Downy Mildew Caused By Hyaloperonospora (Peronospora) spp. on Wild Rocket (Diplotaxis tenuifolia) and Other Crops from Brassicaceae Family.

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Table of Contents

Abstract

Downy mildew is caused by plant parasitic Oomycetes from the genus *Hyaloperonospora* and the genus *Peronospora.* Downy mildew is a common disease of brassicas and closely related cruciferous crops. This thesis surveys downy mildew caused by *Hyaloperonospora (Peronospora)* spp. with focus on the rocket salad species.

The susceptibility experiment was carried out to research the host specificity of plant parasitic Oomycetes *Hyaloperonospora (Peronospora)* spp. on rocket salad (wild rocket, *Diplotaxis tenuifolia* and cultivated rocket salad, *Eruca sativa*) and other chosen crops from the Brassicaceae family (broccoli, cauliflower, swede, oil seeds rape) at the seedlings stage. Seedlings were inoculated with different isolates (frozen plant material from 2008, diseased brassicas and closely related cruciferous crops from the fields in Norway and diseased cruciferous weeds grown close to the fields with the Brassicaceae family crops). The experiment has shown that the pathogen from a certain host plant species was more aggressive on plants of the same species. The pathogen from the host plant *D. tenuifolia* was significantly more aggressive on seedlings of *D. tenuifolia*. The pathogen from the host plant broccoli was more aggressive on broccoli seedlings, but was also capable to infect cauliflower, swede and oilseed rape. The pathogen from host plant swede was more aggressive on seedlings of swede, but disease was observed also on cauliflower and broccoli.

The thesis also covers a survey of the survival of conidia in the air. The spores of *Hyaloperonospora (Peronospora)* spp. were exposed to solar radiation for different time intervals. Afterwards, the viability of these spores was evaluated. Results from the experiment indicate a certain decreasing trend in the viability of spores over the time of their exposure.

The third experimental part surveys whether the overwintering structures of *Hyaloperonospora (Peronospora)* spp. in the soil can cause an infection on the seedlings of *D. tenuifolia*. The soil from the fields, where rocket salad was grown previous years, was sampled. An occurrence of the disease on the seedlings of *D. tenuifolia* was observed. The disease was not observed on the experimental seedlings.

BACKGROUND

In the past years, it has been reported to the Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, several losses due to downy mildew caused by the oomycota *Hyaloperonospora (Peronospora)* spp*.* The rocket salad growers reported significant losses.

Downy mildew is a polycyclic disease caused by different species of *Hyaloperonospora* and *Peronospora* (Göker et al., 2009)**.** The obligate parasite from the genus *Hyaloperonospora* and the genus *Peronospora* is living on brassicas and closely related cruciferous crops. The losses are more severe at the seedlings stage than on mature healthy plants (Koike et al., 2007, Rimmer et al., 2007). Cool and moist conditions are favourable for the disease development (Smith et al., 1988).

Rocket salad crops are used as a fresh newly popular salad mix (D'Antuono et al., 2008). The young leaves of rocket salad crops are harvested. The disease causes quantity and quality losses on the crops. However all aerial parts of the plant can be infected, the symptoms primarily appear on the leaves (Rimmer et al., 2007). Symptoms of the disease such as discoloured lesions and necrotic spots with brown edges can destroyed the quality of the leaves. Also the quantity of the harvest is reduced due to the downy mildew infection. Young seedlings can die as a result of the infection (Rimmer et al., 2007).

In the experiments are rocket salad crops represented by wild rocket, *Diplotaxis tenuifolia* and cultivated rocket salad, *Eruca sativa.* Both species can be grown in Norway. However, the local production of rocket salad was almost restricted by cultivated *D. tenuifolia.* Cultivated rocket salad is grown as a supplement to the production of *D. tenuifolia* in early and late season (source of the information: Bioforsk). Seeds of both rocket plants are available on the Norwegian market. Seeds are sold for example by NORGRO A.S., Brødrene Nelson Frø A.S.

In this thesis is used name cultivated rocket salad for *Eruca sativa* and wild rocket for *Diplotaxis tenuifolia* (Gilardi et al., 2007, D'Antuono et al., 2008).

AIMS OF THESIS

The present study consists of four parts, a Literature Review and three experimental parts. The general aim of this thesis is to broaden a current knowledge base of the oomycota *Hyaloperonospora (Peronospora)* spp. which is causing the downy mildew on the rocket salad crops. Three experimental parts explore different areas of pathology of *Hyaloperonospora (Peronospora)* spp.

The first experiential part is focused on research of a host range of the oomycota *Hyaloperonospora (Peronospora)* spp. at the seedling stage of rocket salad (*D. tenuifolia, E. sativa*) and chosen brassica crops (broccoli, swede, cauliflower, oilseed rape). The goal of this experiment was to identify a host specificity pattern and to confirm a level of aggressiveness of the pathogen by its origin (Rimmer et al.; 2007, Sherriff and Lucas, 1990) on rocket salad crops (*D. tenuifolia* and *E. sativa*) and other chosen members of the Brassicaceae family.

Conidia of the oomycota *Hyaloperonospora (Peronospora)* spp. are spread by wind and water splash. Spread of the spores for long distances is possible, but the survival of spores is not well documented (Nordskog, Hermansen, 2008). The aim of the second experimental part is to research the survival of conidia in the air and to determine an influence of solar radiation on the viability of the spores.

The infection on leaves occurs from the airborne conidia, but the primary infection probably originates from the oospores and may lead to systematic infection (Rimmer et al., 2007). However, the infection of seedlings from the oospores has not been verified under the natural conditions (McMeekin, 1960). The aim of the third experimental part is to examine whether the overwintering structures of *Hyaloperonospora (Peronospora)* spp. in soil can cause an infection on the seedlings of *D. tenuifolia*.

LITERATURE REVIEW

Brassicaceae Family

The Brassicaceae or Cruciferae family contains agricultural and scientific important species. The family is composed of 338 genera and 3 7009 species (Lihová and Marhold, 2006; Rimmer et al., 2007). The genus *Brassica* includes 35 species. Cultivated brassicas comprise six species with great morphological and genetic diversity (Rimmer et al., 2007).

Broccoli (*Brassica oleracea* L. var. *italica* Plenk), cauliflower (*Brassica oleracea* L. var. *botrytis* L.), swede (*Brassica napus* L. subsp. *napobrassica* (L.) Jafri [syn. subsp. *Rapifera*]) and oilseed rape (*Brassica napus* L. subsp. *napus* (L.) Hanelt [syn. subsp. o*leifera,* subsp. *pabularia*] are economically important crops in Norway. Broccoli and cauliflower share the taxon *Brassica oleracea.* The group including cauliflower, broccoli, and sprouting broccoli are designated as inflorescence kales (Rimmer et al., 2007). Oilseed rape and swede belong to the taxon *Brassica napus.*

The leafy crucifer *Eruca sativa* syn. v*esicaria* subsp. *sativa (Miller) Thell.)* belongs to the genus *Eruca.* This old world genus of the tribe *Brassiceae* is composed of four species (Rimmer et al., 2007). *Diplotaxis tenuifolia* (L.) DC belongs to the genus *Diplotaxis*. The genus is composed of over 30 species. *S*everal species of the genus *Diplotaxis* have been used as food crops (D'Antuono et al., 2008). These species were reported as edible as well, *Diplotaxis muralis* (L.) DC, *Diplotaxis erucoides* (L.) DC, *Diplotaxis catholica* (L.) DC, *Diplotaxis harra* (Forsk.) Boiss., *Diplotaxis acris* (Forsk.) Boiss., and *Diplotaxis simplex* (L.) DC (D'Antuono et al., 2008).

Cultivated Rocket Salad, Wild Rocket

Rocket salad is a newly popular leafy crucifer. The use of this plant's young leaves are traditional in the Mediterranean cuisine (Gilardi et al., 2007, D'Antuono et al., 2008). Because of the popular taste, the low amount of calories, and decorative effect, rocket salad is now very often appearing in many other dishes. Rocket salad could be also named as rocket, rucola, arugula, rugula, roquette, ruchetta, cultivated garden rocket and wild rocket (Gilardi et al., 2007). The generic name for rocket salad

can include like *Eruca sativa* and *Diplotaxis tenuifolia* or other plants from mustard family (D'Antuono et al., 2008).

Wild rocket, *Diplotaxis tenuifolia* (L.) DC*,* is a yellow flowering perennial herb. Leaves are thin and spiky. It has a strong pepper flavour. In Norway wild rocket is commonly grown as a rocket salad crop.

Cultivated rocket salad (*Eruca sativa* syn. v*esicaria* subsp. *sativa* (Miller) Thell.) is an annual herb with white flowers. Leaves are thin and spiky. Compared to *D. tenuifolia,* leaves are more rounded and with more mild flavour. As a component of green salad mixes, *E. sativa* is consumed traditionally in the Middle East and in southern Italy (D'Antuono et al., 2008). The plant is also grown as oilseed crop in Asia (Rimmer et al., 2007).

A B

Figure 1. *D. tenuifolia* (A) flowering plant Photo : J. Johansen Hladilová (B) leaf Photo: from the Internet (http://www.thewatercresscompany.co.uk/).

Figure 2. *E. sativa* Photo: A. Mrkvicka, from the Internet (http://flora.nhm-wien.ac.at/Seiten-Arten/Eruca-sativa.htm).

Downy Mildew

Causal Organism

The causal agent of downy mildew is the oomycota *Hyaloperonospora (Peronospora)* spp. Formerly all species of *Hyaloperonospora, Peronospora* living as a obligate parasite on plant tissue of different brassicas and closely related cruciferous crops were merged under one species *Hyaloperonospora parasitica* (Pers.) Constant. (Constantinescu and Fatehi, 2002). Recently for the pathogen living on the species of *Brassica, Raphanus, Sinapis* is considered use of *Hyaloperonospora brassicae (*Gäum.) Göker, Voglmayr, Riethm., Weiss & Oberw., *Peronospora diplotaxidis* Gäum. for the causal agent of the downy mildew on the species of *Diplotaxis tenuifolia, Peronospora erucastri* Gäum. for the causal agent of the downy mildew on the species of *Eruca sativa* (Gaümann, 1918, 1923, Göker et al., 2004).[1)]

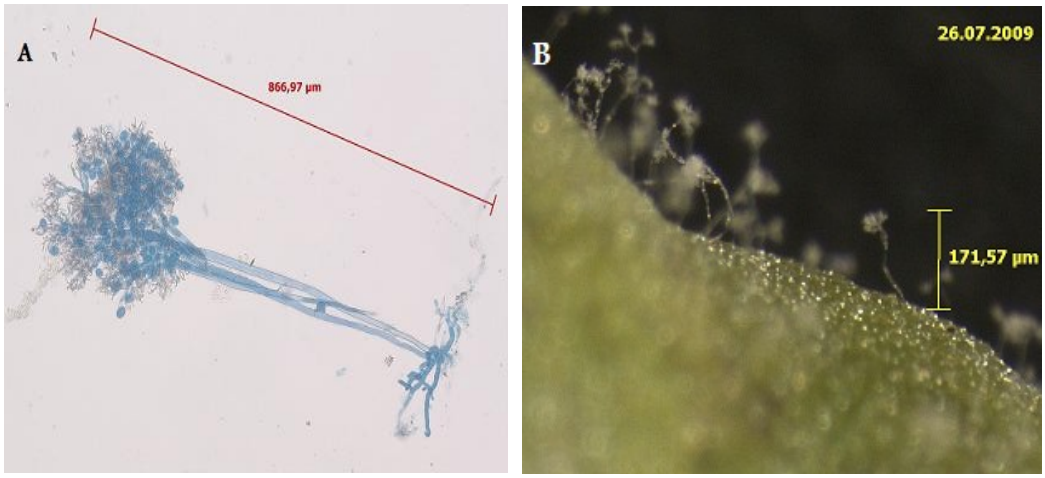


Figure 3. (A) Conidiophores with conidia of *Hyaloperonospora* spp. from the host plant *D. tenuifolia,* (B) Conidiophores of *Hyaloperonospora* spp. on swede leaf *,* Photo: J. Johansen Hladilová.

The pathogen produces conidia on dichotomously branched conidiophores (Figure 3). The conidia lack discharge pores and germinate only by means of a germ tube (Agrios, 2005; Koike et al., 2007; Smith et al., 1988). The pathogen penetrates directly through the anticlinal walls of the epidermal cells (Deacon, 2006; Smith et al., 1988). During the sexual stage, the fungus forms oogonia and antheridium (Figure 4 e). Resting oospore is thick walled, yellow-brown and spherical (Rimmer et al., 2007) (Figure 4 f).

Disease Cycle

The primary infection of the root probably originates from the oospores and may lead to a systematic infection. But the infection on leaves occurs from the airborne conidia. (Rimmer et al., 2007) Conidia on a leaf germinate, make a germ tube and penetrate via appressorium the plant tissue (Slusarenko and Schlaich, 2003) (Figure 4 d). The pathogen forms mycelium and haustoria inside a host tissue. The conidiophores emerge through the stomata (Koike et al. 2007, Smith et al., 1988) (Figure 4 b). The life cycle of the *Hyaloperonospora* spp. species is illustrated on the Slusarenko and Schlaich drawing (Figure 4 a-g).

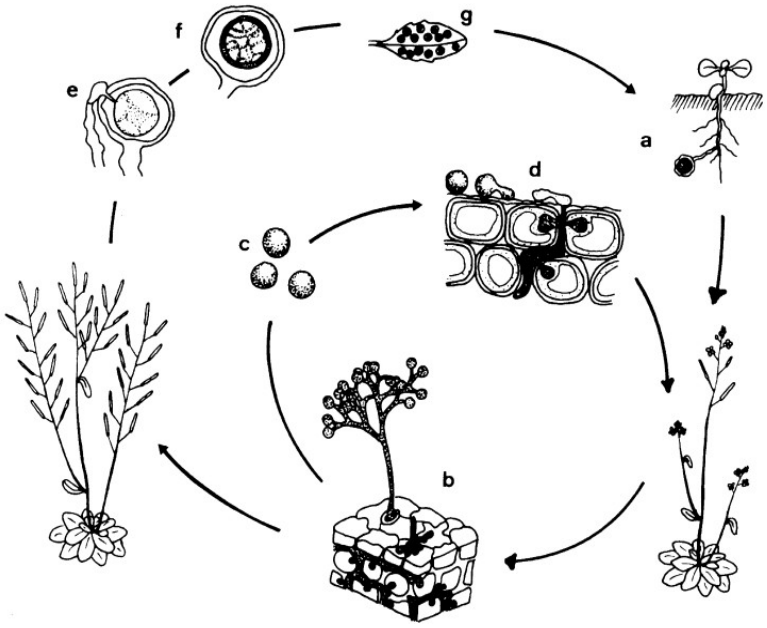


Figure 4. (Slusarenko and Schlaich, 2003) (a) Oospore germinating in soil. (b) Mycelium, haustoria and conidiophore. (c) conidia (d) Germ tube and penetration via appressorium. (e) Antheridium fertilizing on oogonium (f,g) Oospores.

