Resistance of commercial oilseed rape cultivars to Verticillium stem striping disease

In vitro and greenhouse studies

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Resistance of commercial oilseed rape cultivars to Verticillium stem striping disease. *In vitro* and greenhouse studies

Marknadsförda rapssorters tolerans mot kransmögel orsakat av Verticillium. Studerat i växthus och in vitromiljö.

Olof Westergren

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Abstract

Verticillium longisporum is one of the most damaging fungal plant pathogens by causing stem striping disease in oilseed rape production. There are no effective fungicides available, and the management of the pathogen focuses on plant breeding and crop rotation. *Verticillium longisporum* is a hybrid combining the genomes of two different ancestors. One of these ancestors is *Verticillium dahliae* which is a plant pathogen in many cultivated species but not necessarily in *Brassica napus*. *Verticillium longisporum* is mainly a plant pathogen in *B. napus*. The pathogen is soilborne and forms sclerotia that can survive in the soil for many years. The lifecycle is monocyclic and begins with infection of the roots. The pathogen enters the vascular system to further colonize the plant. Symptoms develop late in the growing season. They are often visible as bronze-colored stripes on the stem. Stem striping disease causes yield losses and prematurity of the infected plants.

The aim of this study was to examine 15 oilseed rape cultivars and their tolerance to *V. longisporum.* The ultimate goal was not only to investigate which cultivars were more tolerant than the others, and to determine the reason. Fourteen of the cultivars were modern autumn-sown hybrids, while one was an old spring-sown cultivar from 1983 and used as a reference. The research questions focused on cultivar differences in tolerance and the possible effect of root morphological traits on tolerance to *V. longisporum.* Several methods were used to answer to the research questions. Pot experiments with root-dip inoculation were carried out to evaluate cultivar tolerance to two *V. longisporum* isolates (S22 and S31), both originating from Östergötland, Sweden. Inoculation was carried out two weeks after seed germination at growth stage (BBCH) 12, and symptoms and biomass were measured four weeks after inoculation. To study root architecture, the cultivars were grown in gel-based growth medium for 10 days. Plants were scanned and analyzed using a root image analysis tool.

The results showed significant biomass reductions in general with one isolate being significantly more virulent than the other. The S22 isolate caused significant biomass reductions in all cultivars tested. The old reference cultivar Hanna stood out as more susceptible to the S31 isolate compared to all other cultivars. Cultivars Commodore, Credo, DK Expat, LG Adapt, LG Armada, Triathlon and Janosh showed possible tolerance to mild infection with S31. Biomass reductions did not correlate between the two isolate treatments. Plant symptoms such as dead leaves did not correlate with biomass reductions. When the two isolates were combined, Hanna and LG Armada showed significantly more symptoms than DK Exentric and Triathlon. Root architecture trials showed differences in morphological traits between cultivars. A possible correlation was found between biomass reduction and root architecture. The less virulent isolate was correlated with the total root length. Cultivars with larger total root lengths had less biomass reduction, indicating that a larger root system resulted in more tolerant cultivars. Root morphology may therefore have an effect on mild *V. longisporum* infection.

Populärvetenskaplig sammanfattning

Kransmögel är idag en av de mest allvarliga svampsjukdomarna i svensk rapsodling. Likt klumprotsjuka finns ingen direkt bekämpningsmetod för svampen, bekämpning av kransmögel förlitar sig huvudsakligen på god växtföljd och sortmaterial. Tidigare har kransmögel enkom varit ett svenskt problem och forskningsämne. Allteftersom svampen förekommer mer och mer i rapsodling internationellt har även andra fått upp ögonen för problemet.

Det har gått 55 år sedan Göran Kroeker först beskrev sjukdomen orsakad av patogenen *Verticillium longisporum*. Han kallade fenomenet vissnesjuka. Idag kallar vi det kransmögel. Han beskrev symptomen som synliga först sent i grödans utveckling. Vanligt är att endast ena sidan av stjälken vissnar av från roten och upp medan resterande del av stjälken förblir frisk. Bladen vissnar också och faller till marken i förtid. När fältet sedan är tröskat förblir vilsporer av svampen kvar i fältet, där de kan överleva i tiotals år. Vissa ogräsarter är även potentiella värdar för svampen när inte raps odlas. När det sedan är dags att odla raps nästa gång på fältet ligger vilsporerna redo att angripa och uppförökas. Det må ha gått 55 år sedan de första problemen med kransmögel uppmärksammades, men än så länge gäckar den fortfarande svensk rapsodling.

En större sortprovning genomfördes 2025 i ett fältförsök i Östergötland med fokus på kransmögel. Parallellt har samma sorter testats i växthus och laboratorium på SLU Ultuna i det här examensarbetet. Arbetet har syftat till att under kontrollerade förhållanden jämföra utvalda rapssorter sida vid sida. Hypotesen var att rotsystemets utseende - dess morfologi - har en påverkan på mottagligheten. Hypotesen bygger på att rapssorter som har en rotutveckling som premierar sidorötter framför pålrot får värre symptom.

För att testa hypotesen användes två tillvägagångsätt. Sorterna odlades i krukor där två olika inokuleringar med *Verticillium* jämfördes mot en frisk kontroll. Inokuleringen av plantorna gjordes genom att rötterna doppades vid två-örtbladsstadiet i en bestämd koncentration av svampsporer i vattenlösning. Inokulering betyder att man artificiellt utsätter en planta för en patogen. På så vis får alla sorter samma behandling. De två behandlingarna bestod av isolat av *V. longisporum* tagna från två fält i Östergötland. Isolaten kan man likna vid två olika individer men som genom sin geografiska närhet bör dela många egenskaper. Eller så kan man i alla fall tycka. Det ena isolatet, kallat S22, kommer från närheten av Fågelsta, det andra är S31 och kommer från ett fält nära Vadstena. Exakt vilka fält som proverna kommer från är inte känt. Avståndet mellan de två tätorterna är dock endast 8 kilometer fågelvägen, något som kommer visa sig vara intressant.

För att undersöka rötternas morfologi användes en annan metod. För rotstudierna odlades sorterna på en agarplatta, en nästan genomskinlig odlingsgel. Denna metod har bevisats ge rotutveckling som speglar den i jord. Metoden uppfyller kravet på att kunna jämföra rapssorterna utifrån deras genetiska egenskaper och inte miljömässiga variationer. Rötterna skannades senare och analyserades i ett dataprogram för att mäta rotlängd och räkna antalet rötter.

Försöken i växthus visade stora skillnader mellan isolaten S22 och S31. Fyra veckor efter inokulering med S22 hade den behandlade rapsen cirka 50–80 procent lägre biomassa jämfört med frisk kontroll. För isolatet S31 var denna förlust endast cirka 5–30 procent. Dessa förluster går inte att översätta direkt till skördeförlust i fält. Det de visar är att kransmögel kan påverka en gröda i väldigt olika omfattning, detta på små geografiska avstånd. I laboratoriestudierna användes även en gammal vårrapssort från 80-talet framtagen av W. Weibull AB: Hanna. Sorten inkluderades för att den i forskningssammanhang ofta används och är uppskattad. Den gav också en givande insikt till försöket, även om jämförelsen mot de moderna hybrid höstrapssorterna är lite som att jämföra äpplen och päron. Sorten Hannas motståndskraft mot kransmögel var inte sämre för S22 än de moderna sorterna. För S31 visade den sig dock mycket sämre i jämförelse med moderna sorter. De moderna sorterna har alltså resistensegenskaper som Hanna saknar för en typ av kransmögel men inte till den andra. Fler svar ger tyvärr inte denna studie på detta.

Till de grundläggande frågeställningarna: Vilka sorter stod sig bäst mot kransmögel och finns det en korrelation med rötternas morfologi? Försöken i växthus visade på små sortskillnader, det fanns knappt några signifikanta skillnader. Endast den gamla sorten Hanna stack ut för S31 som mycket mer mottaglig för kransmögel. Commodore, Credo, DK Expat, LG Adapt, LG Armada, Triathlon och Janosh hade ingen signifikant reduktion av biomassa inokulerade med S31, de påvisade alltså en möjlig tolerans vid milda angrepp. Symptomen av sjukdomen på plantorna räknat som döda blad korrelerade inte med förlusten av biomassa. Sorterna som visade mest symptom var LG Armada och den gamla sorten Hanna hopräknat för både S22 och S31. De sorterna som visade minst symptom var Triathlon och DK Exentric.

Rotstudierna visade att det fanns skillnader mellan sorterna och att vissa sorter allokerade mer på sidorötter än pålrot. Det fanns alltså inget samband mellan längden på sidorötterna och längden på pålroten mellan sorterna. Att vissa sorter hade mer sidorötter berodde alltså inte på att de hade ett större rotsystem överlag. Försöket i växthus visade endast på ett svagt potentiellt samband mellan rotsystemets morfologi och mottagligheten av kransmögel. Sambandet var mellan rotsystemets totala storlek och angreppet av isolatet S31. Det var dock ett stort rotsystem som gav till fördel att ge hög tolerans mot *V. longisporum*. Fler sorter hade dock behövts testas för att ge säkrare resultat. Att det inte fanns tydliga samband mellan sidorötter och mottaglighet är till fördel för framtida förädling. Tidigare sortstudier har nämligen visat att rapssorter med mer sidorötter har högre skördepotential.

Sammanfattningsvis visade arbetet att det ännu finns mycket att studera om *V. longisporum* och kransmögel som den orsakar i raps. Arbetet visar att skördeförluster av kransmögel kan slå väldigt olika beroende på patogenens genetik. Populationerna av *V. longisporum* som finns i fält kan vara väldigt olika varandra. Detta försvårar också potentiellt framtida resistensförädling då patogenen är väldigt diversifierad. Problemet bottnar också i att det inte finns någon rapsförädlare i Sverige som ser till våra populationer av *V. longisporum* främst i sin resistensförädling.

