The state-of-the-art knowledge on Fusarium in onions, especially focused on the research done in the Netherlands

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Grower Summary

The state-of-the-art knowledge on Fusarium in onions, especially focused on the research done in the Netherlands

Headline

- Fusarium basal rot, caused by a special form of the common soil fungus Fusarium oxysporum, is an increasing threat to onion production in temperate regions including the UK. The special form can only be distinguished from the common form by its pathogenicity to onions. Morphologically different forms of the fungus are not distinguishable.
- Presently, control measures are not available in practice. Eradication of the disease from infected soils is not likely to be possible. Potential disease management tools include tolerant varieties, crop rotation, soil amendments with organic material and hygienic measures.
- Detection of the fungus in soils and planting material is a key issue to develop effective disease management on a field and farm level. Good opportunities are offered by genetic techniques such as DNA finger printing supported by PCR.

Background and expected deliverables

Fusarium basal rot is a disease caused by the soil borne fungus Fusarium oxysporum. This globally occurring fungus can be found in all soils. Special forms of this fungus exist that each have their own host range. The fungus is a common threat to onion production in warm climates. In countries like the United States, Italy and France Fusarium basal rot has a long history. In the nineties the disease became apparent in a restricted area in a temperature climate country, the Netherlands. At present, the disease occurs in several onion production areas in the Netherlands and in the UK as well.

The fungus can infect roots of growing onion plants and from there infect basal plates and bulb onion tissue but it can also attack basal plates directly from the soil. The root system of infected plants deteriorates more as infection proceeds. This becomes apparent from wilting

symptoms and leaf die back. Infected plants can die during growth or appear as rotten bulbs at harvest or during storage. The fungus can survive in soil with thick-walled resting spores and on plant debris in the soil. Studies on the longevity of these spores are not known, but it is likely that the fungus can remain in the soil for a very long time to the extent that it cannot be expected that the fungus will disappear completely from infected commercial production fields.

In the Netherlands, research was carried out in the period between 1994 and 2004. The research was directed at:

- Testing current and new varieties for tolerance to disease attack.
- Development and testing of a pathogenicity test and a bio-assay as instruments to determine pathogenicity of isolates and disease potential of commercial production fields.

During the research, investigations were also done on the pathogenicity of Fusarium avenaceum and on the relation between Fusarium basal rot and the cracking of onion basal plates. Also, the bio-assay was used to carry out investigations on the development of disease potential of commercial fields in relation to the number of years with non-host crops.

It was the objective of the current review to indicate possibilities for future research and to develop effective disease management tools. Recommendations are as follows:

- Development and identification of tolerant varieties.
- Development of a reliable and cost-effective detection procedure based on DNA fingerprinting for soils and planting materials.
- Investigating the population dynamics of the fungus to establish the decline of disease potential of infected soils and the increase of disease potential after growing tolerant or non tolerant varieties.
- Development of practical tools for disease management such as organic soil amendments and, possibly, chemical treatments.

Summary of the review

The review was based on research carried out in the Netherlands in the period 1994-2004 and on supporting literature research. The research consisted of:

- Experimental trials on variety tolerance.
- Development of a pathogenicity test.
- Development of a bio-assay.
- Studies on population dynamics.
- Studying the pathogenicity of Fusarium avenaceum.
- Establishing the relationship between cracking basal plates and Fusarium basal rot.

The variety trials were carried out with the following varieties: Summit, Hyfield, Hysam, Spirit, Balstora, Mundial, Recorra, Takmark, Takstar, Sundance, Daytona and Taleto. The varieties Sundance and Daytona were bred for the American market as tolerant varieties while Takmark and Takstar were Japanse varieties with reported tolerance level. Takmark, Recorra and Mundial had the highest tolerance levels, but still suffered from disease on highly infected soils. The variety Takmark had not good storage ability. The variety was early maturing. The tolerant varieties Recorra and Mundial were not assessed for their storability.

A pathogenicity test was developed for survival of seedling and fungal growth under sterile conditions. The test procedure is described in the review. Results were not stable enough to have a good reliability. More development work seems necessary.

A bio-assay was set up to determine the disease potential of soil samples. The test was carried out in a greenhouse and consisted of 8 small pots filled with the sampled soil and 20 onion seeds per pot of a non-tolerant variety. This procedure should increase the likeliness of infection between fungus and host plant. This bio–assay appeared to take too much time (not cost-effective) to carry out and was too variable.

Using the bio-assay, sampled soil from commercial production fields with a known history of non host crops (varying from 0-8 years) was investigated for its disease potential. The objective was to find the number of years with no Allium crops that would decrease disease potential to a level that would be safe enough to grow a tolerant or susceptible onion variety. The bio-assay appeared to have too large a variability to be reliable and the decline of disease potential seemed to be very slow.

Besides Fusarium basal rot from Fusarium oxysporum, the disease was also reported from onions grown from sets in a particular year being caused by Fusarium avenaceum. The studies showed that this is a weak pathogen to onion and that the fungus is not likely to produce a significant disease level in a healthy crop.

In the Netherlands, the phenomenon of cracking basal plates was noticed in the period that Fusarium basal rot became more established. Farmers and advisors questioned a relation between both phenomena. Our research showed that more Fusarium basal rot did not coincide with more cracked basal plates. Therefore, this phenomenon seems not to be linked to Fusarium oxysporum.

1 Science section

1.1 Introduction

Nowadays, UK onion growers are confronted with a relative new and steadily increasing problem, Fusarium basal rot, caused by Fusarium oxysporum f.sp. cepa. The disease is common in countries with warmer climates such as the USA, France and Italy. The last few decades the fungus has extended into more temperate regions like the Netherlands and the UK. Because the fungus is known to have relatively high optimum growth temperatures, it is tempting to speculate that the occurrence of the fungus in temperate regions is a consequence of global warming. However, mutation of the fungus could also be at the origin of the observed extension.

Effective and practical measures to control or manage the disease are not available. In the Netherlands, this onion disease is also increasing but seems to have a slightly longer history in this country, especially in certain regions. The financial loss is substantial for Dutch farmers confronted with the problem, in the first place because the yield is lower and because of higher storage costs and secondly because the disease level can ultimately result in fields unsuitable for onion production for many years. This onion disease is well-known in other onion producing countries like the US, Italy and France. This is not surprising as the causal agent, the fungus Fusarium oxysporum, has a growth optimum at higher temperature ranges.

It is the objective of this report to give the current state of relevant knowledge about Fusarium in onions and to identify possible developments with good promise for the future. To meet this goal, knowledge, insights and results that resulted from research carried out in The Netherlands between 1994 and 2004 have been reviewed. A literature search has also been undertaken to complete a picture of the present day knowledge regarding Fusarium oxysporum f.sp. cepae.

1.2 Disease development at different growth stages

Fusarium basal rot in onions is caused by the soil fungus Fusarium oxysporum f.sp. cepae. This fungus is a special form of the general occurring fungus Fusarium oxysporum. In many onion growing areas this fungus causes disease attacks on onions at different stages of growth (Cramer, 2000). Roots can be infected from young stages onwards leading to early plant death. Infections in later growth stages will result in basal rot, either at harvest or in store. On heavily infected fields a Fusarium attack can gradually increase to high levels. Figure 1 shows disease progress in a heavily infected soil in The Netherlands with the spring sown onion variety Hysam. From the figure it is clear that in this soil the most disease incidence occurred during the growing season although the percentage of harvested bulbs with symptoms basal rot varied from 44-81%. Figures 2 and 3 shows pictures basal rot symptoms.