Symptoms

Primarily symptoms of downy mildew are irregular yellow necrotic patches with white fungal growth on young leaves or cotyledons. The growth of conidiophores and conidia is occurring on the under surface of the leaf, but, in favourable conditions, the both sides of the leaf can be affected (Nordskog and Hermansen, 2008; Rimmer et al., 2007; Koike et al., 2007) (Figure 5). The disease is more severe on young plants and this can result in the death of seedlings (Koike et al., 2007)

On older plants can appear brown, greyish or black spots on the surface of a curd or a head of vegetable. The internal tissue is also attacked by systemic infection causing black discolouration, typical on cauliflower or broccoli. Black lesions and internal root discolouration is also typical on radish infected by *Hyaloperonospora* species (Koike et al., 2007)

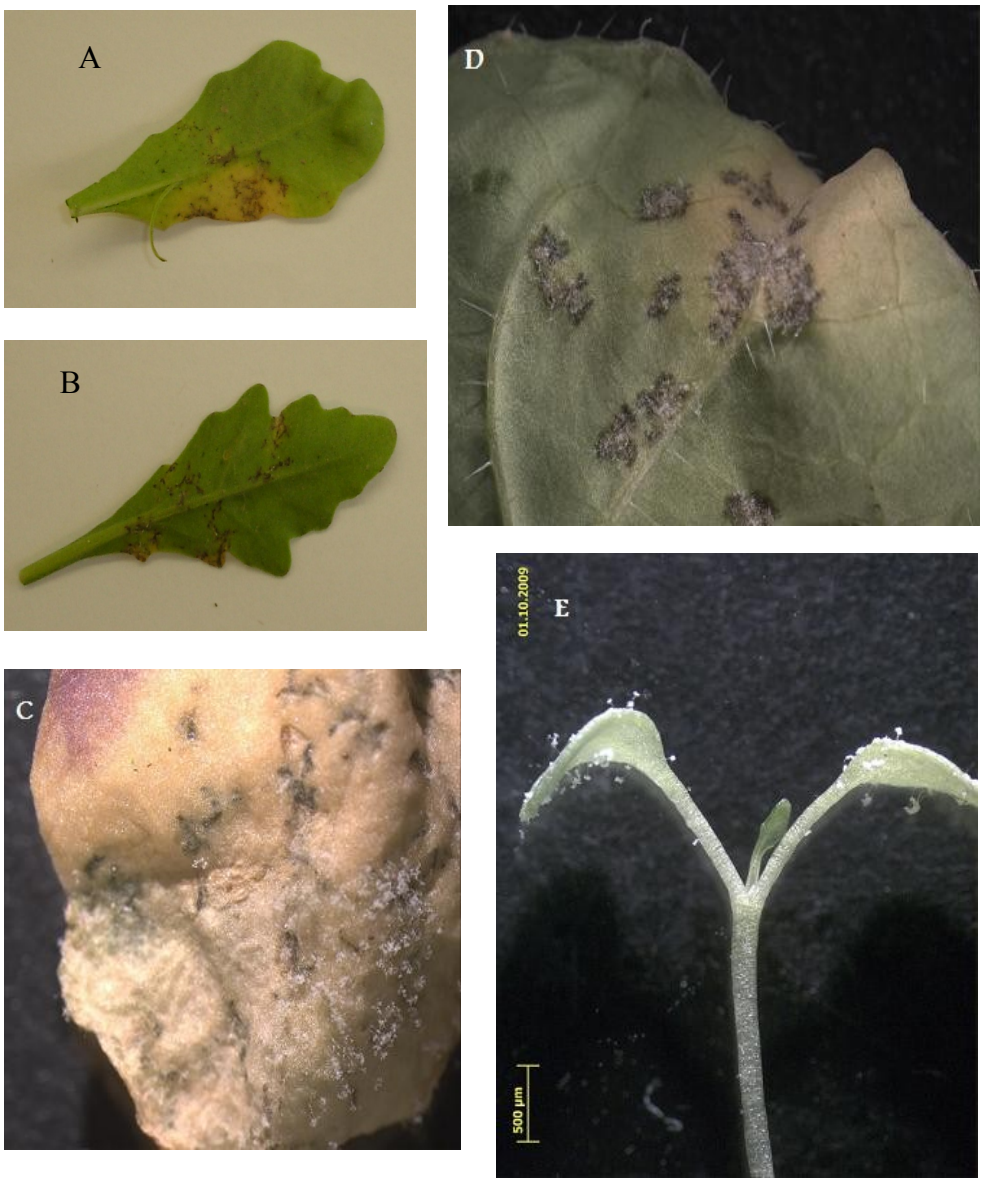


Figure 5. Symptoms of downy mildew (A) (B) Symptoms on *D. tenuifolia* (C) Yellowish, necrotic leaf of *D. tenuifolia* with the sporulating conidiophores (D) under surface of the swede leaf (E) seedling of *D.tenuifolia* covered with the conidiophores*,* Photo: J. Johansen Hladilová.

Taxonomy

The taxonomy of the causal agent from the family Peronosporaceae of downy mildew on the cruciferous crops has been debated from the beginning of its taxonomy and there were several principal changes in the species concepts (Göker et al., 2004, Sherriff and Lucas, 1990). Göker et al., 2004 considers it as the most difficult problem in the taxonomy of the plant parasitic Oomycetes.

However, *Hyaloperonospora (Peronospora)* isolates are morphologically rather unified (Göker et al., 2009, Yerkes and Shaw, 1959), the variation in the host range and recent molecular analyses indicates a narrow species delimitation within the family Peronosporaceae (Göker et al., 2009, Choi et al., 2003).

De Bary, 1863; cited in Yerkes and Shaw, 1959, suggested host specialization of the paraziting *Peronosporas* below the level of host family as a taxonomic criterion. Gaümann, 1918, 1923 delimited 52 species of *Peronospora.* He proposed a taxonomic marker of specialization on host species or genera rather than host families. Gustavsson, 1959 advocated the host range principle in the taxonomy of *Peronosporas* suggested by Gaümann, 1918, 1923. Yerkes and Shaw, 1959 ascribed all *Peronospora* specimens on Brassicaceae to a single aggregate species, *Peronospora parasitica*. Constantinescu and Fatehi, 2002 presented molecular and morphological evidence to split the genus *Peronospora* into three genera, *Peronospora* s. str., *Hyaloperonospora*, and *Perofascia.*

The causal agent of downy mildew on Brassicaceae was represented by merging species *Hyaloperonospora parasitica* (Pers.) Constant. Recently, Göker et al., 2004, 2009 presented phylogenetic studies based on molecular analyses using internal transcribed spacer (ITS) sequences. He confirmed use of the narrow species delimitation and the host range as a taxonomic marker suggested by Gaümann, 1918, 1923. Göker et al., 2004, 2009 again suggested the use of *Hyaloperonospora brassicae* for the causal agent of downy mildew on species of *Brassica, Raphanus, Sinapis. Hyaloperonospora parasitica* shall be restricted to species *Capsella bursa-pastoris.* Another suggestions are for example the use of the species *Peronospora diplotaxidis* for the causal agent of downy mildew on the species of *Diplotaxis tenuifolia, the* species *Peronospora erucastri* for the causal agent of downy mildew on the species of *Eruca sativa* or *Hyaloperonospora arabidopsidis* for the causal agent of the downy mildew of the plant model organism *Arabidopsis thaliana.*

However, the taxonomy of Peronosporaceae is still inconsistent. There are several recent publications (Nordskog, Hermansen, 2008, Rimmer et al., 2007, Koike et al., 2007) which are following the Constantinescu and Fatehi, 2002 taxonomical

suggestions. Rentel et al., 2008 is presenting a study where *Hyaloperonospora parasitica* is considered as a native oomycete pathogen of *Arabidopsis*.

Host Specificity

Downy mildew affects most vegetable, ornamentals, and weeds from the Brassicaceae family (Smith et al., 1988). Downy mildew is common on cabbage, Brussels sprouts, cauliflower, broccoli, marrow-stem kale, kohlrabi, Chinese cabbage, rape, rutabaga and mustard (Rimmer et al., 2007). Koike, 1998 reported an appearance of downy mildew of arugula (*E.sativa*), caused by *Peronospora parasitica,* in California. Romero et al., 2005 came with first report of downy mildew of arugula (*E.sativa*), caused by *Peronospora parasitica,* in Argentina. Downy mildew of wild rocket (*Diplotaxis tenuifolia*), caused by *Peronospora parasitica,* in Italy was reported by Garibaldi et al., 2004.

As described above, from the beginning of the *Peronospora* taxonomy the variation in the host range was realised (Göker et al., 2004, 2009). Göker et al., 2004, 2009 advocated the host range principle in the taxonomy of Peronosporas suggested by Gaümann, 1918, 1923. He is also considering that *Hyaloperonospora* species are not necessarily confined to closely related host plants and that some host species are susceptible to several *Hyaloperonospora* species. Gustavsson, 1959 wrote that the *Peronospora* has been found capable of infecting also species within the other host genera. The different aggressiveness of the pathogen by its origin within *Brassica s*pecies was already presented by Gaümann in 1926.

Not only the host specialization within *Brassica* species was considered. Sherriff and Lucas, 1990 analysed the influence of different *Brassica* species, different cultivars of the same species and geographical distinct of the isolates.

Host-pathogen Interaction

In the 1990s there was established laboratory plant–pathogen interaction model between *Hyaloperonospora parasitica* and *Arbidopsis thaliana* (Rentel et al., 2008). Rentel et al., 2008 and Grouffaud et al., 2008 suggested a defence mechanism

against oomycete, bacterial and viral pathogens where *H. parastica* effector protein ATR13 triggers defence responses.

Epidemiology

Hyaloperonospora spp. survives as oospore in plant debris in the soil. The oospores in the soil are probably the source of primary infection and may lead to a systemic infection (Rimmer et al., 2007, Smith et al., 1988). The infection on leaves occurs from airborne conidia. However the infection of seedlings from oospores has not been verified under natural conditions (Rimmer et al., 2007). Seed transmission is not considered to be relevant for this pathogen (Smith et al., 1988). Cool and moist conditions (temperature between 10 and 15 ^{o}C and relative humidity of 90 to 98%) are favourable for development of the disease (Nordskog, Hermansen, 2008; Paul et al., 1998). The asexual cycle can be completed in 3-4 days at 20°C and with moist conditions. Conidia are often formed at night (Smith et al., 1988).

Conidia of *Hyaloperonospora* spp. are spread by wind and water splash. Conidia can survive a few days on leaves under typical field conditions, but at low temperature and in the absence of moisture can survive more than 100 days (Smith et al., 1988). Spread for long distance by the conidia is possible, but the survival of spores is not well documented (Nordskog, Hermansen, 2008).

Control

Rimmer et al. (2007) is suggesting crop rotation, removal of plant debris and weed control as useful measures. The cultivation of one or only few cultivars of the same species can enhance changes in pathogen populations against host resistance.

Several fungicides (chlorothalonil, dichlofluanid, mixured of cymoxanil and mancozeb, oxadixyl in a mixture with the mancozeb and propamocar) are used for chemical control (Rimmer et al., 2007). The application is important in seedlings stage (Smith et al., 1988). Measures for reducing humidity can be applied, especially in greenhouse.

HOST SUSCEPTIBILITY

INTRODUCTION

Downy mildew is caused by plant parasitic Oomycetes from the genus *Hyaloperonospora* and the genus *Peronospora*. The pathogen infects several species from the Brassicaceae family. However, the isolates from the different brassica species are usually more virulent on the species of the origin (Rimmer et al., 2007). The variation of the host range within the family Peronosporaceae and the use of the host specificity as a taxonomic criterion is debated (Göker et al., 2009, Choi et al., 2003).

The experiment investigates the host range of *Hyaloperonospora* spp. at the cotyledon stage of brassicas and closely related cruciferous crops. The hypothesis is that the level of the aggressiveness of the pathogen depends on its origin within the Brassicaceae family (Rimmer et al.; 2007, Sherriff and Lucas, 1990). The question is whether the population of the pathogen *Hyaloperonospora* spp. on different brassica species might infect rocket salad species and vice versa and how much this infection is severe.

The cotyledons susceptibility test can determine differences in aggressiveness of different isolates. Paul et al. 1998 used a cotyledon test to differentiate winter oilseed rape cultivars according to their susceptibility to *Hyaloperonospora (Peronospora)* spp. Gilardi et al., 2007 has used the susceptibility test on seedlings of *E. sativa* and *D. tenuifolia* to investigate resistance of different rocket cultivars to *Fusarium oxysporum* under artificial inoculation conditions.

The experiment (the cotyledons susceptibility test) consists of two parts. Part 1 is preliminary to Part 2. Paul et al. 1998 confirmed that a long-term preservation of *Hyaloperonospora (Peronospora)* spp. in a freezer was possible. The Part 1 is also testing the use of the frozen material as a source of primary inoculum.

METHODS AND MATERIAL

Part 1 and Part 2 differ in their source of primary inoculum.

Source of primary inoculum of downy mildew:

Part 1:

- frozen samples of the diseased Brassicaceae family crops (swede, broccoli, cauliflower, wild rocket) from fields in Norway (Lier, Røyken, Østfold), sampled by the Bioforsk in 2008.

Part 2:

- fresh samples of the diseased Brassicaceae family crops (swede, broccoli, cauliflower, wild rocket) from fields in Norway (Lier, Røyken, Østfold), sampled by the Bioforsk in 2009,
- diseased cruciferous weeds grown in the vicinity of the fields with the Brassicaceae family crops in Norway (Lier, Røyken, Østfold), sampled by the Bioforsk in 2009 .

There are other differences between Part 1 and Part 2. In general, in Part 1 are used basic methods and materials, including a disease assessment. Part 2 is elaborated further.

Part 1

Cultivation of Seedlings

Seeds of four to five species from the Brassicaceae family were used. The seedlings of wild rocket salad (*Diplotaxis tenuifolia*), cultivated rocket salad (*Eruca sativa*), cauliflower (*Brassica oleracea* var. *botrytis*), broccoli (*Brassica oleracea* var. *italica)* and swede (*Brassica napus* subsp. *napobrassica*) were cultivated in this part of the experiment.

Seeds were planted in soil (P-jord) contained in 7 cm x 7 cm x 8 cm plastic pots with 10-15 seedlings (15 seeds) in average. *D. tenuifolia* was planted with higher density of the seedlings, approximately 20-30 seeds per pot. Seedlings were grown 10-12 days at 20 to 22°C with a 16 hour photoperiod (Nordskog, 2006). The term *culture* in this thesis refers to a culture of seedlings in one pot. The *culture* contains seedlings of the same species and the same age.

One set contained four to five cultures of the seedlings (pots with seedlings). The pots were placed in the plastic boxes with the transparent top cover 15 x 37 x 10 cm (Figure 6) Twelve sets (R1, R2, R3, R4, S1, S2, B1, B3, B4, B5, B6, B7) were infected. The other four sets (K2, K3, K4, K5) were planted and used as a control set.

The sets R1, B1, S1 consisted of four pots with cultures of seedlings of *D. tenuifolia*, *E. sativa*, broccoli and cauliflower. The sets R2, R3, R4, S2, B5, B3, B4, B6, B7 and K2, K3, K4, K5 contained five pots with cultures of seedlings of *D. tenuifolia*, *E. sativa*, broccoli cauliflower and swedes.

Figure 6. Host Susceptibility Test - Part I- The set with five cultures (pots) with the seedlings of cultivated rocket salad (*E. sativa*), wild rocket (*D. tenuifolia*), broccoli, cauliflower and swede.) Photo: J. Johansen Hladilová

Source of Primary Inoculum

Twelve isolates of different Brassicaceae family vegetables, infected by downy mildew, were sampled in Norway in 2008. These samples were stored in a freezer at -17°C for several months and they were used as a source of the primary inoculum. Four isolates were from host plant *D.tenuifolia* (r1, r2, r3, r4), two isolates were from host plant swede (*s*1, s2) and six isolates were from host plant broccoli (b1, b3, b4, b5, b6, b7), Table 1.

Table 1. Host Susceptibility Test - Part I- Information about twelve isolates (identification number, originated host plant, region, place and date of sampling).

Isolate	Origin host plant	Identification number	Region, Place	Date of sampling
r1	*D.tenuifolia*	B108/Lier08001	Buskerud, Gjerdal	05.05.2008
r2	*D.tenuifolia*	B0608	Buskerud, Røyse	06.08.2008
r3	*D.tenuifolia*	B0208	Jerdal, Røyken	28.05.2008
r4	*D.tenuifolia* DI 902	B0508	Buskerud, Huseby (Børresh Huseby Gård	25.06.2008
b1	Broccoli Varieties: Marathon	R0208	Rogaland, Ase	12.07.2008
b3	Broccoli, Varieties: Marathon	R0308	Rogaland, Ase, Jonas sør	05.08.2008
b4	Broccoli Varieties: Ironman	Ø0408	Østfold, Kubberød v/rideb	11.09.2008
b5	Broccoli Varieties: Ironman	Ø0508	Østfold, Kubberød nedre	11.09.2008
b6	Broccoli Varieties: Ironman	Ø0608	Østfold, Kirken	11.09.2008
b7	Broccoli Varieties: Ironman	Ø0808	Østfold, Granli mot Alby	11.09.2008
s1	Swede	Ø0208	Østfold, Frogn, Fjøser	24.07.2008
s2	Swede	R0108	Rogaland, Orre	01.07.2008

Spore Harvesting

Frozen material was placed in a 50 ml plastic tube. Then the conidia were harvested by adding 20 ml sterile distilled water and then the tube was shaken by hand. The spore suspension was used after parts of plants were removed.

Spore concentration: Maximum of the spores from the frozen material was used. The spore concentration was usually between 0,1 and 2 x 10^4 conidia/ml or less. The concentration was estimated by using microscopy techniques and a hemocytometer (Table 2).

The spore suspension were sprayed on the test seedlings. 15-20 ml of the spore suspension was used per one set. The control set was sprayed by distilled water.

Table 2. Host Susceptibility Test - Part 1. Concentration of spore suspension used for the inoculation on tested seedlings.

