

COST863  
WG2 and WG3 Joint SGM

Plant health in changing environment

MTT Agrifood Research Finland, Jokioinen  
19-20 May, 2008



## Programme

### **COST 863 WG 2 and WG 3 Joint SGM** ***Plant health in changing environment***

MTT Agrifood Research Finland, Jokioinen 19-20 May, 2008

#### ***Monday 19th May***

9.00	Welcome to MTT	Director Ilkka P. Laurila
9.30	Ecological aspects of <i>Xanthomonas fragariae</i> in strawberry: survival in crop debris in soil and distribution in the field	Jan van der Wolf
10.00	Detection of <i>Xanthomonas fragariae</i> and study of its association with the strawberry plant	Jane Debode
10.30	Coffee	
11.00	<i>Colletotrichum acutatum</i> in Norway	Arne Stensvand
11.30	Detection and monitoring of <i>Colletotrichum acutatum</i> on strawberry during the latent phase of the infection	Jane Debode
12.00	<i>Colletotrichum acutatum</i> - infection and survival in alternative hosts	Päivi Parikka
12.30	Lunch	
13.45	Protocols of nuclear plants maintenance and certified strawberry plant production in Finland	Jaana Laamanen
14.15	The situation of healthy plant propagation and availability of healthy strawberry plants in Sweden.	Elisabet Nilsson
14.45	Plant health inspection and control of certified nursery production	Mirkka Kokkola
15.15	Coffee	
15.45	Main phytosanitary problems on strawberry in France	Alain Bardet
16.15	Understanding the epidemiology of <i>Phytophthora cactorum</i> (strawberry crown rot) in strawberry plant propagation	Angela Berrie
16.45	Resistance of strawberry cultivars to <i>Phytophthora cactorum</i>	Päivi Parikka
17.15.	Discussion on production of healthy plants and managing quarantine diseases	
19.30	Dinner at Restaurant Forssan Klubi	

***Tuesday 20th May***

8.15	Viruses and phytoplasmas infecting strawberry	Josef Špak
8.45	French research programme about powdery mildew of strawberry	Alain Bardet
9.15	Current status of fungal diseases in strawberry in Lithuania	Laimutis Raudonis
9.45	Coffee	
10.15	Designing strategies to control grey mould in strawberry cultivation using decision support systems	Bert Evenhuis
10.45	Biological control of grey mould ( <i>Botrytis cinerea</i> ) in strawberry	Christer Olsson
11.15	The effect of PGPR (Plant Growth Promoting Bacteria) as a biological control agent on <i>Botrytis</i> in strawberry	Sezai Ercisli
11.45	Use of chitosan and other resistance inducers to control postharvest gray mold of strawberries	Gianfranco Romanazzi
12.15	Biological soil disinfestation to control <i>Verticillium dahliae</i> in strawberries	Bert Evenhuis
12.45	Lunch	
13.45	Discussion and closing	

## List of participants

### COST

#### participants

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#### Other participants

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Riitta Kempainen	MTT Agrifood Research Finland, Plant Production Research FI-31600 Jokioinen	Finland
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## Ecological aspects of *Xanthomonas fragariae* in strawberry: survival in crop debris and distribution in the field

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Marjanne de Weerd & Arjen Speksnijder  
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*Xanthomonas fragariae* is the causative agent of angular leaf spot disease of strawberry. Although the pathogen is widely disseminated in Europe, for propagation material it is still considered as a quarantine disease and therefore put on the EPPO A2 list. It is causing significant economic damage, not because of direct losses, but because of required statutory measures, which include surveys, hygienic protocols and eradication of infected plant material.

If multiple infections occur and entire fields have to be destroyed, plant material often is sprayed with an herbicide and ploughed under. The first part of our study was conducted to investigate which herbicide is most appropriate for haulm destruction and for what period *X. fragariae* can survive in plant debris in soil. Inoculated, symptomatic plants (cv Elsanta) were treated with Round up, Grammoxone, Duplosan or water. Two weeks after haulm destruction leaves were harvested. Leaves and the remaining plant parts were separately put in net bags and buried in crates with a peaty soil representative for strawberry production. Crates were stored at temperatures prevailing in the Netherlands during the winter of 2006/2007 (6-11 °C). Grammoxone most effectively killed the strawberry plants and enhanced decomposition of plant material in soil. *X. fragariae* could be detected with immunofluorescence cell-staining (IF) in leaves immediately upon storage in soil, but not after 3 or 6 months (n=4). The pathogen could be detected in other crop debris 4.5 month after storage in one out of four samples, but not after 6 months. Bait plants (strawberry, cv. Elsanta) planted on soil containing infected plant material which had been stored for 11 weeks, did not develop symptoms and after homogenizing roots of bait plants, no IF-positive samples were found. The survival of *X. fragariae* in crop debris in soil during the winter in the Netherlands is therefore considered unlikely.

For surveys on fields with symptomless propagation plants or with a low incidence of symptomatic plants, there is a strong need for a reliable sampling protocol, to ascertain the actual infection incidence. As a first step in the development of a sampling protocol, in November 2007, two naturally infected fields (A and B) with propagation plants were sampled containing a low number of foci of diseased plants (respectively 3 and 12). Per infected field one plot of ca. 100 m<sup>2</sup> was sampled with a symptomatic plant in the centre. Per plot, one plant per m<sup>2</sup> was sampled. From each plant, all compound leaves were analyzed individually using immunofluorescence cell-staining. From the symptomatic plant, leaflets were analyzed individually. Field A showed an aggregated distribution of infected plants. Only 70 out of 621 compound leaves were infected. In field B all but two plants were infected, and 434 out of 732 compound leaves. In both fields, older leaves were more often infected than younger leaves. The opposite may be true earlier in the growing season. Currently, in the EU, 10 compound leaves per 2000 m<sup>2</sup> are sampled. Using this protocol, the probability of detecting the infection would have been 59% in field B and 11% in field A, if we presume the same distribution pattern to apply for at least 200 m<sup>2</sup>.