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Abbreviations

Abbreviation	Description
BBCH	Growth stages based on Zadok's scale for cereals
SA	Salicylic acid, a signalling molecule
JA	Jasmonic acid, a signalling molecule
ET	Ethylene, a signalling molecule
PAMP	Pathogen associated molecule patterns
HR	Hypersensitive response
PTI	Pathogen triggered immunity
ETI	Effector triggered immunity
TSW	Thousand seed weight
DPI	Days post inoculation
Mock	Not inoculated plants, used as a reference
N_{LR}	Number of lateral roots
$\sum L_{LR}$	Sum of lateral root length
TRL	Total root length

1. Introduction

During recent years, oilseed rape has been grown on around 4 percent of Swedish arable land (Olsson 2024). It is the most important oil crop grown in the country. Several plant diseases have significant impact on the cultivation of oilseed rape in Sweden as well as in other parts of the world. Some of the most damaging and important plant diseases are caused by the pathogens *Sclerotina sclerotiorum, Plasmodiophora brassicae, Leptosphaeria maculans* and *Verticillium longisporum.*

Verticillium longisporum in oilseed rape was first described in 1969 by Göran Kroecker who described it as a wilt disease caused by *Verticillium dahliae* (Kroeker 1970). Later, the name was changed to Verticillium stem striping disease as it rarely causes wilting symptoms. The disease is considered to negatively affect both the thousand kernel weight and oil content. Yield losses vary, and can range from 10 percent up to 50 percent in Europe and Canada (Dunker et al. 2008; Wang et al. 2023).

The pathogen *V. longisporum* is a hybrid of the known *V. dahliae,* and a species of unknown origin (Fogelqvist et al. 2018). Fogelqvist et. al., (2018) confirmed that *V. longisporum* has three different origins with one common parent, called A1. The A1 species is combined with three different genomes to separately form the lineages A1/D1, A1/D2 and A1/D3 (Inderbitzin et al. 2011). The D2 and D3 are *V. dahliae* lineages, while A1 and D1 do not correspond to any known *Verticillium* species (Inderbitzin et al. 2011b). Analyses of A1/D1 isolates showed that the size of the *V. longisporum* genome was about twice that of *V. dahliae*, a large and complex genome compared with other Ascomycetes. The large genome gives the fungus a large repertoire of gene expression capabilities (Fogelqvist et al. 2018).

Today, there are differences in tolerance among oilseed rape cultivars. (Depotter et al. 2019). There are also cultivars showing limited signs of disease. The field trials conducted by Depotter et al. (2019) demonstrated that substantial yield reductions can be achieved in cultivars that do not exhibit significant disease symptoms during the scoring process. The impact of the pathogen can vary significantly depending on annual variations.

2. Aims of the study

The objective of this study was to investigate the degree of tolerance or susceptibility in 14 different commercial oilseed rape cultivars against *V. longisporum*. There are no effective control strategies against the pathogen, and management of the pathogen in practice therefore focuses on crop rotation and crop breeding. More specifically, the aim of this study is not only to find out which oilseed rape cultivars that are more tolerant, but also to identify possible factors that increase the tolerance. There is a hypothesis that cultivars with a greater number of lateral roots are more susceptible to the disease. Other possible approaches to understanding the background of variations in susceptibility include correlating susceptibility with signaling molecules or defence gene expression.

Research questions

- Do the contemporary commercial oilseed rape cultivars show any different in tolerance against *V. longisporum*?

- Is there a correlation between root architecture and tolerance against *V. longisporum* in oilseed rape cultivars?

- Is there a correlation between the expression of defence genes and tolerance against *V. longisporum* in oilseed rape cultivars?

3. Background

There are three factors needed for a plant to have a disease, and it is shown by the disease triangle in Figure 1. This includes a pathogen, its host and the environment. To understand the disease, it is important to keep the disease triangle in mind. If any of the three crucial factors are missing, the disease will not occur. In crop management, successful disease control requires targeting and affecting at least one of these three factors to alter the disease pressure. A common management method is for example to apply fungicides to hinder pathogen development (affecting pathogen abundance). Another approach is to grow tolerant or resistant cultivars (affecting the factor host).

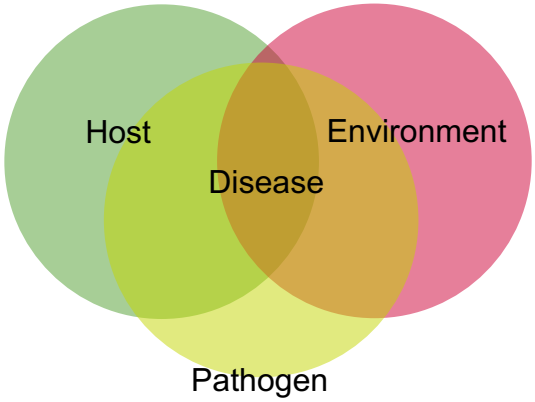

Figure 1: The disease triangle with the three factors for disease in a plant.

3.1 Disease cycle

Verticillium longisporum is a fungal species belonging to the phylum Ascomycota. This fungus is a pathogen affecting oilseed rape production and causes a disease known as Verticillium stem striping. Previously, the common name for this disease was Verticillium wilt. Depending on the severity of disease caused by the pathogen, yield losses in individual plants can reach up to 80 percent in field trials (Dunker et al. 2008).

Verticillium longisporum is a soilborne and monocyclic disease (Figure 2) (Depotter et al. 2016). The microsclerotia formed during host plant ripening are persistent and are able to stay dormant in the soil for several years (Heale & Karapapa 1999). Microsclerotia are induced to germinate by the root exudates from host plants. The infection of a host plant begins with the fungus penetrating the lateral roots,

growing towards the plant's vascular system, and entering the xylem. From the root xylem fungal produced conidia which can travel upwards in the plant and infect the whole plant xylem system.

For systemic spread in the plant, the initial flowering (BBCH 60) is of great importance (Zhou et al. 2006; Dunker et al. 2008). The plant growth rate also plays a role in disease development. Later flowering individuals of *Arabidopsis thaliana* have been shown to develop more severe disease than earlier flowering samples. Additionally, longer flowering periods have been shortened by *V. longisporum* (Häffner et al. 2010). The same experiment also showed that stunting caused by the pathogen correlates well with time till maturity, with slowly maturing plants being more severely stunted than faster developing ones. In addition to flowering period and development rate being crucial for systematic spread, very susceptible cultivars of *B. napus* have been shown to be able to be systematically infested by *V. longisporum* at earlier growth stages (Dunker et al. 2008).

The pathogen has the potential to reduce the transport of plant sap in the plant vascular system, thereby affecting plant growth. In later crop growing stages and plant senescence, the pathogen starts producing microsclerotia outside of the xylem. Microsclerotia are subsequently released into the soil during decomposition of plant residues.

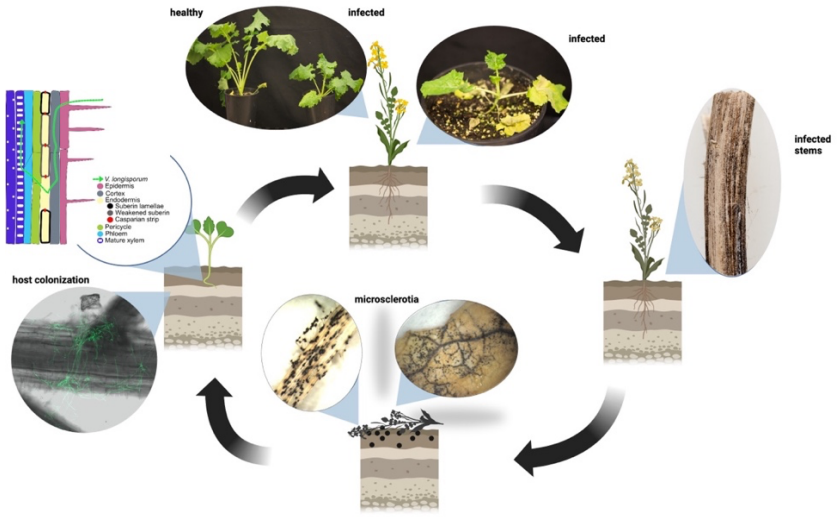

Figure 2: Disease cycle of V. longisporum in oilseed rape (Tzelepis 2025). Bottom part of the cycle resembles microsclerotia dormant in the soil, microsclerotia are left with harvest residue. The cycle continues following the arrows with a new host plant inducing the spores to germinate. During plant growth the pathogen spreads in the host through its vascular system. In pot experiments the growth of inoculated plants are stunted as seen in the top part of the figure. In field conditions the symptoms are visible as brown coloured stipes along the stem. At plant senescence, right side of the figure, the microsclerotia are once again formed.

Verticillium longisporum is spread mainly through soil or plant material. Seed transmission is possible in spring cultivars but has not been confirmed in autumn-sown cultivars (Zheng et al. 2019). *Verticillium longisporum* is present in many soils all over Europe. A study concluded that the A1/D1 lineage is the most common in European soils, although it is diverse and consists of several subclades (Vega-Marin et al. 2025). The same study concluded that there are geographical differences between isolates. Swedish, Latvian and German isolates formed one subgroup, whereas English and French isolates formed another genetic group ((Vega-Marin et al. 2025).

3.2 Symptoms

The symptoms first become visible to the naked eye during later stages of plant development. Symptoms occur from the roots and up to the stem, often only on one side. This phenomenon gives the pathogen its characteristic brown-coloured stripes along the stem, while the rest of the stem remains symptom free. By cutting/scraping the stem, it is possible to find discoloration in the plant's vascular system prior to symptom appearance from the outside of the stem. An earlier detection of the pathogen is thereby possible. During plant senescence, microsclerotia are formed, with black spots appearing in the affected brown areas. The pathogen causes earlier plant senescence. The effect of earlier plant senescence is thought to shorten the seed-filling period, resulting in lower thousand seed weight (TSW) (Gladders 2009).

3.3 Crop management

Being a persistent soilborne pathogen abundant in the vascular system, there are no fungicide options for controlling *V. longisporum* in today's agriculture. Including break crops between susceptible hosts is one strategy to reduce disease pressure in the soil. One study found that nitrate availability in the root also affects the colonialization of *V. longisporum* in host tissue (Dölfors et al. 2024). Nitrogen availability also affects the length of lateral roots, with better nitrogen availability resulting in longer lateral roots (Louvieaux et al. 2020). Lateral roots being the entry point of *V. longisporum* may affect plant colonization if the root system is larger (Zhou et al. 2006). Nitrogen fertilizer strategies limiting nitrate availability in plant tissue can therefore impact colonization of the pathogen. On the other hand, lower nitrogen availability affects the yield potential negatively (Louvieaux et al. 2020).