Isolate	Set	Concentration of 20 ml with spore suspension (n x 10 000 spore/ml)
r1	R1	0,4
b1	B1	0,1
s1	S1	n ‹ 0,1[a]
r2	R2	1,8
s2	S2	unknown
r3	R3	1,9
b3	B3	n ‹ 0,1[a]
b4	B4	0,1
r4	R4	0,2
b5	B5	0,1
b6	B6	n ‹ 0,1[a]
b7	B7	0,3

a …. n ‹ 0,1, n (number of spores) is lower than 1 000 per ml. The number of the spores was very small for an estimation of spore concentration with use of a microscope. Any spores were not found.

Inoculation on Seedlings and Promotion of Asexual Sporulation

The seedlings were incubated for 7-10 days at 20 to 22°C to allow infection. The seedlings were incubated over night with 100% RH to promote the asexual sporulation.

Disease Assessment

The main indicator for the evaluation was an occurrence of the conidiophores of *Hyaloperonospora* spp. on the tested seedlings. **Positive**, if the conidiophores of *Hyaloperonospora* spp. occurred and **negative**, if did not.

Part 2

Cultivation of Seedlings

The seedlings of six species from the Brassicaceae family were used. The seedlings of wild rocket salad, cultivated rocket salad, cauliflower, broccoli, swede and oilseed rape were cultivated.

Seeds were planted in soil (P-jord) contained in 7 cm x 7 cm x 8 cm plastic pots. *E. sativa*, broccoli, swede, cauliflower and oilseed rape cultures consist of 15-20 seedlings (20 seeds) in average. *D. tenuifolia* were planted with a higher density of the seedlings, approximately 30-40 seeds.

The seedlings were grown 8-12 days at 20 to 22°C with a 16 hour photoperiod. One set contained six pots with cultures of seedlings, which were placed in the plastic boxes 20 x 34 x 7 cm with the transparent top cover (Figure 7).

Two types of sets were used. *Multi-species set* and *mono-species set*. *Multi-species set* refers to the set consisting of pots with seedlings of six different species (wild rocket salad, cultivated rocket salad, cauliflower, broccoli, swede and oilseed rape). *Mono-species set* refers to the set consisting of pots with seedlings of one species**.**

Figure 7. Host Susceptibility - Part 2. The sets with six different cultures (six pots with the seedlings). Photo: J. Johansen Hladilová

Source of Primary Inoculum

Fresh samples of the infected crops from the Brassicaceae family (swede, broccoli, cauliflower, wild rocket) and cruciferous weeds *(*C*apsula bursa pastoris* and *Thlaspi arvense)* came from the fields of brassica crops and near-by area in Norway (Lier, Røyken, Østfold) in 2009 were used. The plant material was sampled by local extension officers and sent by mail to the Bioforsk.

Following twenty isolates was used: b11, b13, b22, c14, r6, r8, r9, r12, r15, r16, r17, r18, r19, s21, s7, s10, w18, w19, w20, w23. Nine isolates (r6, r8, r9, r12, r15, r16, r17, r18, r19) were obtained from the host plant wild rocket (*D.tenuifolia*), three isolates (b11, b13, b22) were from the host plant broccoli, three isolates (s21, s7, s10) were from the host plant swede, one isolate (c14) was from the host plant cauliflower and four isolates (w18, w19, w20, w23) were from cruciferous weeds (Table 4.).

Table 4. Host Susceptibility - Part 2. Information about received 20 isolates (identification number, originated host plant, region, place, and date of sampling).

Isolate	Origin host plant	Identification number	Region, Place	Date of sampling
r6	*D. tenuifolia*	R0902	Rogaland	20-22.6.2009
r8	*D. tenuifolia*	R0903	Rogaland, Randaberg	30.06.2009
r9	*D. tenuifolia*	B0309	Villingstad	02.07.2009
r12	*D. tenuifolia* Variety: Adw.seed 907	B0906	Røyse, Elstøen Gartneri	12.08.2009
r15	*D. tenuifolia*		Huseby Lier	24.08.2009
r16	*D. tenuifolia*		Lianes gård	24.08.2009
r17	*D. tenuifolia*		Thor Graff	24.08.2009
r18	*D. tenuifolia*		Røyse, Elstøen Gartneri, sampled at Thor Graff pakkeriet	02.09.2009
r19	*D. tenuifolia*	R0908	Rogaland	04.09.2009
b11	Broccoli Variety: Lord	R0904	Rogaland	18.08.2009
b13	Broccoli	R0906	Rogaland Rygg, Randaberg, Elnar Hanasand	19.08.2009
b22	Broccoli	R0909	Rogaland, Randaberg, Goa	03.09.2009
c14	Cauliflower Variety: Nemo	R0905	Rogaland, Rygg, Randaberg, Elnar Hanasand	19.08.2009
s7	Swede	Ø0902	Østfold	29.06.2009
s10	Swede	Ø0902	Østfold, Frogn,Hopes ad, Andreas Wiig	06.08.2009
s21	Swede	R0907	Rogaland	04.09.2009
w18	Weed (*Capsula bursa pastoris*)		Lianes gård	24.08.2009
w19	weed (*Capsula bursa pastoris)*		Thor Graff gård	24.08.2009
w20	weed (*Capsula bursa pastoris* and *Thlaspi arvense*)		Thor Graff gård, ecological fields	24.08.2009
w23	Weeds (*Capsula bursa pastoris*)	B0910	Lier	2?. 9.2009

Treatment of Isolate

Within max. 5 days after sampling, the spores were harvested and clean cultures of seedlings were infected with the spore suspension.

Spore Harvesting

Infected parts of the plant with sporulating lesions were placed in a 50 ml plastic tube. Conidia were harvested by adding sterile distilled water (for one set with six pots with seedlings was usually used 20 ml of the spore suspension) and then shaking with the tube by hand. The spore suspension was used after parts of plants were removed. Maximum of the spores from the received plant material were used to achieve sufficient amount of the inoculum for the final COMPLETE set.

Establishment of Sufficient Amount of Inoculum

To achieve a sufficient amount of the inoculum for the final COMPLETE set, the spore suspension had to be sprayed on minimum one set with 3-6 pots with seedlings of the same host plant species as the used isolate. Figure 8 is showing two ways of the used procedure to infect the seedlings.

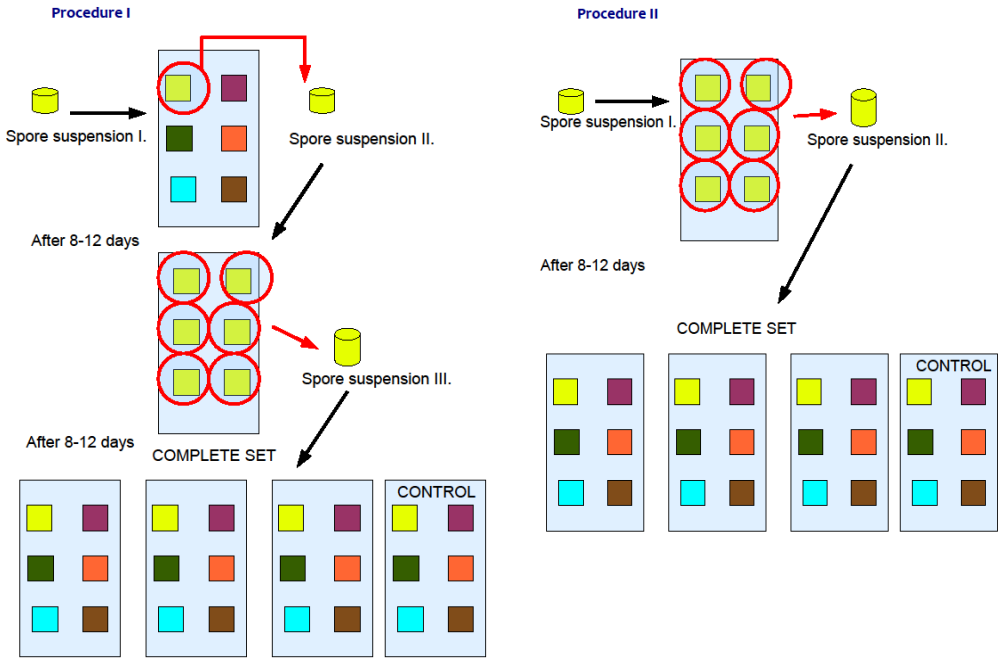


Figure 8. Schematic drawing of two procedures to infect seedlings utilized the fresh material. Each colour represents one plant species. Procedure I: First, the available multi-species set is infected. Spores from host plant species of the isolate are harvested and reinoculated on the mono-species set (host plant species of the isolate). Spores from the mono-species set are harvested and inoculated on the final COMPLETE sets (three multi-species sets plus one control set). Procedure II: First the available mono-species set (host plant species of the isolate) is infected. Spores from mono-species set are harvested and inoculated on the final COMPLETE sets (three multi-species set plus one control set).

Inoculation on COMPLETE SET

Spore suspensions were sprayed on seedlings. The spore suspension from each isolate was inoculated on three sets with six different species. The fourth set was a control set. 20 ml of the spore suspension was sprayed per one set. (For one COMPLETE SET was used 60 ml of the spore suspension plus 20 ml of distilled water for the control set).

Concentration of spore suspensions was between 0,2 and 1 x 10^4 conidia/ml (Table 5). Microscopy techniques and a hemocytometer were used to estimate the spore concentration.

After a period of 7 days to 12 days the seedlings were incubated over night and with 100% RH to allow infection. The incubation time was extended and a promotion of asexual sporulation was repeated, when needed.

Table 5. The concentrations of the spore suspension for the final COMPLETE SET´s inoculation.

Isolate	Set	Concentration of 20 ml with spore suspension (n x 10 000 spore/ml)
r6	R6a, R6b, R6c	0,2
s7	S7a, S7b, S7c	1,0
s10	S10a, S10b, S10c	0,7
r8	R8a, R8b, R8c	0,8
b11	B11a, B11b, B11c	0,8
b13	B13a, B13b, B13c	0,7
r15	R15a, R15b, R15c	0,3
r16	R16a, R16b, R16c	0,25
r17	R17a, R17b, R17c	0,45
r18	R18a, R18b, R18c	0,35
b22	B22a,B22b, B22c	0,4

Disease Assessment

The main indicator for the evaluation was an appearance of the conidiophores of *Hyaloperonospora (Peronospora)* spp. on the experimental seedlings. **Positive**, if the conidiophores of the fungus occurred and **negative**, if did not.

Further, the observation of **symptoms** is included. The following rating scale was used:

3 Positive, an appearance of the conidiophores was observed. An area of the plant tissue covered by the conidiophores of *Hyaloperonospora (Peronospora)* spp. or the lesions with conidiophores were observed.

2 Same symptoms (yellowish lesions, darker spots) as on the seedlings with the rating scale 3, but any sporulating conidiophores were not observed.

1 No significant symptoms, non specific lesions, spots, necrotic areas were observed.

0 Negative, no symptoms, no sporulating conidiophores

0, 1 and 2 are considered as **negative**, negative for the pathogen *Hyaloperonospora (Peronospora)* spp. Even though there were observed symptoms of plant disease on seedlings, there were not observed the sporulating conidiophores of *Hyaloperonospora (Peronospora)* spp.

Intensity of disease were further described by:

a. Quantitative assessment:

1. **The plant disease incidence** (Madden et al., 2007). The percentage of diseased seedlings (individuals). From 0 to 100 %. Zero is corresponding to no seedlings with infection in one observed culture (healthy culture), 100 % to an occurrence of disease on all seedlings of one culture.
2. **The leaf disease incidence** (Madden et al., 2007). The number of disease leaves on the infected plant.
3. **The mean leaf disease severity** (Madden et al., 2007). The percentage of diseased area on one diseased leaf in average. From 0 to 100 %. Zero is corresponding to a healthy leaf, 100 % to an appearance of disease on whole leaf.

b. Qualitative assessment:

1. **Symptoms.** Description of the symptoms on observed plant.

2. **Location of symptoms, sporulating conidiophores.** Location of conidiophores on observed plant (conidiophores observed on a leaf, a stem, lower or upper side of the leaf etc.)

RESULTS

Part 1

The disease was observed on six sets (R1, S1, B4, B5, B6, B7) from twelve infected sets. Set R1 was infected by the isolates from the host plant *D. tenuifolia* and sporulation appeared only on seedlings of *D. tenuifolia*. Sets B4, B5, B6, B7, infected by the isolates from the host plant broccoli, were more aggressive on seedlings of broccoli. Only pots with the seedlings of broccoli were positive**.**

The set S1 was infected by the isolate from the host plant swede and was aggressive on broccoli and cauliflower. The pots with broccoli and cauliflower were positive. This set did not contain the pot with seedlings of swede.

Downy mildew was not observed on B1, R3, B3, R4, R2 and S2. On set R2 and set S2 were observed the fungal growth, but not *Hyaloperonospora (Peronospora)* spp., probably white rust (*Albugo candida*) or grey mould (*Botrytis cinerea*).

Part 2

Only eleven isolates (b11, b13, b22, r6, r8, r15, r16, r17, r18, s7, s10) from twenty received isolates (b11, b13, b22, c14, r6, r8, r9, r12, r15, r16, r17, r18, r19, s21, s7, s10, w18, w19, w20, w23) were successfully reinfected on the final COMPLETE set.

<u>COMPLETE Sets</u>

The occurrence of the disease and amount of the disease vary within cultures (pots with seedlings) and sets. Two main differences are affecting the observed results:

- **plant species of planted seedlings**
- **plant species of the host plant of the used isolate**.

D. tenuifolia Isolates

Main indicator. In all infected sets (R6a, R6b, R6c, R8a, R8b, R8c, R15a, R15b, R15c, R16a, R16b, R16c, R17a, R17b, R17c, R18a, R18b, R18c) the cultures of *D. tenuifolia* were positive. Other cultures were negative with one exception. The swede culture

was positive in the set R8a (one cotyledon leaf covered by conidiophores was observed).

Observation of symptoms. The symptoms were observed on all cultures (pots with seedlings) of *D. tenuifolia* in 18 infected sets (R6a, R6b, R6c, R8a, R8b, R8c, R15a, R15b, R15c, R16a, R16b, R16c, R17a, R17b, R17c, R18a, R18b, R18c). The similar symptoms were observed on all cultures of *D. tenuifolia* infected with the isolates r6, r8, r15, r16, r17, r18. White mycelium, sporulating conidiophores, conidiophores were observed on both cotyledon leaves and on the stem. The occurrence of conidiophores could be accompanied with dark or yellowish lesions. The yellowish lesions were more significant with development of the disease, over time, and with repeating of a promotion of asexual sporulation. The cotyledon leaves became necrotic, yellowish with dark spots. White conidiophores were observed often where the seedlings grew close to each other (a dense sowing).

The disease symptoms were observed also on the other planted species. The symptoms were observed on the cultures of *E. sativa* in five sets (R16a, R15c, R18a, R18b and R18c), on the cultures of broccoli in eight sets (R15a, R15b, R16a, R16c, R17a, R17b, R17c and R18c), on the cultures of swede in two sets (R8a, R15a), on the cultures of cauliflower in ten sets (R15a, R15c, R15b, R16a, R16c, R17a, R17b, R17c, R18b and R18c) and on the cultures of oilseed rape in two sets (R15c and R18c). The rating scale of symptoms is summarized in Table 6. With exception of six cultures (pots with seedlings), the observation of symptoms was rated by number **1** (no significant symptoms, observed lesions, spots, necrotic areas). Those six cultures were broccoli from sets R16a, R16c and cauliflower from sets R16c, R15a, R15b, R15c and were rated by number **2** (observed symptoms without observation of the sporulating conidiophores). The symptoms were dark spots on both cotyledons (dark olive-green spots on cauliflower cotyledons), but no conidiophores were observed.

Mean leaf disease severity. The mean leaf disease severity was in average 50% (minimum 20%, maximum 80%, median 45%) on diseased cultures of *D. tenuifolia*. The mean leaf disease severity was in average 3% on diseased cultures of cauliflower. The mean leaf disease severity was in average 5% on diseased cultures of broccoli. The

correlation coefficient of the mean leaf disease severity and the used spore concentration is 0,13. There is weak association between variables.

Plant disease incidence. The plant disease incidence was on diseased cultures of *D. tenuifolia* in average 37 % (minimum 7%, maximum 80%, median 30%). The plant disease incidence was estimated on diseased cultures of cauliflower in average 30%. The plant disease incidence was estimated on the diseased cultures of broccoli in average 20%.