Thanks are indebted to the Dutch Ministry of Agriculture, Nature and Food Quality for granting the projects.

## Detection of *Xanthomonas fragariae* and study of its association with the strawberry plant

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*Xanthomonas fragariae* (Xf) is the causal agent of angular leafspot on strawberry and is considered a quarantine organism in strawberry propagation material within the EU. The occurrence of latent (symptomless) infections complicates the screening of planting material. A real-time PCR protocol for the detection of latent Xf infections was developed. Primers and probe sequences were based on a DNA fragment amplified in the Xf-specific multiplex PCR of Pooler *et al.*, 1996. Specificity of the designed assay was tested with an extended range of Xf collection strains and isolates, with other *Xanthomonas* spp. and with unidentified bacterial isolates from strawberry plants. High detection sensitivity is a prerequisite for studying latent and quiescent infections and the conditions that trigger pathogenic growth and expression of symptoms. Therefore, the DNeasy Plant Mini Kit (Qiagen) was adapted to allow preparation of PCR-quality DNA from 100 mg of fresh strawberry tissue. Extra measures were taken to purify the DNA extracts and approve PCR efficiency. The final protocol enabled Xf detection in undiluted DNA extracts from strawberry tissue, while the default sample preparation and real-time PCR mix, required a 100 fold dilution of the DNA sample to obtain reliable PCR amplification. As low as 300 colony forming units in a 100 mg of strawberry leaf material were detected using the developed detection protocol. Currently, the developed real-time PCR assay, combined with epifluorescence microscopy using fluorescently labelled Xf strains, is applied to study the *in planta* propagation of Xf during its infection cycle.

## Colletotrichum acutatum in Norway

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Black spot caused by *Colletotrichum acutatum* is occasionally found in strawberry fields in Norway. It was detected for the first time in 1999 and found in certified production in 2002. More than half of the certified nurseries, including the elite plant station, had symptomless infections of *C. acutatum* in strawberry planting material the following two years. A renovation programme was carried out, and in 2005 and 2006 *C. acutatum* was found in only one certified nursery. However, in 2007 latent infections of *C. acutatum* was detected in four certified strawberry plant nurseries. As been reported before from Norway, *C. acutatum* is present in almost all fruit and berry species grown in the country and has also been found on many ornamentals and in weeds, but it is a quarantine organism only in strawberry. It is particularly severe in cherry and apple. We have detected the perfect stage of the fungus in highbush blueberry, and when isolating the fungus we find a high frequency of isolates forming the perfect stage in culture. When doing genetic analysis (AFLP), the Norwegian isolates from strawberry separated in two groups. One set of isolates grouped together with strawberry isolates of *C. acutatum* from other countries, and the other set grouped with Norwegian isolates from several hosts, with a majority of isolates from sweet- and sour cherry. The latter group had more than 90% similarity between the polymorphic bands. There was also a third, very genetically diverse group of isolates that included most of the perithecia forming isolates. Further research will reveal if isolates from the different groups may vary in their aggressiveness to strawberry and if it will be possible to distinguish between them in simple molecular tests.

## Detection and monitoring of *Colletotrichum acutatum* on strawberry during the latent phase of the infection

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*Colletotrichum* species cause significant losses in strawberry production worldwide. Symptoms include anthracnose fruit rot, crown rot and lesions on stolons that may girdle runners of daughter plants. A Belgian survey during 2007 revealed that these symptoms are caused primarily by *C. acutatum*. Isolates of this pathogen displayed high levels of morphologic, genetic and pathogenic diversity.

Latent infections are the main cause of dissemination of *C. acutatum*. To better understand this latent presence, we developed a real-time PCR assay for *C. acutatum* using primers designed to the ribosomal DNA internal transcribed spacer 1 (rDNA ITS1). Using TaqMan technology, the assay could reliably detect as little as 50 fg genomic DNA, 100 copies target DNA, or 25 conidia. In addition, the assay recognized *C. acutatum* isolates from various intraspecific molecular groups, while no amplification was observed with several other *Colletotrichum* species or other strawberry pathogens, indicating the specificity of this real-time PCR. Subsequently, detection and quantification of *C. acutatum* was demonstrated in artificially and naturally infected strawberry leaves. First, we were able to detect *C. acutatum* in plant mixes of which only 0.001% of the tissue was infected by *C. acutatum*. Second, real-time PCR analysis of leaf samples taken at various times after inoculation indicated that the assay allows monitoring of growth progression of *C. acutatum* during the latent phase of the infection. This real-time PCR-mediated monitoring of the pathogen was well-correlated with microscopic data and indicated an effect of leaf age on pathogen growth. Finally, the assay allowed detection of *C. acutatum* in symptomless strawberry leaves collected from production fields and planting material.

In conclusion, real-time PCR analysis may advance our understanding of the dynamics of *C. acutatum* during the latent phase of the infection. Ultimately, this might lead to changes in current control strategies, including more targeted fungicide applications.

## *Colletotrichum acutatum*- infection and survival in alternative hosts

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*Colletotrichum acutatum* can survive in strawberry residues in nordic conditions over one winter, maybe even for almost two years. In Finland, the authorities give orders to destroy plants infected by *C. acutatum* to avoid further spread of the pathogen. The pathogen may survive in the remaining weeds or plant debris. The aim of this study is to investigate the role of weeds and fallow plants and their debris as alternative hosts for *C. acutatum* in Finnish conditions.

Altogether 19 weed species, both annuals and perennials were in trials in 2006-2007. Annual species *Chenopodium album*, *Lapsana communis*, *Senecio vulgaris*, *Stellaria media*, *Spergula arvensis*, *Viola arvensis*, *Capsella bursa-pastoris* and *Persicaria lapathifolia* and perennial or biennial species *Tripleurospermum maritimum*, *Stachys* sp., *Matricaria discoidea*, *Taraxacum vulgare*, *Plantago major*, *Rumex longilolius*, *R. acetosa*, *Mentha* sp., *Valeriana* sp, *Epilobium angustifolium* and *Trifolium repens* were tested. Some plant species used in fallowing were also included in trials: *Carum carvi*, *Phacelia tanacetifolia*, *Brassica juncea*, *Lupinus angustifolius* and *Helianthus annuus*. The plants were inoculated with *C. acutatum* spore suspension in a greenhouse.