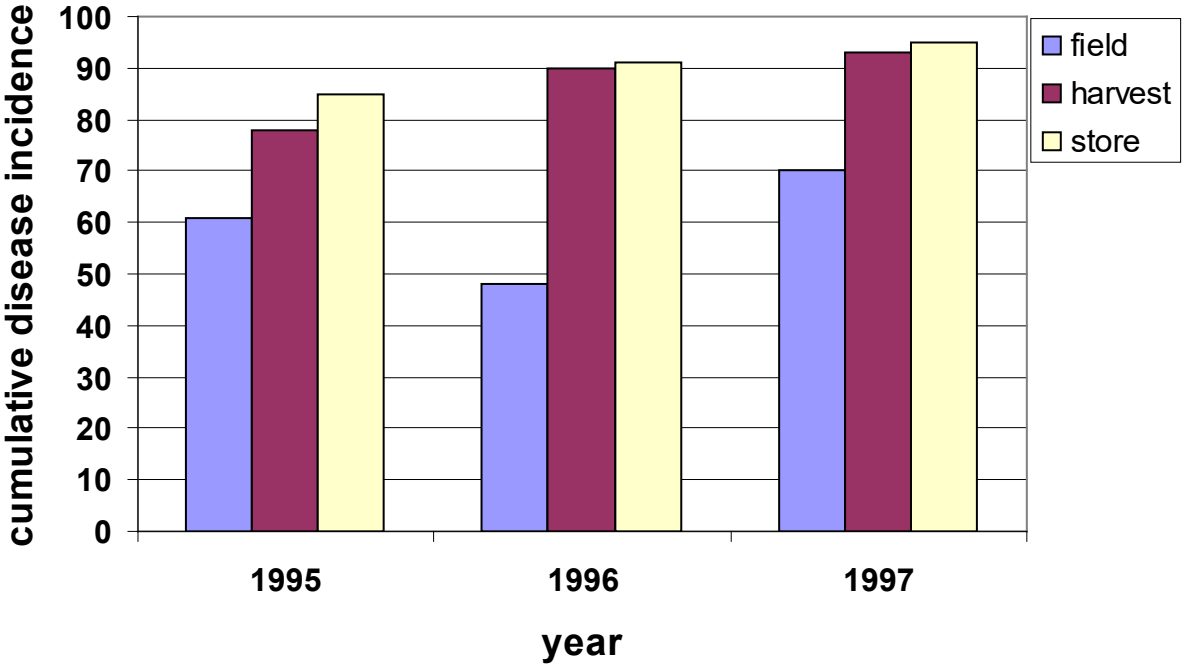

Figure 1. Cumulative disease incidence in cv Hysam on a heavily infected clay soil in the Netherlands

Infection by the fungus in the field can be recognized by wilting of the onion plant following attack of the root system. This symptom is seen earlier under dry conditions than when wet. Discoloration of the basal plate and a rot extending into the bulb are distinct symptoms of the disease. Under wet conditions the fungus can produce white mycelium on the basal plate. This symptom can easily be confused with onion white rot. A farmer was visited whilst looking for

Figure 2.Fusarium basal rot.

good experimental fields for a white rot research program, having reported heavy infection of one of his field by Sclerotium cepivorum. Indeed, the symptoms of the plants looked very much like onion white rot, but sclerotia could not be found in the soil and sclerotia would not form on the diseased plant in storage under wet conditions. This left the conclusion that the grower was suffering from Fusarium attack without knowing it.

This illustrates the importance of good identification methods.

Figure 3. Onions suffering from wilt symptoms.

The severity of infection is to a large degree dependent on the time of infection and plant stress factors. This was shown in a pot trial in Lelystad carried out in 1995, in which seeds, transplants (both Hyfield) and sets (Rocado) were sown or planted in artificially infected soil. Figure 4 shows that plants that are weakened and growing under stress conditions (transplants) were highly susceptible whereas onions from sets showed no wilt at all. However, disease symptoms do not necessarily mean that infection did not influence plant growth. The fresh weight of the healthy plants harvested in the 1995 pot trial was 10-30% lower with plants from seeds or transplants on infected versus not infected soil. A possible

explanation could be that plants that did not wilt or rot suffered from root infection, had to develop new roots to replace infected ones, and thus showed retarded growth.

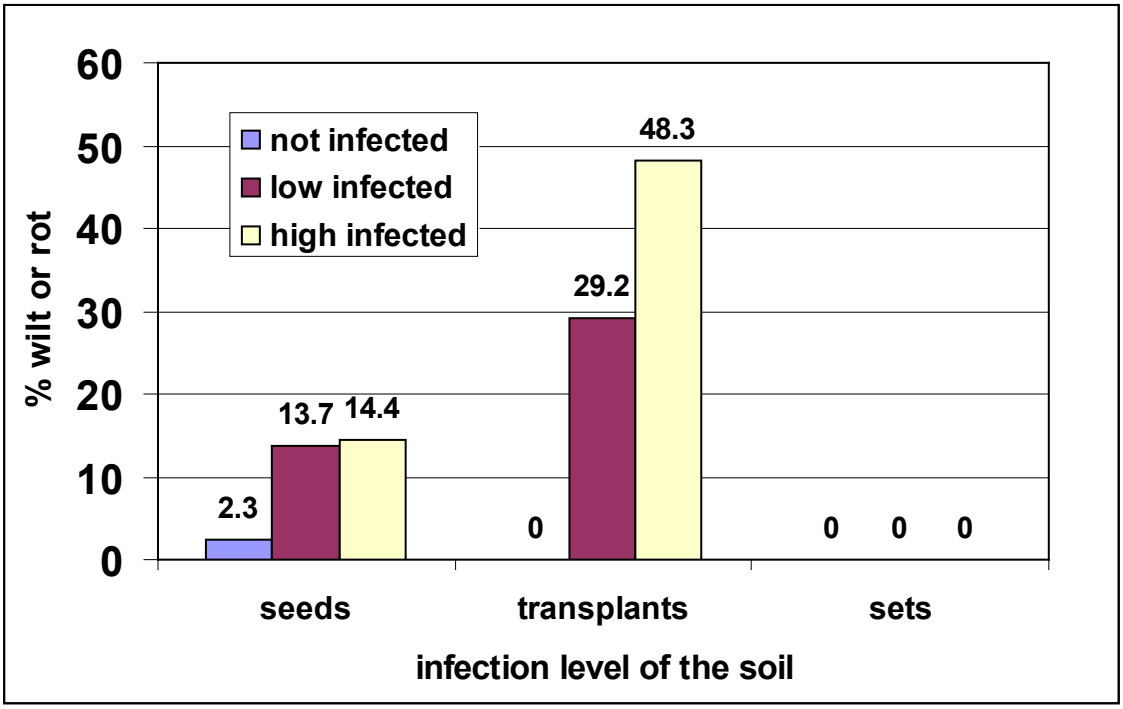

Figure 4. Influence of plant condition of infection.

In the Netherlands, the phenomenon of cracked basal plates was increasingly observed together with an increase in Fusarium basal rot incidents. These developments were assumed to be a symptom of Fusarium attack. In 1998 and 1999 both symptoms were recorded in the same field and resulted as per Figure 5. From this it is clear that both symptoms were not related. Most probably cracking of the basal plate is caused by other factors than Fusarium infection.

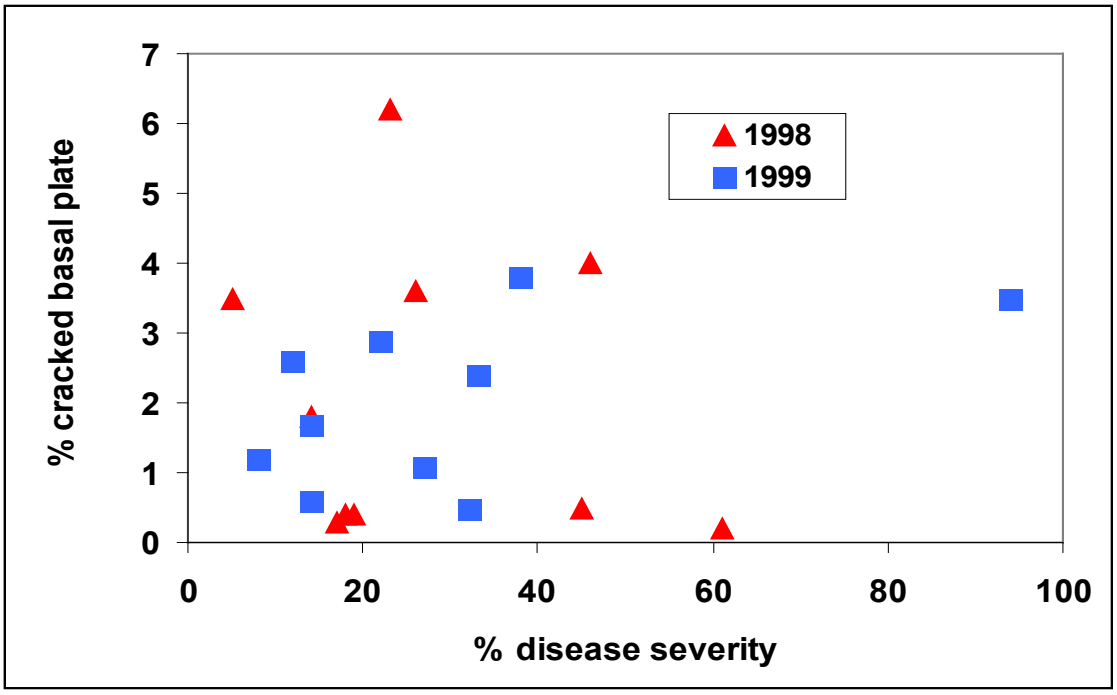

Figure 5. Relation between basal plate rot and cracked basal plates.

