
F. redolens					11
Fusarium sp.					1
Penicillium sp.					2
Bacteria				1	
Excluded sample*					1
Total	4	2	7	8	27

*excluded sample includes inadequate DNA samples, pure cultivation samples destroyed by mites as well as samples where the fungi simply did not grow.

Symptoms of *F. oxysporum* infection on onions from the second sampling varied from no symptoms at all to varied degrees of basal rot (Table 7). Symptoms were observed in 64.5% of the onions infected with *F. oxysporum* or with a double infection including *F. oxysporum.* The symptoms shown on the basal plate of the onions varied from dry and brown to soft, rotten and decomposed. Some of the confirmed infected onions did not show any symptoms at all. The colour of the diseased tissue varied from light brown with streaks of pink, grey, yellow or black to grey/purplish colour or a grey/black/blue tone. The rotten tissue had a gunge like structure and a putrid smell came from those onions in which the infection had proceeded far. White mycelium grew on the inner scales on one of the onions. The attributes of the outside of the onion varied depending on the infection, usually it was soft and rotten but in some cases it looked fine, but there were still symptoms on the basal plate and tissue when the onion was cut in half. The infection appeared to spread in the onion bulb in three different ways; either from the basal plate up the middle of the onion and then outward (Figure 5a), or from the basal plate and up in the outer scales and then inward (Figure 5b). The third way is from the basal plate and evenly upward in the bulb (Figure 5c). Three of the onions from the second sampling were mechanically damaged in the field and one of those had a double infection of both *F. oxysporum* and *Penicillium*. The other two bulbs were infected with bacteria.

Figure 5. *Symptoms of* F. oxysporum *showing different disease progresses A) infection from within and outward B) infection proceeded from the outer scales and inward C) infection evenly from the basal plate and upward (Photo Sara Lager).*

The onions infected with *Penicillium* sp. showed symptoms of rotten dark grey and black bulb tissue with white spots and a discoloured brown basal plate. These onions were rotten from the basal plate upwards. One onion which was infected by *Penicillium* sp. showed no other symptoms than a dry and light brown basal plate. The onions infected by *Sclerotium cepivorum* showed symptoms of dark grey and brown rotten bulb tissue and black sclerotia could be seen on the outer scales. The basal plate was discoloured brown or had decayed. The onion tissue infected by *T. atroviride* was light brown, grey and black with dark green mouldy growth on the outside of the onion. The basal plate was discoloured brown and the onions were rotten from bottom upward along the outer scales.

The onion from which only bacteria grew was in different conditions. The tissue of one bacterial infected onion was black and rotten, whereas another bulb had only a dry and discoloured light brown basal plate and streaks of rotten tissue on one side. Several onions infected by the different fungi and bacteria did not show any symptoms at all (table 7).

Table 7. *Distribution of the pathogenic fungal species and bacteria identified on onions from the second sampling with or without disease symptoms.*

	symptoms No.	Symptomless No.	Total No.
F. oxysporum	17	8	25
F. oxysporum/Sclerotium cepivorum	1	0	1
F. oxysporum/Penicillium sp.	1	3	4
*F. oxysporum/*bacteria	1	0	1
Penicillium sp.	4	29	33
Sclerotium cepivorum	4	0	4
Bacteria	4	31	35
excluded sample*	5	11	16

*excluded sample includes inadequate DNA samples, pure cultivation samples destroyed by mites as well as samples where the fungi simply did not grow.

Interviews of onion producers

Five of the eight onion producers have experienced a problem with the onion quality during last the years. These are referred to as the OQ-producers. The problems experienced were onion rot, allium white rot, wilting and depression spots. The three producers without significant large problems are referred to as the WQ-producers and they all started their onion production within the last six years whereas the OQ- producers had grown onions the last 15-80 years (Table 8). All the OQ-producers said that the problems started during growth in the field and one of them also had experienced a development of disease during storage. The OQ-producers had a shorter crop rotation period during the 1990s with an average of 4.3 years between onion crops. They had now lengthened the period to an average of 5.6 years. One producer among the OQ-producers did not think a longer crop rotation would make a difference as the soil is infested anyway.