All cultures (pots with seedlings) from the control sets (R6 Control, R8 Control, R17 Control, R16 Control, R15 Control, R18 Control) were negative and no symptoms were observed. The correlation coefficient of the mean leaf disease severity and the used spore concentration is 0,29. There is weak association between variables.

Table 6. The rating scale (from 0 to 3) of symptoms of *Hyaloperonospora* spp. on final COMPLETE sets infected with the isolates from the host plant D.*tenuifolia* (r6, r8, r17, r16, r15, r18)

Isolate	Set (6 pots with seedlings sowed and infected in the same time)	*D.tenuifolia*	*E. sativa*	broccoli	cauliflower	swede	oilseed rape
r6	R6 a	3	0	0	0	0	0
	R6 b	3	0	0	0	0	0
	R6 c	3	0	0	0	0	0
	Average	**3**	**0**	**0**	**0**	**0**	**0**
r8	R8 a	3	0	0	0	1	0
	R8 b	3	0	0	0	0	0
	R8 c	3	0	0	0	0	0
	Average	**3**	**0**	**0**	**0**	**0,33**	**0**
r17	R17 a	3	0	1	1	0	0
	R17 b	3	0	1	1	0	0
	R17 c	3	0	1	1	0	0
	Average	**3**	**0**	**1**	**1**	**0**	**0**
r16	R16 a	3	1	2	1	0	0
	R16 b	3	0	0	0	0	0
	R16 c	3	0	2	2	0	0
	Average	**3**	**0,33**	**1,33**	**1**	**0**	**0**
r15	R15 a	3	0	1	2	1	0
	R15 b	3	0	1	2	0	0
	R15 c	3	1	0	2	0	1
	Average	**3**	**0,33**	**0,67**	**2**	**0,33**	**0,33**
r18	R18 a	3	1	0	0	0	0
	R18 b	3	1	0	1	0	0
	R18 c	3	1	1	1	0	1
	Average	**3**	**1**	**0,33**	**0,67**	**0**	**0,33**

Swede Isotates

Main indicator. In all six infected sets (S7a, S7b, S7c, S10a, S10b, S10c), the culture of swede was positive. The cultures of broccoli were positive in all sets infected with S7 (S7a, S7b, S7c,) and also on the sets S10b and S10c (the set S10a was negative). The cultures of cauliflower in all sets infected with S7 (S7a, S7b, S7c) and also on set S10a were positive (the sets S10b and S10c were negative). In all infected sets (S7a, S7b,

S7c, S10a, S10b, S10c) the cultures of *D. tenuifolia, E.sativa* and oilseed rape were negative.

Observation of symptoms. The symptoms, rated by number **3** or **2**, were observed on all cultures of swede, cauliflower and broccoli in all infected sets (S7a, S7b, S7c, S10a, S10b, S10c). The symptoms were rated by number **3** on all six cultures of swede (S7a, S7b, S7c, S10a, S10b, S10c), on 4 cultures of cauliflower (S7a, S7b, S7c, S10a) and five cultures of broccoli (S7a, S7b, S7c, S10b, S10c).

Small yellowish lesions with dark edge and conidiophores on older leaves were observed on diseased swede plants (the mean leaf disease severity was 5-10 %). The cotyledons were often covered with sporulating conidiophores (small cotyledons had 100% leaf disease severity) and later became yellowish and necrotic. The conidiophores were observed on both sides of the leaf and also on a stem of the seedling. Symptoms were observed on one or both cotyledon leaves.

Dark spots were observed on diseased broccoli plants. White conidiophores were observed on both sides of cotyledons and also on the stem. The symptoms were not observed on older leaves, only on cotyledons.

Dark olive green spots were observed on cotyledon leaves on diseased cauliflower plants. The occurrence of the sporualing conidiophores were not abundant. The symptoms were not observed on older leaves or on stem, only on cotyledons.

The disease symptoms, rated by number **2** or **1,** were observed also on the cultures of oilseed rape. The symptoms on oilseed rape were observed on five sets (S7b, S7c, S10a, S10b, S10c). The symptoms rated by number **2** were observed on two sets infected with the isolate s7 (S7b and S7c). The symptoms, rated by number **1,** were observed on all sets infected with the isolate s10 (S10a, S10b, S10c). Dark lesions on mainly cotyledons, but also on older leaves, were observed on diseased oil seed rape seedlings. There were no sporulating conidiophores observed. Non-specific symptoms, rated by number **1**, were observed on the seedlings of cultivated rocket salad (*E. sativa*) in one set (S10a). The rating scale of symptoms is summarized in Table7.

Mean leaf disease severity. The mean leaf disease severity was in average 70 % (minimum 40%, maximum 100%, median 67%) on diseased cultures of swede. The

mean leaf disease severity was in average 14% (minimum 5%, maximum 25%, median 12%) on diseased cultures of cauliflower. The mean leaf disease severity was in average 22% (minimum 15%, maximum 40%, median 17,5%) on diseased cultures of broccoli.

The plant disease incidence. The plant disease incidence was in average 65% (minimum 40%, maximum 90%, median 70%) on diseased cultures of swede. The plant disease incidence was estimated in average 55% (minimum 20%, maximum 90%, median 55%) on diseased cultures of cauliflower. The plant disease incidence was estimated in average 60% (minimum 20%, maximum 80%, median 75%) on diseased cultures of broccoli.

All cultures (pots with seedlings) from the control sets (S7 Control, S10 Control) were negative and no symptoms were observed.

Table 7. The rating scale (from 0 to 3) of symptoms of *Hyaloperonospora* spp. on final COMPLETE sets infected with isolates from host plant swede (s7, s10)

Isolate	Set (6 pots with seedlings sowed and infected in the same time)	*D.tenuifolia*	*E. sativa*	broccoli	cauliflower	swede	oilseed rape
s7	S7 a	0	0	3	3	3	0
	S7 b	0	0	3	3	3	2
	S7 c	0	0	3	3	3	2
	Average	**0**	**0**	**3**	**3**	**3**	**1,33**
s10	S10 a	0	1	2	3	3	1
	S10 b	0	0	3	2	3	1
	S10 c	0	1	3	2	3	1
	Average	**0**	**0,67**	**2,67**	**2,33**	**3**	**1**

Broccoli isolates

Main indicator. In all nine infected sets (B11a, B11b, B11c, B13a, B13b, B13c, B22a, B22b, B22c), the culture of broccoli was positive. The cultures of cauliflower were positive in all sets infected with B22 (B22a, B22b, B22c) and also on three other sets (B11a, B11b, B13a). The cultures of swede were positive in four sets (B11a, B13c, B22a,

B22c). The cultures of oilseed rape were positive in two sets (B13a, B22b). In all infected sets (B11a, B11b, B11c, B13a, B13b, B13c, B22a, B22b, B22c) the culture of *D. tenuifolia* and *E.sativa* was negative.

Observation of symptoms. The symptoms, rated by number **3** or **2**, were observed on all cultures of swede, cauliflower and broccoli in all infected sets (B11a, B11b, B11c, B13a, B13b, B13c, B22a, B22b, B22c).

The diseased broccoli cotyledons were covered with white conidiophores. There were also observed darker lesions, but the leaves were not yellowish or necrotic. The conidiophores were observed on both sides of cotyledon leaves and also on the stem. The symptoms were not observed on older leaves, only on cotyledons. Usually both cotyledon leaves were affected.

Dark olive green spots were observed on cotyledons of diseased cauliflower plants. The occurrence of the conidiophores was not abundant. The conidiophores were observed on the lower part of the dark spots on the leaf and also on the stem. The symptoms were observed on cotyledon leaves, one or both leaves were affected.

Dark spots and yellowish areas were also observed on diseased swede plants. The occurrence of the conidiophores was not abundant. The conidiophores were observed on the lower part of the leaf and also on the stem. The symptoms were observed on one or both cotyledon leaves, but also on older leaves. Small young plants were covered with conidiophores.

The disease symptoms, rated by number **3, 2** or **1,** were observed on cultures of oilseed rape. The symptoms on oilseed rape were not observed only on one set (B13b) from nine infected sets. The symptoms rated by number **3** were observed on two sets (B13a, B22b). The symptoms rated by number **2** were observed on three sets (B11b, B22a, B22c).

The symptoms, rated by number **1**, were observed on culture of cultivated rocket salad (*Eruca sativa*) in three sets (B13a, B13b, B11c).

The rating scale of symptoms is summarized in Table 8.

Mean leaf disease severity. The mean leaf disease severity was on diseased cultures of broccoli in average 50 %(minimum 25%, maximum 70%, median 50%). The mean leaf disease severity was on diseased cultures of cauliflower in average 9% (minimum 2%, maximum 20%, median 10%). The mean leaf disease severity was on diseased cultures of swede in average 7% (minimum 1%, maximum 10%, median 7%).The mean leaf disease severity was on diseased cultures of oilseed rape in average 6% (minimum 2%, maximum 10%, median 5%).

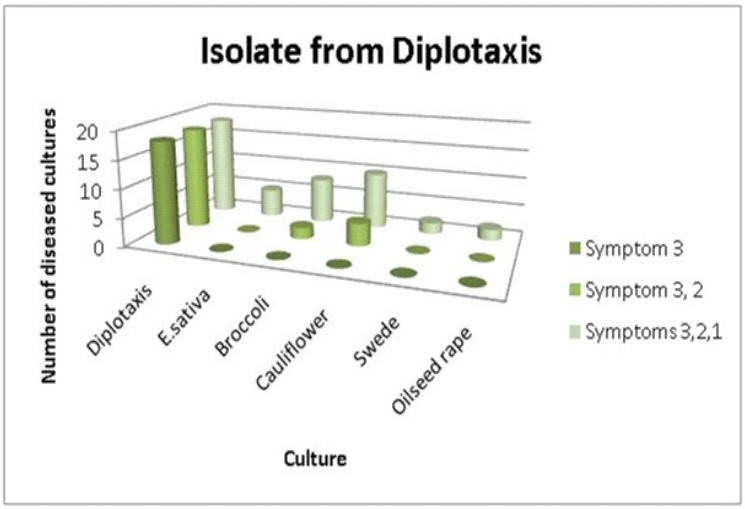
The plant disease incidence. The plant disease incidence was on diseased cultures of broccoli in average 80 % (minimum 55%, maximum 100%, median 80%). The plant disease incidence was estimated on diseased cultures of cauliflower in average 55% (minimum 40%, maximum 80%, median 55%). The plant disease incidence was estimated on diseased cultures of swede in average 24% (minimum 5%, maximum 55%, median 20%). The plant disease incidence was estimated on diseased cultures of oilseed rape in average 4% (minimum 1%, maximum 10%, median 5%).

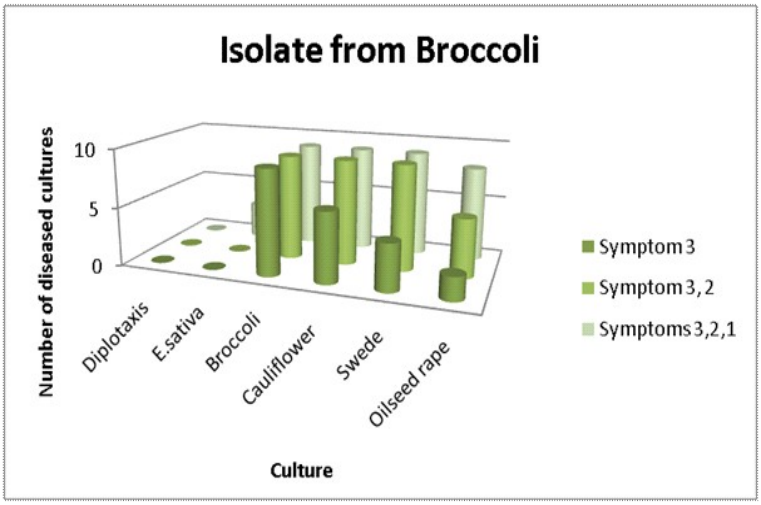
All cultures (pots with seedlings) from the control sets (B13 Control, B11 Control, B22 Control) were negative and no symptoms were observed.

The frequency of the diseased cultures with relationship to the origin species of the isolate is demonstrated in Figure 9.

Table 8. The rating scale (from 0 to 3) of symptoms of *Hyaloperonospora* spp. on final COMPLETE sets infected with isolates from host plant broccoli (b13, b11, b22)

Isolate	Set (6 pots with seedlings sowed and infected in the same time)	*D.tenuifolia*	*E. sativa*	broccoli	cauliflower	swede	oilseed rape
b13	B13 a	0	1	3	3	2	3
	B13 b	0	1	3	2	2	0
	B13 c	0	1	3	2	3	1
	Average	**0**	**1**	**3**	**2,33**	**2,33**	**1,33**
b11	B11 a	0	1	3	3	3	1
	B11 b	0	1	3	3	2	2
	B11 c	0	1	3	2	2	1
	Average	**0**	**1**	**3**	**2,67**	**2,33**	**1,33**
b22	B22 a	0	0	3	3	3	2
	B22 b	0	0	3	3	2	3
	B22 c	0	0	3	3	3	2
	Average	**0**	**0**	**3**	**3**	**2,67**	**2,33**





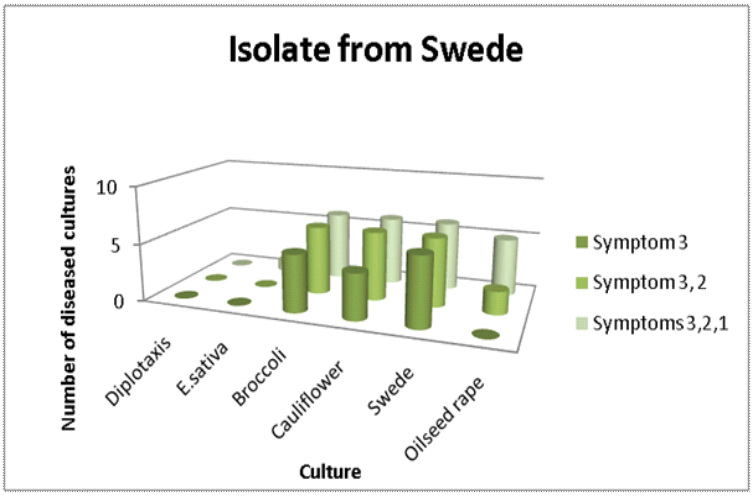


Figure 9. The frequency of the diseased cultures (seedlings of one species in one pot) with relationship to the origin species of the isolate. Data from eleve isolates infected on COMPLETE sets. The COMPLETE set refers to three sets with six cultures of *D. tenuifolia*, *E. sativa*, broccoli, cauliflower, swede and oilseed rape, plus one control set with six cultures).

Other Infected Sets

The procedure of receiving sufficient amount of inoculum (Figure 8) for the final COMPLETE set demanded to infect one or several preparatory sets. Observation of disease symptoms, age of the seedlings and time of inoculation and a promotion of asexual sporulation were documented. This documentation was done together on 93 sets (Table 9). 44 sets of them were COMPLETE sets (*multi-species sets)*. The rest of the sets were 49 preparatory sets. The results are described in detail above in this chapter. The preparatory sets were both *mono-species* and *multi-species sets*.

Table 9. List of documented planted sets of Host Susceptibility– Part 2

Isolate	Preparatory sets	COMPLETE set
r6	R6.1a, R6.1b, R6.2a, R6.3a, R6.3b, R6.4a, R6.4b	Yes
s7	S7.1a, S7.1b, S7.2a, S7.2a, S7.3a, S7.3b	Yes
s10	S10.1a, S10.1b	Yes
r8	R8.1a, R8.1b, R8.2a, R8.2b, R8.2.1,R8.2.2, R8.2.3a, R8.2.3b	Yes
b11	B11.1a, B11.1b	Yes
b13	B13.1a, B13.1b, B13.2	Yes
r15	R15.1, R15.2	Yes
r16	R16.1	Yes
r17	R17.1	Yes
r18	R18.1	Yes
b22	B22.1	Yes
r9	R9.1a, R9.1b, R9.2a, R9.2b, R9.2.1a, R9.2.1b	No
r12	R12.1a, R12.1b	No
c14	C14.1	No
w18	W18.1	No
w19	W19.1	No
w20	W20.1	No
r19	R19.1	No
s21	S21.1	No
w23	W23.1	No

The host susceptibility experiment was defined by the sets including different species. Eighteen sets from preparatory sets were *multi species sets* (R6.1a, R6.1b, R8.1a,

R8.1b, R9.1a, R9.1b, B11.1a, B11.1b, B13.1a, B13.1b, S7.1a, S7.1b, S10.1a, S10.1b, W19, W18, W20, W23). Disease evaluation of those sets was simplified and focused only on a main indicator, i.e. occurrence of conidiophores of *Hyaloperonospora (Peronospora) spp.* There were six sets (R6.1a, R6.1b, R8.1a, R8.1b, R9.1a, R9.1b) infected with the isolate from host plant *D.tenuifolia*, four sets (B11.1a, B11.1b, B13.1a, B13.1b) infected with the isolate from host plant broccoli, four sets (S7.1a, S7.1b, S10.1a, S10.1b) infected with the isolate from host plant swede and four sets (W19, W18, W20, W23) infected with the isolate from weed host plant, *Capsula bursa pastoris* or *Thlaspi arvense*.