1.3 Description of the causal agent: life cycle

Fusarium oxysporum f.sp. cepae can survive in soil either in the form of mycelium on organic material in the soil or as resting spores called chlamydospores and microconidia. After germination, the fungus first grows externally on the onion root before entering after which the fungus colonizes the root and grows upwards towards basal plate and into the bulb scales. The last step in the infection process can take weeks or months. The fungus can produce new chlamydospores within the infected root tissue and microconidia in infected bulb tissue.

Fusarium oxysporum has a high optimum temperature for growth. In laboratory tests optimum temperatures were found between 24 and 27°C (Entwistle, 1990). In the field, plant attack is higher at higher temperatures. Nevertheless, in a highly infested field in The Netherlands onions were wilting and dying as early as mid June when soil temperatures had not reached the optimum temperature range.

Survival of the pathogen in soil in absence of the plant host is possible on organic material, but the fungus has to compete here with other (saprophytic) fungi. The duration of survival of the fungus on the basis of chlamydospores or as saprophytic mycelium is unknown. Research

results on this were not found.

As the fungus is soil borne, it can easily spread with planting material on which soil is attached or by agricultural machines leaving an infested field and not cleaned. The general means of spread of F. oxysporum are described by Nelson in 1981. In his paper, the spread by seed be it externally or internally is seen as the most important potential dissemination pathway. An example is presented by Gracia-Garza (1999) with coca seeds. The authors showed that Fusarium oxysporum could be detected in symptomatic as well as asymptomatic plants. For Fusarium oxysporum in onion a study is presented by Köycü and Özer (1997). They could detect the fungus in less than 1% of the seeds originating from one out of 7 regions studies. It is not known whether the detected isolates were pathogenic. It is not known if this is significant for current day practical conditions. Seed certification and seed treatment with fungicides may have some influence but this has never been investigated. In the Netherlands, areas with the heaviest Fusarium infection have a long history of intensive onion production. The origin of this infection is unknown.

1.4 Tolerance in onion varieties

Worldwide, tolerance in onion varieties is considered the best way to fight Fusarium wilt. In countries with a long history of basal plate rot in onions, much research effort has been carried out to find tolerant varieties (Fantino & Schiavi, 1987; Holz & Knox-Davies, 1974; Retig et al, 1971; Entwistle, 1990). In The Netherlands, several field experiments have been carried out to study the possible tolerance of commercially used varieties. This testing procedure takes a long time and is costly, but less time consuming methods, as used by Holz & Knox-Davies (1974), do not simulate natural infection conditions satisfactorily.

In these experiments, varieties developed for different environmental conditions but known for their Fusarium tolerance were included.

Table 1. Results of a variety trial in 1996

Variety	Yield (t/ha)	% plant loss on the field	% basal rot at harvest	% rot in store
Balstora	31	59	79	17
Daytona	58	38	58	7
Hyfield	28	64	85	21
Hysam	34	48	81	13
Spirit	45	42	77	15
Summit	26	65	84	24
Sundance	59	23	49	4
Taleto	26	62	82	21

In Tables 1 and 2 the results are presented of a variety trail carried out in 1996 and 1997 on a heavily infested field. From both tables it is clear that disease severity was very high, even in tolerant varieties such as Daytona, Sundance, Takstar and Takmark, the first two varieties being developed for the American market and the last two for the Japanese market. Although these varieties suffer less from basal rot, yields were still low, especially in 1997. Moreover, under Dutch conditions the Japanese varieties appeared to be not suitable for storage (bolt onions) and can only be used for direct consumption. The reason that yields in 1997 were much lower than in 1996 and that disease severity was higher, was that the 1997 trial was carried out on the same site as in 1996. The 1996 trial gave the fungus the opportunity to increase in population resulting in higher disease level in the following year.

In both years the first disease symptoms appeared very early. In 1996 first wilting was observed on June 19 while in 1997 3.4% wilted plants were found on June 17. At this time high temperatures had not occurred frequently, leading to the conclusion that disease level in the soil must have been very high.

Table 2. Results of a variety trial in 1997

Variety	Yield (t/ha)	% plant loss on the field	% basal rot at harvest	% rot in store
Balstora	5	63	79	46
Takstar	21	13	61	24
Hysam	6	70	77	23
Spirit	14	39	63	19
Taleto	6	72	79	26
Summit	3	62	88	20
Hyfield	2	72	92	27
Daytona	10	42	71	25
Sundance	14	34	62	22
Takmark	24	21	53	28

In 1998 and 1999 more field experiments were carried out in 5 commercial onion fields (A-E) in the area in the Netherlands where Fusarium wilt is most severe. Two varieties, Summit and Takmark that displayed differences in tolerance level in 1996 and 1997 were compared. Figure 6 shows that disease severity differed much between fields and that the higher tolerance level of Takmark versus Summit is apparent in almost all fields. However, the variability in tolerance level between both varieties is also clear. This could reflect variability in the infection process, including factors such as soil and weather conditions and Fusarium oxysporum population density and virulence. As all fields (A – E) were situated within a restricted distance from each other (2 km), population differences are the most probable reason. Similar differences were found in Lilies (Löffler et al, 1995).

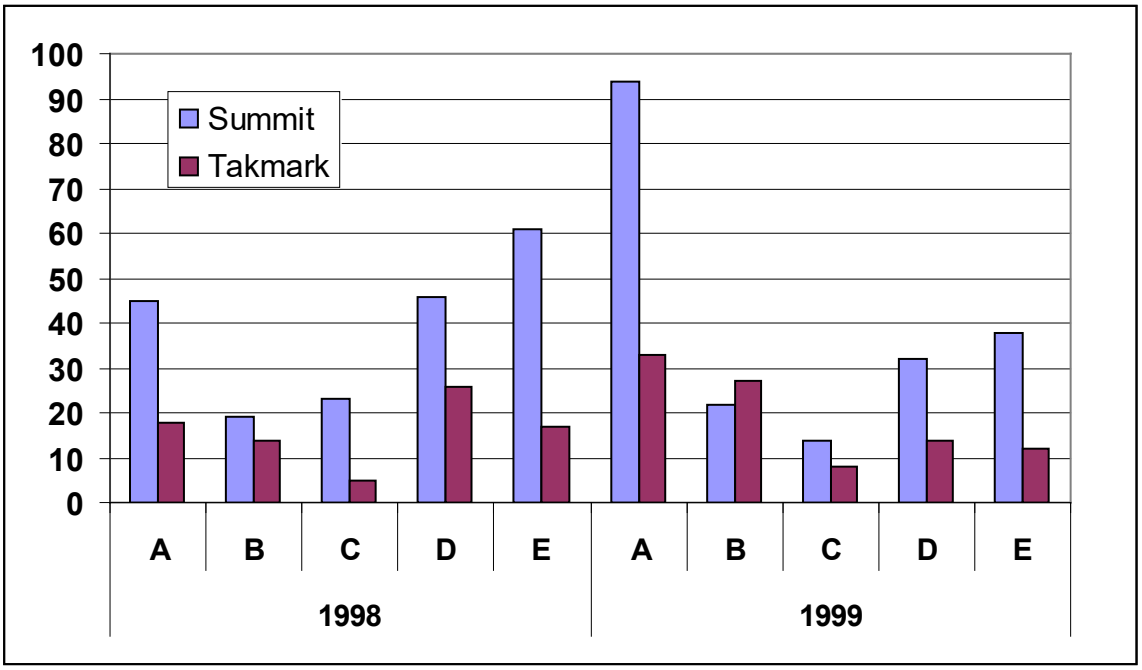

Figure 6. Disease severity (wilt plus rot; Y-axis) on five commercial onion fields in two years with two varieties (X-axis).