All OQ-producers planted seed onions. Four of them used to plant bulb sets 2-8 years ago. One OQ-producer still plants bulb onions as well as seeds. All of the WQ-producers used seeds. There were no main differences in the other crops grown by the onion producers. These crops were cereals, forage, brown beans, pies, potatoes, oil-seed rape and corn. Three of the onion producers grew sugar beets more than five years ago, they were all OQ-producers. Two of the OQ-producers did not experience a problem with onion maggots, although one of them used insecticides as a prevention method. One OQ-producer experienced a very small problem with onion maggot while the last two OQ-producers saw it as a large problem. None of the WQ-producers experienced a problem with onion maggots.

All OQ-producers experienced that the problems with onion quality start in the field. Two producers experienced no change in disease frequency during storage *i.e.* if the onions were healthy when stored, they were healthy when they were taken out and sold. One of the producers thought that the quality problems start in the storage as well as in the field *i.e.* healthy onions were stored and had symptoms after storage. The WQ-producers experienced no decline in onion quality during storage.

The additional interview revealed that the majority of all the producers who produced onions previous years thought the quality of the onions harvested 2010 was better than an average year.

Table 8. *Information about the onion production system. "WQ" stands for Without onion Quality problems and "OQ" stands for Onion Quality problems.*

Producer by location	Problem		Start of production	Onion in crop rotation	Years since bulb onions used
	Field	Storage			
Skärlöv	No (WQ)	No	2009	Never	Not used
Stenåsa	No (WQ)	No	2010	Never	Not used
Kastlösa	No (WQ)	No	2004	Never	Not used
Smedby	Yes (OQ)	No	1975	6-10 years	2
Råkskogen	Yes (OQ)	Yes	1975	4-5 years	7
Väderstad	Yes (OQ)	No	1930th	~5 years	2
Bjärlinge/ Hammarby (N & S)	Yes (OQ)	No	1970th	5-6 years	0 (still planting)
Kåtorp	Yes (OQ)	No	1995	~5 years	5

Discussion

The result from both the first and second sampling showed that *F. oxysporum* is an important pathogen which infects and gives symptoms on onions on Öland. The first sampling showed that *F. redolens*, which is closely related to *F. oxysporum*, is also a pathogenic fungus on onions on Öland.

The difference in morphology of the isolates observed in this study (appendix 1 figure III) depends on adaptations to the cultural environment growing conditions (Booth, 1971; Geiser *et al.*, 2004). Using morphology to correctly determine the *Fusarium* species demands the culture environment to be standardised. Therefore it was easier and the method gave more correct results when the PCR and sequencing methods were used instead. The isolates with *F. avenaceum* used as reference isolates could have been contaminated as there was no match with the *F. avenaceum* JIA primers, but this is probably not the case. There is also a small chance that the primers could have been defect.

A reinfection of onion plants according to the Koch postulate would verify the pathogenic properties of the *Fusarium* species. However, it is important to consider that the stored isolates may be less pathogenic than the original culture as *Fusarium* species change phenotypically when preserved (Burgess *et al.*, 1994).

The selection of the isolates from the first sampling became defective as several samples were considered contaminated without a thorough analysis, hence there were no *Sclerotina* sp. or *Penicillium* sp., as well as very little bacterial growth recorded amongst the onions from the first sampling. Bacteria can be pathogenic on onions and it is therefore difficult to determine whether the isolates with bacteria were contaminated or if there was an actual bacterial infection on the onion. Therefore, it may be considered that the percentages of *F. oxysporum* and *F. redolens* from the first sampling were elevated in comparison to the second sampling.

The result from the fungal infected onions does not show how large percentage of the onions in the field that are infected as the sampling was biased and the study was performed with a qualitative and not quantitative purpose. The sampling was biased in order to sample onions with a full range of visible symptoms on the onions. There are several ways of sampling and information about which percentage of the onions were infected could have been obtained with a randomised sampling. The biased sampling does however indicate which fungi that infect the onions.