D.tenuifolia Isolates

The cultures of *D. tenuifolia* were positive on five from six sets infected by the isolate from host plant *D. tenuifolia*. The other plant species cultures (broccoli, *E. sativa,* cauliflower, swede) were negative. All cultures (pots with seedlings) were negative on one set.

Swede Isolates

The cultures of swede, broccoli, cauliflower were positive on both two sets infected with the isolate s7 (S7.1a, S7.1b). The cultures of *D. tenuifolia* and *E. sativa* were negative.

The cultures of swede were positive on both two sets infected with the isolate s10 (S10.1a, S10.1b). The culture of broccoli was positive only on the set S10.1a. The cultures of *D. tenuifolia* and *E.sativa,* cauliflower and oilseed rape were negative.

Broccoli Isolates

The cultures of broccoli were positive on all four preparatory sets infected with the isolates b11 and b13. The other cultures of *D.tenuifolia, E.sativa,* cauliflower, swede and oilseed rape were negative.

Isolates of Weed Host Plant

All seedlings were negative on all four preparatory sets infected with the isolates w19, w18, w20, w23.

Comprehensive Results of Sets Infected with Isolates from Host Plant *D.tenuifolia*, Swede and Broccoli

The main indicator (an appearance of the conidiophores of *Hyaloperonospora (Peronospora)* spp on the experimental seedlings) was evaluated on 53 sets of Part 1 and Part 2 (COMPLETE sets and multi-species preparatory sets), Figure 10.

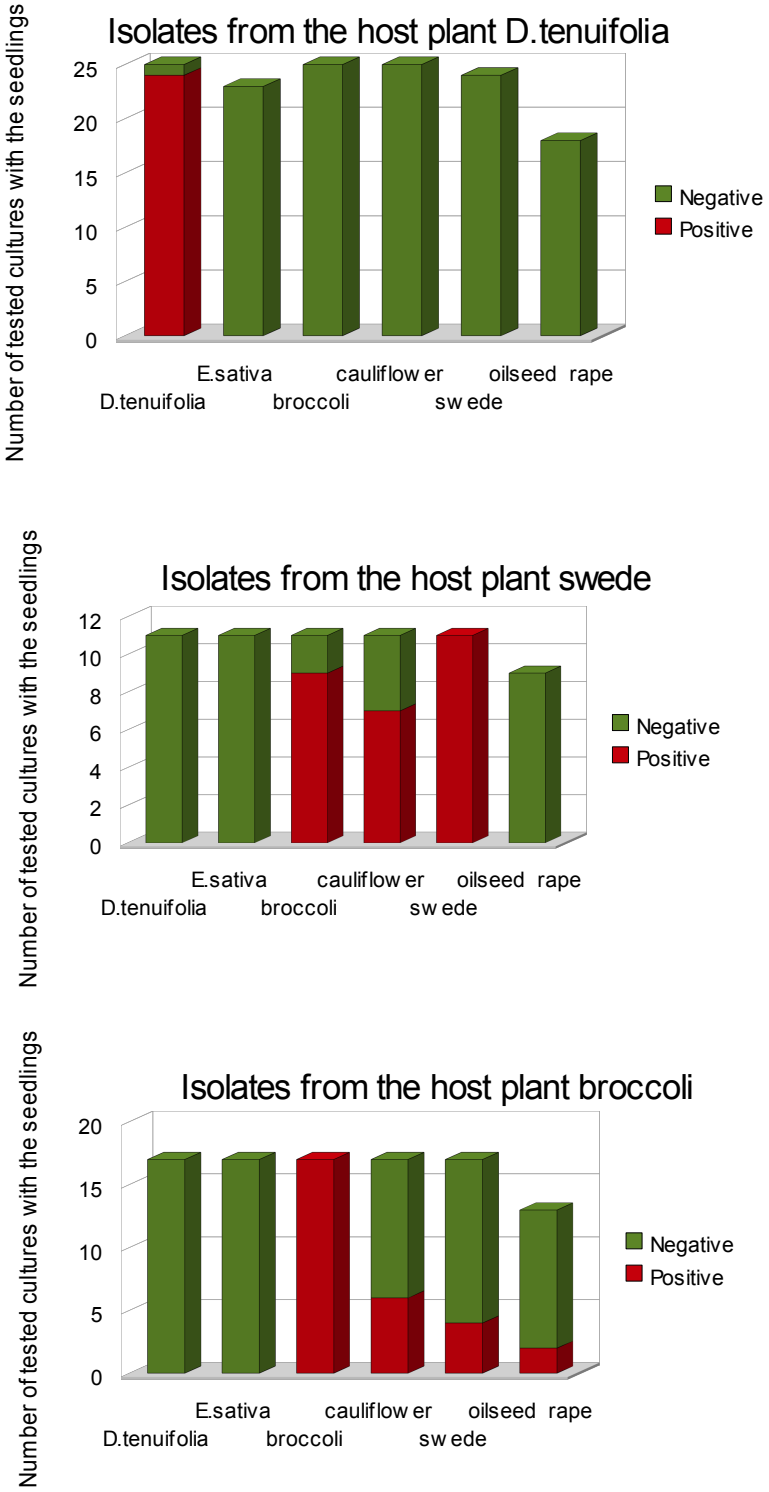


Figure 10. The frequency of the positive and negative cultures (pots with seedlings) from all multi-species sets infected with isolates from host plant *D.tenuifolia*, broccoli, swede.

DISCUSSION

Source of Primary Inoculum

In the first part of the experiment, Part 1, the inoculum was from frozen material. Paul et al. 1998 confirmed that long-term preservation of *Hyaloperonospora (Peronospora)* spp. in a freezer at -21°C for a period of 1 year using cryoprotective agents was possible. However the cryoprotective agents were not applied, the use of the frozen material as source of the primary inoculum has shown to be possible. But the successful inoculation and development of the pathogen from frozen material was much lower than from fresh material. Asexual sporulation of *Hyaloperonospora (Peronospora)* spp. was observed on 50% of the infected sets with material from a freezer. Asexual sporulation of *Hyaloperonospora (Peronospora)* spp. was observed on 94% of the infected sets with fresh material (Part 2). The storage of infected plant material in a freezer for several months can influence the quality of the spores. Paul et al. 1998 concluded that germination of spores of *Hyaloperonospora (Peronospora)* spp. for at least 12 months in a freezer in a solution of glycerine was reduced up to 79%. Also quality of the sampled material (for example enough sporulating lesions on a leaf) or decreased aggressiveness of the pathogen can influence an occurrence of the disease.

Spore Concentration

Silué et al., 1996 used concentration of the suspension 2 x 10^4 conidia/ml, Sousa et al., 1996 5 x 10^5 conidia/ml. However, an abundant sporulation on the sets infected with concentration of spore suspension 0,1 x 10^4 conidia/ml was observed. Disease appeared on the inoculated seedlings with the spore concentration lower than 0,1 x 10^4 conidia/ml. The infection was not severe, only a few plants were infected. The weak correlation between the concentration of spore suspension and the mean leaf disease severity and between the concentration of spore suspension and the plant disease incidence on sets infected with isolates from *D.tenuifolia* within COMPLETE sets can indicate no significant influence on the spore concentration.

Mean Leaf Disease Severity, Plant Disease Incidence

The percentage received from mean leaf disease severity and plant disease incidence gives very rounded number. The estimation is based on eye observation of the person evaluating the experiment. Observer´s estimates can be imprecise and inaccurate (Parker et *al.*, 1995). But together with rating scale of the symptoms can show the trend in aggressiveness of the pathogen.

Host Specificity

The hypothesis was that there is a different aggressiveness of the pathogen by its origin (Rimmer et al.; 2007, Sherriff and Lucas, 1990) on chosen members of Brassicaceae family. Results from Part 1 and Part 2 has shown that the pathogen *Hyaloperonospora (Peronospora)* spp. from a certain host plant species was more aggressive on the same plant species.

The first testing part of this experiment has shown that a certain host specificity pattern was already markable. Downy mildew was observed on six from twelve infected sets in the Part 1. The pathogen *Hyaloperonospora (Peronospora)* spp. from one plant species was more aggressive on the host plant species on five sets (R1, B4, B5, B6, B7). R1 was infected by the isolates from *D.tenuifolia* and sporulation has occurred only on *D.tenuifolia*. The same was observed on B4, B5, B6 and B7, where the isolates originated from broccoli were more aggressive on seedlings of broccoli.

The results of eleven COMPLETE sets has shown that the pathogen from the host plant *D. tenuifolia* was significantly more aggressive on cultures of *D. tenuifolia*. All infected cultures of *D. tenuifolia* were positive, the conidiophores of *Hyaloperonospora (Peronospora)* spp. were observed. The other cultures were negative with one exception. (This exception can be also explained by a mistake with the working practice. More isolates were used in the same time and there is a possibility of infecting from other isolate.)

Some symptoms were observed also on all other planted species. But only on broccoli and cauliflower cultures in the sets infected with isolate from host plant *D.tenuifolia* were symptoms rated by number 2.

The pathogen from host plant swede was more aggressive on the cultures of swede on eleven COMPLETE sets. But disease symptoms were also observed on broccoli and cauliflower. The symptoms were rated by number 3 on all six cultures of swede, on four cultures of cauliflower and five cultures of broccoli. The mean leaf disease severity and the plant disease incidence was significantly higher on swede. Aggressiveness of the pathogen on broccoli and cauliflower seems to be higher on broccoli, but the differences are not significant.

Also the pathogen from the host plant broccoli was significantly more aggressive on the cultures of broccoli. The disease was also observed on cauliflower, swede and oilseed rape. The mean leaf disease severity and the plant disease incidence were significantly higher on broccoli. The second highest mean leaf disease severity and plant disease incidence were on cauliflower plants.

Results of the experiment has shown that *E. sativa* was resistant against the pathogen isolated from the host plant *D. tenuifolia* and also against the pathogen isolated from the host plant broccoli and swede.

The results from preparatory sets are not full-valued as compared to the COMPLETE sets. But results from preparatory sets also confirms the host specificity pattern of the pathogen.

However, the symptoms of the disease vary within the cultures (pots with seedlings) and the sets. Few statements can be generalized:

- The disease is severe on the cotyledon leaves.
- Both cotyledons do not always get affected together. Often, only one cotyledon leaf was observed with severe infection, while the another cotyledon leaf remained healthy.
- There was a common occurrence of sporulating conidiophores on the stem of the seedlings.
- The younger, smaller plants were usually more affected with disease. Seedlings were covered with the sporulating conidiophores. The leaf disease severity was 100%.

- Usually the plant tissue was covered first with white conidiophores and over time the leaf became yellowish and necrotic. The occurrence of the sporualting conidiophores decreased over time.
- Disease symptoms were often observed on true leaves of swede. But the leaf disease severity was low. Conidiophores and symptoms on true leaves were seldom observed on the other plant species.

The results of the experiment are confirming the theory of the narrow species delimitation within the family Peronosporaceae (Göker et al., 2009, Choi et al., 2003). The evidence of the host specificity pattern of the different members of the Brassicaceae family isolates supports the use of the host range as a taxonomic marker proposed by Gaümann, 1918, 1923; Göker et al., 2004; Choi et al., 2003.

Göker et al., 2004 concluded that some host species are susceptible to several *Hyaloperonospora* species. The *Peronospora* has been also found capable of infecting species within the other host genera (Gaümann,1926; Gustavsson, 1959). The results of the isolates obtained from swede and broccoli can confirm that aggressiveness of the pathogen *Hyaloperonospora (Peronospora)* spp. is influenced by host plant species, but also by host plant taxon or genus within the Brassicaceae family.

SURVIVAL OF CONIDIA OF *Hyaloperonospora (Peronospora)* spp. IN AIR

INTRODUCTION

Conidia of *Hyaloperonospora (Peronospora)* spp. are spread by wind and water splash. Spread for long distance is possible, but the survival of spores is not well documented (Nordskog, Hermansen, 2008). The solar radiation together with relative humidity and temperature is affecting the spore survival (Le et al., 2009). The experiment was carried out to determinate an influence of solar radiation on germination of spores of *Hyaloperonospora* (*Peronospora*) spp. over time of the exposure. The spores were harvested from the host plant swede and wild rocket (*Diplotaxis tenuifolia*).

METHODS AND MATERIAL

Cultivating Cultures of Wild Rocket and Swede

Seedlings of two species, wild rocket (*Diplotaxis tenuifolia*) and swede were cultivated. Seeds were planted in soil (P-jord) contained in 7 cm x 7 cm x 8 cm plastic pots. Wild rocket cultures consist of 30-40 seedlings in average, swede cultures consist of 15-20 seedlings (20 seeds) in average. Seedlings were grown 8-14 days at 20 to 22°C with a 16 hour photoperiod.

Isolates

Spores of *Hyaloperonospora (Peronospora)* spp. from two isolates, r6 and s7, were used (Table 4). The isolate was infected on seedlings of same species as origin host plant species. The isolate r6 was infected and reinfected on wild rocket cultures and s7 was infected and reinfected on cultures of swede.

Inoculation on Seedlings

The seedlings were incubated for 7-10 days at 20 to 22°C to allow infection. The incubation time was extended and a promotion of asexual sporulation was repeated 2 or 3 times on some of the sets.

Promotion of Asexual Sporulation

The seedlings were incubated over night with 100% RH to promote asexual sporulation. The leaves with the sporulating conidiophores were harvested the day of the experiment.

Swede. The plant disease incidence of swede cultures was rather high (approximately 70%). The characteristic yellowish lesions with the brown edge and the sporulating conidiophores on the surface of the leaf were observed. The leaf disease severity was only around 5%.

D.tenuifolia. The sufficient amount of the leaves with the sporulation was promoted on the sets where the sporulation was done for the first time. Cotyledons were covered by the white conidiophores, the plant disease incidence was around 50% and the leaf disease severity around 60%.

Placement of Spores on Membrane Paper

The spores were transferred on membrane filter papers (47-mm diameter, 0,45-µm pore, mixed cellulose esters membrane filters). The transfer was done by a contact of the side of the leaf with sporulating conidiophores and the membrane papers (Le et al., 2009). One or two leaves of swede were used for the spore transfer per one membrane paper. Several cotyledon leaves of *D. tenuifolia* were used for the spores transfer on one membrane paper.

Solar Exposure

The membrane papers with spores were expose to solar radiation up to 9 hours. They were placed on the racks in either direct radiation or shadow (Le et al., 2009). The number of germinating spores were evaluated after exposure of 60 minutes, 120 minutes, 180 minutes, 240 minutes, 300 minutes, 420 minutes and 540 minutes. The control membrane papers were not exposed to solar radiation.

Together 60 Petri dishes with harvested spores of *Hyaloperonospora (Peronospora)* spp. were prepared. Fifteen dishes contained spores from the host plant wild rocket (one dish was a control sample, seven dishes were exposed to sun and seven dishes were placed in shadow) and 45 dishes contained harvested spores from the host plant

swede (one control, three replicates of seven dishes exposed to sun and seven dishes placed in shadow).

The solar experiment was carried out on 12.8.2009, 19.8.2009 and on 9.9.2009. (Appendix III contains information about weather conditions on 12.8.2009, 19.8.2009 and on 9.9.2009). Only the experiment on 12.8.2009 was evaluated.

Treatment and Incubation of Exposed Spores

The membrane papers were placed in a moist chamber for 15 minutes and spores were transferred to a Petri dish with water agar (1,5%) for 24 hours (Le et al., 2009).

Assessment of Viability of Spores

The spore germination on each Petri dish was suppressed by applying lacto fuschin after incubation for 24 hours. The viability of the spores on a particular dish was assessed by determination of a percentage of the germinating spores. The spores were counted with aid of a microscope. The upper limit of the counted spores on a dish was 300 spores. The dishes with spores were counted twice. First time, germinating spores were considered the spores with all sizes of a germ tube or a significant formation of a germ tube. Second time, only spores with a long germ tube were considered as germinating spores.