In 2002 and 2003 additional variety trials were conducted in the Flevopolder area in Holland from where the first reports of Fusarium originated (Tables 3 and 4). In these trials some varieties were used that were not available in the period 1996-1999. The tolerance level of the variety Takmark was equalled by the variety Recorra in 2003, with Mundial approaching the tolerance level of Takmark.

Table 3. Results of variety testing on an infected field in the Flevolpolder area in 2002.

Variety	Yield (t/ha)	% plant loss on the field	% basal rot at harvest	% rot at harvest + in store
Summit	17	32	47	61
Spirit	37	19	23	28
Takmark	53	12	4	7
Mundial	44	13	7	16

Table 4. Results of variety testing on an infected field in the Flevopolder area in 2003.

Variety	Yield (t/ha)	% plant loss on the field	% basal rot at harvest	% rot in store + in store
Summit	4	89	63	82
Spirit	21	58	30	46
Takmark	30	17	10	23
Recorra	40	26	11	25
Mundial	28	32	12	27

According to literature data (Entwistle, 1990; Abawi & Lorbeer, 1971b), infection of both roots and basal plates (discoloration) occurs equally in both tolerant and susceptible varieties. In tolerant varieties the fungus would not have the capacity to grow beyond the basal plate and enter the fleshy bulb material. This means that tolerant varieties will probably loose some yield potential growing on infested soil. Together with the fact that some rot still occurs at harvest and during storage, the use of tolerant varieties (assumed that they are adapted to the local growing and climatic conditions) should only be part of a total disease control strategy.

1.5 Development of a bio-assay

As part of the Fusarium research program in the Netherlands efforts were made to develop a bio-assay. The objective was to have a reliable and cost-effective bio-assay to determine the disease potential of a commercial production field. Eventually, such a bio-assay could be used to advise on onion growing and variety choice. Several planting materials were investigated to set up this bio-assay:

- First, onion transplants were considered. They gave good and severe disease symptoms, but soil infection with a weak pathogen like Fusarium avenaceum (see further) resulted in the same symptoms. It was concluded that an assay using transplants could not distinguish between Fusarium oxysporum and this fungus.
- Secondly, sets were used. It became clear that sets did not produce enough symptoms. This may have been caused by the ability of this planting material to produce roots at a faster rate and hence to a greater recovery potential of plants grown from sets.
- Thirdly, seeds were used. Using this material, a fungus like F. avenaceum could not

produce symptoms. On a soil artificially infected with a pathogenic isolate of Fusarium oxysporum, plants started to wilt and die about 15 days after emergence and this gradually increased to 87% after 30 days. To increase disease development, small pots (290 ml) were used with 20 seeds per pot (high encounter probability of roots and spores of the fungus). The assay was carried out at a constant temperature of 25oC.

The bio-assay with seeds was tested on soil from 5 commercial onion fields in two years. For each soil sample 8 pots were used in the bio-assay. On each field 20 samples were taken from different sites. On the same sites onions were grown after sampling. In this way, 100 comparisons were realized between the bio-assay result and disease severity in the field. The relation found together with the reliability is shown in Figure 7.

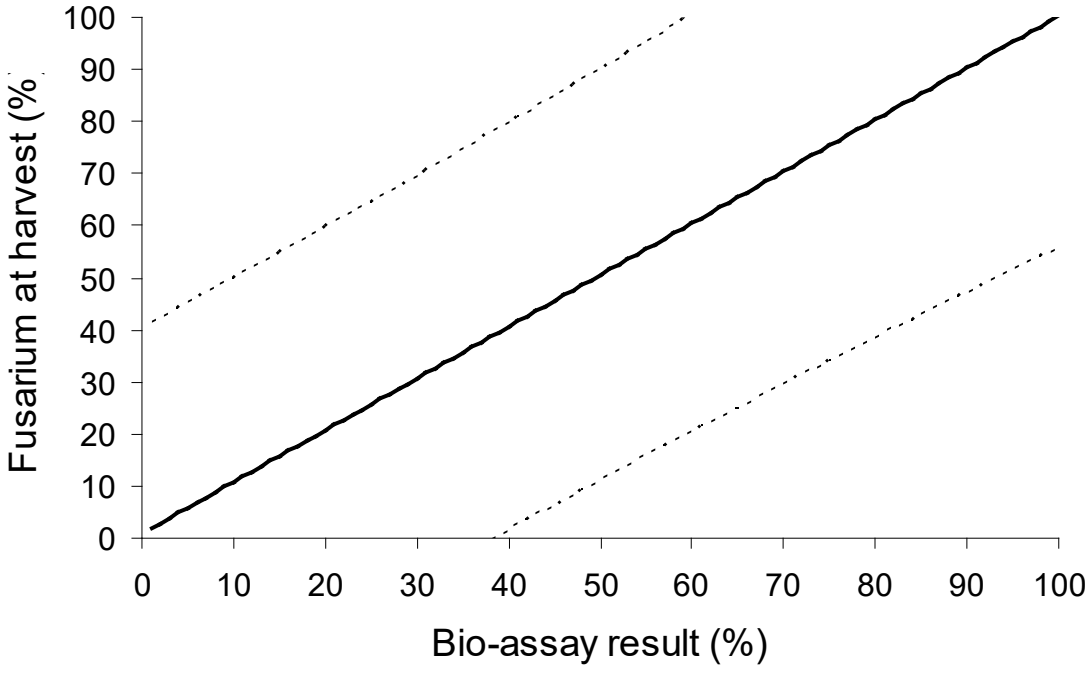

Figure 7. Established relation between bio-assay result and Fusarium incidence until harvest (continuous line) and the 95% confidence limits (dotted lines).

Figure 7 makes clear that the reliability of the bio-assay was not good enough to predict Fusarium basal rot to occur.

1.6 Disease prevention and control

Diseases caused by Fusarium oxysporum are difficult to control as are most diseases from soil borne fungi. Chemical control based on soil treatment (such as soil disinfection) are not a very promising route as these treatments tend to be expensive and there is a tendency to reduce soil treatments at a European level. Worldwide disease management of Fusarium basal rot is based on tolerant varieties. Other measures should be used to develop effective management strategies. In this chapter all possible management alternatives are summed up.

- Avoidance of fields with a recent basal rot incidence. As a reliable and cost-effective detection procedure is not yet available, it is not possible to analyze soil samples in order to determine an infection level. This leaves past onion growing history as the only reliable method to determine whether or not disease could be expected.
- If infected plants are found, the destruction of these plants together with healthy looking plants surrounding them could limit dissemination of the fungus through the field as mentioned by Pataky (1988) in a Fusarium control manual for ornamentals. The removal of these plants could slow down disease development in the field.
- Steam sterilizing the soil and realizing a temperature of over 80^{o}C for at least 30 minutes, or 70^{o}C for more than 1 hour (Pataky, 1988). This procedure is expensive and not cost-effective for onion production. In combination with other diseases to control, this could result in some control of Fusarium basal rot. However, no data on the effect on Fusarium basal rot have been found.
- Chemical control with fungicides. Tebuconazole gives a reduction of fusarium when the soil is treated before onions are sown. This treatment is not allowed in the Netherlands. In China, carbendazim, thiophanate and thiophanate-methyl are fungicides that have been reported to control Fusarium wilt on watermelons. An organo-copper fungicide called homodemycine (HDE) has also been proven to be effective in China.The pathogen is able to develop resistance to these fungicides (Chupp and Sherf, 1960; Li and Liu, 1990).
- Use of tolerant varieties in infected fields. There is variation in susceptibility to the disease with different varieties, none of which are completely immune. In the Netherlands tolerant varieties are available (Takmark and Takstar). However, they are not widely used because storability is poor. One question is whether tolerant and non-tolerant varieties stimulate disease increase in the same way or if there is a difference. To answer this

question, soil has been sampled before and after growth of several varieties in a field experiment in 2002 and 2003. The sampled soil was tested in a bio-assay (see above) of which the results are expressed as the percentage of dead or wilted plants. The results of this procedure are shown in Table 5. The figures show that the infection did not increase after growing Takmark or Spirit but increased after growing the varieties Summit, Recorra and Mundial. It must be stated here that results were highly variable and that the bio-assay does not have a high level of reliability.

Table 5. Bio-assay results on infection level increase after onion growing.

