

Hygiene protocol Fusarium in lettuce

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framework of:**

***IWT 140984 Integrated control strategy of fungi and
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 - Inagro
 - Research Station for Vegetable Cultivation (PSKW)
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HYGIENE PROTOCOL for *FUSARIUM*

Hygiene is essential throughout the business!

Provide disinfection mats

- At each **company entrance**, and at **every door to the growing area**
- Mats must be large enough to be **impossible to bypass**
- **Everybody and every vehicle/trolley** must walk or drive over the mat
- Mats must be **thoroughly wetted** with potassium hydroxide solution (1-2%, pH>10)



Everything that goes into the glasshouse must be disinfected

- Provide facilities for washing/disinfecting **hands** before entry to glasshouses
- Provide clean **shoes and clothing** or use disposable overshoes, overalls and gloves
- Allow only essential **visitors** and provide them with clean footwear and clothing
- Disinfect **work utensils** such as knives with 100 ppm sodium hypochlorite or 70-80% alcohol
- Clean materials with as hot water as possible under high pressure, removing all **organic residues**
- Machines from other companies (e.g. **contractors**) must be clean and disinfected
- Only allow cleaned **packaging material**
- Only use **seedlings** from a grower who takes control measures against Fusarium; inspect the plants

Avoid dispersal within the glasshouse

- Organic matter, soil particles and dust spread the disease, keep **paths** clean
- Remove all **crop residues** between crops
- **Inspect** the crop regularly and send plants with suspect Fusarium symptoms for diagnosis
- Disinfect **recirculated water** with UV, ozone or heat
- **Use different growing material** for each section/compartment; regular disinfection helps
- Always **work in the same direction**



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Hygiene protocol

Fusarium oxysporum f. sp. *lactucae* in lettuce

1 Introduction

In the Netherlands, an increase in outbreaks of *Fusarium* was observed in soil-based cultivation of glasshouse lettuce during the winter season of 2014/2015. Losses increased during the course of 2015 and by the autumn it was estimated that 50-60% of Dutch glasshouse lettuce production was lightly or heavily contaminated (Source: Hygiene Protocol *Fusarium*, Green Agro Control).

In Belgium, the first reports of large areas of lettuce showing wilting occurred in 2015. After sampling the cause was shown to be *Fusarium oxysporum* f. sp. *lactucae*. In 2017, the problem was identified throughout Flanders (Antwerp, East Flanders and West Flanders) with the highest levels of infection in the provinces of Antwerp and East Flanders. The affected area has increased and is now estimated at 12% of the total area. In both the Netherlands and Belgium, the problem so far only exists in soil-based year-round glasshouse lettuce production. Research by the NVWA (Dutch Food and Consumer Product Safety Authority) has shown that a new race of *Fusarium oxysporum* f. sp. *lactucae* has been found. This new race deviates from race 1 that occurs in outdoor production in Spain and Italy. The race found in Belgium is still being studied by Ghent University (Phytopathology Laboratory).

To address the new *Fusarium* threat, solutions must be sought using hygiene, resistance and (biological) plant protection products. Steam sterilisation of soil can also help to reduce contamination. Resistant varieties for this soil-bound fungus are not available in the short term, but may provide the only solution.

To limit further spread as much as possible, hygiene measures are necessary.

This hygiene protocol for limiting as far as possible the occurrence and spread of *Fusarium oxysporum* f. sp. *lactucae*, is available thanks to collaboration between the partners of the 'FUNSLA' project. Use was made of the Hygiene Protocol drawn up in the Netherlands by Groen Agro Control in collaboration with LTO Glaskracht Nederland.

2. *Fusarium*

Fusarium oxysporum is a common soil-borne fungus and has various sub-species (formae specialis), which can affect about 80 plant species. Each sub-species is specialised to one or more host plants. The disease occurs in cucumber, paprika, tomato, lettuce, melon, banana, various potted plants and cut flowers. The plant pathogenic sub-species 'forma specialis', in lettuce is *Fusarium oxysporum* f. sp. *lactucae*.