The impact of *V. longisporum* in crop management depends on several factors. With the disease triangle in mind (Figure 1), the choice of cultivar and year-dependent factors influence the severity of disease. Crop yield losses vary between years and cultivars (Depotter et al. 2019). Annual environmental differences can have an impact on disease severity. From one year to the next, the same cultivar can show

different degrees of symptom development. The same level of symptom severity can also result in different levels of crop yield losses.

3.4 Root architecture

For soilborne pathogens, the host plant root system is an essential feature in its ability to cause disease. It is part of the environment for pathogen infection. Studies have shown that the size of the root system for *Brassica* species can correlate with disease severity caused by soilborne pathogens. For *Plasmodiophora brassicae,* the causal agent of clubroot disease, it has been found that *Brassica nigra* exhibited greater tolerance than *Brassica napus* (Fredua-Agyeman et al. 2019), and that *B. napus* has a significantly larger root system than *B. nigra* (Yang et al. 2024). *Verticillium longisporum* may exhibit a higher disease index when inoculated on larger plants of *B. napus* with larger root systems (Cui et al. 2023). The amount of branching by lateral roots can also have an impact on disease severity. Different pathogen species initiate endodermal penetration on different parts of the root system. In terms of difference between the closely related pathogens *V. longisporum and V. dahliae,* the former initiates infection in the lateral roots, while the latter primarily affects on the main taproot (Zhou et al. 2006). Not only root system size, but also morphology, can influence pathogen virulence. Root architecture is dependent on soil texture, nutrient availability, genotype, water availability etcetera. Studying one of these aspects relies on all other variables being consistent between treatments. Focusing on differences in root morphology among different genotypes requires the same growing conditions for all samples.

3.5 Plant defence mechanisms

Extensive studying of plant defence mechanisms has been made in *Arabidopsis thaliana*, which belongs to the same family, Brassicaceae as oilseed rape *B. napus*. As a model species it can be used to evaluate different defence mechanism treatments effective against *V. longisporum*. The genomes of *B. napus* and *A. thaliana* share extensive conserved regions. One study showed that the total sequence identity is 86 percent between the two species – meaning that traits of the *Brassica napus* species can be widely studied in *A. thaliana* (Parkin et al. 2005). During pathogen infection there are different compounds used as signaling by the plant to induce plant pathogenesis-related proteins (PRs) production. Examples of these signaling compounds are salicylic acid (SA), ethylene (ET) and jasmonic acid (JA) (van Loon et al. 2006).

3.5.1 Signaling Pathways

Salicylic acid (SA) is a plant hormone, so called phytohormone, that functions as a signaling molecule to activate systematic acquired resistance (SAR) against plant

pathogens (Ratzinger et al. 2009). A negative impact on expression of SA in a plant, either by reduced production or function, may increase susceptibility to plant pathogens. On the other hand, when SA is artificially supplemented it has a positive effect on plant pathogen resistance (Loake & Grant 2007).

Both Ethylene and Jasmonic acid act on necrotrophic pathogens rather than biotrophic (Alkooranee et al. 2015). Necrotrophic pathogens are not affected by HR, which instead benefit their infection development.

Signaling molecules act in a complex manner, interacting in both antagonistic and synergistic ways, depending on the conditions. For example the NPR1 monomer through SA signaling has an inhibiting effect on JA signaling (Beckers & Spoel 2006). A study has shown that applications of JA and SA to plants in low concentrations are synergic and expressions of defence related genes PDF1.2 and PR1 were enhanced(Mur et al. 2006). The same study confirmed that higher concentrations of the signaling molecules induce the production of Reactive oxygen species (ROS) causing Hypersensitive response (HR) with plant cell death as the ultimate result.

3.5.2 Gene for gene resistance

To exemplify gene-for-gene resistance in *B. napus,* another pathogen can be used that triggers signaling pathways. Infection with the ascomycete *Erysiphe cruciferarum* in *B. napus* resulted in a strong response and upregulation of defence related genes (Alkooranee et al. 2015). *PR-1* and *PR-2* genes regulated by the SA-signaling pathway and the *PR-3* and *PDF1.2* genes regulated by the JA/ET-signaling pathway all peaked in gene expression 6-10 days post inoculation (dpi). The defence genes *PR-1, PR-2* and *PDF1.2* were all preceded by the response and upregulation of *CHI570* and *CHI620* enzymes regarded to also be regulated by the JA/ET pathway (Alkooranee et al. 2015). The *PR-3* gene, a basic chitinase, was upregulated with a local peak 1 dpi. Chitinases break down chitin, a component in fungal cell walls.

3.5.3 Resistance mechanisms against *V. longisporum*

Resistance or tolerance can be both quantitative and qualitative. Quantitative means that several mechanisms determine the tolerance of a cultivar. Each of the genes involved in quantitative resistance have single-handed minor effects on plant resistance. However, when multiple genes each contribute to partial resistance, they can collectively have a significant impact on the plant's overall resilience. Qualitative resistance means that resistance and susceptibility can be compared with an on/off switch, either resistant or susceptible, a single R gene can imply total resistance to a single pathogen species or race. Single R genes controlling plant

resistance can rapidly be overcome by new races of pathogens, making qualitative resistance a brittle system. *Verticillium spp.* interacts with the host in a complex manner, and no single resistance pathway has not been recognized. Susceptibility or tolerance relies on a quantitative pathosystem. Deletion of single genes in oilseed rape cultivars can on the other hand have a qualitative resistance impact on *V. longisporum* pathogenicity (Jacott et al. 2024).

The root cortex of a young root tissue is the main point of attack for the pathogen, with older tissue having a physical barrier that is more difficult to penetrate in the endodermis. Resistance against *V. dahliae* in *B. napus* can be attributed to a physical barrier that inhibit an infection, whereas no such effect has been observed against *V. longisporum* (Eynck et al. 2007, 2009). Lignification of the cortex as a part of pathogen triggered immunity (PTI) is not considered sufficient to fully prevent infection in any *B. napus* cultivar (Häffner & Diederichsen 2016). To overcome PTI, pathogens secrete so called effectors to hinder recognition. In general effectors suppress the effect of PTI. Suppression can be done by binding for example to chitin protecting fungal hyphae from plant recognition (Häffner & Diederichsen 2016). In response to effectors plants with tolerance or resistance recognize the effectors with resistance proteins as for example already mentioned *PR1* and *PR2*. Effector triggered immunity (ETI) is a second level of defence over PTI. A plant response showcasing resistance with ETI can be through HR when attacked by a biotrophic pathogen. The immune plant then sacrifices cells to evade pathogens. Pathogens can in response to ETI also evolve new effectors which can suppress and overcome the plant immunity (Rafiei et al. 2022).

Studies of the *V. longisporum* genome have found 80 possible genes coding for different effectors in a *V. longisporum* strain, isolated from heavily infested soils in Sweden (Fogelqvist et al. 2018). A study also showed the importance of one of these effectors, named VL3320. Overexpressing this effector made the disease symptoms more severe upon plant infection. It is not fully understood how the VL3320 effector interacts with the plant other than its part of the pathogen virulence (Rafiei et al. 2022).

Levels of signaling molecules in *B. napus* inoculated with *V. longisporum* have been studied showing that SA was upregulated but JA did not show any response (Ratzinger et al. 2009). Higher concentrations of SA in the plant tissue were correlated with the reduced shoot length - a symptom caused by *V. longsiporum*. Plants showing more symptoms, unsurprisingly, also had a larger fungal pathogen biomass. More susceptible plants thus had a greater SA response signaling for the expression of the PR-1 and PR-2 defence proteins.

4. Materials & Methods

This work included three sets of experiments. The cultivars included in the study are listed (Table 1). Number 1-14 are all winter oilseed cultivars that are either in commercial use or under evaluation for commercialization. Number 15, Hanna, is an older spring oilseed cultivar which was introduced by W Weibull AB in 1982. Hanna was one of the first "00-cultivars" meaning that it had low concentrations of glucosinolates and erucic acids (Meyer 1997). Hanna is known for its low resistance to *V. longisporum* and is therefore often used in greenhouse experiments as a control for the infection process.

Table 1: Cultivars included in the experiments. All except Hanna are hybrids and either in commercial use or being tested for future use. Showing thousand seed weight (TSW) of all cultivars when provided.

Nr	Cultivar	TSW (g)
1	Hemma	5,11
2	Maverick	4,94
3	Helypse	5,3
4	LG Adapt	6,27
5	Triathlon	5,8
6	Eriksen	5,43
7	Dompteur	4,57
8	DK Expat	4,82
9	DK Exentric	4,71
10	LG Armada	4,93
11	Commodore	5,28
12	Credo	6,75
13	Janosh	5,27
14	Karat	5,46
15	Hanna	-

4.1 *Verticillium longisporum* isolates

The two fungal isolates used in this study both come from fields located in Östergötland Sweden. The S22 isolate has its origin from a field in the vicinity of Fågelsta and S31 from Vadstena. The linear distance between the two urban areas is only 8 kilometers. To inoculate plants, conidia of both isolates were produced. by streaking the PDA plates with mycelia and incubated for 14 days in darkness at 20^0C. To harvest the conidia for plant inoculation distilled water was added to each plate and then rubbed with a spreader tool. The water solution was filtered through miracloth, and the concentration of conidia was calculated. The concentration was then adjusted to 10^7 conidia/ml.

4.2 Pot experiments

Pot experiments consisted of 18 replicates of each treatment (S22, S31 and mock), each replicate representing one plant. For each treatment, plants accordingly were dipped either in conidial suspension or just in water for mock inoculation. The plants grew in a controlled environment greenhouse with 16 hours of daylight and 18^0C-23^0C. The soil used was Hasselfors^{TM} krukjord with a pH of 5,5-6,5, the soil included added macro and micronutrients.