*C. acutatum* sporulated on old leaves of all the artificially infected weed species but not on the young leaves and tops of all the respective species. Old leaves of *Tripleurospermum*, *Chenopodium*, *Lapsana*, *Plantago*, *Capsella*, *Viola*, *Epilobium* and *Rumex* were heavily infected while the fungus was not recovered on young parts of *Epilobium*, *Viola*, *Senecio* and *Lapsana*. Young leaves of fallow plants *Phacelia tanacetifolia* and *Brassica juncea* had infection, but *C. acutatum* was recovered only old leaves of *Helianthus annuus* and *Lupinus* sp. Infection in young leaves could be superficial, but in old leaves the fungus easily colonized senescent tissues. *C. acutatum* caused necrotic lesions on some plant species like *Plantago major* and *P. tanacetifolia*. The fungus was able to survive in infected debris of some weeds on soil surface over one summer. *C. acutatum* sporulated in the debris of *Carum* and *Phacelia* after the trial period of 4 months. Inoculation with the infected debris of *Capsella* caused black spot symptoms in petioles of bait plants and *C. acutatum* was also isolated of the bait plants inoculated with *Capsella* and *Epilobium*. In PCR analysis of petiole bases and upper parts of crowns of the bait plants, positive reactions were observed of plants inoculated with *Epilobium*, *Phacelia*, *Carum*, *Tripleurospermum*, *Plantago*, *Taraxacum* and *Capsella*.

The result shows that weed debris is a potential source of inoculum for strawberry plants still after some months on soil surface in warm conditions.

## Protocols of nuclear plants maintenance and certified strawberry plant production in Finland

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Certified plant production started in Finland 1976. Today it is regulated by the degree of certified production and propagation material given by Ministry of Agriculture and Forestry in 2006. This degree includes berry, fruit and landscaping plants. The Finnish Food Safety Authority (Evira) supervises all the production chain. Nearly 20 million strawberry plants are planted every year in Finland, including about 3.5 million Finnish certified plants.

In Finland certified production chain is short, consisting of three steps: nuclear plants, elite plants and certified plants. Nuclear plants are approved by Evira Plant Health Unit. MTT Plant Production Research at Laukaa is responsible for nuclear plant disease testing, therapy treatments, plant maintenance as well as elite plant production.

According to the Finnish degree the nuclear plant candidates are tested against 24 different plant enemies. Testing methods are selected by taking into account the standards of European and Mediterranean Plant Protection Organization (EPPO) and the recommendations of Evira Plant Health Unit. Therapy treatments, like heat therapy, chemotherapy and cryotherapy are used when necessary. Nuclear plants are preserved *in vitro*. The cultures are renewed annually from potted plants grown in isolated greenhouses or from cryopreserved buds. True to type tests based to berry and runner productivity, are made in greenhouse and when needed in field.

MTT Plant Production Research at Laukaa produces about 50 thousand micropropagated strawberry elite plants per year by using nuclear plant as mother stock. In 2008 six Finnish nurseries are authorized certified strawberry plant producers. From each elite plant they produce about 70 runner plants which are marketed as certified plants. They have mainly been pot plants, also production of low-priced frigo plants has recently started. Certified production includes 10 strawberry cultivars tested to survive in Finnish climate. Four of these cultivars are bred in MTT Horticulture at Piikkiö.

During recent years strawberry farmers have decreased their own plant production and have started to import of frigo or waiting bed plants instead. Purchase of imported strawberry plants has increased due to easy availability of fast cropping plant material and low plant price. This trend has had its drawbacks: harmful diseases in the bargain.

In spite of changed markets Finnish certified production has hold its position and is highly ranked. Finnish certified strawberry plant production has many advantages: a compact production chain, modern protocols and long experience on each level of certified production. High standard of production can be measured by minor incidence of disease or pest problems. Limited number of fungicides and pesticides complicates the production in Finland and other Scandinavian countries. The only alternative is high discipline in hygiene and other cultural methods.

# The situation of healthy plant, propagation and availability of healthy strawberry plants in Sweden

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The Swedish certification scheme for strawberry plants has been in action since the beginning of 1980: s and kept the strawberry growing free from serious soil and plant borne diseases. The Swedish Elite Plant Station follows the guidelines for certification, published in the directive, today SJVFS 2004:79 from the Swedish Board of Agriculture and has its background in the EPPO-schemes.

Characteristic for the Swedish certification system is: No tolerance: for P&D (listed in SJVFS 2004:79). The field inspection is not only visual, samples of plants are taken and sent to analyse laboratory. The number of propagation steps is restricted and therefore micropropagation is used.

In many years the threat for serious P&D prevented the berry growers to buy plants not produced within the Swedish certification system. But after the entry to EU some of them took the risk and bought plants abroad. Pretreatment with efficient available chemicals seems to have been successful and the trade has increased. Today the Swedish certified plant production is fourth of the production 10 years ago.

In Sweden we have a good working certification system for plant production on a high level, but the production has gradually decreased. The traditional production is bare rooted plants dug up in the spring just before the delivery. The plant producers did not change to the production of frigo plants when the strawberry growers asked for this plant quality and they lost market shares. The production of frigo plants is not very easy in our climate. The trend is that we have got fewer but bigger strawberry growers and the big growers require a uniform outer plant quality and an efficient logistic. In many cases the plant propagators out of Sweden are better in the marketing. Some growers avoid to getting serious diseases by having their own nursery and their mother plants come originally from the Elite Plant Station. The changes in the trade have affected the situation for Swedish certified production. Now we have only 3-4 propagators with a total area of 3-4 hectares comparing to 934 hectares for the Dutch plant growers. The number of plants produced in Sweden is 2-3 million per year comparing to 400-500 million plants within the system in the Netherlands. Each year 15-20 million plants are planted in Sweden.