RESULTS

First counting

Solar experiment on 12.8.2009:

Table 10. The first counting of the solar experiment on 12.8.2009. Number of observed spores on a particular dish.

TIME	0 minute	60 minutes	120 minutes	180 minutes	240 minutes	300 minutes	420 minutes	540 minutes
SH								
D.t.	332	319	326	353	320	-	220	357
Swe 1	58	160	406	213	124	120	371	104
Swe 2	316	120	414	99	325	-	71	171
Swe 3	168	161	750	112	80	223	323	-
SUN								
D.t.	332	323	313	322	493	340	378	148
Swe 1	58	93	385	63	79	40	139	224
Swe 2	316	33	447	283	202	27	77	29
Swe 3	168	76	48	347	124	37	34	5

SH...Treatment of the spores, the spores placed under shelter
SUN...Treatment of the spores, the spores exposed to direct solar radiation
D.t....Host plant *Diplotaxis tenuifolia*
Swe...Host plant swede

Table 11. Percentage of germinating spores on each dish of the solar experiment on 12.8.2009. First counting, germinating spores are considered all spores with all sizes of germinating tube or a significant formation of the germ tube.

Time		0 min	60 min	120 min	180 min	240 min	300 min	420 min	540 min
Host plant	Treatment								
D. tenuifolia	Shelter	56	67	29	76	46	-	21	27
D. tenuifolia	Sun	56	86	65	57	37	60	21	30
swede 1	Shelter	43	25	31	33	71	43	54	37
swede 2	Shelter	64	49	34	31	62	-	46	46
swede 3	Shelter	42	78	39	31	59	75	74	-
swede 1	Sun	43	66	10	16	49	25	17	28
swede 2	Sun	64	27	14	54	36	63	21	14
swede 3	Sun	42	13	21	48	42	32	44	60

Figure 11.1 shows the mean curve of percentage of germinating spores versus time. The germination dropped at 120 minutes and the curve seems to have a second climax at 240 minutes. After 240 minutes the mean of germinating spores per dish seems to decrease again. It can be used a linear fitted line to see a trend of the curve. There is a certain negative trend in a germination of the spores over time (Figure 11.2.). Also correlation coefficient of the percentage of germinating spores and time is -0,493 and P-Value = 0,215 (Appendix IV, Output from MINITAB). The probability that H_0 (the samples come from uncorrelated population) is true, seems to be rather high. There is an weak correlation between variables. The negative correlation coefficient indicates negative proportionality.

The box plot (Figure 11.3.) is graphically showing the distribution of data about percentage of germinating spores over time. There is big variation in our obtained data. Standard deviation of percentage of germinating spores was high (Appendix IV, Output from MINITAB).

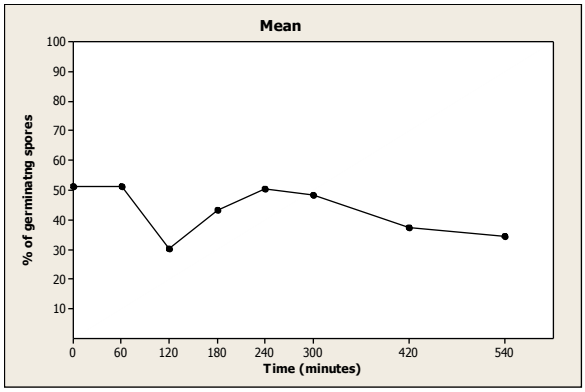


Figure 11.1. Percentage of germinating spores over time. Mean of percentage of germinating spores of *Hyaloperonospora* spp. exposed to solar radiation on 12.8.2009.

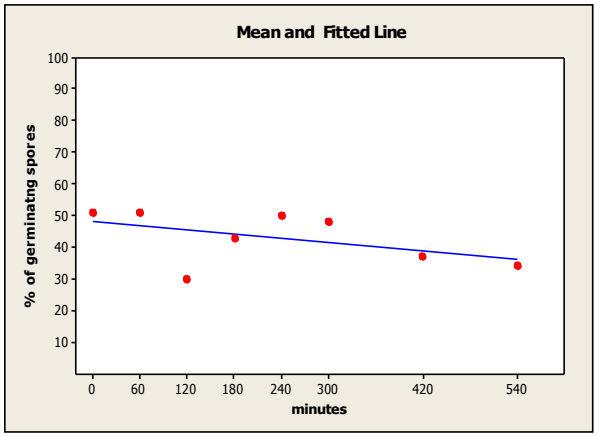


Figure 11.2. Scatter plot with regression. Percentage of germinating spores over time. Mean of germinating spores of *Hyaloperonospora* spp. exposed to solar radiation on 12.8.2009.

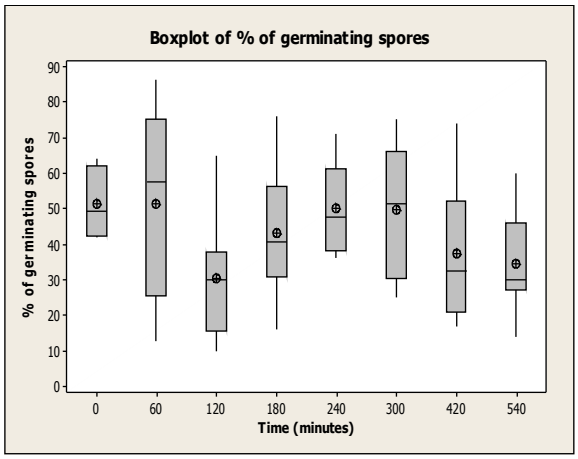


Figure 11.3. Box plot. Distribution of data of percentage of germinating spores over time. Spores were exposed to solar radiation on 12.8.2009. The symbol in the box plot is a mean of germinating spores of *Hyaloperonospora* spp.

Data from the solar experiment can also be analysed with regards to two factors. The first factor is an influence of the treatment of experimental membrane papers (sun versus shadow). The second factor is a different host plant (swede versus *D.tenuifolia*).

The percentage of germinating spores versus time from membrane papers exposed to direct **sun** and percentage of germinating spores versus time from membrane papers placed in **shadow** are plotted on Figure 12.1**.** The curve of percentage of germinating spores exposed to shadow has first climax at 60 minutes. The curve drops after 120 minutes. Second climax in the germination of the spores is after 240 minutes. The curve of spores exposed to sun is more smooth and after 60 minutes the germination of spores is decreasing. The trend line (the linear trend line was again used) of the curve of spore exposed to sun is more decreasing over time.
Both, the correlation coefficient of the percentage of germinating spores exposed to **sun** and time and the correlation coefficient of the percentage of germinating spores exposed to radiation in **shadow** and time indicate negative proportionality. (Appendix IV, Output from MINITAB). The association between viability of spores exposed to **sun** and time seems to be stronger then the relationship between viability of spores placed in **shadow** and time.

Data from two treatments (shelter, sun) were analysed by using statistical methods to compare similarity of means. The 2-sample T-test can be used, if the data are normally distributed. Data are normally distributed (Appendix IV, Output from MINITAB). The P-Value is bigger than 0.05 hence the data provide no strong evidence against the null hypothesis ("H_0 = the sample came from population with same mean"). P-Value was 0,08803 for data. The test has not indicated significant effect on treatments. But the 2-sample T-test has indicated that there were effects of treatments when the two highest and the two lowest values were eliminated. The P-value was 0.04985.

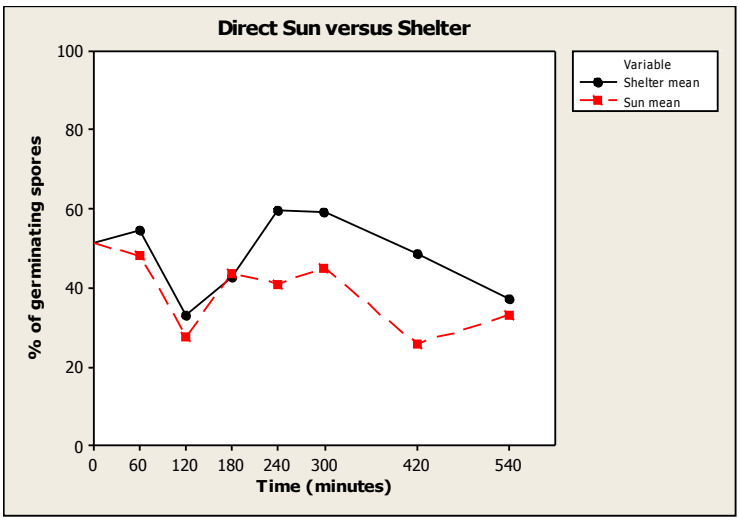


Figure 12.1. Percentage of germinating spores over time exposed to solar radiation on 12.8.2009. Mean of germinating spores exposed to direct sun versus mean of germinating spores influenced by radiation in shadow.

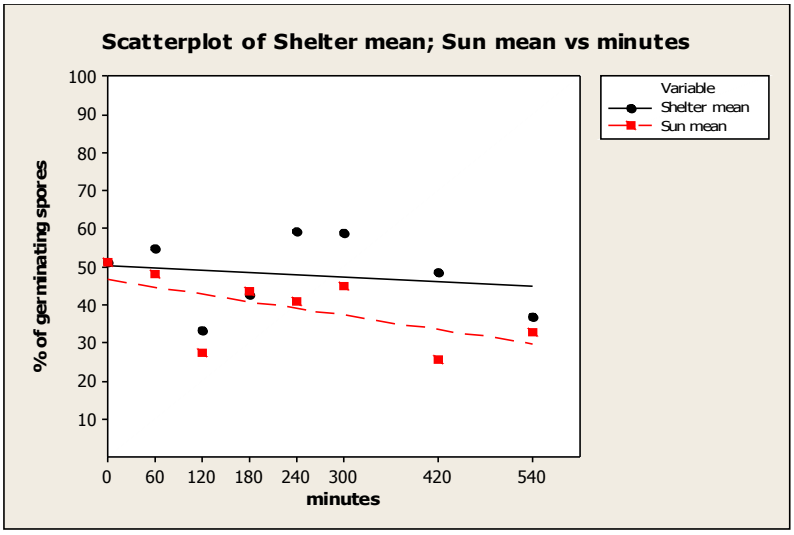


Figure 12.2. Scatter plot with regression. Mean of germinating spores exposed to direct sun versus mean of germinating spores influenced by radiation in shadow. The spores were exposed to solar radiation on 12.8.2009.

The percentage of germinating spores from *D.tenuifolia* versus time and percentage of germinating spores from swede versus time is plotted on Figure 13.1. The curve of *D.tenuifolia* vary at the beginning, but after a climax at 60 minutes the percentage is decreasing. The curve of spores from swede has a climax at 240 minutes.

Data from different species (*D.tenuifolia*, swede) were analysed by using statistical methods to compare similarity of means. The 2-sample T-test can be used, if the data are normally distributed. Data are normally distributed (Appendix IV, Output from MINITAB) and data were transformed to be independent of time. The P-Value is bigger than 0.05 hence the data provide no strong evidence against the null hypothesis ("H0 = the sample came from population with same mean"). P-Value was 0.2346 for data dependent on the time and the P-value was 0.1726 for the transformed data. The 2-sample T-test has indicated that there was no significant effect of species of the host plant.

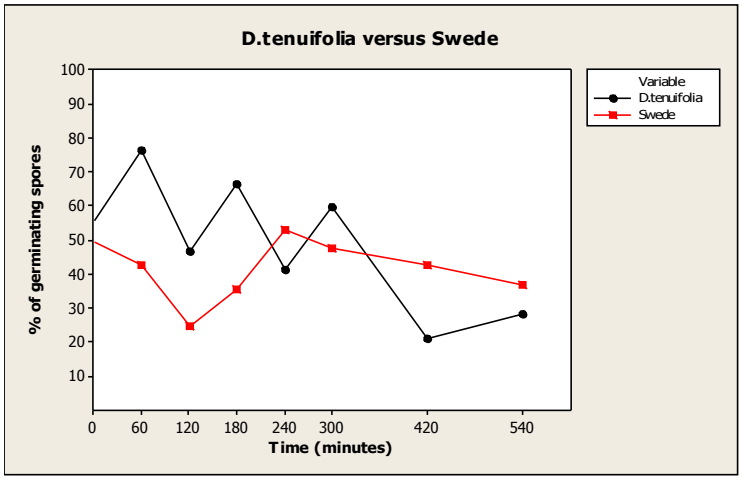


Figure 13.1. Percentage of germinating spores over time. Mean of germinating spores from *Hyaloperonospora(Peronospora)* spp. on *D.tenuifolia* versus mean of the germinating spores from *Hyaloperonospora(Peronospora)* spp. on swede.

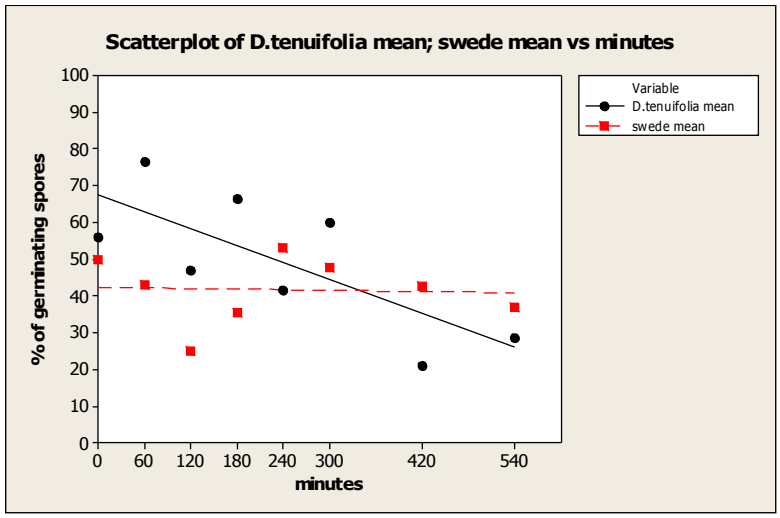


Figure 13.2. Scatter plot with regression. Mean of germinating spores from *H.parasitica* on *D.tenuifolia* versus mean of germinating spores from *Hyaloperonospora* spp. on swede.

Second counting

During the second counting germinating spores are considered those spores which have a long significant germination tube. Obtained data were very inconsistent and it was difficult to undertake some statistical analysis (Table 12, Table 13).

Table 12. The second counting of the solar experiment. Number of observed spores on each experimental dish from the experiment on 12.8.2009.

TIME	0 minute	60 minutes	120 minutes	180 minutes	240 minutes	300 minutes	420 minutes	540 minutes
SH								
D.t.	320	310	300	192	208	-	32	149
Swe 1	3	97	300	13	11	64	219	3
Swe 2	863	98	304	13	127	18	36	17
Swe 3	0	0	151	21	5	5	12	-
SUN								
D.t.	320	0	125	300	145	300	167	5
Swe 1	3	172	330	10	1	0	19	120
Swe 2	863	34	324	142	128	0	5	0
Swe 3	0	64	52	415	3	0	1	7

SH...Treatment of the spores, spores placed under shelter
SUN...Treatment of the spores, spores exposed to direct solar radiation
D.t....Host plant of the oomycota, *Diplotaxis tenuifolia*
Swe...Host plant of the oomycota, swede

Table 13. Percentage of the germinating spores on each dish from the second counting of the solar experiment on 12.8.2009. Second counting, only spore with long significant germ tube is considered as a germinating spore.

Time		0 min	60 min	120 min	180 min	240 min	300 min	420 min	540 min
Host plant	Treatment								
D. tenuifolia	Shelter	13,75	51,29	0,33	6,25	7,21	0	0	0
D. tenuifolia	Sun	0	0	5,33	0	0	6,25	66,21	0
swede 1	Shelter	6,26	0	0,66	7,69	1,57	0	41,67	0
swede 2	Shelter	0		56,29	0	0	0	16,67	0
swede 3	Shelter	13,75	0	1,6	0,67	0	0,33	0	0
swede 1	Sun	0	16,28	16,36	0	0	0	0	0
swede 2	Sun	6,26	11,76	1,23	25,35	0	0	0	0
swede 3	Sun	0	0	5,77	36,63	0	0	0	0

DISCUSSION

The solar experiment was depending on the coordination between weather conditions and available sufficient amount of plant material with sporulating conidiophores. However the experiment was done 3 times, only the experiment on 12.8.2009 was evaluated.

The reasons for the choice were following:

- On 12.8.2009 were favourable weather conditions (Appendix V, meteorological data as temperature, global radiation or relative humidity from VIPS database http://www.vips-landbruk.no/).
- It was possible to harvest plant material for 15 samples with spores of *Hyaloperonospora (Peronospora) s*pp. from *D. tenuifolia* and for 45 samples with spores of *Hyaloperonospora* spp. from swede.