There was no documentation of the status of the onion roots in this study since some onions where uprooted at the time of the second sampling. Documentation would therefore have been inaccurate as it could not be known whether the roots were detached due to the uprooting or due to fungal infection. Neither was there any documentation of the status of the leaves since it would be difficult to know if the chlorosis and necrosis depended on the ripening of the onion or if they were symptoms of disease.

The *Fusarium* species found in this study coincides with the studies of Bayraktar and Dolar (2011) and Galván *et al.* 2008 as follows: *F. oxysporum* seems to be the most frequently occurring species in this study which agrees with the result in Bayraktar and Dolar (2011) and Galván *et al.* (2008). Only a very small percentage of the onions were infected with *F. culmorum* which also confirms the findings of Galván *et al.* 2008 and Bayraktar and Dolar 2010, which indicates that *F. culmorum* is not as important as a pathogen as *F. oxysporum*. *F. redolens* however, had an intermediate importance in the current study, which is similar to the

result in a study by Bayraktar and Dolar (2010), who found that *F. redolens* was the fifth out of seven most important *Fusarium* species analysed.

The symptoms recognised during the current study match the symptoms described by others. The colour of the bulb tissue infected by *F. oxysporum* was recorded in the current study and was found to be highly variable *i.e.* light brown with streaks of pink, grey, yellow or black to grey/purplish colour or a grey/black/blue tone. The onion bulb tissue infected by *F. oxysporum* was only described as "discoloured" by Schwartz & Mohan (1995) and Cramer (2000), whereas the infected bulbs were described as reddish-brown in the study by Tahvonen (1981). The heavily infected basal plate of the infected onions was easily detachable from the rest of the bulb, which was also observed by Cramer (2000). Of all the onions infected by *F. oxysporum*, 35.7% appeared symptomless. Infected onions without any symptoms were also described by Brayford (1996). This is very interesting as it supports the fact that one of the producers could store healthy looking onions in the autumn and the onions showed symptoms when taken out of storage. There is also a minor risk that the isolates were contaminated, but this is considered to be negligible since such a high proportion of the onions had positive amplification without displaying any visible symptoms of fungal infection during sample preparation.

The species *Trametes versicolor*, *Trichoderma atroviride*, *Bionectria ochroleuca* and *Penicillium canescense* have shown to have biocontrol properties (appendix 3) (Ruiz-Dueñas & Martínez, 1996; Harman *et al.*, 2004; Yohalem *et al.*, 2004; Nicoletti *et al.*, 2007). *Trametes versicolor* have shown to have an antagonistic effect on *F. oxysporum* f. sp. *lycopersici* and may also have an effect on *F. oxysporum* f. sp. *cepae.*

The interviews revealed that onion producers, which have produced onions for more than six years, experienced a problem with onion rot. These producers have taken action to decrease the problem such as longer crop rotation and change from planting small bulb sets to planting seeds. Bulb sets have a higher risk of being contaminated with soil-borne fungi and altogether have a higher potential of being infected, as it is a vegetative propagation (Gunnel Andersson, pers. comm.[*]).

The study includes an analysis of potential background factors such as seeding process and crop rotation etc. To make a proper statistical analysis of the impact of potential background and risk factors, would require a larger group of onion producers serving as reference to obtain sufficient statistical power. However, the onion producers on Öland are few and the producers visited during this survey represented approximately 70% of the total number of the onion growers on Öland.

The chlamydospores have the best survival rate of all the *F. oxysporum* spores, especially in cold climate such as the Swedish winters. It is therefore most likely that these are the spores found in soils used for onion production in Sweden. A large content of spores, especially chlamydospores has been built up in the soils on Öland as the soils have been used for onion production on Öland for many years may and as the chlamydospores can survive for a very long time in the soil. The accumulation of chlamydospores could be the explanation to why some producers experience a problem with onion quality. This is supported by the outcome of the interviews (table 8) where five producers experiencing problems with onion quality have produced onions for 15-80 years, while the producers without onion quality problem only

[*] Gunnel Andersson, Plant protection advisor, Swedish Board of Agriculture, Kalmar, Personal communication. 2011-05-05

produced onions for 1-6 years. Hence, a long crop rotation period is very important to reduce the level of chlamydospores and to improve onion quality by reducing *F. oxysporoum* inoculum. The crop rotation has been lengthened during the last years and this might be the reason why the onion harvest during 2010 was considered good by the onion producers.