Another change is the evaluation of new varieties. Some years ago all new cultivars were first tested in official trials with plants from the Elite Plant Station. This procedure delayed the circulation of new diseases. The Elite Plant Station now concentrate on local varieties not available in the international trade and on a range of species (softfruits, fruit and woody ornamentals) special adapted to our climate.

Our geographic site is an advantage and could be more used in plant production. Up to now neither *Xanthomonas fragariae* nor *Colletotrichum acutatum* are spread in Sweden. Soil fumigation has not been used, instead field and crop rotation are used.

In the control of the certified plant production there has been no problem the last years. The plant material is sent to approved laboratories for analysis pointed out by the National Board of Agriculture. Different laboratories are used, mostly abroad.

To use certified plant material is a matter of course. The growers buy certified plants but they are not always aware of that the meaning of the grades can vary between the countries.

In strawberry growing we will always find P&D and these depend of which chemicals the growers are allowed to use. Over the last years in Sweden we have got increasing problems with strawberry mites (*Phytonemus pallidus*) and an old pathogen is coming back, leaf nematodes (*Aphelenchoides fragariae*). Different fungus, maybe crown rot- leather rot (*Phytophthora cactorum*) cause the infections. Many symptoms are disguised by spraying with chemicals without knowing which fungus is causing the infection.

## Plant health inspections of strawberry and control of certified nursery production

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The Plant Health Unit of Evira is responsible for preventing the establishment of quarantine organisms in Finland and controlling the quality and health of planting material. To fulfil these duties it plans and carries out inspections of domestic plant production, public green and forestry sites, marketing, import and export of plants and controls pest eradication measures. The inspections are performed by Evira's own phytosanitary inspectors and inspectors of 15 Employment and Economic Development Centres. In 2007 a total number of 2700 inspection visits were carried out.

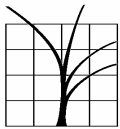
Strawberry planting material of domestic or foreign origin is always inspected and sampled when plant lots intended for marketing are met in garden centres, suppliers' warehouses etc. Market control challenges phytosanitary authorities because plants are transported freely and rapidly inside the EU and only spot checks can be made. For this reason a survey for quarantine organisms is also carried out at strawberry farms during the cropping season.

There are only few producers of strawberry CAC-plants (plants meeting the minimum requirements) in Finland. CAC-plants are always inspected and sampled when they are met in the inspections of production places.

Domestic certified production of strawberry plants is inspected at least twice during the growing season. Plants and their mother plants are sampled systematically for testing of potential quarantine and quality affecting pests.

Plant samples are analysed in the Plant Quarantine Laboratory of Evira. In 2007 the total number of strawberry pest analyses was 663. The pests of current interest are *Colletotrichum acutatum*, *Xanthomonas fragariae*, *Phytophthora fragariae*, *P. cactorum*, *Verticillium* spp., *Phytonemus pallidus*, *Aphelenchoides* spp. and *Ditylenhus dipsaci*. Soil samples from nurseries are analysed for potato cyst and virus vector nematodes. In 2007 the health of inspected plants was good. Of the regulated pests only *P. cactorum* was detected in one sample and *P. pallidus* in two samples. *C. acutatum* is the only strawberry quarantine pest which has been found in Finland. It has never been detected in domestic plants.

Certified production of propagation and planting material is regulated by the statute 9/06 of MAF. The statute gives detailed orders concerning e.g. quality assurance, propagation material and production itself. It also defines the duties of phytosanitary authorities, elite plant suppliers and nurseries. A quality assurance system including written standard operation procedures is required for authorization to produce certified material. Quality assurance systems as well as applications for approval of nuclear plants are checked and approved by Evira. Production places are also inspected by Evira before authorization.



In France, 80% of the strawberry production area are under tunnel and 11% are in soil less cultural systems. The strawberry plants have many pests and disease, and the new cultural practices increase the susceptibility of strawberries. In France strawberry growers have less and less registered pesticides. These last years a new pest, *Duponchelia fovealis* (butterfly) cause many damages on young plants.

The main disease is the powdery mildew. It is an important problem for the new practices, soil less systems and so for the ever bearing and day neutral cultivars. The disease affects leaves, flowers and fruits reducing yield and fruit quality. The 4 fungicides registered are not really efficient when the disease is set up.

The *Botrytis cinerea* is a serious problem in open field strawberry and during raining. Under tunnel the control is generally easy with 2 or 3 fungicides during flowering and with good practices (opening tunnel to ventilate). This pathogen attacks also the crown and in soil less systems is more difficult to control it.

The anthracnose (*Colletotrichum accutatum*) may be serious in open field culture and in nursery areas. Only one fungicide is registered and it is generally efficient.

Against *Phytophthora cactorum* and *Verticillium fragariae* there is none registered fungicide. The growers have to use healthy plants in a healthy field.

The larvae of *Duponchelia fovealis*, a small caterpillar, is a new pest in south of France. It causes serious damage on leaves and fruits. The only way to protect strawberries is to use biological control agents (specific nematodes).

The species of aphids are numerous and many of its are pesticide-resistant. Only 4 aphicides are registered, but its are not really efficient against all the species. Aphid become again an important problem.

The thrips is an important problem in all the cultural practices and the 2 registered pesticides are not generally enough efficient.

Against mites (*Tetranychus urticae*), 9 pesticides are registered but some of its are only efficient on egg and larvae or larvae and adults and only one kills egg, larvae and adult. Damages are mainly serious for tunnel practices and in summer.

The whitefly (*Trialeurodes*) appears in the forced crops and damage is a decrease of fruit quality caused by a fungus (fumagine) growing on the whitefly-honeydew. The control of this insect is very difficult.

Beside the chemical controls, actually many studies are in progress to involve the biological control of these insects and mites, with predators or parasitic wasps.