Figure 1. Infected lettuce plant with brown vascular bundles (Source: PSKW)

In 1955, a sub-species of *Fusarium oxysporum* was reported in lettuce for the first time in Japan. This *forma specialis* was also found in other Asian countries and the US. In 2002 the fungus was found in Europe, initially in Italy and then in Portugal in 2004. First infections in southern France were reported in 2014.

Up to 2017, 3 races (sub-species) were known. In 2017 it was established that the race that occurs in the Netherlands deviates from these 3 races and was named as race 4. Whether we are also dealing with this race 4 in Belgium is currently being determined.

F. oxysporum is unique in that the fungus produces three types of asexual spores; macroconidia, microconidia and chlamydospores. The macroconidia and microconidia are produced in infected plants on the stem surface and can spread to neighbouring plants. Chlamydospores have thick cell walls and can survive in the soil for prolonged periods. They are formed when the growing conditions for the fungus deteriorate or when the plant dies.

Mycelium or spores that germinate can penetrate the root. Subsequently, the mycelium will end up in the vascular bundles and form microconidia that are further transported in the plant. Again, these microconidia will germinate and block the vascular bundles, causing visible wilting symptoms. Eventually the fungus also grows outside on the stem and makes macroconidia. Chlamydospores can then be formed from old mycelium or macroconidia. These survival structures are very persistent; therefore all plant material must be removed between crops. They survive on the ground, on glasshouse stands and equipment, in organic matter and in the soil. If they germinate, new infections occur.



Figure 2. Wilting of soil-based glasshouse lettuce (Source: PSKW)

Fusarium oxysporum also lives on dead plant material if the host plant is not present. This enables the fungus to survive indefinitely in the soil. There is currently no solution available to eliminate *Fusarium* from the soil.

Although seed does not seem to play an important role in spreading infection, seed transfer cannot be completely dismissed. Dutch seed companies have never been able to demonstrate the fungus in lettuce seed. In the event of a possible contamination, it is important to immediately implement hygiene measures to prevent further spread.

3. Contamination and spread

- *Fusarium* infects the plant through the roots and grows upwards through the xylem vessels. Since the xylem is responsible for transport of water and nutrients from the roots to the leaves; blocked xylem vessels cause the plant to wither and die.
- No root damage is necessary, for example by root centipede or nematodes, to cause an infection. The *Fusarium* fungus can affect the plant independently. Root damage can increase a fungal attack.
- The fungus affects *Lactuca sativa*: iceberg lettuce, butterhead lettuce, oak leaf lettuce, Batavia, lollo bionda, lollo rossa, Roman lettuce, multileaf varieties. Some varieties show an increased tolerance.
- Above-ground spread of the fungus mainly takes place via infected plant material, plant residues, through crop treatments and water droplets. Over medium and short distances, the disease can also be spread through contaminated soil (earth that sticks to machines, tools, crates, pallets) and even through the air. Spread over long distances may occur via seed.



Figure 3. Necrosis in the vascular bundles caused by *Fusarium oxysporum f. sp. lactucae* (Source: Green Agro Control)

- The fungus can survive in the ground for several years. The optimal soil temperature for fungal development is 24-28°C. The first symptoms appear in spring as soil temperatures rise. Observations in Belgium show that infections can occur at 15°C.
- Research shows that sensitive varieties are damaged more at higher temperatures. It is therefore difficult to indicate the general temperature range for lettuce, but in warmer growing periods it may be beneficial to plant resistant varieties.

4. Symptoms

After planting in soil, signs of infection begin with yellowing, then wilting and die-back. Older plants can survive, but development lags behind uninfected plants.



Figure 4. Retarded growth caused by *Fusarium oxysporum f. sp. lactucae* (Source: PSKW)

- Infected plants usually show a red-brown necrotic discolouration in the root base.
- Vascular discolouration is often seen in leaf veins.