4.2.1 Seed pre-germination and plant inoculation

Seeds were first surface sterilized with 6-14 percent sodium hypochlorite for 3 minutes. The sodium hypochlorite was then rinsed off with autoclave water, the seeds were then spread out to germinate in a darkroom on wet filter paper in sealed petri dishes for two days under greenhouse conditions. Seeds which had germinated were then planted in a nursery with capacity for 150 plants per each cultivar. The nursery consists of small pots with soil with a single seed per small pot. The plants grew in the nursery for 14 days, until to develop two true leaves. 54 plants were needed for each cultivar and were selected from the nursery to ensure homogeneity between treatments and cultivars. Treatments with conidia from isolates S22 and S31 were done with root dipping technique for 15 minutes. In the root dipping technique, plants were removed from the nursery and the loose soil was washed off to expose the roots. Then, the roots were dipped into a conidial suspension or in water for mock inoculation. Root dipping inoculation has been proven to be a method giving severe symptoms to plants in greenhouse experiments (Koike et al. 1994).

Plants were harvested four weeks dpi. The plants were around BBCH 18 at that time, meaning that 8 true leaves had developed. The aboveground fresh weight and degree of symptoms were measured at harvest. Symptoms were measured as the number of leaves per plant that were green, yellow and dead. Yellow and dead leaves indicating disease symptoms. Three batches of pot experiments were conducted to make the work feasible. In every batch the cultivar ‘’Maverick’’ was included as a bridge to evaluate a potential batch effect.

4.3 *In vitro* experiments

In vitro experiments were conducted to evaluate expression of the resistant genes *PR1, PR2, PR3, PDF1.2, CHI570* and *CHI620* in different oilseed rape cultivars in response to pathogen inoculation. Seeds were first surface sterilized with sodium hypochlorite for 3 minutes as described above. The trial consisted of two plants per replicate and in total nine replicates per cultivar. Of the nine replicates six were inoculated with S31 isolate and three were mock. The seeds were slightly submerged in fresh Murashige and Skoog (MS) with added sucrose, plates had

dimensions 120x120mm (Figure 3A). The growth medium contained 15g/L of sucrose, 2,2g/L MS and 6,1g/L plant agar. During the growth period plates were kept upright in a growth room with a temperature of 22ºC and 16 hours of light per day, during dark hours the temperature was 20ºC. The plates were put in an upright position in a rack. Seven days post seeding the plants were extracted from the plate and inoculated with root-dipping technique with a concentration of $3,5*10^6$ conidia/ml. After 15 minutes the inoculated plants were replanted back in the MS (Figure 3). Mock treatments were inoculated with water. Three dpi roots and 2 cm of stem was harvested and collected in tubes.

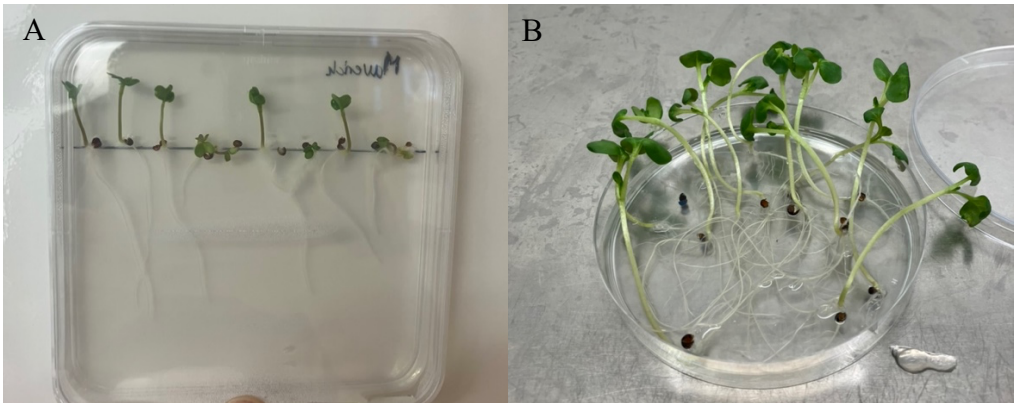

Figure 3: A: In vitro growth of oilseed rape seedlings. B: Inoculation method of oilseed rape seedlings in vitro.

To prepare for RNA extraction the samples were freeze dried. After drying samples were stored in -70 °C. For RNA extraction, the plant material was crushed using small glass pebbles that were added in the tubes to homogenize the tissue. RNA extraction was conducted using the Spectrum™ Plant total RNA kit (Sigma-Aldrich Co. LLC), according to the manufacturer instructions.

After RNA extraction a procedure followed to remove the remaining gDNA from the samples. 1000 ng of RNA was transferred from the original sample to a PCR tube, 2 µL DNA buffer and water was also added to reach a total volume of 20 µL. DNase was added to the samples to degrade gDNA in a 37 °C heating cabinet. After 30 minutes EDTA was added and to inactivate the DNase, and samples were incubated in a PCR machine for 10 minutes at 70°C. Afterwards the samples were put back in -70°C. The final step to prepare the samples for RT-qPCR was to prepare cDNA with reverse transcription of the mature mRNA. For that reason the Bio-Rad iScript™ cDNA synthesis kit was used and the reaction protocol for incubation as described by the manufacturer.

Last step of the *in vitro* experiment was to run RT-qPCR to determine the concentration of resistant genes. The RT-qPCR machine uses fluorescence attached

to completed DNA strands to measure expression of different genes. By using specific primers, the expression of each defence gene can be determined. The expression of *GADPH* was used as a reference, which is a common gene in plants coding for an enzyme breaking down glucose.

4.4 Root architecture

Root architecture experiment was performed using 12x12 cm agar plates and scanning intact plants. The growth medium contained 15g/L of sucrose, 2,2g/L MS and 6,1g/L plant agar. Two plates containing 5 seeds each were prepared for every cultivar and set to grow in a controlled growth room, 16 hours of daylight at 22ºC, during dark hours the temperature was set to 20ºC. The seeds were first surface sterilized in 6-14 percent sodium hypochlorite for 3 minutes. After surface sterilization they were placed and fully submerged in the growth medium. The plants were then set to grow for 9 days in the same setup as the *in vitro* experiment but with less seeds per plate (Figure 3A).

After extraction from the growing medium plants were scanned in an Epson V800 flatbed scanner with a resolution of 1200dpi. The images were then processed in Rhizovision software (Seethepalli & York 2021). Root morphology traits that have been proven to vary between genotypes are the number of lateral roots (N_{LR}), sum of lateral root length ($\sum L_{LR}$) and total root length (TRL) (Dunker et al. 2008; Kupcsik et al. 2021). These parameters are studied to confirm or contradict the hypothesis that root morphological traits of rapeseed cultivars have an impact on disease severity.

4.5 Statistical analysis

Three software were used to conduct the statistical analysis. Minitab™ was used to do statistical analysis of root architecture data (Minitab, LLC 2021). This software was used to perform One-way Anova with 95% confidence. Comparisons were run assuming equal variance with Tukey test, based on median values. For pot experiments, assessing cultivar and treatment effect on growth variables statistics software Rstudio was used (R Core Team 2022). Data were modelled using Generalized Linear Mixed Models (GLMM). GLMM was used instead of LMM because the data were not normally distributed. Regression analyses were used to correlate root architecture data with pot experiment data. Microsoft Excel analysis ToolPak add-in was used to do linear regression analysis (Microsoft Corporation 2025).

5. Results

5.1 Pot experiments

To evaluate the resistance/tolerance of 14 commercial oilseed rape cultivars in *Verticillium longisporum*, pot experiments were conducted. Plants were grown for four weeks in a greenhouse, and symptom development was evaluated. The results from pot experiments gave significant biomass reductions across all cultivars inoculated with the S22 isolate. The loss of biomass was in general significantly greater for plants inoculated with S22 than S31 (Table 2). The pattern was confirmed for all cultivars except the reference spring oilseed rape ‘’Hanna’’ which showed no significant difference in biomass reduction between both inoculum (Table 2). This spring cultivar was also the only one reaching stem elongation (BBCH 30), while all other cultivars only reached the leaf development stage (BBCH 16-19).

Combining all three experimental batches and all cultivars, the results showed that S22, and in most of the cases S31, significantly reduced plant weight (Table 2). The batch effects between harvests were minimal. Results for biomass reduction with S31 treatment showed greater cultivar differences than S22 biomass reductions (Figure 4). Standard deviation was larger for inoculated plants compared to mock treated plants. The spread of data was thus larger with fungal inoculation compared to healthy plants. Hanna had significantly higher biomass reductions than other cultivars for S31. Commodore, Credo, DK Expat, LG Adapt, LG Armada, Triathlon and Janosh showed no significant biomass reduction comparing S31 infection with mock treatment (Table 2). Further, cultivars Commodore, Credo and Janosh even showed a negative biomass reduction for S31 (Figure 5), meaning that the biomass of S31 was higher than for mock, but the effect was not statistically significant.