Another pests can cause damages on strawberries without efficient way to control its. Cyclamen mite or nematodes can cause dwarfism. Larvae of weevils or several caterpillars can cause generally minor damages in strawberries.

In open field and in autumn some foliar damages can appear cause by fungus like *Ramularia tulasei* or *Zythia fragariae* or by bacteria *Xanthomonas fragariae*. Generally fungicides formulated with copper applied in the first symptoms are active.

## Understanding the epidemiology of *Phytophthora cactorum* (strawberry crown rot) in strawberry plant propagation

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Crown rot caused by the fungus *Phytophthora cactorum* is an important disease of strawberries causing significant losses in both fruit production and plant propagation, especially in crops grown under protection. Complete crop loss in fruit production can occur under favourable warm, wet conditions in susceptible cultivars. Similarly, the presence of crown rot in propagation stocks can result in rejection of the whole stock. The fungus *P. cactorum* has a world-wide distribution and a wide host range and also causes leather rot on strawberry. A recent report (2004) indicates that isolates can be separated genetically into two groups: isolates that cause crown rot are specific to strawberry crown rot. Isolates of *P. cactorum* from other hosts and from leather rot do not cause crown rot.

The fungus produces several spore types – asexual spores (sporangia) that are mainly involved in infecting plants and initiating new epidemics, asexual resting spores (chlamydospores) that are thick walled and can survive adverse conditions and sexual spores (oospores) that are also thick walled and capable of surviving adverse conditions. The resting spores can remain in soil and plant debris for many years. The fungus is favoured by warm wet conditions (optimum temperature 25°C for infection and development). A warm period with prolonged wet is essential for infection. High temperatures and water stress appear to enhance disease development and / or symptom expression. The disease is introduced into new crops by planting into infected soil or into areas contaminated by infected debris or water or by planting infected planting material. Inoculum dispersal and disease spread within plantations is by water splash. Cultivars vary in susceptibility to crown rot. Tamella, Pearl, Opal and Elsanta are very susceptible. Hapil and Cambridge Favourite are much less susceptible. Most of the problems with the disease are associated with the very susceptible cultivars which are also those most favoured by the market.

Many of the current problems with crown rot are associated with attempts to produce disease-free planting material of the more susceptible cultivars and especially of the everbearers. These problems are complicated by the ability of the fungus to exist in apparently healthy planting material as latent (symptomless) infected tissue, which will manifest itself at a later stage of plant production or during fruit production. The production of healthy planting material is crucial to the control of the disease in fruiting beds. Therefore a reliable test that can detect symptomless infection in planting material is an essential part of plant production. Current methods of detection use highly sensitive, specific real-time PCR methods and more traditional methods of detection by floating petiole bases in water to encourage fungal development and sporulation. The critical issue may be simply one of sampling and testing a sufficient number of plants

In the UK, production of healthy planting material is initiated from stock mother plants via micropropagation. Previous work by Harris and Bell at East Malling, which was confirmed in more recent studies, indicated that any crown rot present in stock plants would develop in the media during micropropagation and that surviving plants were crown rot-free. Real-time PCR tests conducted on the surviving plants did not detect any crown rot.

In recent cases of crown rot developing in propagated plant production has been *via* micropropagation and, despite negative tests on the weaned plants, crown rot has developed in the later stages of production. What is not clear is the origin of the disease and the possible failure of the sampling and test procedures to detect the problem.

## Screening Plant Resistance to *Phytophthora cactorum*

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*Phytophthora cactorum* was first detected to cause crown rot and plant death on strawberry in Finland in 1990. The disease caused severe plant losses in fruit production and nurseries. Resistance of strawberry cultivars to crown rot has been evaluated at MTT Agrifood Research Finland since 1993 using the crown test and the NFT test. By 2003, about 60 cultivars had been tested in greenhouse by different methods.

The crown test was first used for screening cultivar resistance. The nutrient film technique (NFT) has been used at MTT for screening for resistance to crown rot since 1998. The two methods, crown test and NFT test, have been compared with the same strawberry cultivars in similar greenhouse conditions. Most of the cultivars tested showed the same level of resistance in the crown and NFT tests. The amount of zoospores in the inoculum used did not affect the results.

The NFT system is, however, expensive and the viability of *Phytophthora* zoospores can be reduced or completely lost if the hydroponic environment contains substances that are harmful to them. Inoculation by dipping the tray plants in *P. cactorum* inoculum was investigated to solve this problem. The inoculum concentration was the same as previously used in the NFT test. Inoculation of small runner plants rooted in peat substrate in Vefi pots seemed to give the same result as addition of sporangia to circulating irrigation water, but symptom expression was delayed during the first two test weeks. According to the tests on cultivars 'Jonsok', 'Kimberly' and 'Polka', inoculation in Vefi pots with zoospores is effective and can save costs in the greenhouse environment.

Crown rot developed within 4 weeks and a longer test time did not change the level of infection. However, the peat substrate used in tray pots should be free from fungal antagonists which can prevent *Phytophthora* infection of plants. Inorganic substrates, rockwool and vermiculite, are safe alternatives to peat.

# Viruses and phytoplasmas infecting strawberry

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Seven aphid-transmitted viruses were reported in strawberry - *Strawberry crinkle virus* (SCV), SMYEV, *Strawberry mottle virus* (SMoV), *Strawberry vein banding virus* (SVBV), *Strawberry pseudo mild yellow edge virus* (SPMYEV), *Strawberry chlorotic fleck virus* (SCFV), and *Strawberry latent C virus* (SLCV). SCV, SMYEV, SMoV, and SVBV have been considered the most economically important viruses of strawberry in the majority of production areas (Martin & Tzanetakis 2006). The primary aphid vector is *Chaetosiphon fragaefolii*. Nematode-transmitted viruses - *Tomato ringspot virus* (ToRSV), *Raspberry ringspot virus* (RpRSV), *Strawberry latent ringspot virus* (SLRSV), *Arabidopsis mosaic virus* (ArMV) and *Tomato black ring virus* (TBRV) have wide host ranges and can cause significant losses in strawberry. Properties of *Tobacco necrosis virus* (TNV), ilarviruses *Strawberry necrotic shock virus*, (SNSV), *Fragaria chiloensis latent virus* (FCILV), and *Apple mosaic virus* (ApMV) and several other unclassified viruses e.g. *Fragaria chiloensis cryptic virus* (FCICV) were reviewed by (Martin & Tzanetakis 2006). Crinivirus and Closterovirus infections transmitted by *Trialeurodes* and *Bemisia* whiteflies were described in USA as a possible emerging problem. Currently, RT-PCR methods are available for the detection of most of the viruses known to infect strawberry, however with limitations caused by genetic diversity of some strains.

