Blackleg in winter oilseed rape

A study of the causal pathogens *Plenodomus lingam* and *Plenodomus biglobosus*

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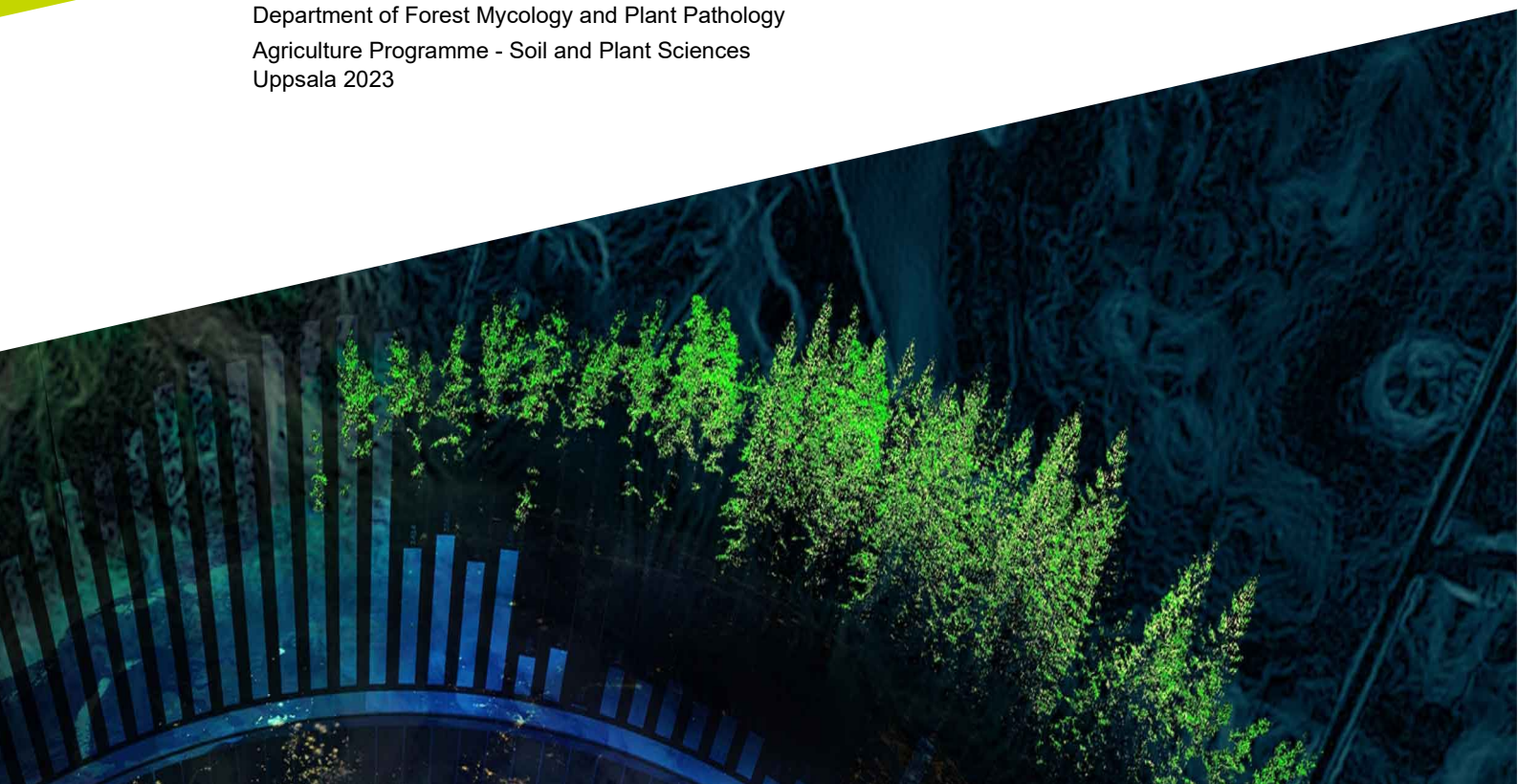

Black legin winter oilseed rape- A study of causal pathogens *Plenodomus lingam* and *Plenodomus biglobosus* in Sweden

Torröta i höstraps- En studie om orsakande patogenerna Plendomus lingam *och* Plendomus biglobosus *i Sverige*

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Abstract

Over the past two decades Swedish oilseed rape production has increased significantly, primarily due to the introduction of new and improved varities and increase in profitability. This crop has become a crucial component of both food and feed production. Climate change poses new challanges for crop production. Altough blackleg, caused by the two fungal pathogens *Plenodomus lingam* and *Plenodomus biglobosus*, is not a new disease in Sweden, it is expected to thrive in a warmer climate. The two pathogens coexist in the oilseed rape plant and share most of the life cycle, and it is the differences in their life cycles that allow them to coexist. They differ in the appearance of symptoms, with *P. lingam* causing the more severe stem canker and *P. biglobosus* causing less severe stem lesions. This study aimed to investigate the presence of *P. lingam* and *P. biglobosus* in Swedish winter oilseed rape and to investigate whether the presence of disease symptoms correlate with the molecular detection of the pathogens. Stem samples of four varieties of winter oilseed rape were collected in summer July 2023 from the Rural Economy and Agricultural Societies' field trial in Skåne. Two of the varieties carried the blackleg resistance gene *Rlm7*. Disease score was made on the cross section and the surface on the base part and on the upper part of the stem. The presence of the two pathogens were then quantified using a species-specific ddPCR assay. The results showed that both *P. lingam* and *P. biglobosus* are present in Swedish oilseed rape fields and that the prevalence differ among varieties. The results aslo showed that *P. lingam* dominates in the basepart and the upper part of the stem. The number of gene copies for *P. lingam* are equal between the upper part and the base part, and the same applies to *P. biglobosus*. This contradicts the theory that, *P. biglobosus* should mainly be found in the upper part of the stem. Further, the proportion between the two species changes between the autumn and the summer. Both in the autumn and in the summer *P.lingam* was dominating over *P.bigobosus* and was on a high level while *P. bigobosus* inceres in the summer compared to the autumn. Furhermore, high incidence and severity of blackleg in the fall negatively affects the yield.