Variety	Bio-assay result		% increase
	Before sowing	After harvest	
Summit	22	31	41%
Spirit	26	30	15%
Takmark	20	22	10%
Recorra	17	25	47%
Mundial	22	29	32%

- A standard disease management tool regarding soil borne pathogens is not growing host plants for a certain number of years. The question is to what extent this control measure is effective in the case of Fusarium basal rot. Fusarium oxysporum species can mostly survive in soil by chlamydospores in plant debris (Nelson, 1981).These are thick-walled spores that are assumed to be able to survive for many years, although no data were found to give insight into the survival rate of chlamydospores of Fusarium oxysporum. Furthermore, the fungus could also be able to survive on plant debris as mycelium. Data are lacking to indicate the required number of non-host years that are effective for the population level to decline to sufficiently low numbers. A rough indication in practice is a period of 3-4 years but no data were found to back up this advice. In the Netherlands we have done research on soils with a Fusarium basal rot history of 0-8 years old. The bio-assay earlier was used to find an indication of disease level. Results were variable and no disease decline could be detected (Figure 8). An example of survival of a pathogenic Fusarium oxysporum comes from Blok & Bollen (1996). They carried out research in the Netherlands on early decline in asparagus fields caused by Fusarium oxysporum f. sp. asparagi. The fungus survives asparagus-free periods for at least 20 years and was found to be present in soil up to 1 m deep, showing the long survival potential for the fungus.

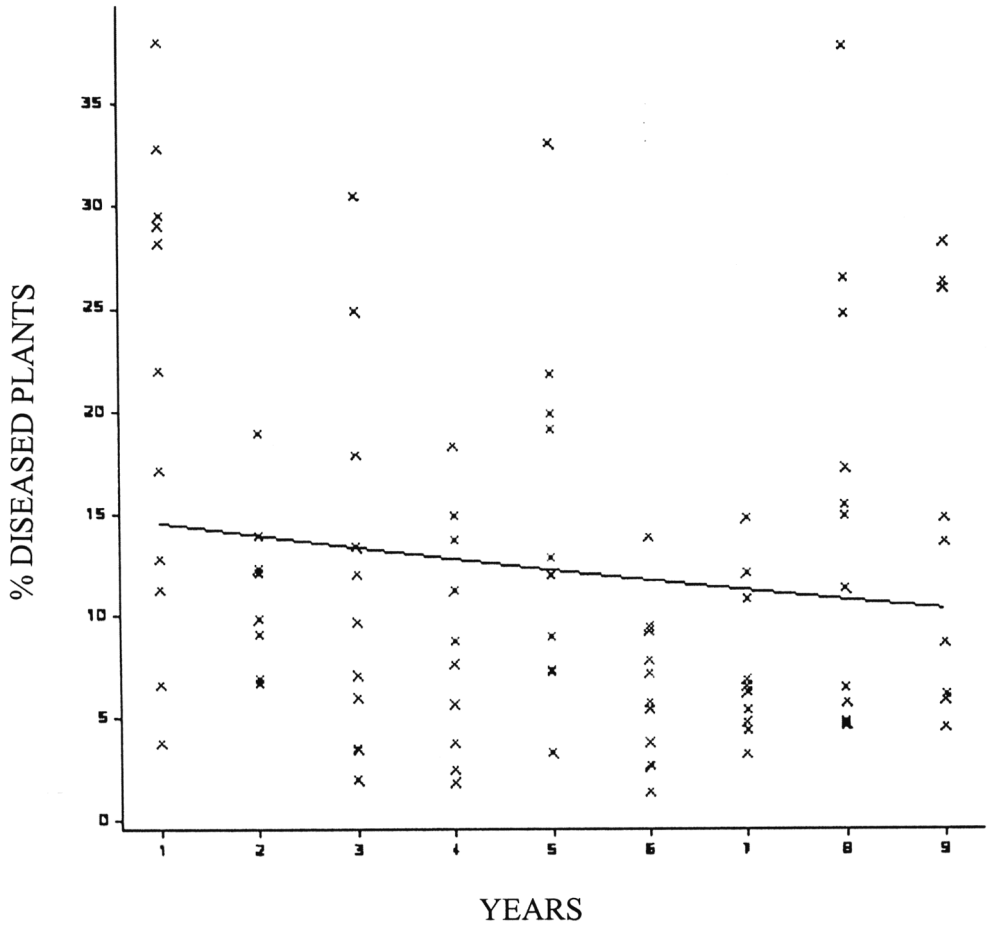

Figure 8. The effect of the number of years without onion growing (X axis) on the % diseased onions plants in the bio-assay as caused by Fusarium infection (Y axis) in 2003

- A set of management tools can be identified for prevention of disease spread:
    - The best possible prevention of soil infection by Fusarium oxysporum f.sp. cepae would be to only use planting materials that are free from inoculum. It is likely that the disease can spread by attachment to or infection of planting material such as sets. In the Netherlands, a system is in place with NAK-T where sets are certified free from disease. Sets from fields with disease incidence must not be used. However, a detection procedure on sets to ascertain the absence of pathogenic Fusarium (for instance PCR-based genetic techniques) could further limit the risk of disease spread. Moreover, it cannot be ruled out that the disease could spread with other planting material as well as onion sets. Theoretically, soil attached to potato seed, tulip bulbs or other planting material could also produce a potential risk.
    - In years with very low onion prices it can happen that produce cannot be sold.

This could result in higher volumes of onion waste. It would be wise to prevent this waste from ending up on non-infected soils.

- Furthermore, prevention of disease spread can be based on the principle of good farm hygiene. The fungus can spread with soil attached to machinery and tools, boots etc. when moving from an infected field to a not infected one (Mace et al., 1981).
- It is known that some Fusarium oxysporum fungi can spread with irrigation water (Kommedahl et al 1970). In the case of Fusarium oxysporum f.sp. cepae no records can be found to support this disease dissemination, but it cannot be ruled out.

- In countries with a warm summer climate, soil solarization alone or in combination with fumigants is effective in controling pathogenic soil fungi. In France, soil solarization has not proven to be effective enough for completely eliminating the inoculum from soil in the case of Fusarium oxysporum f. sp. dianthi. The addition of small quantities of fumigants (metham sodium or methyl bromide) significantly improves its efficacy. Satisfactory crop growth up to the second year of culture was possible (Cebolla et al., 1993). However, this method is not cost-effective for onion growing and not applicable in temperate regions.
- Organic soil amendments could possibly be effective in controlling diseases caused by Fusarium oxysporum. Amendments such as mustard (Brassica juncea) residues, rice husks, oyster shell powder, urea, KNO3, Ca superphosphate, mineral ash, wheat straw, clover straw, onion residues, coffee hulls, oilcakes from groundnut, mustard, sesame, cotton seeds, stalks of sunflower, alfalfa and Hungarian vetch (Vicia pannonica) were found very effective for the control of Fusarium wilt disease of bean, celery, melon, peas, radish and tomato caused by Fusarium oxysporum. In Figure 9 the effect of sunflower, alfalfa and vetch on basal rot in a crop folowing soil amendment, are shown (Ozer et al, 2000). The author suggested that the effects could be explained on the basis of different parameters such as nitrogen level, microbial population and soil pH. Other research reports on the effectiveness of organic residues comes from Smolinska (2000) who carried out research on Fusarium oxysporum f.sp.lycopersici in tomato and found significant effects on both chlamydospore survival and disease severity (Smolinska, 2000).

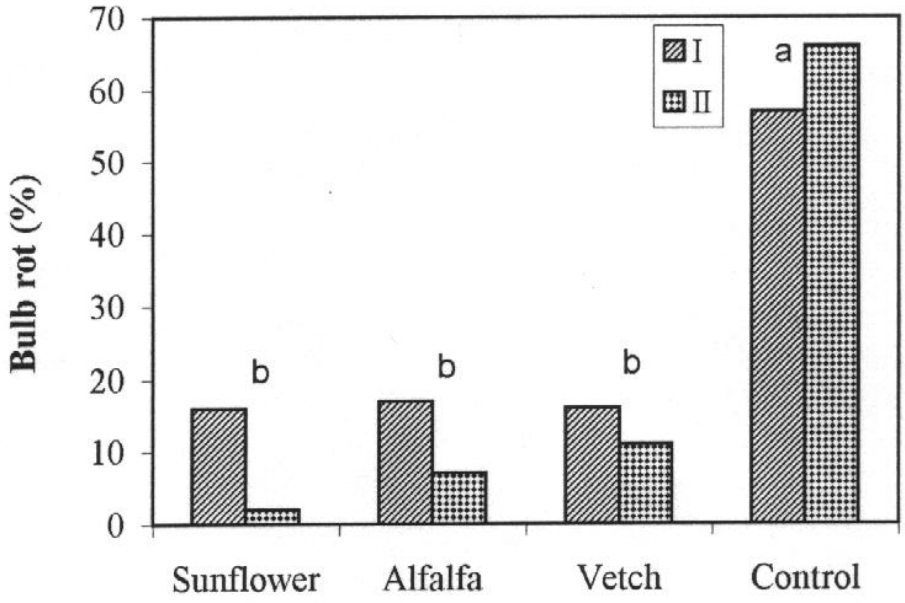

Figure 9. Onion bulb rot incidence (%) in the soils amended with stalks of different plants in two experimental areas. Columns with the same letter do not differ significantly (P=0.05) (figure copied from Ozer et al., 2002).