Figure 5. Symptoms of wilting in butterhead lettuce (Source: Rijk Zwaan)

5. Control measures

The best control measure against *Fusarium* wilt is to plant cultivars that are fully resistant against the prevalent race. Unfortunately, these are outdoor varieties and not suitable for glasshouse production. There is information about and experience with partially resistant varieties. Tests already show that the following varieties are partially resistant:

- Intermediate and high-resistance varieties:
 - Butterhead lettuce: 42-120 RZ (Rijk Zwaan), Emilina (outdoor-type, southern Europe, Rijk Zwaan), Sandalina (outdoor-type, southern Europe, Rijk Zwaan). Note: these partially-resistant varieties require a fully-adapted cultivation technique.
 - Lollo bionda: Lugano (Rijk Zwaan), Livorno (Rijk Zwaan), Limeira (Rijk Zwaan)
 - Lollo rossa: Satine (Rijk Zwaan), Athmos (Rijk Zwaan), Soltero (Nunhems)
 - Multileaf types: Codex (Salanova crispy, Rijk Zwaan), Haflex (Salanova crispy, Rijk Zwaan), Triplex and Wintex (Salanova crispy red), Extemp (Salanova crispy green)
 - Green oak leaf lettuce: Kitonia (Rijk Zwaan)
 - Red oak leaf lettuce: Xerafin (Salanova oak red, Rijk Zwaan)
 - Roman lettuce: Actina (Syngenta Seeds)
 - Batavia: Yacht (Salanova, Rijk Zwaan)
 - Crunchy types: 41-673 RZ (Rijk Zwaan), 41-692 RZ (Rijk Zwaan)

- Sensitive varieties:
 - Butterhead lettuce: All common types under glass.
 - Lollo bionda: Lozano (Rijk Zwaan)
 - Multileaf types: Vicinity (Salanova, Rijk Zwaan)
 - Green oak leaf lettuce: Cook (Salanova, Rijk Zwaan)
 - Red oak leaf lettuce: Rouxaï (Rijk Zwaan), Saturdaï (Rijk Zwaan), Eventaï (Rijk Zwaan), Soupiraï (Rijk Zwaan), Xandra (Salanova, Rijk Zwaan)
 - Roman lettuce: Maximus (Rijk Zwaan)
 - Batavia: Othilie (Rijk Zwaan), Vessel (Salanova, Rijk Zwaan), Bobal (Salanova, Rijk Zwaan)



Figure 6. Sandalina (see photo) and Emilina require an adapted cultivation technique (Source: PSKW)

- Crop rotation: Research has shown that *Fusarium oxysporum* f. sp. *lactucae* can also colonize roots of tomato, broccoli, cauliflower and spinach, but without visible symptoms. *Fusarium oxysporum* f. sp. *lactucae* can also survive on plant residues, enabling inoculum of the pathogen to increase. It therefore seems that crop rotation does not offer a solution for the control of *Fusarium*.
- Chemical crop protection products: no products are currently permitted for the control of *Fusarium* in lettuce in Belgium. The FUNSLA project began a programme to investigate products that might have an inhibitory effect against *Fusarium*. Azoxystrobin, present in the products Ortiva, Amistar and Mirador, and permitted in protected lettuce production against *Rhizoctonia* (in Belgium) showed minor effects at the beginning of cultivation against *Fusarium*, but at the end of the cropping cycle, treated lettuce was fully infected.

- Biological crop protection products: Efficacy tests undertaken in the FUNSLA project, showed that no product was able to control the disease, but there were slight differences in weight and root development. For example, a slight positive effect was observed for Prestop (32% *Gliocladium catenulatum* J1446) and various products based on *Trichoderma* when the first application was done by application to modules or at the plant nursery. Prestop and Trianium P (*Trichoderma harzianum* T-22) already have approval in protected lettuce production in Belgium. When the *Fusarium oxysporum* infection is still very low, such as after steaming in the first crop, the results may be better. Trianium P was applied 1 week after sowing with 600 g in 100 litres of water per 100m². 300 g of product were applied to trays just before planting with 100 litres of water per hectare. We recommend not to combine Rizolex (tolclofos methyl) and products based on *Trichoderma* in the same tank, but they can be used separately on the soil. Prestop and products based on *Trichoderma* are not recommended to be tank-mixed.
- Cultivation period: Soil temperature has a major influence on the development of *Fusarium oxysporum* f. sp. *lactucae*; i.e. lower soil temperatures significantly reduce the risk of contamination and crop losses, so choose a cultivation period that is least suitable for the development of *Fusarium*. Based on experience in Belgian companies, we can assert that harvesting between 1 January and 1 March gives fewer symptoms. However, this is always dependent on the soil temperature. Research shows that plants showed visible wilting symptoms in the summer 10-20 days after infection, whereas in the winter symptoms took 45-50 days to develop.
- Variety choice: Grow less sensitive varieties during warmer growing periods.
- Hydroponic cultivation: Several trials are currently under way to determine whether *Fusarium oxysporum* also easily affects lettuce grown hydroponically. Results apparently depend on how the artificial infection is applied. No natural damage has so far been observed in the Netherlands or Belgium, but there is no guarantee that *Fusarium* will not occur. *Fusarium* is a water-loving fungus and can spread very quickly in a crop in water. When decontamination equipment can run continuously, hydroponic lettuce may be an option for the future, but high investment costs relative to lettuce prices determine system viability.
- Hygiene measures: For the time being, these are the only measures to prevent contamination and prevent spread in the event of an existing contamination.