Further work is necessary to establish to which extent the harvested onions are infected by *F. oxysporum, F. redolens* and *F. culmorum,* and to find out if that amount is rising or declining from one year to the next. It would also be interesting to investigate the reasons behind this potential severity of fusarium basal rot. Future studies are required in order to investigate whether the fungal species with antagonistic properties can be used as biological control of onion pathogens.

Conclusion

Fusarium oxysporum and *F. redolens* are the two most common *Fusarium* species found on onions from Öland. *F. culmorum* was also identified but only on one out of 181 onions. The symptoms of *F. oxysporum* in the onions in this study were similar to the symptoms described in earlier studies *i.e.* basal rot starting at the basal plate as well as spreading up in the scales resulting in a discoloured and watery bulb tissue. One interesting observation was that 35.7% of all the onions infected with *F. oxysporum* were symptomless. The reason behind the experienced increase in quality problems in onion production on Öland might be the result of a shorter crop rotation period, accumulation of chlamydospores in the soil and perhaps due to planting infected bulb sets.

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Appendix 1

Fusarium fungal species

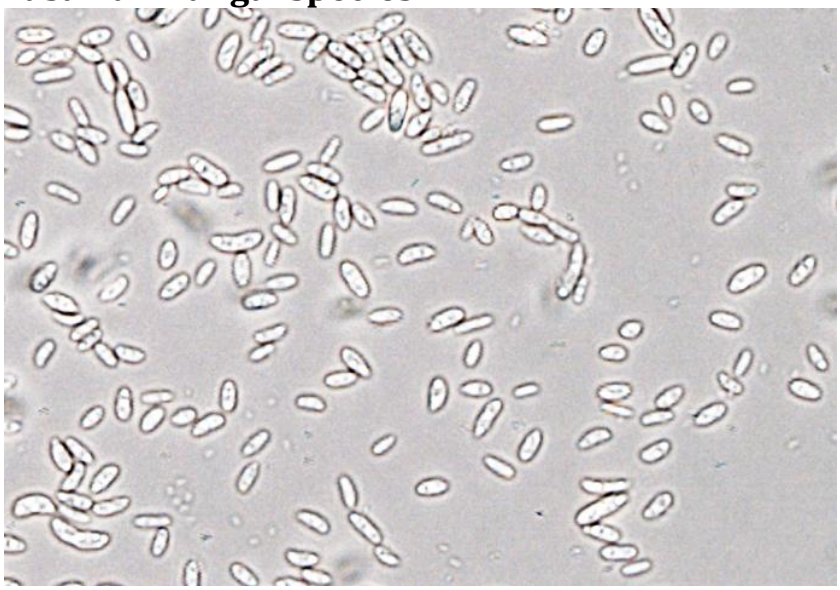

Figure I. *Microconidia of* Fusarium oxysporum. *The average size of the conidia is 6.7µm.*