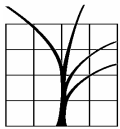
Phytoplasmas induce usually severe symptoms including gradual extinction of affected part or the whole plant. **Strawberry green petal** is probably the most frequent phytoplasma disease (aster yellows group, ribosomal subgroup 16SrI-C). Symptoms of phyllody strawberry flowers reported in commercial strawberry in North America, Europe, Japan and Russia were caused by phytoplasmas of both ribosomal eastern and western strain of aster yellows. **Strawberry phyllody** associated with phytoplasmas belonging to aster yellows group, 16SrI-B and 16SrI-C ribosomal subgroup was found in Italy. Phytoplasma bodies were observed also in plants showing **strawberry lethal decline** and **yellows symptoms** in western Washington, British Columbia, Oregon and Australia, respectively. Non-classified phytoplasmas associated with **strawberry witch's broom and multiplier diseases** were noted in North America and in the Netherlands. Recently, strawberry lethal yellows phytoplasma and '*Candidatus* Phytoplasma fragariae' were included in stolbur group, 16SrXII-C and 16SrXII-E ribosomal subgroups, respectively.

Strawberry plants with vigorous growth, regular shape and colour of leaves, high quantity of healthy-looking flowers and runners which did not produce fruits were recorded in the Czech Republic. The disease was named **strawberry sterility**. Biological experiments, transmission electron microscopy and molecular methods identified the presence of strawberry mottle virus together with mixed phytoplasma infection of aster yellows (subgroup 16SrI-B) and clover phyllody (subgroup 16SrI-C).

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**Ctifl**



## French research programme on powdery mildew of strawberry

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The fungus *Podosphaera aphanis* causes a disease usually known as powdery mildew. It is now the main aerial disease in all nursery and fruit production areas. The disease affects leaves, runners, flowers and fruits at all times of the life cycle. Patches of white, powdery fungus mycelium may appear on the undersides of leaves as the disease progresses. Patches of white can also appear on scapes, green fruits or mature fruits. It tends to be more serious with the new cultural practices of forced soilless crops for year-round production.

The strategies to control powdery mildew are limited: most varieties are susceptible and few are tolerant or resistant, the range of phytosanitary chemicals is narrow and often inefficient in case of strong fungus pressure, the anticipation of fungus attacks is often inaccurate because powdery mildew biology is inadequately known and, finally, alternative control methods require further study.

In this context, an interdisciplinary proposal was born in 2006, carried out by Ctifl, who is in charge of the project, in collaboration with INRA (French national research centre), Hortis Aquitaine (regional experimental station) and six departmental chambers of agriculture.

These studies are made also in close cooperation with French strawberry breeders - Ciref Cv, Darbonne, Marionnet, Angier - and the network of regional experiment stations: Aprel, Arelpal, Cate, SECL, Sefra, SEHBS, Selt-LCL.

This three-year project comprises four complementary parts.

Part one aims to improve knowledge on the biology, parasitic circle and epidemiology of powdery mildew.

Part two will focus on existing tolerance or resistance within the pool of strawberry varieties.

Part three will study the susceptibility of the fungus to specific fungicides.

Part four will prospect alternatives using new practices and new types of chemical activity.

The project will run over a three-year period, from 2006 to the end of 2008.

# Current status of the research of strawberry diseases and its management in Lithuania

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There are near 1000 hectares of commercial strawberry plantations in Lithuania. Several years ago the growing of strawberries for deep freezing and processing was developed in Lithuania. The most common strawberry cultivar for market was 'Senga Sengana'. At present, the strawberry market is growing up and berry export is being expanded in Lithuania and it is very important to select only few productive strawberry cultivars for market. There were investigated different strawberry cultivars at the Lithuanian Institute of Horticulture (LIH) during different periods and cultivars 'Honeoye', 'Elkat', 'Polka', 'Pandora' were recommended for commercial growing.

The main factor restricting strawberry production is the yield losses up to 15–92% or even whole plantations - due to fungal diseases in many countries. The most common diseases in Lithuania are grey mould caused by *Botrytis cinerea*, root diseases caused by *Phytophthora* spp., *Verticillium* spp., *Rhizoctonia* spp., *Fusarium* spp., *Phythium* spp., leaf diseases caused by *Mycosphaerella fragariae* (leaf spot) and *Marssonina potentillae* (leaf scorch). Powdery mildew caused by *Sphaerotheca macularis* is more common only in protected an indoor growing strawberry.

Pesticide use is restricted or not allowed at all because of high ecological risk. Therefore, the resistant varieties could become one of the factors in strawberry for disease management. The incidence of fungal leaf diseases 'Saulenė', 'Honeoye', 'Kent', 'Elkat', 'Polka', 'Dangė', 'Senga Sengana', 'Pegasus', 'Bogota' and 'Pandora' was evaluated in 2004-2005 under field conditions at the LIH. The lowest incidence of *M. fragariae* was recorded on strawberries 'Dangė', 'Elkat' and 'Pegasus' during both experimental years. Disease prevalence ranged on average from 43 to 46 % and disease intensity from 14 to 15.7 %. The most damaged (disease incidence 70%) were 'Honeyoe', and 'Senga Sengana', meanwhile 'Kent', 'Saulenė', 'Bogota', 'Polka', 'Pandora' were damaged by 51–68%. 'Elkat' and 'Polka' were the most damaged (disease prevalence was 68-69% and intensity 36–46%) by leaf scorch (*M. potentillae*), meanwhile the lowest incidence of leaf scorch was recorded on 'Bogota'.