6. Industrial hygiene

Total industrial hygiene is necessary; once infected there is no cure to get rid of Fusarium. A clean start is important to prevent contamination in the new crop. Important: the right working order and the right priorities.

Important:

- Basic rule: Start clean and stay clean!
- Everything that needs to be disinfected must first be thoroughly cleaned. Organic material neutralizes disinfectants, so they are no longer active. That is why everything must first be sprayed clean or cleaned before disinfection can take place.
- Always refer to the label instructions for use when using disinfectants. Do not let anyone (accidentally) go from a dirty to a clean area.
- Disinfectants give the best results at high RH, high temperature (20°C), on clean surfaces and with long exposure time (from 30 minutes to 16 hours, depending on the agent used). The best policy is to ensure that everything stays wet for as long as possible.

Re-cropping

1. Remove old crop and all organic material. Organic material is the main source of infection. Shredding crop residues causes direct contamination of the soil.
2. After removing all organic material, spray the production area (equipment and structures) with disinfectant. (Soil obviously cannot be sprayed clean).
3. Spray glasshouse structures with water to remove dust from the glasshouse and parts. In case of heavy contamination, also add sodium hypochlorite to the water. Use a minimum of 3,000 litres of solution per hectare. Concentration of chlorine: 100 ppm (100 ml per 1000 litres of water).
4. Disinfect the entire watering system: Pipes, sprinkler caps, etc. can be contaminated. If there is any fungus (spores) present, then new plants will be re-infected immediately after planting.
5. Chemical soil disinfection: Experience shows us that soil disinfection with chloropicrin or 1,3-dichloropropene is inadequately effective. The efficacy of products based on metam is currently being investigated.

6. Steaming: Soil steam treatment can significantly reduce the amount of *Fusarium* in the soil, but it cannot completely eliminate the pathogen. So there is still a risk of infection for subsequent lettuce crops. It is therefore important to combine control measures such as steam treatment with other measures. According to the literature, a temperature of 70°C for 30 minutes is optimal, and kills a large proportion of the pathogen. Best results are obtained by vacuum steaming because *Fusarium* can also survive in deeper soil layers. *Fusarium* is less likely to survive in anaerobic environments, e.g. those caused by groundwater.
7. Disinfectants can be used to kill fungal contamination on screen cloths – first open the screen cloth by 75% to lower it. Contractors use a combination of hydrogen peroxide and peracetic acid as fog treatment (e.g. Sopuroxid 15, see Table 1).
8. Clean and disinfect all equipment; machines (spading machine, forklift, planting machine), knives, tools, trays, packaging material, spraying equipment, etc. Authorised products require a recognition of category PT (product type) 2 and are listed in Table 1 (Belgium).
9. Clean other business premises. Destroy or wash all clothing and footwear used in the old crop at a high temperature (90°C).
10. Provide soil nutrition and grow a more resistant crop variety.