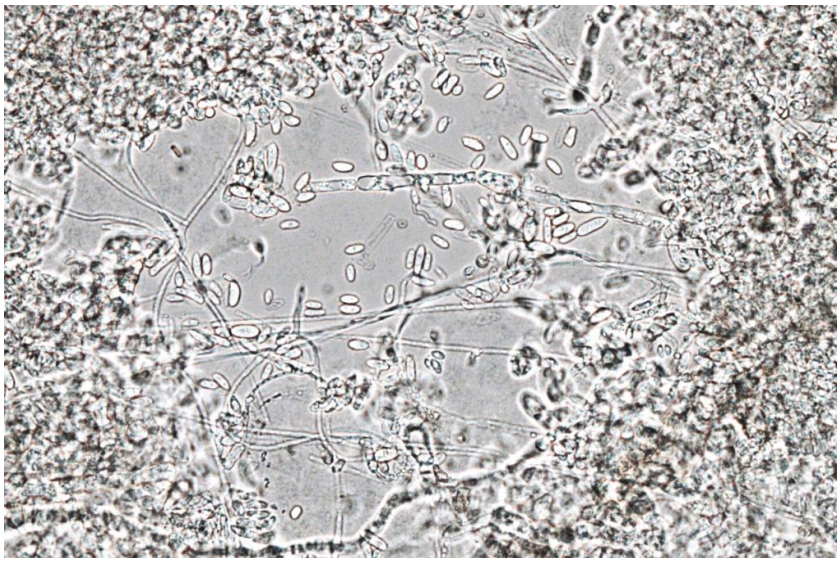

Figure II. *Microconidia of* Fusarium redolens. *The average size of the conidia is 7.4µm.*

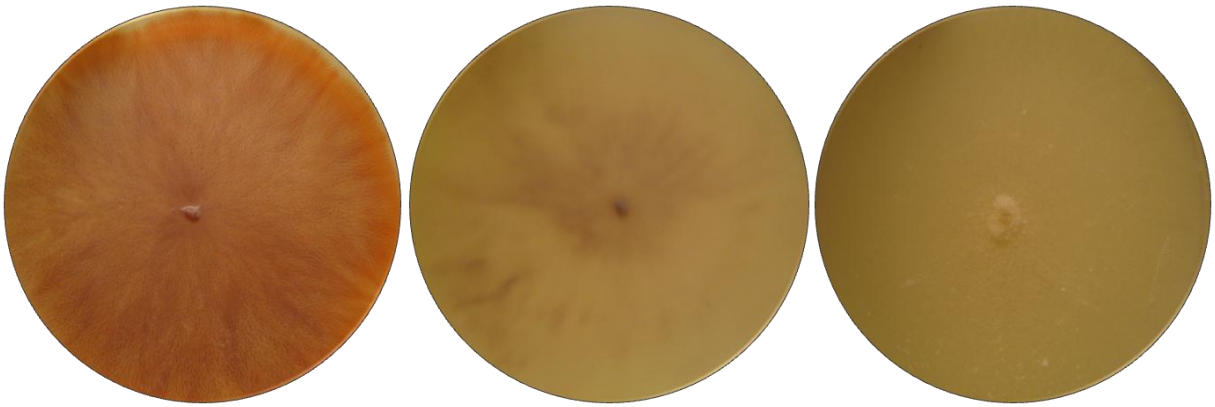

Figure III. *Three different phenotypes of* F. oxysporum *when the isolates are grown on agar.*

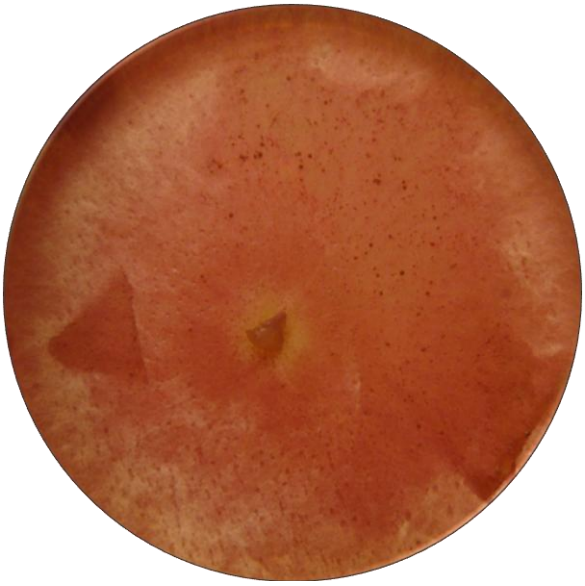

Figure IV. *A double infection of* F. oxysporum *and* F. culmorum. *Some mycelium has been removed.*