- The phenomenon of soil suppressiveness regarding Fusarium wilt in several crop species has been known for 70 years. Knowledge of the underlying principle could open perspectives for Fusarium wilt control. In some soils the disease did not occur although Fusarium oxysporum f.sp. cepae was present together with a susceptible host and suitable climatic conditions. In France, research has pointed out that non-pathogenic Fusarium oxysporum isolates play an important role in the mechanism underlying soil suppressiveness. Suppressive soils are low in iron avaliability (Alabouvette et al, 1993). In The Netherlands, Blok et al (1997) have shown that the pre-colonization of soil with non-pathogenic isolates of Fusarium oxysporum reduced root rot severity of asparagus plants by more than 50%. Although soil suppressiveness has been the basis of much research, it has so far not produced practically usable control measures. However, it seems promising for improvement of understanding the phenomenon as it can extend our knowledge on biological and non-biological interactions in disease outbreaks.

1.7 Other Fusarium species

During 1994 in the Netherlands many onions from sets appeared to suffer from Fusarium wilt. From diseased bulbs the fungus Fusarium avenaceum was frequently isolated. Following this, pot trials were done with Fusarium isolates to study the possible pathogenicity of the fungus. In a 1996 pot trial non-infested soil was artificially inoculated with one of the isolates. The results are presented in Table 6.

Table 6. Effect of Fusarium avenaceum

Soil infestation	% wilted or dead plants
No	5
Light	15
Heavy	25

Another pot trial carried out in 1996 showed that within the population of Fusarium avenaceum differences in pathogenicity existed. Both isolates were originating from rotten onion bulbs. Different levels of artificial soil infestation were used. In Table 7 the results are presented, showing that there was a large difference between two isolates, one (004) being pathogenic and the other (019) not. Also, the Table shows that at the end of the experiment the healthy looking plants on infested soil did not weigh less than those on non-infested soil.

Table 7. Influence of different isolates of Fusarium avenaceum.

Isolate	Soil infestation level	% wilted or dead plants	Fresh weight of healthy plants (mg)
no	0 %	10	1040
004	25%	43	895
	50%	37	1036
	75%	57	1064
	100%	50	819
019	25%	7	1022
	50%	10	903
	75%	0	1032
	100%	3	1242

With Fusarium oxysporum this is different: healthy plants on Fusarium oxysporum infested soil weigh less. This is shown in Figure 10 based on results of the second 1996 pot trial in which also an artificial infection with a Fusarium oxysporum f.sp. isolate was used. From these data it is clear that the pathogenesis of Fusarium avenaceum and Fusarium oxysporum is different. This is all the more clear from Figure 11 based on the second 1996 pot trial in which a susceptible (Hyfield) and a tolerant (Sundance) variety was grown on soil artificially infested with either a pathogenic and non pathogenic isolate of F. avenaceum (Fav004 respectively Fav019) and a pathogenic isolate of F. oxysporum (Foc016). The tolerance of the variety Sundance only appeared to be valid for attack by F. oxysporum and not for attack by F. avenaceum.

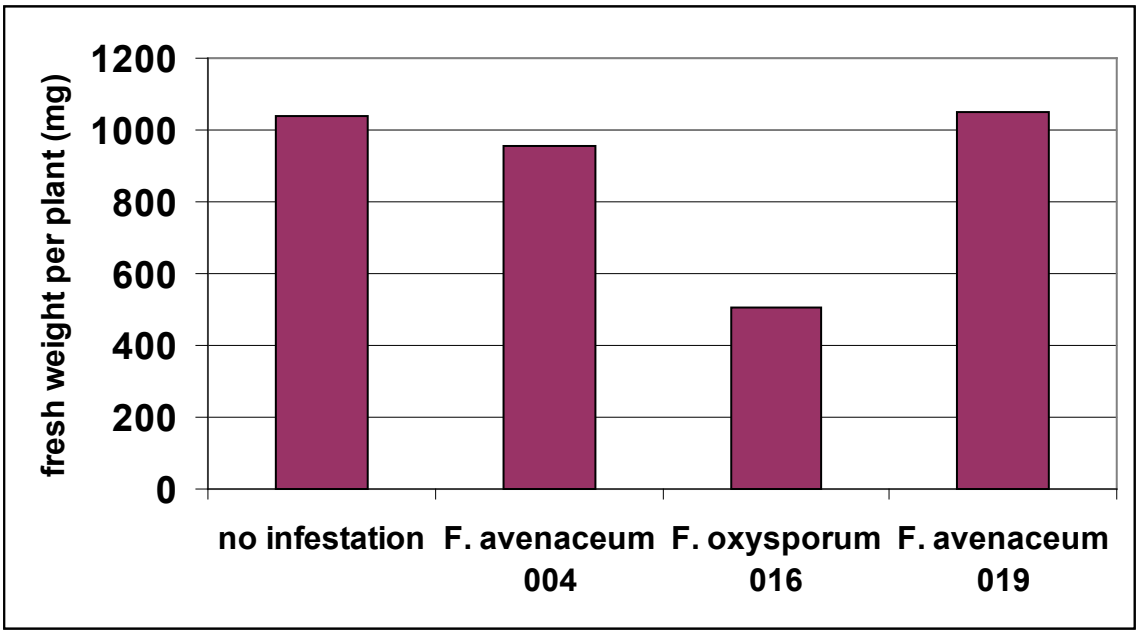

Figure 10. Influence of soil infestation with different isolates on fresh weight of healthy looking plants