*Table 2: Weight of fresh biomass of each cultivar and treatment. Weight as mean value in grams with standard deviation (SD). Significant difference (sign.) comparing treatment S22 and S31 with mock only within the same cultivar. Tukey model, Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.*

	Mock		S22			S31		
Cultivar	**Mean (g)**	**SD**	**Mean (g)**	**SD**	**Sign.**	**Mean (g)**	**SD**	**Sign.**
Commodore	42.34	3.57	24.92	6.45	***	43.26	7.7	
Credo	40.53	5.95	25.08	9.26	***	44.7	10.61	
DK Exentric	51.83	4.34	14.87	7.18	***	38.93	9.5	*
DK Expat	50.15	6.58	15.55	6.76	***	41.99	8.02	
Dompteur	51.69	5.89	16.27	9.4	***	39.95	4.72	*
Eriksen	58.66	6.88	21.15	11.4	***	42.02	10.01	***
Hanna	63.51	8	30.82	9.46	***	35.15	4.3	***
Helypse	60.45	7.71	24.1	7.35	***	48.05	7.88	*
Hemma	55.77	5.63	19.21	8.2	***	41.93	9.91	**
Janosh	50.98	8.05	22.49	4.98	***	52.52	5.67	
Karat	54.34	11.22	18.46	6.14	***	41.72	8.77	*
LG Adapt	57.55	10.68	20.16	10.1	***	50.4	6	
LG Armada	56.31	6.86	21.04	8.06	***	49.7	7.25	
Maverick	53.86	8.96	16.49	7.29	***	45.27	8.33	***
Triathlon	51.48	10.22	17.91	6.6	***	42.56	7.53	

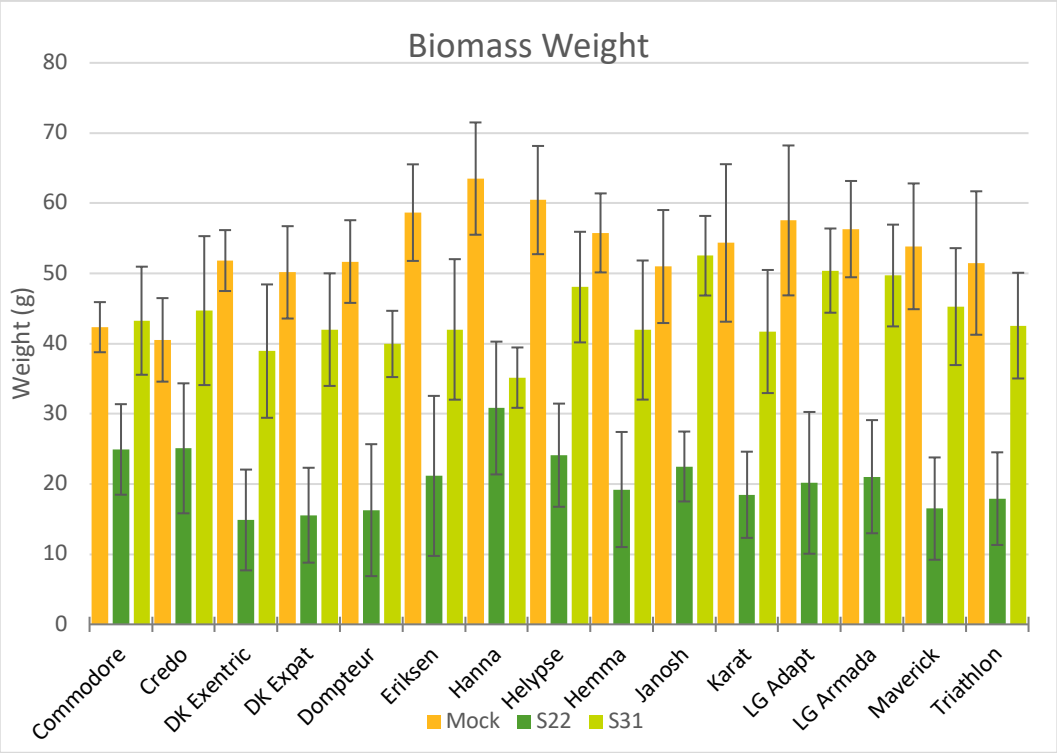

Figure 4: Biomass of all cultivars and treatments in grams fresh weight. Error bars represent standard deviation. Treatments separated by colour. All cultivars except Hanna follow the same pattern with weight of S22 being significantly lower compared to S31.

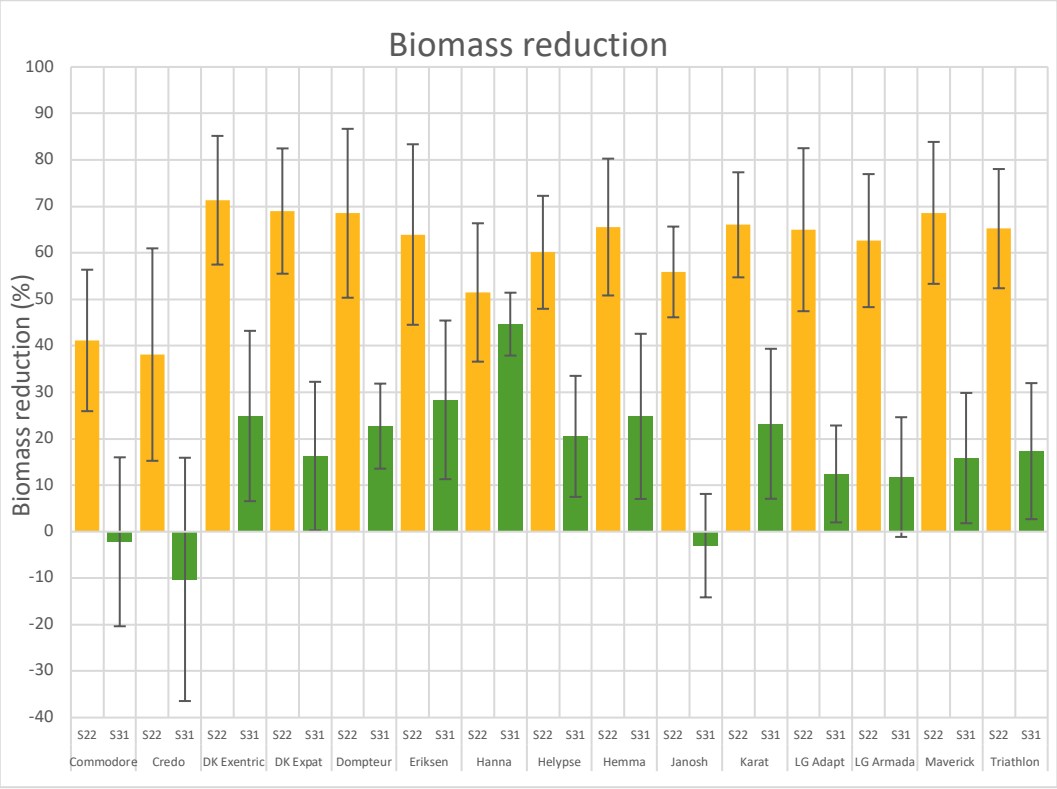

Figure 5: Weight reductions (%) of different cultivars. Calculated as loss of biomass in percent between mock and treatments S22/S31. No complete significance can be tested due to negative values as well as the data being composite values.

5.1.1 Symptoms evaluation

Plants inoculated with S22 showed clear symptoms in all cultivars (Figure 6). Visible symptoms included stunted growth and the number of dead leaves. For S31, the symptoms were not always clear. When mock and S31 were compared side by side, it was usually possible to distinguish between the two. However, for certain cultivars, such as Credo and Janosh, it was not possible to distinguish between mock and S31 infected plants based on visual assessment. Credo was one of the cultivars with the lowest biomass reduction. Credo also had a more compact growth pattern than mock. The biomass of Credo was the lowest measured compared with that of other mock-treated cultivars (Figure 6).

The total number of leaves, green, yellow and dead, did not vary between mock and inoculated plants (Figure 7), and the growth stage at harvest point was accordingly not affected by fungal infection. However, the growth was affected by stunting with smaller leaves and shorter petiole especially upon infection with the S22 isolate. Wilt was also an apparent symptom. As previously mentioned the total number of leaves does not vary between treatments. The number of dead leaves in S22 and S31 treatment is therefore a measure of symptoms (Figure 8). Between the different batches the cultivar Maverick indicated that there was some but small variance. In mock treatment there were no yellow leaves in the first harvest. The other two harvests, yellow leaves occurred in mock as well. The reason for what is regarded as symptoms in mock treatment by yellow leaves is explained by shading of lower leaves. The last batch also had an overall lower weight of biomass and overall lower reductions of biomass than previous batches. This commonly occurs in early spring, when the days become longer and artificial light in the greenhouse is replaced by natural sunlight.

The results showed correlation between S22 and S31 as it has been observed for the weight reduction. The S22 isolate had more severe symptoms as well as lower biomass. Symptoms such as dead leaves were more abundant in inoculated plants (Figure 8). The cultivars Commodore, Credo, DK Expat, LG Adapt, LG Armada, Triathlon, and Janosh exhibited tolerance to *V. longisporum*, as previously mention upon mild infection with the S31 isolate . However, these cultivars exhibited symptoms. The number of dead leaves was significantly higher for S22 and S31 compared to mock (Figure 8). The results showed that LG Armada and Hanna had significantly more dead leaves than DK Exentric and Triathlon.

Figure 6: Representative plants of cultivars Credo, Hanna, LG Armada and Janosh showing visual symptoms on inoculated plants and mock as a reference. Credo with a negative biomass reduction (not significant) show successful inoculation as dead leaves in S31 treatment.