- The germination of spores were counted twice. The first counting and the second counting was done on all experimental dishes from experiment on 12.8.2009.

The obtained data from the experiment did not give significant results. The results are not in contradiction with the hypothesis that the solar radiation together with relative humidity and temperature affects the spore survival (Le et al., 2009). In general, the viability of the spores was slightly decreasing over the time of their exposure. Results indicated that two different treatments (sun, shadow) had influence on the percentage of the germination spores over time and that different host plant species (*D. tenuifolia*, swede) had no influence on the percentage of the germination spores over time. The high standard deviation of the data, especially up to 300 minutes, probably would be reduced if more data would have been received to perform an analysis with.

On the other hand there were rather few data to undertake a statistical analysis. There were exposed only one replicate of the membrane paper with spores from the host plant *D. tenuifolia* and three replicates of spores from the host plant swede on each interval (60 minutes, 120 minutes, 180 minutes, 240 minutes, 300 minutes, 420 minutes and 540 minutes) and two different treatments (sun or shadow). Unfortunately the statistical presumption that three replicates of swede give better results than one replicate of *D. tenuifolia* is not applicable. There was an influence of poor quality of sporulating plant material from swede. The experimental dishes often contained very few spores. It was difficult to find any spores.

The right procedure of counting (the determination of the germ tube) and good spore material are essential factors for good results of the experiment.

The spores were exposed to solar radiation for time intervals from 1 to 9 hours. According Smith et al., 1988, conidia can survive a few days on leaves under typical field conditions, but at low temperature and in the absence of moist can survive more than 100 days. If the viability of spores is decreasing after several days, the influence of solar radiation on the viability of spores can be negligible within 9 hours. The results has shown no strong relationship between time of exposure and the viability of the spores.

Data from the second counting were evaluated later and were reduced by contamination of the plates with mould. To undertake statistical analysis was not giving reasonable results. But the high percentage of germinating spores at 420 minutes exposure to direct sun indicates that there is a low influence on the viability of the spores within 7 hours of exposure to direct sun.

SURVIVAL OF OVERWINTERING STRUCTURES OF *Hyaloperonospora (Peronospora)* spp. IN SOIL

INTRODUCTION

The downy mildew infection caused by *Hyaloperonospora (Peronospora)* spp. on leaves occurs from the airborne conidia, but the primary infection probably originates from oospores and may lead to systematic infection. The infection of seedlings from oospores has not been verified under natural conditions (Rimmer et al., 2007, McMeekin, 1960).

The soil experiment was carried out to survey whether the overwintering structures of *Hyaloperonospora (Peronospora)* spp. can cause infection on the seedlings. The occurrence of the infection on planted seedlings of wild rocket (*Diplotaxis tenuifolia*) on the sampled soil can indicate the presence of the overwintering structures of *Hyaloperonospora (Peronospora)* spp. causing the primary infection.

METHODS AND MATERIAL

Collecting of Soil Samples

Nine soil samples (N1a, N1b, N2, N3, N4a, N4b, N4c, N5 and control sample) were collected from the farm where rocket salad was grown and where downy mildew caused by *Hyaloperonospora* (*Peronospora)* spp. was observed. The samples were collected on 8.6.2009.

Information about the place of the sampling

Place: Thor Graff Farm, Hurumveien 13 A, 3440 Røyken

Region: Røyken Kommune

Field characterization: Vegetable production

Soil: Albeluvisol, Cambisols [2)]

Geology: Thor Graff farm seems to be in close distance to the W of a prominent N-S directed normal fault. This normal fault is of Permian age, but was a little rejuvenated in Cenozoic. This fault separates the western block (with Thor Graff land) from the eastern block.

The western block consists, most likely of old basement rocks, Proterozoic gneissic granites with relatively high content of alkali, which may be explained by metasomatic or migmatite processes.

The locality may have a thin cover of weathering product (eluvia, deluvia, with relicts of Cenozoic and Recent sands and argillites). All patchy and having zero to several metres of thickness. These weathering products and soils rest on the Proterozoic crystalline basement are influenced by the compositions of this basement rocks (possibly gneissic granites with relatively high contents of alkali; relatively pure groundwater with slightly increased content of iron, and Al-hydroxides) (Gleditsch, 1952; Olesen et al., 2008; Ramberg et al., 2008; Nyborg and Solbakken, 2003; Arnoldussen, 2005).

Information about samples

The samples was taken from five fields where *D. tenuifolia* was grown last year. The other crops grown in the vicinity of the fields with lettuce and spinach. The field number 5 is not closely located to the fields number 1-4 (Table 14).

Table 14. The information about the soil samples collected from the farm with occurrence of downy mildew disease

Field	Sample	Method of sampling	Amount	Previous soil treatment
Field 1	N1a	Two rows of the field were sampled. Soil were collected on the edge of the row and from the middle of the row in each approximately 5 m.	9 litres	Sprayed with pesticides
	N1b	Two rows of the field were sampled. Soil were collected on the edge of the row and from the middle of the row in each approximately 5 m.	9 litres	Sprayed with pesticides
Field 2	N2	One row of the field number 2 was sampled. Soil were collected on the edge of the row and from the middle of the row approximately in each 5 m.	9 litres	Sprayed with pesticides
Field 3	N3	One row of the field number 3 was sampled. Soil were collected on the edge of the row and from the middle of the row approximately in each 5 m.	8 litres	Sprayed with pesticides
Field 4	N4a	Method of the sampling: Two rows of the field number 4 was sampled. Soil were collected on the edge of the row and from the middle of the row in each 5 m from both sides.	8 litres	Sprayed with pesticides
	N4b	One row of the field number 4 was sampled. Soil were collected on the edge of the row and from the middle of the row in each 5 m.	10 litres	Probably not sprayed with pesticides
	N4c	The lower part of the row. Soil were collected on the edge of the row and from the middle of the row in each 1,5 m from both side.	5 litres	No sprayed with pesticides or very late in the season
Field 5	N5	Two rows of the field were sampled. Soil were collected on the edge of the row and from the middle of the row in each 5 m.	5 litres	Sprayed with pesticides
	Control	Sampled randomly	7 litres	Not used for vegetable production for many years

Preparation of Soil Samples

All soil sampled from the field were measured and sieved. (The sieve about 0,4 mm x 0,4 mm were used to homogenize the soil structure and prepare the soil for the experimental use).

Cultivating of Rocket Salad Seedlings

Seeds of *D. tenuifolia* were planted in sampled soil contained in three plastic trays, 50 cm x 30 cm x 7 cm. Standard planting soil (P-jord) was used to complete the trays. The geotexitilie was used to prevent mixing of the sampled soil and P-jord soil. Seeds were sowed in rows with distance 12-15 cm with 0,09 gram seeds per one sowed row in average. In 21 trays (N1a, N1b, N2, N3, N4a, N4b, control sample P-jord) were sowed about 3 rows and in 9 trays (N4c, N5, control sample with soil from the field) were sowed about 2 rows. Two control samples were planted. One control sample in standard planting soil (P-jord) and other control sample in soil collected from the field.

All 30 trays were randomly placed (Table 15) on a sunny place in the experimental fields of the UMB (Norwegian University of Life Sciences) area in the vicinity of the Ås church (Figure 14). The trays were watered in regular intervals.

Figure 14. The experimental trays placed in the in the vicinity of the Ås church. Photo: J. Johansen Hladilová

Table 15. The schema of the placement of the experimental trays from the soil experiment.

Sample 4b III.	Sample 1a III.	Sample 4c III.	Sample 1b I.	Sample 3 III.	Sample 1b III.
Sample 1a II.	Sample 4c I.	Sample 2 III.	Control Sample P-jord III.	Sample 3 I.	Sample 4a I.
Sample 1b II.	Sample 1a I.	Sample 4a III.	Sample 2 I.	Sample 5 I.	Sample 4c II.
Sample 5 II.	Sample 3 II.	Control Sample field III.	Control Sample field I.	Sample 2 II.	Control Sample P-jord I.
Sample 4a II.	Sample 4b II.	Control Sample P-jord II.	Sample 4b I.	Control Sample field II.	Sample 5 III.

Evaluation

The seedlings of *D. tenuifolia* on 30 trays were regularly observed and checked for the symptoms of downy mildew.

RESULTS

No symptoms of downy mildew caused by *Hyaloperonospora* (*Peronospora)* spp. were observed. The germination of seeds and growth of the seedlings was poor in the trays with the sampled soil. Table 16 is showing the number of plants after first period of growing (3-4 weeks) on each tray. The average number of the plants per one row was 7 plants on the trays with sampled soil from the fields. The average number of the plants per one row was 17 plants on the control trays with sampled soil. The average number of the plants per one row was 34 plants on the control trays with garden soil (P-jord), Figure 15(A,B).

A

B

Figure 15. (A) The control tray with garden soil (P-jord), 3-4 weeks after sowing of seeds of *D.tenuifolia* (B) The control tray containing soil from field, 3-4 weeks after sowing of seeds of *D.tenuifolia*.

After 7-8 weeks the plants of *D.tenuifolia* died and weed plants dominated on the experimental trays. White rust caused by *Albugo candia* on weed plants *Capsula bursa pastoris* were observed.

Table 16. The number of plants after first period of growing (3-4 weeks) on each sowed row on each experimental trays. In 21 trays (soil sample N1a, N1b, N2, N3, N4a, N4b, control sample P-jord) were sowed seeds of *D.tenuifolia* in 3 rows and in 9 trays (soil sample N4c, N5, control sample with soil from the field) seeds of *D.tenuifolia* in 2 rows.

Sample 4b III.			Sample 1a III.			Sample 4c III.		Sample 1b I.			Sample 3 III.			Sample 1b III.			
13	5	12	5	7	17	4	0	6	6	7	12	4	8	7	14	10	
Sample 1a II.			Sample 4c I.			Sample 2 III.		Control Sample P-jord III.			Sample 3 I.			Sample 4a I.			
6	9	9	2	10		7	14	5	32	40	35	7	10	11	3	1	6
Sample 1b II.			Sample 1a I.			Sample 4a III.		Sample 2 I.			Sample 5 I.			Sample 4c II.			
5	8	6	5	0	5	7	5	0	3	10	6	4	8	2	11		
Sample 5 II.			Sample 3 II.			Control Sample field III.			Control Sample field I.			Sample 2 II.			Control Sample P-jord I.		
9	2		8	3	13	5	23	16	31	20		6	6	4	27	34	29
Sample 4a II.			Sample 4b II.			Control Sample P-jord II.			Sample 4b I.			Control Sample field II.			Sample 5 III.		
1	11	6	6	1	2	29	45	37	11	12	11	20	19		1	2	

DISCUSSION

The experiment did not give sufficient results. Growing conditions were not favourable for the growth of wild rocket plants. Disease was not observed on *D.tenuifolia*.

Revision of Methods

Outdoor placement of the experiment. The experimental trays were placed outdoor and they were exposed to natural weather conditions. The intention was to reach natural conditions for the development of the pathogen.

The outdoor placement were not successful. Outdoor weather condition such as heavy rain (Appendix III, climate data from VIPS database http://www.vips-landbruk.no/) destroyed the structure of the soil and plants were not able to grow.

Suggestion for further consideration:

1. Placing the trays in greenhouse.
2. To settle an experiment field on a farm fields with previous occurrence of downy mildew on wild rocket.

Revision of Materials

Soil. The plants at the trays with garden soil were growing better in the first period (3-4 weeks after sowing). The average number of plants was higher on the control sample with garden soil (P-jord) and lower on the trays with the sampled soil. Heavy rainy weather destroyed the structure of the soil and experimental plants were not able to grow in the second period of the growth (4-7 weeks after sowing) .

Size of the trays. The size of the plastic trays (50 cm x 30 cm x 7) cm was probably not suitable. There was limited how much soil from the farm field could be taken. Deeper and smaller trays were more suitable to the amount of the sampled soil. Reducing the number of sowed plants could be also considered.

White rust can occur in association with downy mildew (Rimmer et al., 2007). White rust caused by *Albugo candia* was observed on weed plants *Capsula bursa pastoris* in the experimental trays.

COMPREHENSIVE DISCUSSION AND CONCLUSION

Three experiments were done to survey the pathogen *Hyaloperonospora* spp. on rocket salad crops and other members from the Brassicaceae family. The main focus was on the host range of *Hyaloperonospora* spp., the host susceptibility experiment.

However until recent past the causal agent of downy mildew on Brassicaceae was represented by merging species *Hyaloperonospora parasitica* (Pers.) Constant. (Constantinescu and Fatehi, 2002), the variation of host range within this species was confirmed (Gaümann, 1918, 1923; Sherriff and Lucas, 1990, Silué et al., 1996, Rimmer et al., 2007). The results from the host specificity experiment are in accordance with the again considered narrow species delimitation within the family Peronosporaceae (Göker et al., 2009, Choi et al., 2003). The host specificity experiment has shown that the pathogen from the host plant wild rocket (*D.tenuifolia*) were almost restricted to the same plant species as the species of the origin. Within the genus *Brassica* the pathogen was also most aggressive by its origin (Rimmer et al., 2007), but the pathogen were capable to infect other brassicas. The results lead to a conclusion that the aggressiveness of the pathogen H*yaloperonospora* (*Peronospora*) spp. is influenced by the host plant species (Göker et al., 2004), but also by the host plant taxon or genus within the Brassicaceae family.

Seedlings of *E. sativa* have shown to be resistant against the pathogen isolated from the host plant *D. tenuifolia* and also against the pathogen isolated from the host plant broccoli and swede. The results indicate that *E.sativa* can be recommended as an alternative rocket salad species for producers of rocket salad crops with problems of downy mildew. The further research of downy mildew on *E.sativa* can complete this study.

The results of the solar experiment with spores of *Hyaloperonospora* spp. indicate a moderate negative trend in the viability of spores exposed to solar radiation. A longer time interval of solar exposure and more replicates are recommended for further investigation.

The third experiment has not shown any sufficient results. A revision of methods and materials shall be done to continue the research in this area.

The thesis broadened current knowledge about the oomycota *Hyaloperonospora (Peronospora)* spp. causing the downy mildew disease on rocket salad crops. The results have confirmed the host specificity pattern of the pathogen by its origin (Rimmer et al.; 2007, Sherriff and Lucas, 1990). The thesis also contributed to the discussion about taxonomy. The host range is considered as a taxonomic marker (Gaümann, 1918, 1923, Göker et al., 2004, Choi et al., 2003).

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ONLINE RESOURCES:

1) Index Fungorum, web database of the fungal nomenclature (12.11.2010), www.indexfungorum.org

2) Norsk institutt for skog og landskap, Kart og statistikk, Skog og landskap undersøker og kartlegger Norges arealressurser (12.11.2010), http://www.skogoglandskap.no/temaer/jordsmonn

Appendices

Appendix I: The important Norwegian plants susceptible for downy mildew caused by *Hyaloperonospora (Perenospora) spp*

Table 1: Important Norwegian plants susceptible for downy mildew caused by *Hyaloperonospora (Perenospora) spp.* (Modified from Ramsfjell, 1960; Jørstad, 1964 referred in Nordskog, Hermansen, 2008):

Plant	
Latin name	**English name**
Vegetables	
Brassica napus subsp. *rapifera*	swedes
Brassica oleracea var. *capitata*	cabbage
Brassica oleracea var. *botrytis*	cauliflower
Brassica oleracea var. *sabellica*	kale
Brassica oleracea var. *italica*	broccoli
Brassica napus subsp. *napus*	oilseed rape
Eruca sativa	cultivated rocket salad
Diplotaxis tenuifolia	wild rocket
Other plant and Weed	
Allaria petiolata	garlic mustar
Arabidopsis arenosa	sand rock-cress
Arabidopsis petraea	northern rock-cress
Arabidopsis thaliana	thale cress
Arabis hirsuta	hairy rock-cress
Barbarea stricta	winter cress, yellow rocket
Barbarea vulgaris	bittercress, herb barbara, rocketcress, yellow rocketcress, wound rocket
Berteroa incana	hoary alyssum
Brassica rapa ssp. Campestris	turnip rape
Bunias orientalis	hill mustard, turkish rocket

Capsella bursa-pastoris	shepherd's-purse
Cardamine amara	large bittercress
Cardamine bulbifera	coralroot bittercress
Cardamine flexuosa	woodland bittercress
Cardamine impatiens	narrowleaf bittercress
Cardamine pratensis	cuckoo flower, lady's smock
Descurania sophia	flixweed
Draba glabella	smooth draba
Draba verna	shadflower, nailwort
Erysimum cheiranthoides	treacle-mustard
Erysimum strictum	tall wormseed wallflower
Hesperis matronalis	dame's rocket
Lobularia maritima	sweet alyssum
Matthiola incana var. annua	tenweeks stock
Noccaea caerulescens	alpine penny cress
Raphanus raphanistrum ssp. raphanistrum	wild radish
Rorippa islandica	northern marsh yellowcress
Sisymbrium altissimum	tumble mustard
Sisymbrium officinale	hedge
Thlaspi arvense	field penny-cress
Turritis glabra (Arbis glabra)	tower mustard

Appendix II:

Table 1. The information about the evaluation of 53 sets for the main indicator from the host susceptibility experiment. The main indicator is **positive** or **negative** occurrence of the conidiophores of *Hyaloperonospora (Peronospora) spp* on the experimental seedlings.