Keywords: Blackleg, *Plenodomus lingam*, *Plenodomus biglobosus,* Phoma leaf spot, Steam canker, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*

Sammanfattning

Under de senaste två decennierna har svensk rapsproduktion ökat markant, främst tack vare introduktionen av nya och förbättrade sorter. Höstraps har blivit en viktig komponent i både livsmedels- och foderproduktionen. Klimatförändringarna innebär nya utmaningar för växtodlingen. Även om Phoma, orsakad av de två svamppatogenerna *Plenodomus lingam* och *Plenodomus biglobosus*, inte är en ny sjukdom i Sverige, förväntas den öka i ett varmare klimat. De två patogenerna samexisterar i rapsväxten och delar större delen av livscykeln, samtidigt är det skillnaderna i deras livscykler som gör att de kan samexistera. De skiljer sig åt i uppkomsten av symptom, med *P. lingam* som orsakar den mer allvarliga symptomen och kan orsaka liggraps och *P. biglobosus* som orsakar mindre allvarliga skador på stjälken. Denna studie syftade till att undersöka förekomsten av *P. lingam* och *P. biglobosus* i svensk höstraps och att undersöka om förekomsten av sjukdomar korrelerar med molekylär detektion av patogenerna. Fyra sorter av höstraps samlades in sommaren 2023 från Hushållningsällskapets fältförsök i Skåne. Två av sorterna har resistensgenen *Rlm7.* Sjukdomsgradering gjordes på tvärsnittet och ytan på basdelen och på den övre delen på stammen. Förekomst av de två patogenerna bekräftades och kvantifierades med hjälp av artspecifik ddPCR-analys. Resultatet visade att båda. *P. lingam* och *P. biglobosus* finns i svenska rapsfält och förekomsten skiljer sig åt mellan sorter. Resultatet visade även att *P. lingam* dominerar i basen och den övre delen av stammen. Samma nivå av gencopior av både *P. lingam* och *P. biglobosus* hittas i både basen och övre delen av stjälken. Vilket inte stämmer överens med teorin som säger att *P. biglobosus* huvudsakligen finns i den övre delen av stjälken. Propotionerna mellan de två arterna ändras mellan hösten och sommaren. Både på hösten och sommaren dominerade *P .lingam* över *P. bigobosus.* För *P. bigobosus* ökar förekomsten på sommaren jämfört med hösten. Resultatet visar också att hög förekomst av höstinfektion av *P. lingam* påverkar rapsskörden negativt.

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1. Introduction

Blackleg and phoma leaf spots on oilseed rape *Brassica napus* ssp. *oleifera* is caused by the closely related fungal pathogens, *Plenodomus lingam* (syn. *Leptosphaeria maculans*) and *Plenodomus biglobosus* (syn. *Leptosphearia biglobosa*). The fungus *P. lingam* is associated with stem canker symptoms in the base of the stem, where as *P. biglobosus* is generally considered as less aggressive species, which often induces lesions in the upper part of the stem (Stonard et al., 2010). Incidences with leaf spots caused by these two pathogens in Swedish winter oilseed rape (OSR) have increased in recent years. Due to the improved economy in oilseed rape production, there has been a significant increase in the area of oilseed rape cultivation. Considering the climate changes, the weather is projected to become more favourable for the pathogens and the risk of infection in winter OSR is expected to increase.

1.1 Aims and hypothesis

The aim of this thesis was to gain a better understanding of the epidemiology of the important disease blackleg and phoma leaf spot. Based on four different varieties, the importance of this disease in winter oilseed rape (OSR) was assessed. The molecular detection and quantification of the two pathogenic species were performed by analysing DNA extracted from leaves and stem samples of the four varieties using the Droplet Digital PCR (ddPCR) method. The symptoms on the stems were scored visually in both the stem base and in the upper stem part of each sample.

The following hypotheses tested in this study are as follows:

- Both *Plenodomus lingam* and *Plenodomus biglobosus* are present in Swedish oilseed rape fields and the prevalence differ between varieties.
- *Plenodomus lingam i*s primarily present in the base of the stem whereas, *Plendomus biglobosus* is mainly found in the upper part of the stem.

- The proportion of the two species changes between the autumn and the summer in OSR.
- High incidence and severity of fall-infections of blackleg negatively affects the OSR yield.

2. Background

2.1 Oilseed rape

Oilseed rape, *Brassica napus* ssp. *oleifera*, a member of the Brassicaceae family, is the world's second most important oilseed crop after soybean oil (*Worldwide oilseed production by type 222/23* 2023). The family contains 338 genera with over 3709 cultivated species worldwide (Zheng et al. 2020). Oilseed rape (OSR) has an AACC genome with 2n = 38 and is a hybrid of *Brassica oleracea,* (turnip rape, CC genome, 2n = 18) and *Brassica rapa* (cabbage, (AA genome, 2n=20). The crop was domesticated through selection by humans thousands of years ago (Obermeier et al. 2022). The primary OSR production regions of the world include Canada, Europe, China, India, and Australia (*Rapeseed Explorer* u.å.) . The distrubution of winter and spring OSR depends on the climate. Winter OSR need vernalization to start the flowering, therefore, winter OSR is mostly cultivated in Europe and Asia. Spring OSR do not require vernalization and is grown in northern parts of America, Australia and Europe. The OSR is cultivated in more than 66 countries and reached 35 million hectares. China produces one fifth of the worlds production of OSR, with an amount of 11,9 million tonnes (Zheng & Liu 2022) .

In the 1980s, new high-yielding and hybrid varieties were introduced, leading to increased yields and the opening of new markets in the food and feed sectors in these regions (Zheng et al., 2020). The OSR serves as a crucial preceding or break crop in cereal-dominant areas, playing an important role in improving soil structure and reducing the disease pressure. Low erucic acid and glucosinolate seed content have been selected for over 70 years through extensive global crossbreeding. This has resulted in a decrease in genetic diversity in modern varieties due to bottlenecks created by the selection of these traits. As a consequence, modern varieties are more vulnerable and less resistant to fungal pathogens and insects because of the decreased genetic diversity (Obermeier et al., 2022). The decline in average yields in Europe and Australia is attributed to several factors including the increase in pests and diseases, high temperatures, low precipitation and the restricted use of some chemical products like neonicotinoids, which are now considered illegal (Zheng et al. 2020). In the future, pathogen pressure is expected to increase due to a larger number of pathogens and their increased spread in the Northern Hemisphere, particularly in Northern Europe. The most significant changes in pathogen composition are predicted to occur in spring and autumn (Chaloner et al., 2021)

In Sweden the area under cultivation of OSR and turnip rape for 2022 increased by 29,000 hectares (+27 %) compared to 2021, reaching a provisional total of 135,100 hectares (*Jordbruksverket Jordbruksmarkens användning 2022*). This is the largest area covered by OSR and turnip rape since 1993. Approximately 88 % of the area is devoted to winter OSR, making it the largest oil crop. In 2023, 118,800 hectares of winter OSR are being grown, which is the largest area ever recorded (*Jordbruksverket Jordbruksmarkens användning 2022*) .