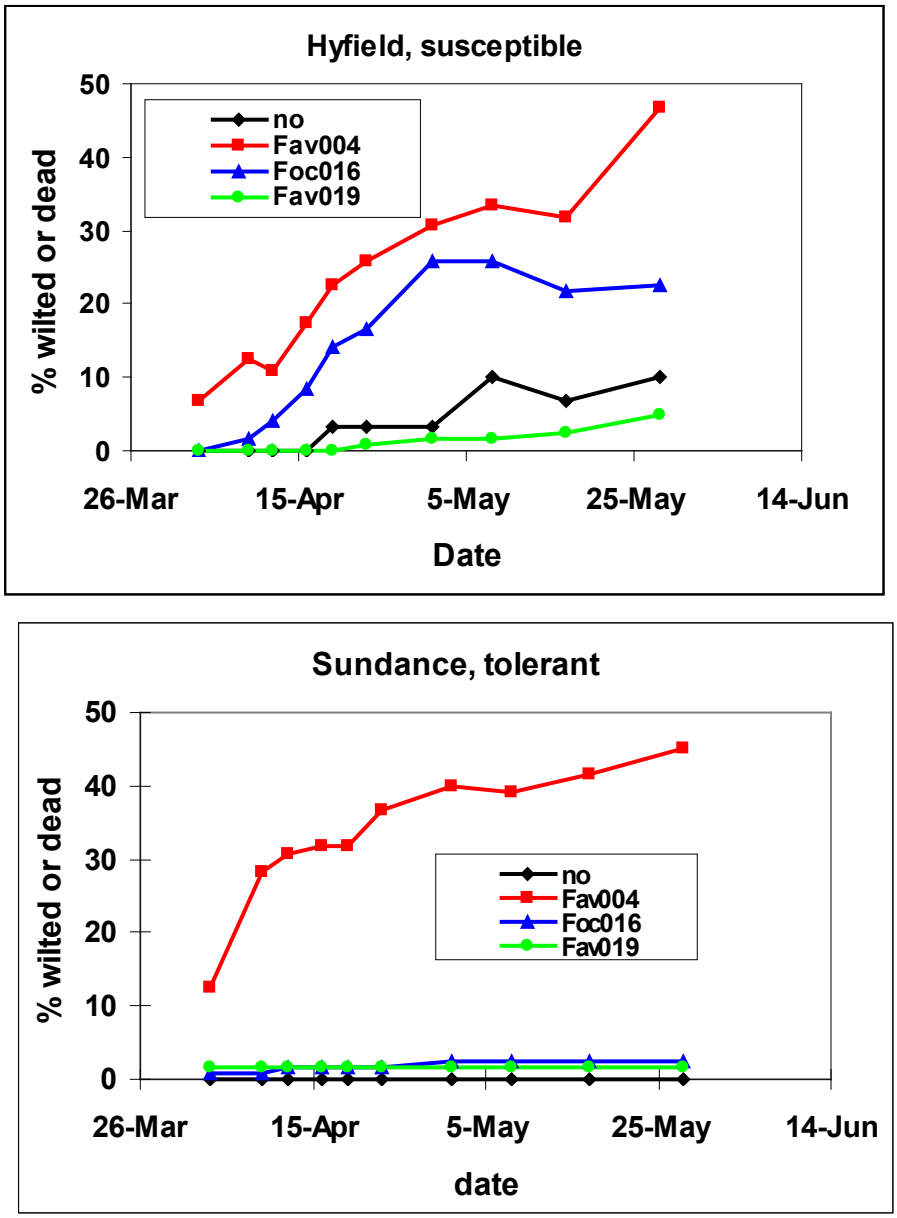

Figure 11. Disease development by Fusarium avenaceum (Fav) and Fusarium oxysporum (Foc) in a susceptible and tolerant onion variety.

In both 1996 pot trials, transplants were used and in subsequent pot trials where sets or seeds were used, F. avenaceum did not produce wilt symptoms. Fusarium avenaceum can therefore be described as a weak pathogen. This was underlined by a trial in 1995 on a commercial onion field in the southwest of the Netherlands where in the previous year wilt problems with sets were encountered. In this trial no wilt became evident (<0.5%). Possibly, the 1994 wilt

problems in commercial onion production from sets originated from the fact that in that year wet weather conditions retarded planting for a long time while after delivery the sets were probably stored on farms under conditions that increased the vulnerability of the sets to infection with F. avenaceum.

1.8 Research procedures

Having effective research instruments at your disposal, is a prerequisite to yielding good results. For this reason, a paragraph of this report is used to indicate these instruments, their usefulness and their restrictions. Research on a soil-borne fungus like F. oxysporum is not straightforward and research results described with this fungus should always be valued on the basis of the research methods used. For Fusarium research several instruments are available.

The most commonly used method is the detection of F. oxysporum with the aid of selective Komada medium (Komada, 1975) or other selective media (Abawi & Lorbeer, 1971a) and a statistical technique called soil dilution plate count. On Komada medium only F. oxysporum is able to grow at a significant rate. However, also F. avenaceum can form colonies, but this fungus grows more slowly on this medium and can easily be recognized by its appearance. Although the Komada medium is useful to determine the number of colony forming units of F. oxysporum in the soil, the result has no direct bearing on soil infestation with F. oxysporum f.sp.s cepae. If this selective method is used on soil with a known history of basal rot in onions, it can give an indication of the degree of infestation (Abawi & Lorbeer, 1971a). However, if the objective is to determine the infestation level on an arbitrary soil, false interpretations are likely, because the pathogenicity of a colony is not determining its growth pattern on a Komada or other selective medium. This is illustrated by the research carried out by Edel et al (1997) who collected non-pathogenic isolates of Fusarium oxysporum (non-pathogenic to wheat, tomato, flax and melon) using Komada medium. Media such as these are difficult to make and use some chemicals that have highly toxic properties, so care must be taken in the preparation process. Media like the Komada medium have been used widely to study the infection behavior of the fungus in field and controlled conditions.

To carry out research on Fusarium it is necessary to store isolates of the fungus. This can be done in several ways:

- Storage on agar media. Fusarium species are known for their easy loss of morphology and virulence during storage on these media. This was experienced with an isolate recovered in 1995 from a bulb with symptoms of basal rot. In a first pot trial at the beginning of 1995 the isolate caused 50% of onion transplants to wilt and die while in a second pot trial that same year the isolate caused almost no wilting.
- Storage on soil. After the experience described above, the fungus was stored on sterilized soil. In this soil the fungus is stored with its resting spores (chlamydospores). Mutations and loss of virulence are less likely to occur.
- Storage on autoclaved wheat seeds. This method was used by Lacy & Roberts (1982) to produce Fusarium inoculum for artificial inoculation of the soil. There are no records of research on possible decline of pathogenicity of the fungus during storage on this medium.

As there are no morphological characteristics that can be used to determine the pathogenicity of isolates of Fusarium oxysporum, other methods are needed to do so. Bio-assays could be used for this purpose:

- In research directed at developing or testing tolerant onion varieties, a standard bio-assay uses onion transplants. The roots of these transplants are cut at 4 cm under the basal plate and subsequently dipped for some time in a spore suspension (Fantino & Schiavi, 1987) or planted in an artificially infested sterilized soil (Abawi & Lorbeer, 1971b; Retig et al, 1970). However, research records are scarce in which these methods are used with a wide range of isolates of F. oxysporum. Joffe et al (1972) tested 68 F. oxysporum isolates from onion (mostly with disease symptoms) using this test procedure and found that the results were equally distributed in the range 0-100% dead seedling plants. In our research we found that some isolates of F. avenaceum were pathogenic using onion transplants but were not when using seeds or sets as planting material (see further). The planting material used showed great differences in virulence of isolates of the fungus. Transplants are potentially more sensitive to fungal infections as wounds are created in the process. It is therefore at least questionable if the pathogenicity results by Joffe et al (1972) would have been the same if in stead of transplants other planting materials had been used, eg sets or seeds.

- Inoculation of mature bulbs was used as a pathogenicity test by Holz & Knox-Davies (1974). In this test, a spore suspension was placed on wounds that are made artificially in the basal plate. This has been tried at Lelystad but good infections using different isolates were not produced. Again, artificial wounds are used in this test procedure and the creation of these wounds could easily influence test results.
- A test procedure that would exclude artificial wounds as an entry for fungi, would be the inoculation of soil in pots in which seeds or sets are planted. To use this procedure to identify the pathogenicity of a single isolate of the fungus, it would be necessary to use natural soil without basal rot history or sterilized soil. The last possibility would lead to an enormous increase of the fungus in the soil and hence create an abnormal pathogen population, leading to questionable results. The first possibility (natural soil) would introduce the interference of the natural soil flora, possibly due to the phenomenon of natural soil disease suppressiveness.

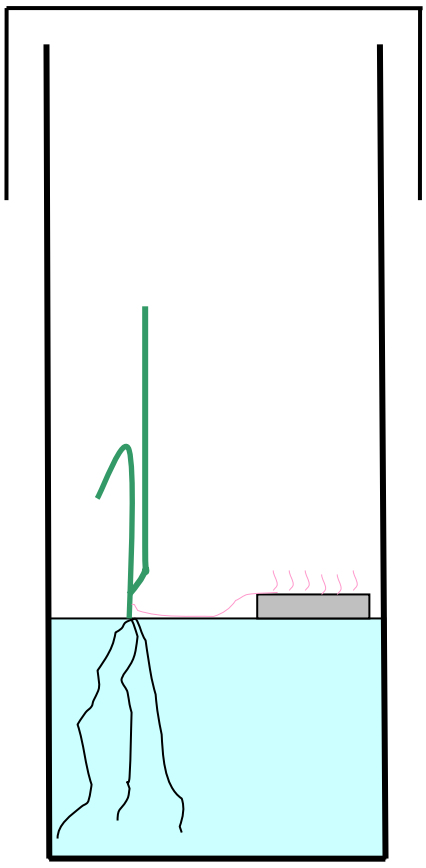

Figure 12. Set up of pathogenicity test for Fusarium oxysporum f.sp. cepae

- In view of the above, a procedure has been tried to develop for identification of the pathogenicity of Fusarium isolates. The procedure consists of a sterilized tube (Ø 25 mm with a volume of 55 ml sealed with a plastic cap, schematized in Figure 12) filled with 18 ml of a growth medium. Onion seeds treated with thiram/carbendazim were germinated under sterile conditions and transferred to the tubes after germination. As soon as the growing plants have one true leaf of 3-4 cm, an agar piece with the fungus was added to the tube. After 20 days the result was observed (onion plants dead or alive). Per isolate 7-10 tubes were used. During the research this test was carried out 6 times using a total of 25 isolates including 11 isolates of Fusarium oxysporum and 7 isolates of

Fusarium avenaceum. The other isolates were Fusarium solani, F. proliferatum, F. equiseti, Penicillium sp. And Rhizoctonia solani of which only F. proliferatum showed pathogenicity killing 7 out of 10 plants in 1 out of 6 experiments. The results were not stable (Table 1), partly caused by loss of virulence during storage but also partly due to unknown influences. Nevertheless, it seems worthwhile to further develop this test procedure as it is quick and cheap. Encouraging is the finding that isolate 022 showed clear pathogenicity in two pot trials (after artificial soil infestation) whereas isolate 025 did not. This was consistent with the results of the pathogenicity test as shown in Table 8.