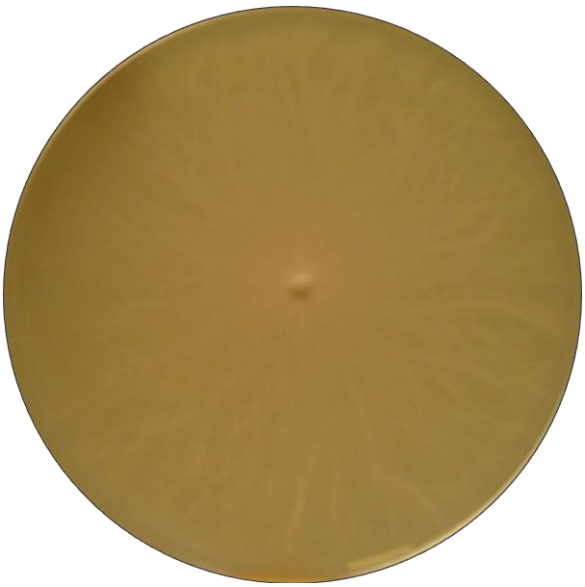

Figure V. *A plate with growth of an isolate with* Fusarium redolens.

Appendix 2

Polymeric chain reactions (PCR)

A small mixture of 10 times the recipe was first mixed to ensure that the PCR process worked with the reference isolates and that no cross matches aroused. The concentrations of the DNA samples used for the PCR were about 0.5 ng DNA μl^{-1}. Ten µl of each DNA sample and ten µl of the PCR mixture for each of *F. oxysporum*, *F. culmorum* and *F. avenaceum* were used for each reaction. The procedure for the TEF and ITS primers were the same as above but with 25 µl of the sample and 25 µl of the PCR mixture. The PCR conditions was 7 minutes at 95°C and then 35 cycles of 95°C for 30 seconds and 62°C for *F. culmorum*, 55°C for *F. oxysporum*, 58°C for *F. avenaceum* and ITS and 53°C for TEF. Each cycle was followed by a 30 second extension and when all the 35 cycles were complete a 7 min extension followed at 72°C.

Table I. PCR mixture recipe per DNA sample.

	F. o. F.c. F.a.	TEF, ITS	Concentration
H_2O	2,94µl	7,35 µl	
Green buffer (10X)	2,0 µl	5 µl	1X
dNTP	2,0 µl	5 µl	0.2 mM
$MgCl_2$	1,2 µl	3 µl	2.75 mM (total conc.)
Primer F	0,8 µl	2 µl	0.2 µM
Primer R	0,8 µl	2 µl	0.2µM
Dream Taq™	0,26 µl	0,65 µl	0.03 U μl^{-1}
DNA sample	10 µl	50 µl	0.5 ng μl^{-1}
Total volume	20 µl	50 µl	

Appendix 3

Non-Fusarium fungal species

Trametes sp.
Trametes sp. is a group of basidiomycota which can cause white rot in hardwood trees (Agrios, 2005). *Trametes versicolor* has proved to produce enzymes which degrade the cell walls of *Fusarium oxysporum* f. sp. *lycopersici* which cause a vascular wilt disease on tomato plants and has therefore potential as biocontrol agent to *Fusarium oxysporum* (Ruiz-Dueñas & Martínez, 1996)

Penicillium sp.
Several *Penicillium* species cause blue mold on onion (Figure A) (Schwartz & Mohan, 1995). Blue mould is a disease which emerges during harvest and storage. *Penicillium* sp. grows on harvest residues in soil and on plant and animal debris. Onion bulbs are usually infected through wounds or damaged bulb tissue. *Penicillium* can also be pathogenic on intact tissue. *Penicillium* sp. can germinate and grow at 15-32°C, but the optimal temperature is between 21-25°C. The fungus prefers a moist environment to grow. The mycelium of *Penicillium* sp. is branched and transparent and has long straight conidiophores that produce several branches. These are the penicilli (brushes) which characterize many *Penicillium* species. The phialides are situated on the end of the penicilli and have long chains of conidia, which are globose to ovoid and can be green, blue or yellow. Some of the primary symptoms appear on the scales. The scales obtain pale yellowish blemishes, watery soft spots and occasionally purplish red stains. At a later stage a green to blue green mould can develop in lesions. When the onion is cut in two, one or several of the scales may be water-soaked and show a light brown to greyish colour. As the infection progresses the bulb becomes soft and usually gives a putrid odour. One species of *Penicillium* which was found in this study is *Penicillium canescens.* This *Penicillium* species produces toxic penicillic acid, which has been found to prohibit germination of corn seeds (Keromnes & Thouvenot, 1985). *Penicillium canescens* has also shown some repressive effect against diseases caused by *Rhizoctonia solani* (Nicoletti *et al.*, 2007).