2.2 The pathogens causing blackleg

The pathogens *P. lingam* and *P. biglobosus* cause blackleg on mainly Brassica species for example *Brassica napus* (oilseed rape, canola), *B. rapa, B. rapa, B. juncea, B. oleracea,* and numerous wild crucifers species (Rouxel & Balesdent 2005). On winter OSR in Europe, North America and Australia the pathogens stand for yield losses of millions of tons (Brachaczek et al. 2021). Phoma stem canker disease also causes important damages in Europe. The two species *P. biglobosus* and *P. lingam* belong to the order Ascomycota, suborder Pezizomycotina, class Dothideomycetes and genus Pleosporales (Kaczmarek & Jedryczka 2011) Through comparative genome sequencing analysis of 19 conserved proteins, *P. lingam* and *P. biglobosus* diverged 22 million years ago (Zou et al. 2019). The analysis also shows that *P. lingam* has two subclasses Brassicae and Lepidii while *P. biglobosus* have six subclasses; Brassicae, Canadensis, Thlaspii, Erysimii, Australensis and Occiaustralensis. The most common species of *P. biglobosus* that have been detected in most OSR regions is Brassicae (Zou et al. 2019). *Plenodomus. lingam* and *P. biglobosus* frequently coexist in Europe and North America, potentially originating from a shared ancestor.

2.2.1 Disease symptoms caused by these two pathogens

In the autumn the initial symptoms occur as phoma lesion spots. These spots are caused by *P. lingam* and are characterized by pale grey colour and numerous pycnidia, whereas *P. biglobosus* has smaller spots with dark margins, a light brown center, and fewer or no pycnidia (Stonard et al., 2010). From the leaf, the spots of the more damaging *P. lingam* cause vascular tissues and grow down to the stem and lead to necrotic stem canker which can lead to lodging (Zou et al. 2019). On the other side, the less damaing *P. biglobosus* causes stem lesions that are limited to the upper stem part and does not cause stem lodging (Zou et al. 2019).

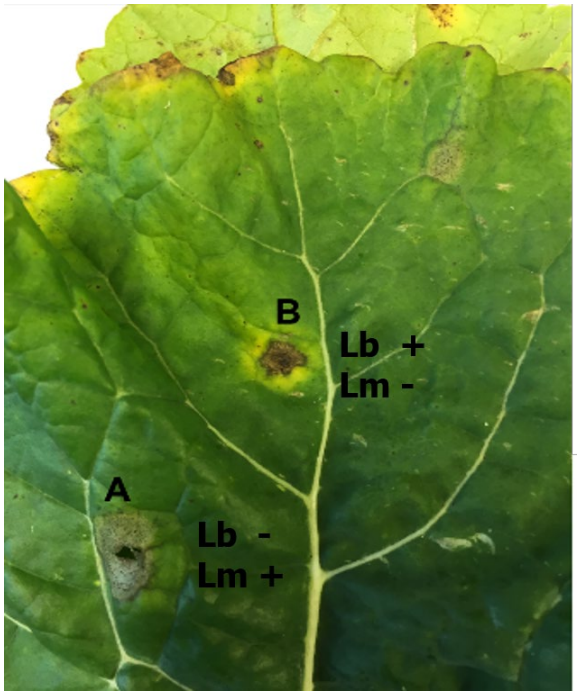

Figure 1*. The two species of phoma leaf spots. Spot A shows* P. lingam *and spot B shows* P. biglobosus *(Omer, Z & Wallenhammar, A-C. 2021).*

In the summer, stem base canker is mainly caused by the more damaging *P. lingam*, while upper stem lesions are mostly connected with the less damaging species *P. biglobosus* (Salam et al. 2007; Stonard et al., 2010). In some cases *P. biglobosus* has been found to cause stem canker in winter OSR in England (Huang et al. 2014) and in Sweden *P. biglobosus* has been detected in the stem base part (Omer & Wallenhammar 2023). In countries such as Poland, high temperatures during the summer can result in significant yield losses caused by *P. biglobosus*, which is otherwise, generally less damaging in winter OSR (Liu et al. 2014).

***Figure 2**. Phoma stem canker in OSR (Brånstrand, I. 2023).*

2.2.2 Life cycle

There are numerous similarities between the lifecycles of *P. lingam* and *P. biglobosus*. The plant infection in Australia, Canada, and Europe is primarily caused by airborne ascospores in autumn. The ascospores are produced during the sexual part of the life cycle (Figure 3). These spores are formed within fruting bodies "pseudothecia" that develop on plant residues from the previous season. Pseudothecia have the ability to persist for over four years on OSR stubble (Brachaczek et al. 2021). During the first three years, it can serve as an inoculum source. At this stage of saprophytic growth, *P. lingam* can produce the phytotoxic metabolite sirodesmin PL, a member of a class of fungal secondary metabolites known as epipolythiodioxopiperazine. *Plenodomus biglobosus* does not produce this metabolite. Sirodesmins can inhibit the growth of several microorganisms including *P. biglobosus* (Elliott et al. 2007).

Trials in both in controlled enviroment and in the fields indicate that both species can coexist on OSR during the cropping season due to different ecological niches (Brachaczek et al. 2021). One significant difference in the ecological niches is the optimum temperature and humity for pseudithecial maturation. For temperatures below 10 ˚C maturation of pseudothecia, *P. lingam* fruiting bodies mature faster than *P. biglobosus* (Kaczmarek & Jedryczka 2011). A consequense of this is the difference in realese of ascospores. In early autumn and winter, *P. lingam* releases its ascospores while *P. biglobosus* releases its ascospores later in winter/spring. This leads to the onset of epidemics at different times (Stonard et al., 2010).

After release, the ascospores can survive for up to 30 days in dry conditions at temperaturs between 5 °C and 20 °C (Kaczmarek & Jedryczka, 2011). Subsequently, the majority of spores are deposited on OSR plants within a radius of 10 km from the source (Zheng et al. 2020). Upon this deposition, ascospores germinate on OSR cotyledons and proceed to penetrate their leaves through stomata or plant wounds. A result of fungal infection is the development of large intercellular spaces between the mesophyll cells. Mycelium of *P. biglobosus* proliferates more rapidly than that of *P. lingam* within the plant (Frąc et al. 2022). The latency period is highly dependent on temperature. Experiments carried out in controlled environments have shown that temperature has an effect on the incubation time. For example, this parameter requires 5 days at 20 °C and 14 days at 8 °C (Kaczmarek & Jedryczka, 2011). The symptoms occur on the leaves as phoma lesion spots. *Plenodomus lingam* spots are characterized by a numerous pycnidia, whereas *P. biglobosus* have spots with fewer or no pycnidia (Stonard et al., 2010).