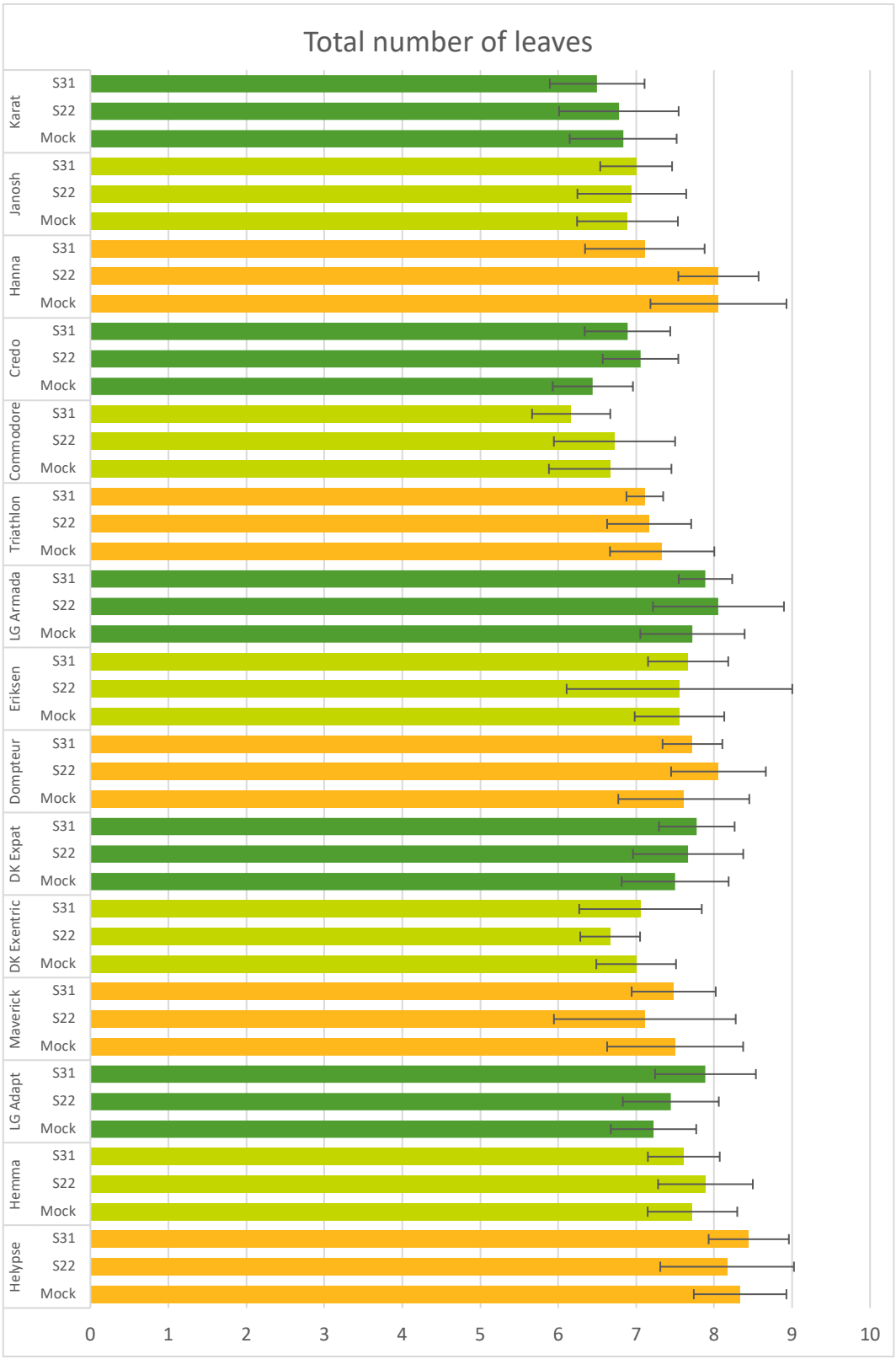

Figure 7: Total number of leaves as green, yellow and dead (x-axis) per cultivar and treatment (y-axis). Cultivars shown with different colours. Error bars represent standard deviation.

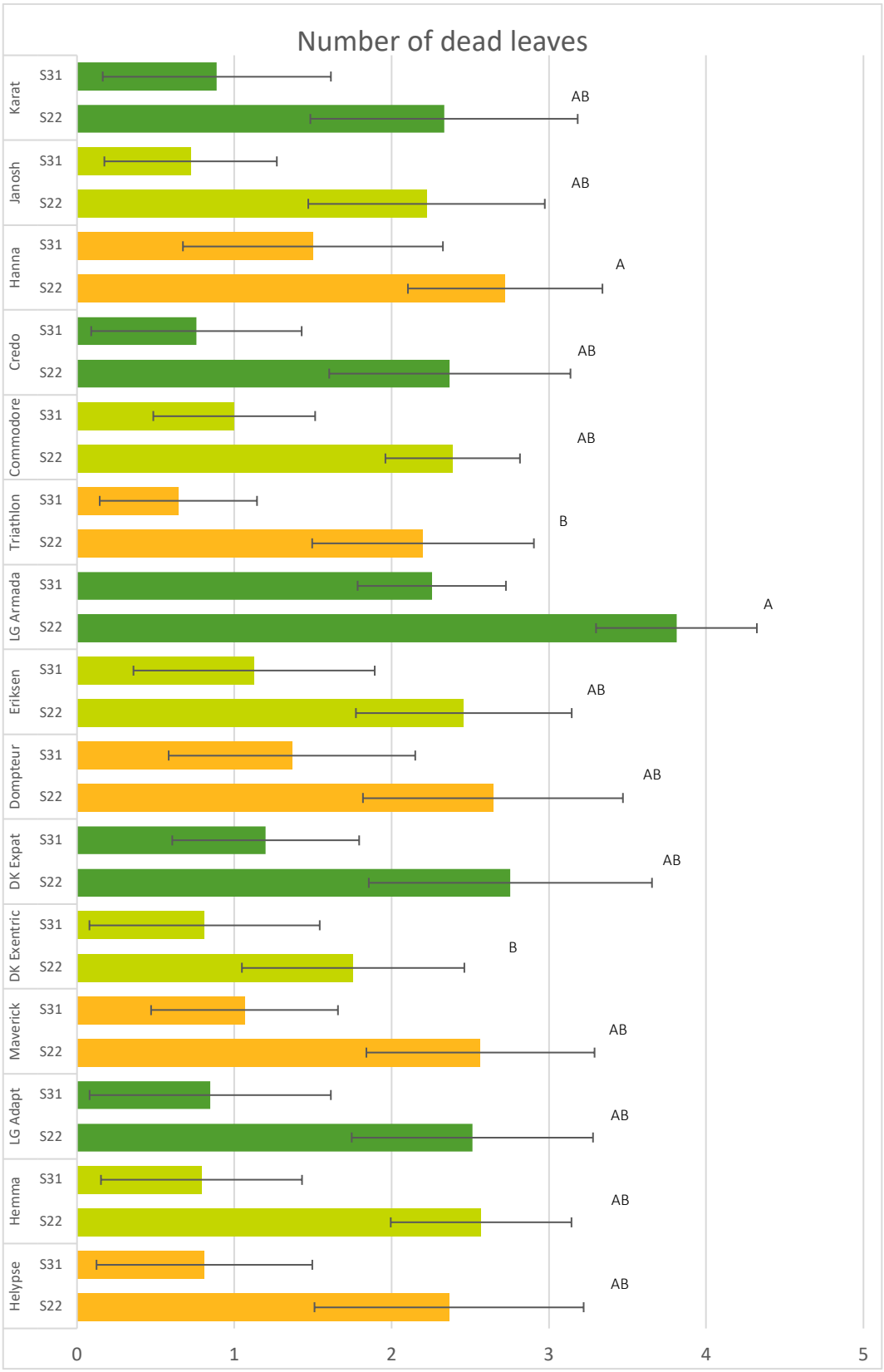

Figure 8: Number of dead leaves per cultivar and treatment. Significance level α = 0.05 by Tukey pairwise comparison. Grouped with letters A-B indicating significant differences comparing cultivars level of symptoms combining both S22 and S31 symptoms. Cultivars sharing one or more letters are not significantly differentiated.

5.2 *In vitro* experiments

The *In vitro* experiment did not proceed as planned and yielded no usable results. The probable cause was a failure in the reverse transcription of RNA into synthesized cDNA.

5.3 Root architecture

All examined traits of root morphology showed significant differences among tested cultivars. The number of replicates varied between cultivars because of, some plants were contaminated and some plants either were too small or seeds had not germinated. The correlations between different morphological traits are weak. There is no strong correlation between N_{LR} (Table 3) and $\sum L_{LR}$ (Table 4)

Table 3: Number of lateral roots (N_{LR}) per plant. N for the number of replicates of each cultivar. Significance level α = 0.05 by Tukey pairwise comparison. P = 0,000, F value 3.26. Grouped with letters A-C indicating significant differences. Cultivars sharing one or more letters are not significantly differentiated.

Cultivar	N	Mean	Grouping		
Karat	6	40.67	A		
Janosh	7	35.29	A		
Helypse	7	33.29	A	B	
Credo	6	31.17	A	B	C
LG Adapt	6	29.83	A	B	C
DK Expat	8	28.5	A	B	C
Hemma	6	27.67	A	B	C
DK Exentric	7	27.29	A	B	C
Triathlon	5	27.2	A	B	C
Maverick	7	27.14	A	B	C
Eriksen	8	27	A	B	C
Commodore	6	25.83	A	B	C
LG Armada	10	25.4	A	B	C
Dompteur	10	19.3		B	C
Hanna	5	13.4			C

The root system showed significant differences among cultivars, with Credo having significantly longer lateral roots compared to Janosh, Eriksen, LG Armada, Maverick, Dompteur and Hanna (Table 4). The total size of the root system has significant differences between cultivars with Credo having a significantly larger root system compared to Hemma, Eriksen, LG Armada, Maverick, Dompteur and Hanna (Table 5).

Table 4: length of lateral roots ($\sum L_{LR}$) per plant in mm. N for the number of replicates of each cultivar. Significance level $\alpha = 0.05$ by Tukey pairwise comparison. P = 0,000, F value 3.21. Grouped with letters A-B indicating significant differences. Cultivars sharing one or more letters are not significantly differentiated.

Cultivar	N	Mean (mm)	Grouping	
Credo	6	248.5	A	
Triathlon	5	198.1	A	B
Karat	6	187.1	A	B
DK Exentric	7	180.2	A	B
LG Adapt	6	171.6	A	B
Helypse	7	166.8	A	B
DK Expat	8	163.9	A	B
Commodore	6	147.8	A	B
Dompteur	10	129		B
Hemma	6	122.9	A	B
LG Armada	10	116.7		B
Janosh	7	115.3		B
Eriksen	8	114.9		B
Hanna	5	79.31		B
Maverick	7	78.9		B

Table 5: Total root length (TRL) in mm. N for the number of replicates of each cultivar. Significance level $\alpha = 0.05$ by Tukey pairwise comparison. P = 0,000, F value 4.07. Grouped with letters A-D indicating significant differences. Cultivars sharing one or more letters are not significantly differentiated.

Cultivar	N	Mean (mm)	Grouping			
Credo	6	380.2	A			
Karat	6	343.4	A	B		
DK Expat	8	314.5	A	B	C	
Triathlon	5	307.7	A	B	C	D
Helypse	7	303.2	A	B	C	D
Commodore	6	288.5	A	B	C	D
DK Exentric	7	288.4	A	B	C	D
LG Adapt	6	279.4	A	B	C	D
Janosh	7	264.2	A	B	C	D
Hemma	6	250.1		B	C	D
Eriksen	8	245.4		B	C	D
LG Armada	10	243.9		B	C	D
Maverick	7	241.6		B	C	D
Dompteur	10	223.3			C	D
Hanna	5	182.0				D

To evaluate if there was a correlation between lateral root length and primary root length a Regression analysis was made (Figure 9). The objective was to understand if the root traits were dependent on the speed of root growth or if cultivars allocated growth to certain morphological traits. The analysis showed no correlation between the length of lateral roots and primary root (Figure 9). The results showed that cultivars allocate root growth to certain traits. They can be classified as either bushy, with a focus on lateral root growth, or deep, with a focus on primary root growth.