Glasshouse setup

1. Ensure that the entire nursery is clean and remains clean. Provide good planning and implementation; do not let anyone (accidentally) go from a dirty to a clean area.
2. Place a large disinfection mat at each operating entrance and all entrances to cultivation areas. Place large mats in such a way that harvest carts/forklifts drive through with all wheels. Walking around or bypassing mats must be impossible. Also ensure that staff with company footwear walk through the mats. Ensure that the mats are always wet. Fill the containers with 1-2% potassium hydroxide solution and keep the containers clean and effective. Mats that are not properly maintained are a source of infection.
3. Only use cleaned containers from the plant propagator.
4. Plants: Start from healthy plant material, ensure an entry check of all incoming plants. The plant material must be grown by a company that also implements measures against *Fusarium*.
5. Use clean shoes and clothing (wash clothes at 90°C!).

During cultivation

1. Deny access to visitors who do not have to be in the glasshouse; lock doors.
2. Lay disinfection mats at entrances that can not be ignored and ensure that they are always soaked with potassium hydroxide: ensure that pH is always above 10.
3. Ensure that employees use clean clothing and that they clean their hands with soap and water before they enter the greenhouse, after which they may be disinfected.
4. Separate harvesting trousers for each section/compartment, regular disinfection.
5. Use separate and disinfected tools for each section/compartment.
6. Always perform crop work in the same working direction.
7. Visitors: provide disposable overboots, disposable overalls and disposable gloves.



Figure 7. Make it clear to visitors that wearing overboots is mandatory

8. Crop control is essential; regularly inspect plants for possible symptoms. Think about your working direction!
9. Have plants with suspicious symptoms examined by a reputable laboratory.
10. Irrigation can spread disease locally through spray water, via contaminated soil or infected plants.
11. When recirculating the feed water: disinfect by heating, UV or ozone.
12. Check the effectiveness of the disinfectant before recirculation is started.

If contamination has occurred

1. Remove infected plants or plants that have come into contact with infected plants in closed plastic bags. It is best to remove contaminated plants, plant and root

remains and a buffer of plants around them (1-2 m²).

2. Mark contaminated places. Pay attention to the order of work: ensure that the zone where infected plants have stood is the last area for crop work.
3. After removing contaminated plants, wash hands thoroughly with soap and if clothing has come into contact with contaminated plant material: remove clothing and wash at a minimum of 90°C with washing powder.

7. Cleaning and disinfection methods

Cleaning agents:

1. Hands: Soap, alcohol gel.
2. Disinfection mats for disinfecting footwear: potassium hydroxide (1-2%).
3. Cleaning pad, machines, blades, etc.: Use as hot water as possible and a high-pressure cleaner and disinfect with sodium hypochlorite (100 ppm).
4. Algae, biofilm: hydrogen peroxide, sodium hypochlorite. Note: do not use sodium hypochlorite in irrigation water during cultivation because of the chlorate issue.
5. 70-80% alcohol to disinfect hands, small surfaces and tools.

Disinfection equipment:

1. Heating: water must be treated for at least 120 seconds at 85°C or 15 seconds at 95°C. The advice for complete disinfection is 180 seconds at 85°C or 30 seconds at 95°C, because it is often not certain which pathogen you are dealing with.
2. UV: minimum dose of 150 mJ / cm².
3. Ozone: 2 to 5 ppm ozone for at least 1 minute or 1 ppm for at least 5 minutes.
4. Ultra-membrane filtration is also suitable for *Fusarium*.

Disinfectants:

Table 1. Authorised disinfectants for treatment of materials and surfaces (PT2) against fungi.

Product	Authorisation number	Active substance	Dosage	Minimum exposure time	Remarks
Menno Florades	10212P/B	benzoic acid	1%	16 hours	For control of plant pathogenic fungi, the surfaces must remain wet for at least 16 hours.
Sopuroxid 15	4797B	peracetic acid, hydrogen peroxide	1%	60 min or longer	corrosive
Horticlean 15 Fort	7716B	peracetic acid, hydrogen peroxide	1%	60 min or longer	corrosive

Always follow the instructions on the label. Disinfectants work best if first the materials and surfaces to be disinfected have been thoroughly cleaned. Always consult the following link:

https://www.health.belgium.be/sites/default/files/uploads/fields/fpshealth_theme_file/list_e_biocides_pdf_site_web_96.pdf

Further information:

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