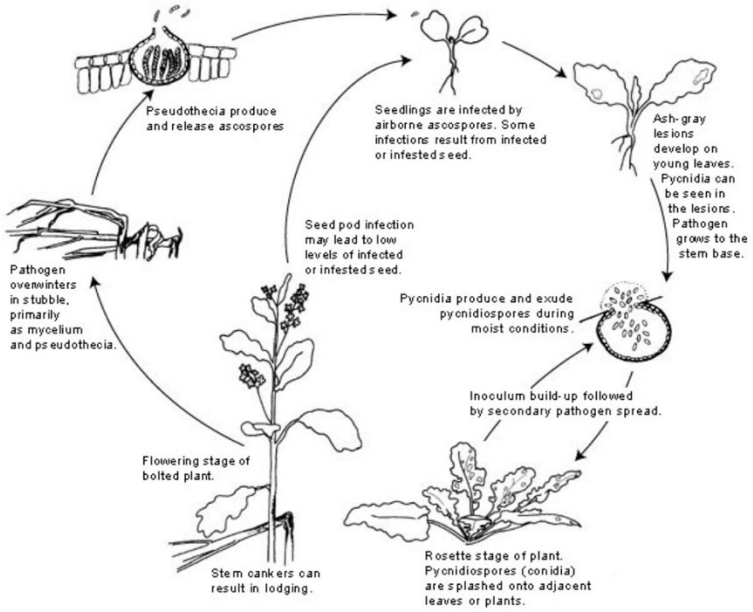

Figure 3. *An overview over the life cycle of* P. lingam *and* P. biblobosus. (Reproduced, by permission, from Ash, G. 2000. Blackleg of oilseed rape. 2000. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2000-1111-01. © The American Phytopathological Society).

Pycnidia is another type of fruiting bodies produced by the two fungal species and they contain pycnidiospores (Figure 3). This represents the asexual stage of the pathogen's life cycle, with pycnidospores serving as secondary inoculum. Pycnidospores are numerous and are transmitted short distances, between 2 to 40 cm, by rain droplets, causing infections on new leaves and in the upper stem part of OSR plants (Travadon et al. 2007). One similarity between ascospores and pycnidospores is that both can infect leaves. However, differences exist in the distance they can spread and the method of spread. Experimental data indicates that ascospores germinate more quickly than pycnidospores under identical environmental conditions. In Europe, stem canker is more closely associated with ascospores than pycnidospores (Huang et al. 2005).

The fungal pathogens spread without visible symptoms from the leaf surface to the stem's tissues. During this stage of the life cycle, the mycelium grows steadily through the veins and eventually reaches the leaf petiole, remaining latent until stem surface symptoms appear. The spots have a dark tone with grey or brown margins, and pycnidia inside may also arise from infected siliques resulting from infected stems.

The occurrence of phoma stem canker or upper stem lesions are attributed to pathogen invasion that hinders the flow of water and nutrients through the veins, causing death of host cell tissues and early maturation of infected plants. Notably, *P. lingam* is related to basal stem canker, resulting in damage to stem cortex wood

and pitch tissues and being highly destructive. Conversely, *P. biglobosus* affects the stem cortex in the upper part of the stem, causing less damage. The reason to the lesions being located at different parts of the stem is due to the the difference in timing of ascopore release (Salam et al. 2007; Stonard et al., 2010). Controlled experiments have indicated that during the flowering stage, *P. lingam* can infiltrate the roots of *B. napus* through xylem vessels. Additionally, *P. biglobosus* inoculum is capable of penetrating the roots via lateral emergence sites (Stonard, et al., 2010).

After harvest, OSR crop residues are the main source of inoculum for the following season, and the pathogen can also grow on previously uncolonized stem parts. Fruit bodies appear later on many stems, and untilled stubble is a suitable environment for the development of the generative stage (Kaczmarek & Jedryczka, 2011). Previous studies indicate that *P. lingam* can survive longer than *P. biglobosus* on buried crop residues (Fitt et al. 2006a). However, in unburied crop residues, *P. biglobosus* dominates after one year. Both species have a longer survival rate on unburied material compared to buried material. Furthermore, field trials have shown that the DNA of *P. lingam* in the top 5 cm of the soil is insignificant three years after a cropping season with OSR. During the first and second years DNA can be identified, though in a reduced quantity in the second year (Stonard, et al., 2010). Another source of inculum is seed infection, which is a potential cause of infected plants (Kaczmarek & Jedryczka, 2011)

Table 1. *The differences between* P. lingam *and* P. biglobosus.

P. lingam	***P. biglobosus***
Leaf infected with numerous of pycnicia in the autumn	Leaf has no or few of pycnicia in the autumn
Fast maturation of pseudothecia below 10 ˚C	Slow maturation of pseudothecia below 10 ˚C
Release of ascospores in autumn/winter	Release of ascospores in winter/spring
Causes the more severe base phoma stem canker	Causes less severe upper stem lesions
Survive longer on buried crop residues	Shorter surival time on buried crop residues

2.3 Control practises

There are several measures that farmers can take to manage and control the phoma leaf spotting and stem canker diseases. To reduce the incidence and severity of *P.*

lingam and *P. biglobosus* in the field, tillage, adjustments to sowing dates, reduction in plant density, chemical treatment, resistant cuktivars and nitrogen management can be implemented (Brachaczek et al., 2021).

2.3.1 Crop roation and stubble management

A four-year break between OSR crops is recommended to reduce the risk of ascospore infection from colonised residues. Growing OSR in closer crop rotations prevents residues from decomposing, causing an increase in crop residue. Results from field trials demonstrate that both rotation and tillage methods lead to a decrease in blackleg (Guo et al., 2005). A rotation including wheat, or a combination of wheat and flax reduced blackleg in both tilled and zero-tilled systems (Guo et al. 2008). Determining whether rotation or tillage have the greatest impact on phoma is difficult, but combining these methods significantly reduces the amount of blackleg inoculum (Guo et al., 2005). Tillage affects *P. lingam* survival in three ways: it promotes the decomposition and fragmentation of infected stubble. It also promotes a more favourable environment for microorganisms by burying them, thereby protecting them from surface desiccation. Moreover, this creates a less conducive environment for pathogen survival and spread when the crop residues are buried (West et al. 2001). In a zero-tillage system, a more diverse rotation has a greater impact on blackleg (Guo et al., 2005). The best approach for managing residues is to rake, bury, or burning them to reduce inoculum (Hwang et al. 2016).