Table 8. Results with the pathogenicity test

Isolate		% plants killed						
Nr	Species	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 8
-	Control	0	0	0	0	0	0	30
002	F. oxysporum				90			
005	F. oxysporum		30					
016	F. oxysporum	100	0	0	60	80	40	
022	F. oxysporum			30	100	100	100	
023	F. oxysporum			40	100			
025	F. oxysporum					20	60	0
026	F. oxysporum					0	30	30
156	F. oxysporum				80	30	20	30
180	F. oxysporum							100
181	F. oxysporum							100
182	F. oxysporum							70
004	F. avenaceum		100	100	80	70	60	
013	F. avenaceum		0		10	0	30	
019	F. avenaceum	90	10	60	100	20	0	
024	F. avenaceum			90	100	100	100	
107	F. avenaceum				100			
145	F. avenaceum				100		90	
171	F. avenaceum				100	100	90	0
020	F. profileratum	0	0	0	40	20	70	

To study measures with potential for basal rot control, a research method can be used as described by Fantino & Schiavi (1987). Artificially inoculated soil was used to study the tolerance level of onion varieties. The authors produced inoculum of an onion pathogenic isolate of F. oxysporum by growing the fungus on autoclaved wheat seed caryopses. The inoculum was placed at 5 cm depth alongside the crop row. This is an effective procedure to test field performance for possible tolerance of onion varieties.

1.9 Detection of Fusarium oxysporum f. sp. cepae

In the previous chapter some methods used in Fusarium research have been described. In this chapter techniques will be addressed that have been developed or are being developed to detect forma speciales of the fungus Fusarium oxysporum. In developing effective management strategies, prevention is a key issue. To prevent infestation or unexpected crop loss due to basal rot, it is essential to have a practical and cost effective detection procedure.

Vegetative compatibility

The first possible procedure could come from the classification framework called Vegetative Compatibility Groups (Leslie, 1993; Leslie, 1996; McCallum et al., 2004). The VCG technique is based on the potential of F. oxysporum isolates to genetically recombine (or: fuse by forming a heterokaryon) (Pulhalla, 1985) and is used to study the relatedness of plant pathogenic fusaria. The presence or absence of the recombination ability in an isolate is used to classify the fungus. The testing procedure is quite simple and cost-effective. The question is whether this classification procedure has any relation to pathogenicity (Leslie, 1996). In some forma speciales of Fusarium oxysporum this is the case, but in others not. A VCG-study with isolates from onion has been carried out by Swift et al (2002). The 19 isolates tested belonged to five VCGs. The authors pointed out that their study was insufficient extensive to draw conclusions. At this point, the usefulness of the VCG procedure to distinguish pathogenic from non-pathogenic isolates of F. oxysporum is not yet determined.

Pathogenicity test combined with soil dilution plate counts

Soil dilution plate counts combined with selective media (see chapter 4) will result in the soil population level of Fusarium oxysporum. To distinguish between pathogenic and non-pathogenic isolates of Fusarium oxysporum a pathogenicity test could be used. According to

what is stated in chapter 4, an effective and reliable pathogenicity test for onion is not available at the moment. Moreover, a procedure such as this would be time consuming and not cost effective. De Cara et al (2004) described the combination of selective media and pathogenicity test for melon and this resulted in a 100% identification of the forma specialis of melon (31isolaties tested) from a known infested field. There is no work using this procedure on fields with no wilt history where non-pathogenic inoculum could be relatively more numerous.

ELISA

The Enzyme-Linked Immunosorbent Assay (ELISA) is a method usually employed in biochemistry to detect the presence of a certain substance in a sample. It utilizes antibodies specific to the substance (antigen); these antibodies are linked to an enzyme which causes a chromogenic or fluorogenic substrate to produce a signal. The method can be employed to detect the presence of viruses and certain fungi in plant or soil samples. In literature no reference was found for using this technique to detect Fusarium oxysporum in onions or other host plants.

PCR (Polymerase Chain Reaction) and DNA fingerprinting

The ability of Fusarium oxysporum isolates to cause basal rot is based on genetic characteristics. Individuals that have a certain behavior in common (such as pathogenicity) also have some part of their genetic material in common. If this part is known then the DNA of a certain isolate can be screened for similarity and thus for its pathogenic ability. This procedure is called DNA fingerprinting. The PCR technique is used to multiply the DNA part that needs to be detected so that small amounts of DNA amidst other material can be detected. This technique has been used widely on plant pathogens including Fusarium oxysporum. For example a testing procedure was developed by Chiocchetti et al (1999) for detection of Fusarium oxysporum f.sp. dianthi, causing wilt in carnation. Six out of eight known races were detected on carnation cuttings. Identification of 98% of isolates of Fusarium oxysporum canariensis (palm wilt) in plant tissue was reached by Plyler et al (1999) while the PCR technique in combination with DNA fingerprinting was used to detect Fusarium oxysporum f.sp.basilici on basil (Chiocchetti et al, 2001). Alves-Santos et al (2002) used the PCR technique to distinguish pathogenic from non pathogenic Fusarium oxysporum on common bean (Phaseolus vulgaris) and reached a 100% identification in roots and stems of bean

plants. De Haan et al. (2000) developed a multiplex PCR assay that gave good discriminating between field isolates of *Fusarium oxysporum f.s. gladiolus* of races 1 and 2 detected on Gladiolus corm material. This PCR assay is currently used by the Bulb Inspection Service when suspected corms are found in the Netherlands.

There are no reports of this technique being applied to Fusarium oxysporum f.sp. cepae. Nevertheless, the PCR procedure has huge promise to develop detection procedures for Fusarium oxysporum f.sp. cepae in planting material as well as soils, because of its reliability and cost-effectiveness.

2 Recommendations for further research and development

To manage Fusarium basal rot properly several tools should be available to the industry:

- Varieties with good tolerance level and at the same time good yield and quality potential. From variety trials it is clear that these varieties are hard to find. A good tolerance level is available with Takmark, Mundial and Recorra, but at least with Takmark storability is not good enough for Dutch circumstances. The search for good varieties should continue together with the breeding companies. For infected fields, these tolerant varieties would be of great help.
- The industry should have a reliable and cost-effective detection procedure at its disposal for both soil samples and plant samples (sets). The PCR plus DNA fingerprinting technique has good promise to fill this gap as witnessed by successes with other formae speciales of Fusarium oxysporum. The further spread of the disease could then be reduced and sets could be certified as being safe in terms of infection.
- As crop rotation is considered an important tool in basal rot disease management, more should be known about the population dynamics of the fungus. So far research in this direction has not been carried out, probably resulting from the lack of a reliable detection method. Development of a PCR plus DNA fingerprinting technique seems a prerequisite to these studies. Related items such as host range, survival rate in the absence of host plants and reproduction capacity on host plants, could be addressed to produce management tools on field and farm level.
- Measures such as organic soil amendments have shown good promise of providing the onion industry with effective control tools. It is recommended that more detailed research is done on this direction.

The development of these tools is costly. At the same time, it is in the interest of most European onion growers to have them at their disposal. It is therefore recommended to coordinate the main onion producing countries in a collaborative approach to provide the industry with these tools. This research should be linked with seed companies, laboratories and research institutes in several European countries.

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