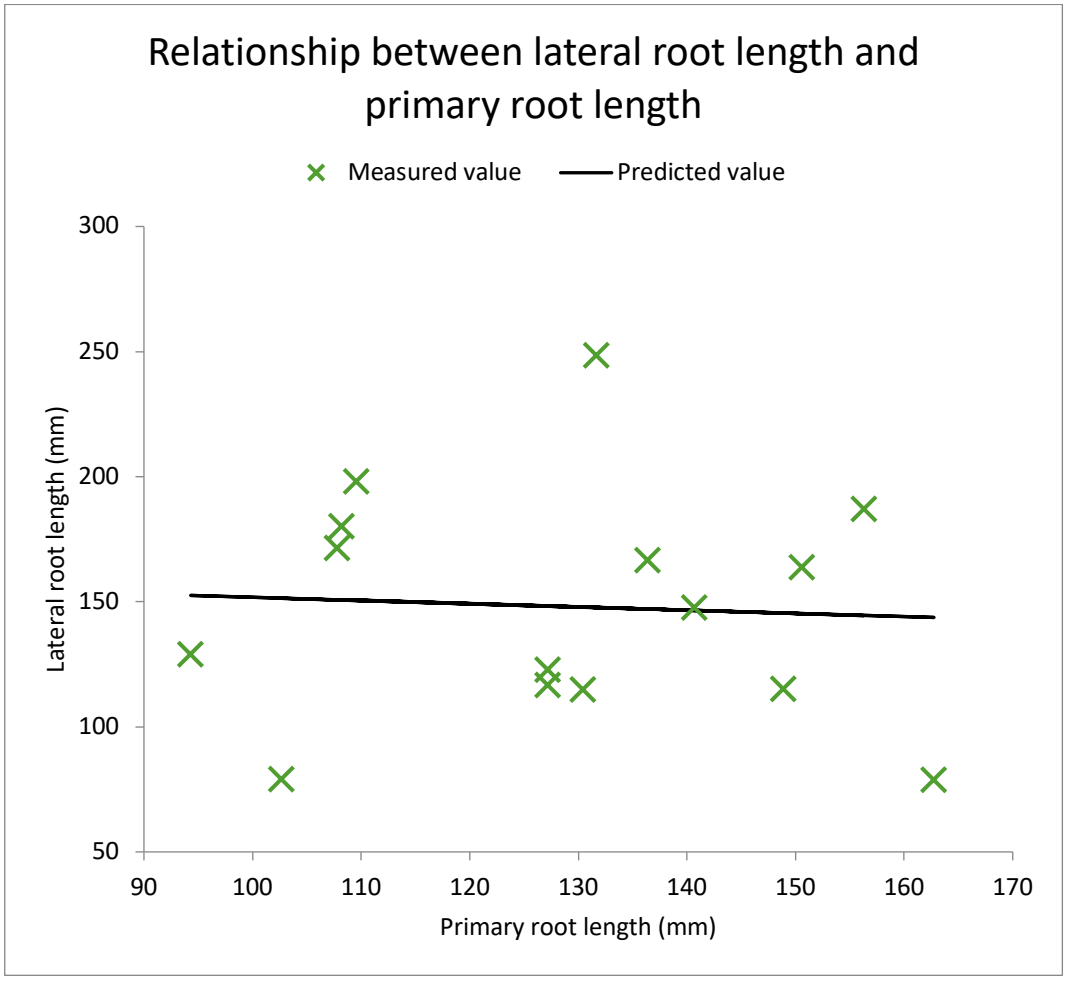

Figure 9: Regression analysis of correlation between length of lateral roots and primary root. R^2 = 0.003, P= 0,837, N = 15. The analysis shows that there is no correlation between the length of lateral roots and primary root.

5.4 Correlation between susceptibility and root architecture

In total, six regression analyses were performed. Number of lateral roots (N_{LR}), sum of lateral root length ($\sum L_{LR}$) or total root length (TRL) were put on the X-axis respectively with S22 or S31 on the Y-axis. Only one possible correlation could be found between root morphology and biomass reduction (Figure 10). The relation between TRL and S31 biomass reduction can be explained with a coefficient of determination of 28,5 percent. This low value means that other factors are also highly determining plant resistance against S31 isolate. The p-value for the model was 0,040 which is statistically significant. This means that the x-value (TRL) has a significant part in determining the y-value (S31 biomass reduction). These data suggest that a longer root system possibly results in higher tolerance against mild *V. longisporum* infection.

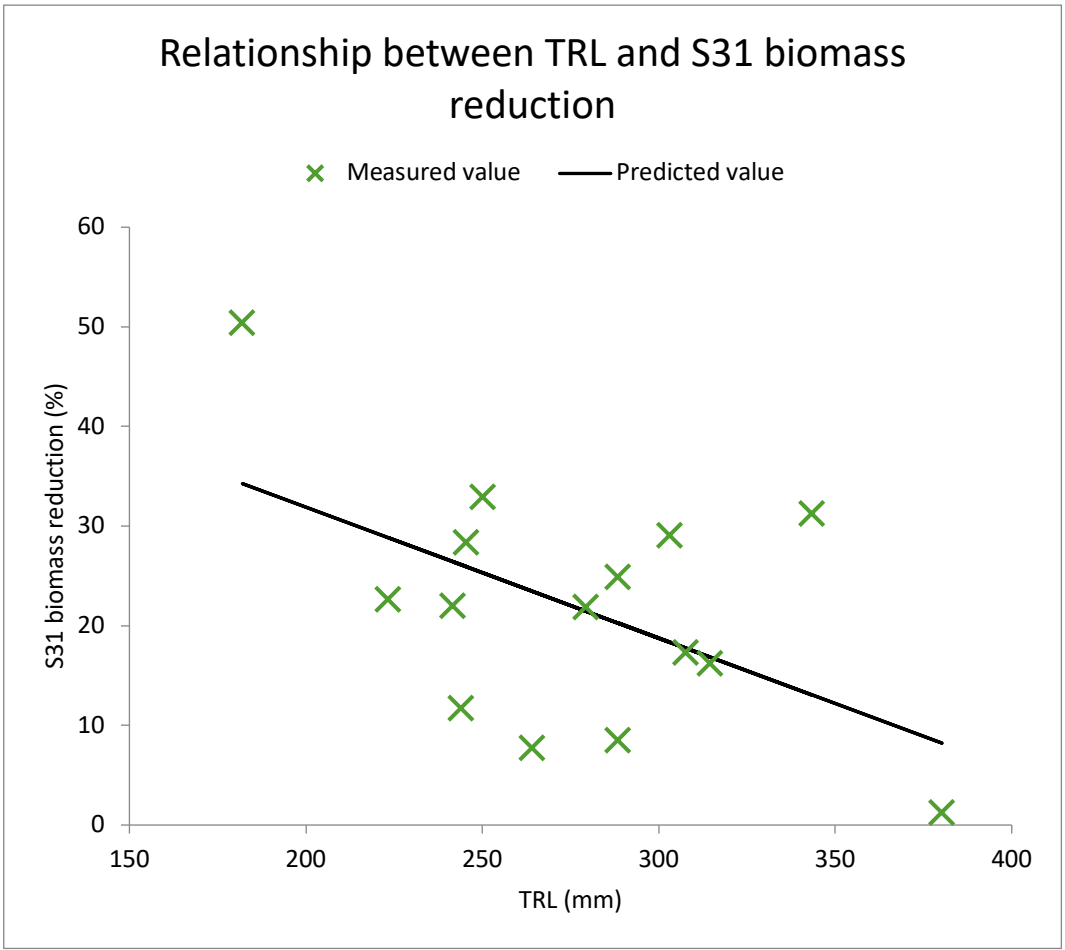

Figure 10: regression analysis of biomass reduction by S31 isolate dependent on Total root length (TRL). R^2 = 0,285, P = 0,040, N=15. The analysis shows a weak correlation between S31 biomass reduction and TRL.

6. Discussion

This study aimed to investigate differences in resistance amongst oilseed rape cultivars to *Verticillium longisporum.* In addition to these experiments, the Swedish seed and rapeseed grower association had a field trial in Östergötland that included all 14 commercial seed cultivars. The location was selected due to a high disease pressure from *V. longisporum.* These experiments in the greenhouse and laboratory can therefore be regarded as complimentary data to the field trial and future understanding of the interaction between *V. longisporum* and oilseed rape.

Three research questions were addressed: first to evaluate cultivars susceptibility to *V. longisporum* through biomass reduction and symptoms*,* secondly to correlate disease severity with root morphological traits, and thirdly to correlate tolerance with expression of plant defense genes. However, the *in-vitro* experiment to assess defense gene expression failed and no results were retrieved.

The results from the greenhouse pot experiments showed significant plant responses upon inoculation with *V. longisporum*. The results also showed that the response differed significantly when the plants were infected with the S22 or S31 V. longisporum isolates. The S22 isolate showed higher virulence than S31. Even though both isolates derived from Östergötland, Sweden, this demonstrates that isolates of this pathogen from nearby locations can exhibit significantly different levels of virulence in *B. napus*. This difference can be caused by the isolates belonging to different subclades. Another study concluded that, in some cases, isolates can be genetically diverse in small geographic areas. (Vega-Marin et al. 2025).

Some of the plants in the assays had symptoms so severe that they could be considered outliers. The reduction in biomass was so significant that the plants could potentially die from disease. In other studies, the conidia concentration used has resulted in plant mortality when the root-dipping technique was employed.(Cui et al. 2023). The same study also concluded that lower concentrations lead to lower mortality rate of plants. When using a virulent isolate as S22 it can therefore be appropriate to use lower concentrations of conidia during inoculation. All cultivars had at least one example of a plant that could have been regarded as almost dead with S22 inoculation (Figure 11).