2.3.2 Sowing date

The timing of sowing can have an impact on the level of pests and pathogenes affecting many crops, including OSR. If infection occurs early after emergence of the OSR plant in autumn, symptoms may be more severe at harvest (Aubertot et al., 2004). Early sowing can provide stronger protection against the release of ascospores in autumn. It should be noted that ascospores are released at specific times in different regions depending on temprature and humidity (Kaczmarek & Jedryczka 2011) (Aubertot et al., 2004).

2.3.3 Fertilizion

The application of fertiliser, predominantly nitrogen, has both positive and negative effects on the occurrence of pests and diseases. In general, a high concentration of nitrogen results in more susceptibe plant to disease. Nitrogen application in the autumn is more favourable to pathogenic organisms, more than nitrogen applied in the spring. Although the reason is not definitive, one theory is that plants fertilized with nitrogen during the autumn are more vulnerable to frost in the winter (Aubertot

et al., 2004). This increases the risk of wounds and allows the pathogen to infect the plant during spring. A nitrogen application in the autumn results in larger plants and leaves, which in turn increases the area susceptible to infection (Aubertot et al., 2004).

2.3.4 Resistant varieties

Resistant varieties are one of the most central parts of disease management. When highly susceptible varieties from China are grown in Europe, it can lead to total yield loss caused by *P. lingam* (Frąc et al. 2022). Additionally, total yield losses may occur when resistance breaks down, as was the case in Australia (Fitt et al. 2006b). The resistance is broken down due to three main factors: sexual recombination, large population size, and high gene flow through the large-scale dissemination of ascospores (Fernando et al., 2007).

Plant cell surface receptors play a crucial role in the plant's defence against pathogens by detecting pathogen-associated molecular patterns (PAMPs) and highly variable pathogen virulence (effector) proteins (Haddadi et al. 2022). Two types of resistance to *P. lingam* have been identified in *B. napus*. The first type of resistance is qualitative resistance, which acts in cotyledons and leaves after penetration of leaves by hyphae from ascospores (Huang et al., 2009). Qualitative resistance acts as a single-gene, race-specific, complete resistance and is effective if the corresponding avirulent allele is dominant in the local *P. lingam* population. Qualitative resistance can lose its effectiveness in commercial cultivars after few growing seasons due to changes in the population of *P. lingam*, which can render the resistance gene ineffective (Huang et al., 2009). Additionally, environmental factors such as temperature can also affect the effectiveness of major gene-mediated qualitative resistance.

Quantitative resistance is the second type of resistance, which acts during the growth stage between initial leaf infection and the formation of stem canker (Huang et al., 2009). Quantitative resistance is determined by multiple genes and is not race-specific, making it more durable than qualitative resistance. When selecting cultivars, those with quantitative resistance are preferred based on disease scores for stem canker obtained from field trials before harvest. Investigating quantitative resistance against *P. lingam* is challenging due to the delayed onset of the disease. However, seedlings can be screened for qualitative resistance in cotyledon tests (Huang et al., 2009).

The breeding companies mainly use major resistance (R) genes to control the incidence of stem canker caused by *P. lingam*. After recent reports of losses of major gene resistance in France and Australia the breeding companies have started

to prioritize cultivars with durable quantitative resistance, (Huang et al., 2009). To date currently, 18 (R) genes have been identified, of which 5 have been cloned for resistance to *P. lingam.* The genes *LepR3, Rlm2, Rlm4, Rlm7*, and *Rlm9* have been cloned from *B. napus* (Haddadi et al., 2022). The genes *LepR3* and *Rlm2* are Receptor Like Proteins (RLP). The genes *Rlm4* and *Rlm7* are allelic to *Rlm9* and encode Wall Associated Kinase-Like (WALK) proteins (Haddadi et al., 2022) and *Rlm4* and *Rlm7* are located on *B. napus* chromosome AO7, and are genetically linked to *Rlm9*. The *P. lingam* effectors *AvrLm4-7* and *AvrLm7* are small secreted cysteine-rich proteins encoded by a single locus, *AvrLm4-7*. A single amino acid change in *AvrLm4-7* masks recognition by *Rlm4* without affecting *Rlm7* function (Haddadi et al., 2022). The most effective gene is *Rlm7* and has been widely deployed in cultivars in Europe and it has been suggested that it is more durable than other *Rlm* genes (Mitrousia et al. 2018). Conversely, no R gene has been identified or cloned for *P. biglobosus* (Huang et al. 2022).

2.3.4.1 Resistant field trials

Field trials show that a greater quantity of *P. biglobosus* was found in both the upper and the basal stem cranking for varieties possessing the effective *Rlm7* gene (Mitrousia et al. 2018). The quantity of *P. lingam* was very low, leading to the conclusion that the stem canker was caused by *P. biglobosus.* The use of significant resistance genes to regulate *P. lingam* offers the opportunity for infection by *P. biglobosus*. Field trials show that in the third year, OSR was grown, and a high quantity of *P. lingam* was detected in a variety that possessed the effective *Rlm7*. This provides evidence that the resistance can be compromised. In this case the resistance overcomed due to sexual recombination in the fungal fruiting bodies on the OSR stubble, which leads to ascospores with higher genetic diversity (Brachaczek et al. 2021). A practical approach to managing both pathogens would be to breed new varieties with a combination of effective R genes for both *P. biglobosus* and *P. lingam* (Huang et al. 2022).

2.3.5 Fungicides

The control of blackleg can be achieved through the use of fungicides. To ensure optimal effectiveness, spraying should be performed in autumn when 10-20 % of the plants show leaf spots caused by *P. lingam*. The release of ascospores determines the ideal time for fungicide application. Spraying in autumn is particularly recommened, because the earlier the pathogens reach the stem, the more severe the stem canker will be at harvest. By spraying in autumn, the fungus can be prevented from reaching the stem. According to Huang et al. (2022), Triazole fungicides are more effective against *P. lingam* than against *P. biglobosus*. Their study shows that azole products have an effect on both *P. lingam* and *P.*

biglobosus. However, Brachaczek et al. (2021) found that *P. biglobosus* requires a higher dose of this fungicide to achieve comparable results to *P. lingam.* As a result, using a product specific to *P. lingam* may favour the growth of *P. biglobosus.* To date no fungicide resistance as been reported but decreased densitivity has emerged in Australia and western Europe against azole products (King et al.2024).