Chemical control of these diseases can be difficult as several effective fungicides are no longer sold in the market or have lost their registration for use on strawberries. Therefore there were investigated different active ingredients of fungicides against diseases in strawberry. A two year trial examined control of different rates of Cyprodinil + Fludioxonil 625 g kg<sup>-1</sup> against grey mould (*Botrytis cinerea*), white leaf spot (*Mycosphaerella fragariae*) and yields of strawberry. An efficiency Cyprodinil + Fludioxonil 625 g kg<sup>-1</sup> 0.75 kg AI ha<sup>-1</sup> against grey mould and white leaf spot ranged from 58.0 till 73.3% and 46.0 – 70.4% respectively. Lower rate 0.625 kg AI ha<sup>-1</sup> of Cyprodinil + Fludioxonil was effective by 56.5 - 58.4% and 46.0 – 66.7% against grey mould and white leaf spot accordingly. The higher effect of Cyprodinil + Fludioxonil was found when disease pressure of grey mould was high and lower effect when disease pressure of white leaf spot was high. Fungicide Signum 334 WG 1.0 kg/ha gave medium control against *Botrytis cinerea* in strawberries (65,0%), and high control against *Mycosphaerella fragariae* (74.0%). Meanwhile higher rate of Signum 334 WG 1.8 kg/ha gave high control against *Botrytis cinerea* (92%) and *Mycosphaerella fragariae* (78%).

European agriculture faced the strict demands to decrease the use of pesticides in order to reduce any human or environmental hazards, therefore the need for evaluation and reduction of the use of pesticides is expressed. To obtain the reduction of the use of fungicides without any significant damage to the crop, the control of strawberry diseases should be based on registration of climatic data, scouting of biotic parameters, infection risks and simulation disease models. Therefore investigations on forecasting systems of strawberry diseases became one of the strategies for the future research. Since 2007 there were started investigations on warning of *Botrytis cinerea*, *Sphaerotheca macularis* and *Phytophthora cactorum* in strawberry using internet based warning system iMETOS (Pessl, Austria) at LIH.

# Designing strategies to control fruit rot in strawberry cultivation using decision support systems

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Grey mould is one of the major diseases in strawberry cultivation. Fungicides to control *Botrytis cinerea* are applied frequently during flowering and sometimes at harvest. Reduction of pesticide use is one of the major aims of the Dutch government policies. Implementation of a Decision Support System (DSS) helps to achieve this goal.

Pin point timing of fungicide application can improve the efficacy of the treatment and reduce the number of spray applications. Several Decision Support Systems can be used as an aid to control grey mould. Predicted weather data to forecast infection risks are used by most of these models. However in strawberry cultivation irrigation is a daily practice. The effect of overhead irrigation on the *Botrytis* infection risk is unknown. This is one of the reasons that strawberry growers are not or infrequently using DSS's. Therefore adaptation of the model to agricultural management is necessary. Different decision rules to improve the model are tested and compared. Field trials were carried out at Applied Plant Research location Vredepeel in 2006 and 2007.

Under low disease pressure DSS BoWaS controlled *Botrytis* fruit rot 62% better than routine applications of fungicides, with a 50 % reduction of fungicide input in 2006. Adding an irrigation or a disease pressure sub – routine did not improve the model under low disease pressure.

BoWaS based on disease pressure and weather resulted in better control of grey mould than the weather based BoWaS, under high disease pressure in 2007. Adding an irrigation rule did not improve the model further. Using the modified BoWaS, fungicide input was reduced with 36% compared to routine applications with the same efficacy. Applying suitable fungicides curatively gave a similar control of grey mould than preventative applications, but fungicide input was reduced 60%.

Implementation of a DSS can be facilitated if a fungicide with a curative mode of action is registered and the model has the possibility to back cast disease infection risks. The effect of overhead irrigation has to be looked into further.

# Biological control of grey mould (*Botrytis cinerea*) in strawberry

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Plant protection was earlier mainly based on the use of fungicides which hamper or kills the different stages of the plant pathogen but also destroys the natural micro flora. The use of fungicides have also contributed to health problems for the growers and for longlasting effects on the environment. During the last decades the consumers demand for healthy food stuff have encouraged the use of some of the natural micro organisms (fungi and bacteria) that normally resides on leaves, flowers and roots for biological control of plant pathogens. The use of fungicides sooner or later will lead to establishment of resistant forms of the pathogen, but the biocontrol organisms have a more varied way of attack e.g. parasitism, competition for food etc. that might not lead to resistant forms and that to a minor extent will affect the positive organisms. The project focused on discovering new microorganisms that could be used as biological control agents against grey mould in strawberry. Bacteria and yeast fungi were isolated from strawberry flowers and fruits. The yeast species were screened in laboratory and in field trials for antagonism against grey mould. Two species were showing very good and reliable results hindering grey mould infection in two years of field testing. Therefore, they became primary candidates for an environmentally friendly alternative to chemical fungicides. The yeast isolates are also resistant/very tolerant to several registered chemical fungicides used in plant protection. This makes it possible to use them together with traditional fungicides in lower doses in order to get both good protection and reduced use of chemicals compared to the traditional use of fungicides. Continuing research will focus on the mechanisms and for commercialisation of the biocontrol agent.