Figure 11: Plant of Eriksen cultivar inoculated with S22. Example of how virulent the pathogen can be on certain plants.

None of the tested cultivars exhibited complete resistance to *V. longisporum* when exposed to both S22 and S31 isolates. A partial resistance can be argued to be present for Commodore, Credo, DK Expat, LG Adapt, LG Armada, Triathlon and Janosh with S31 inoculation (Table 2), (Figure 5). Compared with the mock treatment, Credo, Commodore, and Janosh showed higher biomass in the S31 treatment. Regarding a potential failure of inoculation, it should be noted that they all exhibited symptoms of dead leaves. The difference in biomass was also not significant and especially the biomass of S31 treated plants had large standard deviations. Even though the biomass differs greatly between the S22 and S31 treatments, it is possible that cultivars susceptible to S22 are also susceptible to S31. The opposite hypothesis can be made for tolerant cultivars. A regression analysis of S22 and S31 biomass reductions was performed to evaluate this; however, no correlation between the two treatments was observed.

The older spring cultivar Hanna was significantly more susceptible to S31 than all other cultivars but in line with modern cultivars for S22 (Figure 5). The comparison of Hanna with modern autumn hybrids is not entirely appropriate, as spring cultivars do not need vernalization to reach BBCH 30. Dunker et al. (2008) showed that the systemic spread of *V. longisporum* can start in earlier growth stages than flowering (BBCH 60). Comparing a cultivar which reached a different growth stage is therefore biased and should be done with caution. It is possible that breeding efforts have successfully developed tolerant cultivars against mild *V. longisporum*

infections but not upon severe ones. As earlier mentioned, this work has shown that strains of *V. longisporum* vary greatly in pathogenicity even in small local distances as also stated by Vega-Marin et al. (2025). The fact that there are no Swedish breeders of oilseed rape is a weakness for resistance breeding of these locally diverse and important pathogens.

The biomass reduction had higher variance between cultivars, upon infection with the S31 isolate (Figure 5). That may be due to the lower virulence. The root-dipping method, combined with the concentration of conidia during inoculation, may have been too harsh for the S22 isolate. Even tolerant cultivars could maybe not cope with the highly virulent pathogen in these cases. On the other hand, earlier studies showed that inoculation concentrations need to be at least 10^6 conidia/ml to get sufficient disease pressure (Depotter et al. 2017; Cui et al. 2023). Infections with the S31 isolate may have given a more representative evaluation of cultivar resistance because the symptoms are more moderate. Different spore concentrations could be tested in the future to evaluate which concentrations that show highest variance between cultivars. The optimal concentration used will be dependent on isolate because of the difference in virulence.

During the experiment three different methods for root architecture studying was performed. Two of them with the target to grow plants in pots and extract the roots at BBCH 18. At that time point, the goal was to reflect plant development during autumn under field conditions. These two methods were regarded as not being feasible to perform in this project.

The first of these two methods was to grow the plants in solely vermiculite making them easy to wash off and prepare for scanning. To ensure good plant growth nutrient containing water was added. However, due to the high porosity of the soil, much of the water and nutrients flowed through the pot and into the tray below. As a result, the plants exhibited severe symptoms of nutrient deficiencies until their roots grew below the pots and into the tray below containing nutrient rich water.

The second method was to take mock plants from the regular pot experiment at harvest and prepare these for scanning, these plants had a good vigor. Washing and cleaning these roots was time-consuming, in a scale of jeopardizing the time frame of the project. The roots that were extracted and scanned also exposed another limitation of the scanner. The large, intact root crown was not easily arranged out on a 2D flatbed scanner without roots crossing each other over and causing miscalculations in the root analyzing software Rhizovision (Seethepalli & York 2021).

The last method and the one that is presented in the result section was possible to overcome the limitations of this project. Growing plants in a gel-based medium gives nondestructive root conditions. A concern with the system is the low resemblance of soil conditions and the limitation of the available size of the growing platform (Iyer-Pascuzzi et al. 2010). Root traits have been shown to have strong correlations with genotype in a gel-based growing medium, suggesting that root traits between different cultivars of a crop can be studied in gel-based growing medium (Iyer-Pascuzzi et al. 2010). It also gives several replicates and uniform growing conditions for all cultivars is ensured. For future experiments the number of replicates can favorably be increased with the type of method used in this work to study root architecture. The time effort per replicate was around 20 minutes which was feasible, compared to several hours for the other two methods tested. The roots that did not meet the criteria for analysis was a greater loss than expected. More replicates would have given higher statistical support and in return made it possible to obtain more significant differences between cultivars. Getting more significant differences can make it possible to categorize cultivars if they focus on lateral or primary root growth, important characteristics for the research question. Some cultivars stood out and could possibly be categorized having with more replicates available (Figure 12). Intermediate allocation of resources is represented by DK Expat, primary growth allocation in Maverick and lateral growth allocation in Credo (Figure 12).

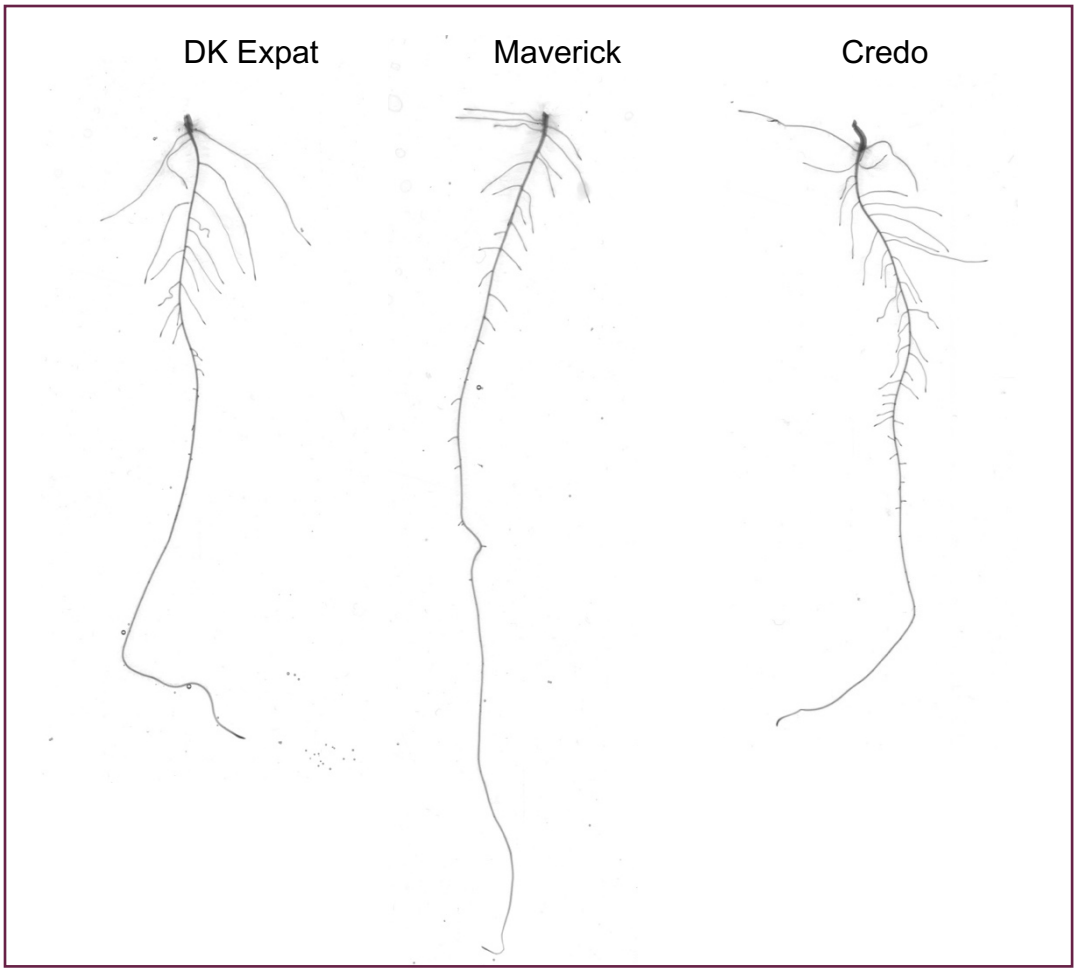

Figure 12: Scanned roots of intermediate root morphology DK Expat, primary root focus Maverick and lateral root focus Credo.

As shown in results section 5.4 there is only one potential correlation found between biomass reduction and TRL (Figure 10). It was a larger root system that was beneficial for plant tolerance against *V. longisporum.* That larger root system was beneficial for *V. longisporum* tolerance could be in contrary to another study suggests that larger root systems have a possibility to result in more severe symptoms upon fungal inoculation (Cui et al. 2023). The study tested two cultivars at different ages. One of the cultivars got more severe symptoms at later inoculation, the results were not significant. To investigate this further a larger number of cultivars should be tested to give higher statistical strength to the regression analysis.

The roots were scanned 9 days post seeding. In early plant development, at the time for root scanning, the TRL may have been affected by growth speed and emergence rate. Even if it can be debated how early root development resembles later plant growth, another study showed that a gel based system similar to the one used in this study gave indicative results for later root development of soil-grown plants

(Kupcsik et al. 2021). The development stage of plants at root scanning and fungal inoculation were the same. This gives insight to the root morphological aspects at the time point of fungal interaction. This is a strength of the work when comparing tolerance and root morphology.

Regarding future breeding of *B. napus* these results are promising. Finding a correlation between larger lengths of lateral roots and disease susceptibility would have conflicted the interest of yield promoting and N-acquisition breeding (Kupcsik et al. 2021).

7. Conclusion

Of the 15 cultivars tested in the greenhouse experiments, no genotype showed full resistance against *V. longisporum*. While some cultivars may have a higher tolerance, several trials must be conducted to confirm this. The virulence of fungal isolates can differ greatly over small geographical distances. This makes it difficult to make secure conclusions about the impact of *V. longisporum* on oilseed rape cultivation, as well as about the resistance of individual cultivars. There are differences in root morphology between cultivars, with some allocating more growth to lateral roots than others. There may be a correlation between cultivars root morphology and tolerance to *V. longisporum*, with cultivars having larger root systems being more tolerant.

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