The use of a growth regulator in OSR aims to redistribute plant growth rather than reduce it. By spraying a growth regulator, the leaf stems, reducing the total leaf length, and keeping the growth point down while increasing the root neck diameter. Several studies conducted abroad and in Sweden have demonstrated these effects (Cederberg 2019). Additionally, root growth is stimulated underground, resulting in more lateral roots and a higher root weight. These parameters enhances the plant's winter hardiness (*Tillväxtreglering i höstraps* 2019). During 2018, products with growth regulating effects were registered in Sweden through reciprocal recognition. The products contain triazoles which have both a growth regulating effect on rape and a fungicidal effect. In Sweden are now Caryx (Metconazole and Mepiquat chloride, BASF) is now registered as a growth regulator while Folicur Xpert (Tebuconazole & Protioconazole, Bayer) and Orius (Tebuconazole, Nufarm) are registered as a fungicides, and they also have a growth regulatory effect (*Tillväxtreglering i höstraps* 2019). The use of these products, and especially Orius and Folicur Xpert, in autumn is one way to manage the disease.

3. Materials and methods

3.1 Samples

Stems from winter OSR plants were collected from the field trial OS27-025-2023-003, located in Simrishamn, Skåne (GPS: 55.51, 14.31), overseen by the Rural Economy and Agricultural Societies. Four different varieties of winter OSR from different breeding companies were sampled, namely Limagrain's LG Scorpion, Syngenta's SY Aliboom, NPZ's Crotora, and Dekalb's DK Plasma. Plasma and Scorpion carries the *P. lingam* resistance gene *Rlm7*, whereas Crotora does not have any known *P. lingam* resistance gene (Gunnarson, n.d.). Alibom have been reported to be vulnerable to *P. lingam* (Syngenta, 2021). The varieties LG Scorpion and DK Plasma are resistant against pod shattering while NPZ´s Crotora and Sy Aliboom are not (Gunnarsson 2021). All four varieties exhibit resistance to clubroot. The trial was left untreated with fungicides and growth regulators that affect *P. biglobosus and P. lingam*.

The trials was sampled twice, in the autumn (E. Brihall pers comm) and in the summer. In the autumn, three plants per plot were collected. The summer sample materials, consisting of fresh winter OSR stems, were collected on the 30th of June 2023. Ten stems per plot (n = 4) from each variety were collected, resulting in a total of 160 stems.

3.1.1 Disease scoring

Disease scoring was carried out on all stems from the field trial in Simrishamn and was conducted according to (*Blackleg | Canola Encyclopedia* 2023). In brief: First blackleg was scored at cross-sections of the stem at 0 cm and 15 cm from the bottom of the stem. The scoring in the cross-section part at 0-15 cm was performed in a 1-6 scale where 1 was no discoloration and 6 was complete discoloration (Table 2). Lesions symptoms on the outside of the stems were also graded in the areas between 0-10 cm and 15-25 cm from the bottom of the stem was performed in a scale of 1-5 (Table 3).

***Table 2**. The grades for the disease scoring on blackleg in the cross section part at 0 cm and 15 cm from the bottom of the stem.*

Grade	Discoluration %
1	0
2	>25
3	25-50
4	51-75
5	<75
6	100

***Table 3**. The grades for the disease scoring on stem canker in the areas between 0-10 cm and 15-25 cm from the bottom of the stem.*

Grade	Canker on circumference
1	No symptoms
2	Weakly developed
3	Less then half circumference
4	More then half circumference
5	Almost the the whole circumference

Results of disease score on individual plants collected in the fall was performed by E. Brihall (pers comm.) from the same location. For each variety three plants in four plots per variety (in total 12 plants) were graded. The first step was to count and record all *P. lingam* spots, followed by the counting and recording of all other spots. Due to the difficulty in visually identifying *P. biglobosus* leaf spots, all other spots that were not classified as *P. lingam* were grouped as 'other spots'. Leaf counting was done in a chronological order, starting from the oldest first true leaf to the youngest leaf, and only spots on the upper side of the leaves were counted. Senescent leaves were recorded, while yellow and rotten leaves were not scored. E. Brihall performed the disease score on the variety LG Scorpion, SY Aliboom, Crotora, DK Plasma.

3.1.2 Disease scoring field

To assess the overall disease pressure in the field, results of field assessment were collected by Carl. Blackert, HS Halland from the sampled and additional field trials OS27-025-2023-001 located in Nyhem, Skåne (GPS: 55.88, 13.05), OS27-025-2023-002 located in Bollerup, Skåne (GPS: 55.49, 14.03) and OS27-025-2023-003, located in Simrishamn, Skåne (GPS: 55.51, 14.31). The coordinates for the fourth field trial are missing but the field trial is located in Rosenhäll and is identical as the three other field trials. The disease assessment was conducted in the autumn 2022 according to Bousset et al. (2015), and reported as spots of *P. lingam*/ m^2/*min.*

The disease score was performed on the varities LG Scorpion, SY Aliboom, Crotora, DK Plasma and a variety mix with the varieties Atora, Dk Expansion H, Artemis (LE 16/316) and Smaragd H. For each field trial there was four blocks of each variety that were tested.

The field trial OS27-025-2023-003, located in Simrishamn, Skåne (GPS: 55.51, 14.31) was harvested 2023-08-12 (*Nordic Field Trial System - Försöksdokumentation: OS7-025-2023-003. Höstraps. Sortförsök resis. mot klumprotsjuka* 2023.) The yield result for OS27-025-2023-001 and OS27-025-2023-002 are not avialble.

3.2 Molecular analysis

3.2.1 DNA extraction

Following disease scoring, the stems from Simrishamn were separated into two parts, the base part (0-10 cm) and the upper part (15-25 cm). Both the upper part and the base part were cutted into 1-2 cm pieces and placed into either 25 or 50 ml Falcon tubes depending on the amount of material. The stem samples were freeze-dried for three to four days, and then ground to a powder using a grinder until of 2-5 mm pieces. The samples were returned to Falcon tubes and were stored at room temperature until DNA extraction.

For each sample, 30 mg tissue of each was placed in an Eppendorf tube with six to ten of both 3 mm and 2 mm glass beads and a small spoonful of ceramics beads.