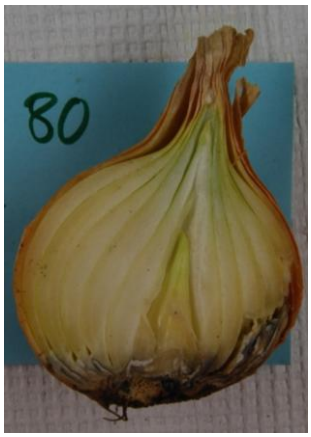

Figure A. Onion with *Penicillium* sp. infection (Photo Sara Lager).

Bionectria ochroleuca
Nielsen *et al.* (2000) showed that *B. ochroleuca* (anamorph *Clonostachys rosea*) reduced the sporulation of *Botrytis aclada* on dead onion leaves under constant moist conditions. *Clonostachys rosea* belongs to the order of hypocreales which are ascomycota (Schroers *et al.*, 1999).

Sclerotium cepivorum
Sclerotium cepivorum causes white rot in onions which is a most harmful and destructive fungal disease of *Allium* species (Schwartz & Mohan, 1995). White mycelia growth on the stem plate and around the stem base is an early sign of *Sclerotium cepivorum* infection (Figure B). Symptoms also occur on the leaves as premature yellowing and as stunting of the plant. The mycelium grows both in the roots and along the root surface to reach the stem plate. Onion plants die fast in the infested areas of the field, after the fungus has reached the stem plate. The fungus reproduces through producing round black sclerotia in decaying onion tissue. The sclerotia can lie inactive in the soil for many years until a host is present nearby. The optimal temperature for germination of the sclerotia is 14-18°C, while the activity of the sclerotia cease at a temperature above 24°C. The mycelium grows fast along the roots and can infect rapidly along the planted row. A temperature of between 5-27°C is required for mycelium growth.

Figure B. Onion with *Sclerotium ceptivorum* infection
(Photo Sara Lager).

Alternaria porri
Alternaria porri causes purple blotch on onion and infect foremost old leaf tissue and occasionally onion bulbs at harvest (Schwartz & Mohan, 1995). Hyphae can infect through wounds or stomata or penetrate epidermis directly. The fungus requires high humidity to sporulate and a temperature of 6-34°C to grow. The growth optimum is at 25°C, whereas very limited growth occurs under 13°C. The symptoms on the leaves are lesions which go from water-soaked to brown to purple. The zone around the lesion is reddish or purple and surrounded with a region of chlorosis. The fungus infects from the neck or through wounds in the fleshy scales. Fungal inhabited bulb tissue turns from deep yellow gradually into wine red.

Trichoderma atroviride

Trichoderma atroviride (Figure C) is one of the *Trichoderma* species which biocontrol properties has been analysed in a number of research projects. *T. atroviride* has been proved to antagonize and eliminate several plant pathogens (Harman *et al.*, 2004). It has, for instance, shown to produce antifungal compounds in bean leaves and thereby reduced grey mould (*Botrytis cinerea*) infection. Root colonization of *Trichoderma* sp. can also increase plant growth and improve plant defence responses.

Figure C. Onion with *Trichoderma atroviride* infection (Photo Sara Lager).

Bacteria

Bacteria can also cause disease in onions but are less long-lived than fungi (Schwartz & Mohan, 1995). Bacteria can only survive a very short time outside living plants and plant residue. When infection occurs, the bacteria enter the onion plants through natural openings or wounds. The symptoms of a bacterial infection are water-soaked spots or streaks in the plant tissue which gradually turn brown, blighting of leaves and rotting of bulbs. Onion infecting bacteria are motile, rod shaped and usually gram negative. They can spread through the movement of water, soil, seeds, infected plant parts, aerosols as well as insect activities and soil on equipment.