	Plant species					
	D.tenuifolia	*E.sativa*	broccoli	cauliflower	swede	oilseed rape
Sets infected with isolates from the host plant *D. tenuifolia*						
Positive	24	0	0	0	0	0
Negative	1	23	25	25	24	18
Number of cultiveted cultures with seedlings	25	23	25	25	24	18
Sets infected with isolates from the host plant broccoli						
Positive	0	0	17	6	4	2
Negative	17	17	0	11	13	11
Number of cultiveted cultures with seedlings	17	17	17	17	17	13
Sets infected with isolates from the host plant swede						
Positive	0	0	9	7	11	0
Negative	11	11	2	4	0	9
Number of cultiveted cultures with seedlings	11	11	11	11	11	9

Table 2. The information about the evaluation of 53 sets for the main indicator from the host susceptibility experiment. The main indicator is positive (number 1) or negative (number 2) occurrence of the conidiophores of *Hyaloperonospora (Peronospora) spp* on the experimental seedlings.

SET	*D.tenuifolia*	E.sativa	broccoli	cauliflower	swede	oilseed rape
R1	1	0	0	0	missing value	missing value
S1	0	0	1	1	missing value	missing value
B4	0	0	1	0	0	missing value
B5	0	0	1	0	0	missing value
B6	0	0	1	0	0	missing value
B7	0	0	1	0	0	missing value
R6 a	1	0	0	0	0	0
R6 b	1	0	0	0	0	0
R6 c	1	0	0	0	0	0
R8 a	1	0	0	0	1	0
R8 b	1	0	0	0	0	0
R8 c	1	0	0	0	0	0
R17 a	1	0	0	0	0	0
R17 b	1	0	0	0	0	0
R17 c	1	0	0	0	0	0
R16 a	1	0	0	0	0	0
R16 b	1	0	0	0	0	0
R16 c	1	0	0	0	0	0
R15 a	1	0	0	0	0	0
R15 b	1	0	0	0	0	0
R15 c	1	0	0	0	0	0
R18 a	1	0	0	0	0	0
R18 b	1	0	0	0	0	0
R18 c	1	0	0	0	0	0
S7 a	0	0	1	1	1	0
S7 b	0	0	1	1	1	0
S7 c	0	0	1	1	1	0
S10 a	0	0	0	1	1	0
S10 b	0	0	1	0	1	0
S10 c	0	0	1	0	1	0
B13 a	0	0	1	1	0	1
B13 b	0	0	1	0	0	0
B13 c	0	0	1	0	1	0
B11 a	0	0	1	1	1	0
B11 b	0	0	1	1	0	0
B11 c	0	0	1	0	0	0
B22 a	0	0	1	1	1	0
B22 b	0	0	1	1	0	1
B22 c	0	0	1	1	1	0
R 6.1 a	1	missing value	0	0	0	missing value
R 6.1 b	1	missing value	0	0	0	missing value
S7.1 a	0	0	1	1	1	missing value
S7.1 b	0	0	1	1	1	missing value
R8.1 a	1	0	0	0	0	missing value
R8.1 b	1	0	0	0	0	missing value
R9.1 a	1	0	0	0	0	missing value
R9.1 b	0	0	0	0	0	missing value
S10.1 a	0	0	1	0	1	0
S10.1b	0	0	0	0	1	0
B11.1a	0	0	1	0	0	0
B11.1b	0	0	1	0	0	0
B13.1a	0	0	1	0	0	0
B13.1b	0	0	1	0	0	0

Appendix III: Meteorological data on 12.8.2009, 19.8.2009 and 9.9.2009. Output from VIPS database (http://www.vips-landbruk.no/)

Table 1. Temperature (mean) in 2 meters (°C) , data from hourly measurement from 10 a.m. til 9 p.m. (the relevant time of the solar experiment).

Time of the measurements	12.8.2009	19.8.2009	9.9.2009
10:00:00	16.3	15.2	18.0
11:00:00	17.0	16.7	18.5
12:00:00	17.9	17.3	19.4
13:00:00	18.7	17.4	20.0
14:00:00	19.5	17.0	20.3
15:00:00	19.8	16.7	20.5
16:00:00	20.3	16.2	20.5
17:00:00	20.1	16.0	20.0
18:00:00	18.5	15.5	19.0
19:00:00	16.7	15.1	16.7
20:00:00	12.3	15.0	12.6
21:00:00	11.4	14.6	13.6

Table 2. Global radiation (W m^{-2}), data from hourly measurement from 10 a.m. til 9 p.m.(the relevant time of the solar experiment).

Time of the measurements	12.8.2009	19.8.2009	9.9.2009
10:00:00	504.8	459.4	433.4
11:00:00	576.7	475.2	514.1
12:00:00	717.0	346.3	569.6
13:00:00	714.0	331.2	574.0
14:00:00	661.3	229.0	552.7
15:00:00	631.7	199.0	471.1
16:00:00	533.6	98.4	387.9
17:00:00	420.1	96.2	263.3
18:00:00	186.8	26.5	130.0
19:00:00	22.4	18.9	23.6
20:00:00	27.2	7.8	0
21:00:00	1.2	0	0

Table 3. Relative humidity(%), data from hourly measurement from 10 a.m. til 9 p.m. (the relevant time of the solar experiment).

Time of the measurements	12.8.2009	19.8.2009	9.9.2009
10:00:00	62.5	65.8	49.9
11:00:00	56.3	63.5	46.8
12:00:00	53.0	63.9	43.7
13:00:00	50.2	66.9	41.8
14:00:00	45.5	70.5	36.6
15:00:00	44.4	67.2	35.5
16:00:00	41.8	71.3	34.1
17:00:00	46.2	75.8	34.0
18:00:00	57.2	73.6	37.5
19:00:00	68.6	77.3	46.6
20:00:00	84.9	81.9	64.6
21:00:00	92	86.3	57.5

Appendix IV: Out put from Statistics Program MINITAB and R, Solar Experiment, First Counting of Spores of *Hyaloperonospora (Perenospora) spp*

1.DISTRIBUTION OF DATA

Descriptive Statistics: % of germinating spores

Variable	Time (minutes)	N	N*	Mean	StDev	Minimum	Q1	Median
% of germinating spores	0	8	0	51,25	9,84	42,00	42,25	49,50
	60	8	0	51,38	27,08	13,00	25,50	57,50
	120	8	0	30,38	17,15	10,00	15,75	30,00
	180	8	0	43,25	19,08	16,00	31,00	40,50
	240	8	0	50,25	12,60	36,00	38,25	47,50
	300	6	0	49,67	19,45	25,00	30,25	51,50
	420	8	0	37,25	20,55	17,00	21,00	32,50
	540	7	0	34,57	14,88	14,00	27,00	30,00

Variable	Time (minutes)	Q3	Maximum
% of germinating spores	0	62,00	64,00
	60	75,25	86,00
	120	37,75	65,00
	180	56,25	76,00
	240	61,25	71,00
	300	66,00	75,00
	420	52,00	74,00
	540	46,00	60,00

2. CORRELATION

Correlations: % of germinating spores; minutes

Pearson correlation of % of germinating spores and minutes = -0,493
P-Value = 0,215

Correlations: Shelter mean; minutes

Pearson correlation of Shelter mean and minutes = -0,191
P-Value = 0,651

Correlations: Sun mean; minutes

Pearson correlation of Sun mean and minutes = -0,596
P-Value = 0,119

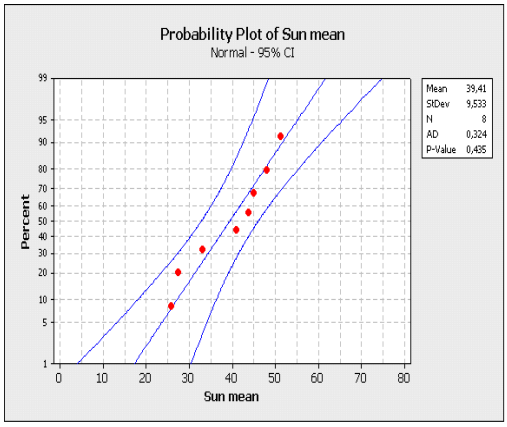
Correlations: D.tenuifolia mean; minutes

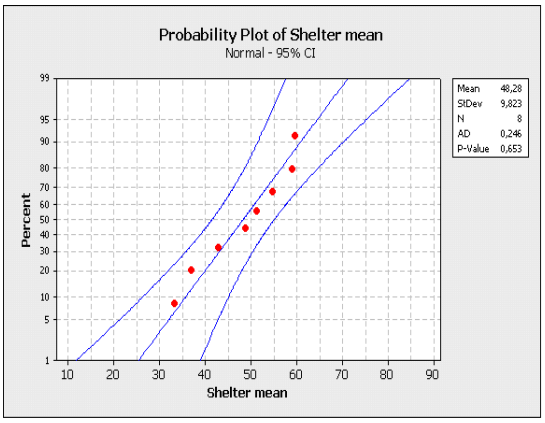
Pearson correlation of D.tenuifolia mean and minutes = -0,743
P-Value = 0,035

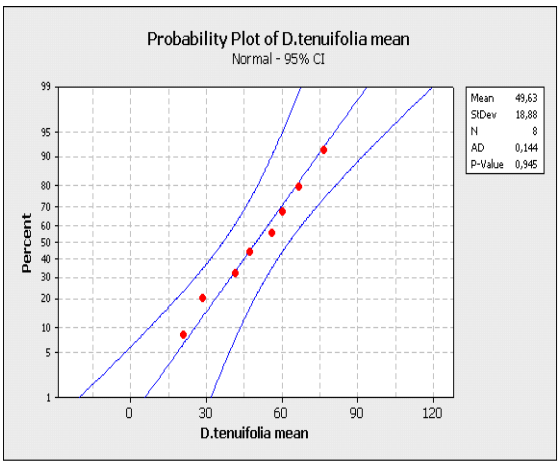
Correlations: swede mean; minutes

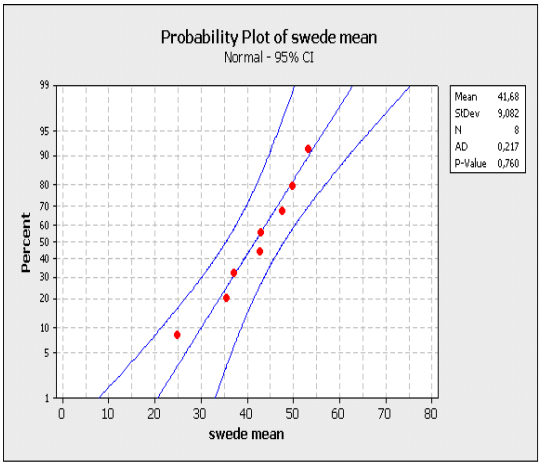
Pearson correlation of swede mean and minutes = -0,055
P-Value = 0,898

3. TESTING WHETHER DATA ARE NORMALLY DISTRIBUTED OR NOT (Sun, Shadow, *D.tenuifolia*, Swede)









P-Value is bigger than 0.05, we cannot reject H0 hypothesis (the samples came from population with normal distribution). Data are normally distributed. We can use the 2-sample T- test for the data.

4. Two- Sample T-Test:

Two-Sample T-Test and CI: Shelter mean; Sun mean

```
Two-sample T for Shelter mean vs Sun mean

              N   Mean  StDev  SE Mean
Shelter mean  8  48,28   9,82      3,5
Sun mean      8  39,41   9,53      3,4


Difference = mu (Shelter mean) - mu (Sun mean)
Estimate for difference:  8,88
95% CI for difference:  (-1,58; 19,33)
T-Test of difference = 0 (vs not =): T-Value = 1,83  P-Value = 0,090  DF = 13
```

Two-Sample T-Test and CI: D.tenuifolia mean; swede mean

```
Two-sample T for D.tenuifolia mean vs swede mean
```

	N	Mean	StDev	SE Mean
D.tenuifolia mean	8	49,6	18,9	6,7
swede mean	8	41,68	9,08	3,2

Difference = mu (D.tenuifolia mean) - mu (swede mean)
Estimate for difference: 7,95
95% CI for difference: (-8,56; 24,45)
T-Test of difference = 0 (vs not =): T-Value = 1,07 P-Value = 0,309 DF = 10

Output from R:
Two-Sample T-Test and CI: D.tenuifolia mean; swede mean , transformed data, (independent data)
t = 1.4453, df = 12.701, p-value = 0.1726
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-3.959984 19.851619

Output from R:
Two-Sample T-Test and CI:data: shelter mean and sun mean
t = 1.8339, df = 13.987, p-value = 0.08803
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
-1.505508 19.255508

Appendix V: Output from VIPS database (http://www.vips-landbruk.no/), Information about Precipitation from May 2009 to September 2009

Table 1: Climate station: Ås, Information about precipitation from May 2009 to September 2009, Interval of measurement: Month.

Measurement Point	Precipitation (mm/ month)
2009-05-01 00:00:00	zero
2009-06-01 00:00:00	29.6
2009-07-01 00:00:00	159.4
2009-08-01 00:00:00	160.2
2009-09-01 00:00:00	28.2
2009-10-01 00:00:00	55.8

Table 2: Climate station: Ås, Information about precipitation (from 20.6. 2009 to 9.8.2009), Interval of measurement: Day.

Measurement Point	Precipitation (mm/day)
2009-06-20 00:00:00	1.2
2009-06-21 00:00:00	0.0
2009-06-22 00:00:00	0.0
2009-06-23 00:00:00	0.0
2009-06-24 00:00:00	0.2
2009-06-25 00:00:00	0.0
2009-06-26 00:00:00	0.0
2009-06-27 00:00:00	0.0
2009-06-28 00:00:00	0.0
2009-06-29 00:00:00	0.0
2009-06-30 00:00:00	0.0
2009-07-01 00:00:00	0.0
2009-07-02 00:00:00	0.0
2009-07-03 00:00:00	9.2
2009-07-04 00:00:00	0.0
2009-07-05 00:00:00	12.4
2009-07-06 00:00:00	7.2
2009-07-07 00:00:00	0.8
2009-07-08 00:00:00	2.2
2009-07-09 00:00:00	8.8
2009-07-10 00:00:00	2.6

Measurement Point	Precipitation (mm/day)
2009-07-11 00:00:00	6.6
2009-07-12 00:00:00	0.0
2009-07-13 00:00:00	6.4
2009-07-14 00:00:00	0.0
2009-07-15 00:00:00	0.8
2009-07-16 00:00:00	8.2
2009-07-17 00:00:00	0.0
2009-07-18 00:00:00	2.8
2009-07-19 00:00:00	13.2
2009-07-20 00:00:00	5.8
2009-07-21 00:00:00	0.2
2009-07-22 00:00:00	2.2
2009-07-23 00:00:00	1.0
2009-07-24 00:00:00	0.0
2009-07-25 00:00:00	4.6
2009-07-26 00:00:00	0.4
2009-07-27 00:00:00	6.4
2009-07-28 00:00:00	4.2
2009-07-29 00:00:00	0.0
2009-07-30 00:00:00	39.4
2009-07-31 00:00:00	14.0
2009-08-01 00:00:00	0.0
2009-08-02 00:00:00	1.0
2009-08-03 00:00:00	26.0
2009-08-04 00:00:00	0.8
2009-08-05 00:00:00	0.0
2009-08-06 00:00:00	0.0
2009-08-07 00:00:00	0.0
2009-08-08 00:00:00	0.6
2009-08-09 00:00:00	5.2

Total measurement of precipitation for Ås climate station for period from 20.06.2009 till 17.7.2009 (28 days)was : **67 mm**.

Total measurement of precipitation for Ås climate station for period from 17.7.2009 till 9.8.2009
(24 days).was : **128 mm**.