DNA was extracted using the NucleoMagR Plant kit according to the manufacturer's guidelines, with some modifications. Specifically, 800 µl of lysis buffer (Buffer MC1) was used instead of 500 µl, and the samples were only extracted once instead of twice. Instead of adding 80 µl of MC6, 100 µl was added to column 6 in the Maelstrom plate. After DNA extraction, the DNA concentration was measured, and the samples were placed in the -20 °C.

3.2.2 Droplet digital PCR (ddPCR)

The optimal DNA concentration was tested in ddPCR analysis before analyzing all the samples. The DNA was diluted to 5 and 20 ng μl^{-1} and finally, DNA from samples was diluted with ddH_2O to the concentration of 10 ng μl^{-1} . DNA in samples with a concentration <10 ng μl^{-1} was not diluted. DNA from one *P. lingam* isolate (1-0,001 ng μl^{-1}) and one *P. biglobosus* isolate (1-0,001 ng μl^{-1}) was included in each reaction as a positive control and ddH_2O was used as negative control. Droplet

Digital PCR was performed with the Bio-Rad system according to Adam (2020). Each assay was performed seperately using CFX real-time machine (*Droplet Digital PCR (ddPCR) – Bio-Rad 2023*).The occurrence of the *P. lingam* and *P. biglobosus* DNA in each sample was analysed in separate reactions. The reaction mix contained: 5.5 µl supermix, 1.1 µl each of forward and reverse primers and 0.55 µl of probe (Jacques et al. 2021, Table 4), 3.75 µl MQ water and 10 µl DNA. Two technical replicates were prepared for each sample The ddPCR methodology comprises a series of steps, the initial stage of which is a pre-PCR. The samples were placed into Automated Droplet Generator from BioRad which uses specially developed reagents and microfluidics to partition each sample into 20000 nl-sixed droplets. Target of interest and background are distributed randomly into the droplets during the partitioning process (*Droplet Digital PCR (ddPCR) – Bio-Rad 2023*). The second step is the PCR reaction, performed with the PCR machine Applied Biosysems ProFlex (Thermo Fisher). PCR perfromed with the following reaction conditions; 95 °C for 10 minutes (1x); 95°C for 15 seconds then 60°C for *P. lingam* and 62 °C for *P. biglobosus* for 1 minute (40x); 98°C for 10 min (1x); 4 °C (∞). The third step was the post-PCR, Droplet Reading. The nucleic acid targets were amplified using PCR in droplets, which were then analysed individually using a two-colour detection system in the QX200 Droplet Reader. The droplet reader's autosampler picks up each droplet from the PCR plate's wells, and the droplets are spaced out individually for fluorescence reading. Fluorescence in two channels is then measured for each individual droplet. Positive droplets which contain at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets (*Droplet Digital PCR (ddPCR) – Bio-Rad 2023*).

Then, for ddPCR Data Analysis, the ddPCR data was viewed in a 1-D plot with each droplet from the sample plotted on a graph of flourucence intensity and droplet number *Droplet Digital PCR (ddPCR) – Bio-Rad 2023*). A manual threshold line was set at 6000 to differentiate between the positive and negative droplets.

The number of respective gene copies of each of the fungal species was calculated according to the following. First the number of gene copies was multiplied with 22 (total volyme per reaction) and divided with 10 (the DNA volume) to get gene copies per reaction (gene copies μl^{-1} in each reaction). The avarage gene copies per reaction was calculated of the two technical replicates for each sample. Gene copies per DNA concentration (measured) was calculated, gene copies per reaction divided with the diluted DNA concentration 10 ng μl^{-1} the result was multiplied with the measured DNA concentration from the DNA extraction for each sample. The value of gene copies per DNA concentration was multiplied with the measured weight for each sample to get gene copies per gram sampled tissue.

Table 4. *Sequences of* P. lingam *'brassicae' (LMB) and* P. biglobosus *'brassicae' (LBB) specific primers and probes (Jacques, et al. 2021).*

Assay	Primer	Sequence (5ˡ-3ˡ)	Annealing tempreture °C
LMB	LMB_EF1_F5	TGGACACTTCTTCTTGACAA	60
	LMB_EF1_R5	GGTCTCGAACTTCCAGAG	
	LMB_P	TACCACGTTCACGCTCGGCC	
LBB	LBB_ACT_F1	TTGAGAGCGGTGGCATCCA	62
	LBB_ACT_R2	CACCAGACTGTGTCTTTGTC	
	LBB_P	ACGATGTTGCCGTAGAGGTCTTTC	

3.3 Statistical analysis

To investigate if there was a relationship between the visual and molecular disease detection, a linear regression analysis was performed and the best best-fitting linear equation and the R^2 value describing the proportion of the variance in the variables were calculated. To visualize the differences in presence of the two pathogens in autumn and in summer and in the upper part and the base part for the four varieties included, a box-plot diagram was produced.

For the autumn leaf samples, gene copies of *P. lingam* and *P. biglobosus* were plotted against spots of *P. lingam* or "other spots". T-tests were also performed in R-Studio, and a p-value was presented.

To assess the correlation between disease incidence and yield, scatter plots were made and R^2 value describing the correlation between the yield and precence of *P. lingam* gene copies in the autumn and in the summer and also the colleration between the yield and *P .lingam* spots in the autumn.

4. Results

4.1 Disease severity

The disease score of the leaf spots on leaves sampled in autumn differed between the four varieties in the field trial in Simrishamn (Figure 4). Plasma and Scoripon, the two varieties carrying the plant resistance gene R*lm7*, also had the lowest number of *P. lingam* spots. The value of ”other spots” is higher than that of *P. lingam* for each variety.