## PGPR as biological control of *Botrytis cinerea* and biofertilizer agents in strawberry cv. Fern

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Plant growth-promoting rhizobacteria (PGPR) play a vital role in crop protection and growth promotion. This group comprises, for example, the bacteria *Azotobacter*, *Azospirillum*, *Bacillus*, *Clostridium*, *Pseudomonas* and *Streptomyces*. This study started in 2007 year aimed to use of PGPR both biocontrol and biofertilizer agent in strawberry production in Eastern Anatolia of Turkey. Around 198 microorganisms were tested against against grey mold in laboratory conditions. Among them 8 out of 198 which had >3 cm inhibition zone in petri dishes on agar medium were selected. Those are T33 *Bacillus pumilis*, MFD-1 *Bacillus lentimorbus*, MFD-20 *Bacillus subtilis*, MFD-21 *Bacillus subtilis*, MFD-113 *Pantoea agglomerans*, MFD-81 *Enterobacter intermedius*, MFD-Ü1 *Bacillus subtilis*, MDF-U2 *Bacillus subtilis*. The strawberry, *Fragaria × ananassa*, cv. Fern were inoculated or left uninoculated with these microorganisms in greenhouse condition. The results were promising for preventing botrytis in strawberry. In field treatments the previously selected microorganisms as biocontrol and biofertilizer agent namely, *Bacillus subtilis* M3, *Bacillus subtilis* OSU-142 and *Pseudomonas putida* BA-8 were used. The inoculation of bacteria was increased plant growth and productivity. The all bacteria used increased antioxidant activity and in particular total phenolic content in fruit, which was the highest in *Pseudomonas putida* BA-8 (44.18 mgGAE/g DW). The combination of all three bacteria increased the leaf area in strawberry cv. Fern compared to control. All bacteria increased plant nutrient elements uptake from soils. In field conditions grey mold were not developed.

# Effectiveness of chitosan and other resistance inducers IN the control of postharvest gray mold of strawberry

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Strawberry (*Fragaria × ananassa* Duch.) is an especially perishable fruit, as it is susceptible to mechanical injury, desiccation, decay and physiological disorders during storage. Gray mold, caused by *Botrytis cinerea* Pers., is the main postharvest disease of the crop, being able to induce severe losses of fruit both in the field and during long-distance transport and storage. Gray mold symptoms on the fruit arise from infection of the floral parts in the field or contact with rotting berries, a phenomenon better known as nesting. *B. cinerea* can also develop at low temperatures, shortening the potential length of storage and marketing. The control of strawberry gray mold can be achieved by physical, biological and chemical means. To meet the growing consumer demand for food without preservatives, several efforts have been focused on reduction of fungicide applications using new alternative means, including the use of resistance inducers. Among these, there are hypobaric treatments and natural compounds like chitosan, which can form an antimicrobial film on the fruit surface. Moreover, several other resistance inducers are available on the market or are currently under investigation. A 4 h hypobaric treatment at 0.25 atm (190 mm Hg) carried out on fruit soon after harvest decreased gray mold incidence more than 40%. Presumably this treatment reduces the ethylene content of the fruit, and although further mechanisms of action of the treatment are still unknown, induced resistance appears to be responsible for decay control. Chitosan is a natural and biodegradable biopolymer. It is an ideal preservative coating for fresh fruit and vegetables because of its film-forming and biochemical properties, and it prolongs storage life and controls gray mold on several fruit, included strawberry. The biopolymer has antifungal activities against a wide range of fungi, included *B. cinerea*, and it is can induce resistance in plant tissues to different pathogens. It was applied as preharvest and postharvest treatment for the control of gray mold of strawberry in storage. In the field trials, chitosan was sprayed at 0.1, 0.5 and 1.0% solutions on strawberries at three vegetative stages (full bloom, green fruit and whitening fruit); untreated strawberries or those sprayed with a fungicide were used as controls. Chitosan decreased gray mold, with the greatest reductions at 1.0%. The best results were obtained by treatment at the whitening stage. In postharvest trials, the strawberries were dipped in chitosan solutions at 0.1, 0.25, 0.5 and 1.0%, and again the highest concentration provided the greatest gray mold protection. Postharvest immersion of strawberries in a commercial chitosan formulation, called Chito Plant, and in other resistance inducers (Bion, Algition, Fitocalcio, Calit, Latic and Xedabio) significantly reduced gray mold. Chitosan treatment increased phenylalanine ammonia-lyase (PAL) activity and decreased the hydrogen peroxide content in the strawberry, showing an eliciting and antioxidant activity.

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## Biological soil disinfestation to control *Verticillium dahliae* in strawberries

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*Verticillium* wilt causes major yield losses in strawberries worldwide. The pathogen can be controlled effectively by soil fumigation with methyl bromide. This chemical, however, is banned completely in the European Union and, therefore, alternative control methods are needed. A method, developed at the end of the 20<sup>th</sup> century is described which controls not only *Verticillium dahliae* but also free-living nematodes, like *Pratylenchus* spp., which promote the *Verticillium* wilt.

With biological soil disinfestation a green manure crop (40 ton per ha) or green biomass is homogeneously incorporated into the soil layer that has to be disinfested. Then the field is lightly compacted and irrigated. Subsequently, the soil is covered with a thick plastic film to restrict oxygen diffusion. The soil is left covered for 6-10 weeks in summer.

After covering the soil, anaerobic conditions develop which last for several weeks. Under these conditions toxic fermentation products are formed. This is thought to be the main cause of pathogen killing. In repeated field experiments, biological soil disinfestation was found to reduce survival of several persistent soilborne pathogenic fungi and nematodes.

Biological soil disinfestation of an experimental field in 1999 was followed by three months of strawberry production from cold stored waiting bed plants in 2000 and by a perennial strawberry crop in 2001. The *Verticillium* inoculum density was decreased with 75 %. In 2000 no differences in the growth of the crop were observed. In 2001 the wilting decreased after biological soil disinfestation and subsequently yield increased. Two farmers applied biological soil disinfestation on their fields and compared it with a control field. One farmer could prevent a crop failure with biological soil disinfestation (the control plot yielded only 30% of the disinfested plot), while the other farmer had an 85 % reduction in inoculum density but had almost no disease.

Research on biological soil disinfestation to control other plant pathogens is planned.