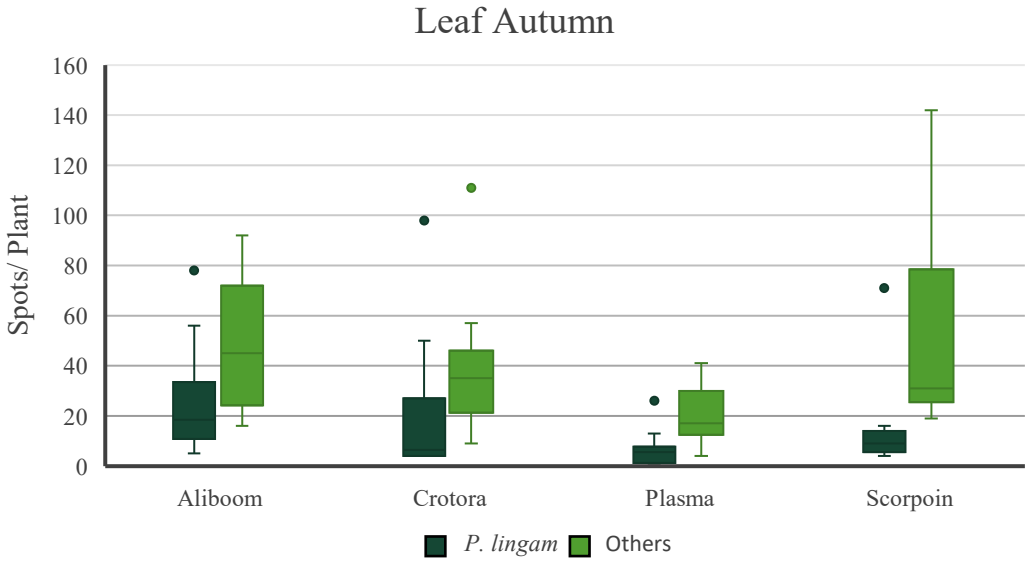

Figure 4. *Disease scores for leaf samples collected in autumn 2022, graded by E.Brihall. The x-axis displays the varieties Aliboom, Crotora, Plasma, and Scorpion. The y-axis displays the spot values, with dark green representing the value of* P. lingam *spots and light green representing the value of “other spots”.*

The results of the field disease scoring in the fall assessed as spots on OSR leaves in the Simrishamn field trial shows that Aliboom had the highest value of *P. lingam* spots, while the other three varieties had the same value as the variety mix (Figure 5). The results from the disease score for the total four field trials *Nyhem, Bollerup, Skåne and Rosenhäll* showed that the highest average number of spots were reproted from Aliboom and Scorpion, while the lowest number of spots were recorded on Plasma (Figure 6).

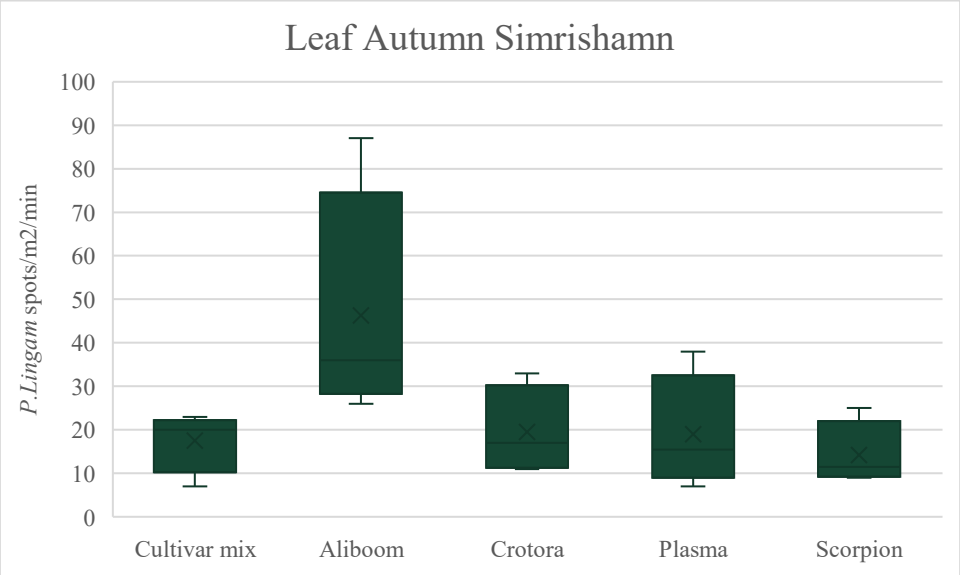

Figure 5. *Field disease assessment score conducted by C. Blackert, HS Halland, in autumn 2022. The x-axis displays the varieties Aliboom, Crotora, Plasma, and Scorpion, as well as the results for a reference in the form of a cultivar mixture including four different varieties. The y-axis displays the count of* P. lingam *spots/m²/min. The standard deviation for the cultivars are as follows: Cultivar mix 7,1, Aliboom 27,6, Crotora 10,3, Plasma 13,3, and Scorpion 7,4,*

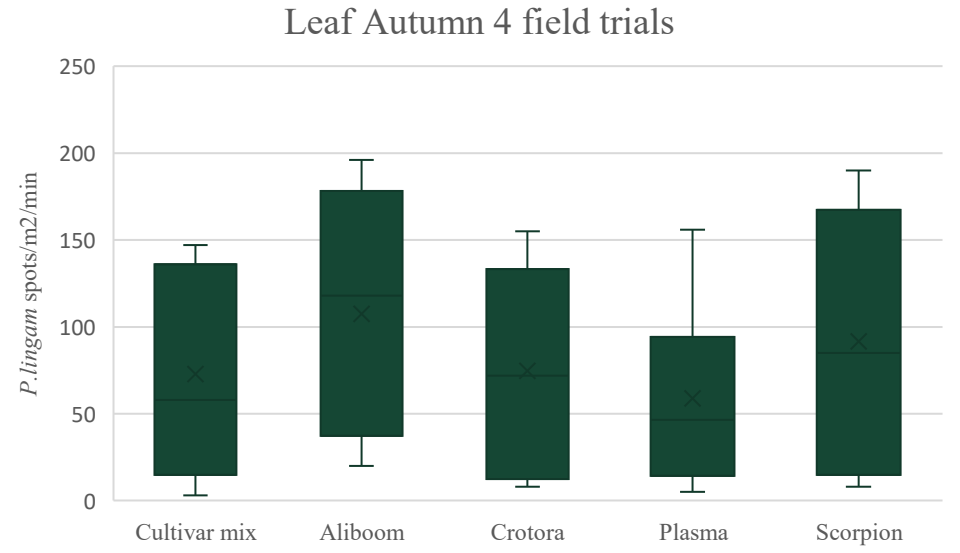

Figure 6. *Disease field assessment score by C. Blackert, HS Halland, from four field trials within the same series in Skåne in autumn 2022 for the disease score field. The x-axis shows the varieties Aliboom, Crotora, Plasma, Scorpion, and a reference in the form of a cultivar mixture including four different varieties. The y-axis shows the count* of P. lingam *spots/m²/min.. The standard deviation for the cultivars are as follows: Cultivar mix 61,0, Aliboom 70,4, Crotora 61,0, Plasma 51,7, and Scorpion 78,1,*

The results of the disease scores for the stem samples shows that the scores for both cross-section and surface are higher in the base part compared to the upper part of the stem for all four varieties (Figure 7). Additionally, all four varieties have lower scores for cross section compared to surface in the upper part. The score for disease on the surface is higher compared to the cross section in both the upper part and the base part for all varieties, except for Aliboom.

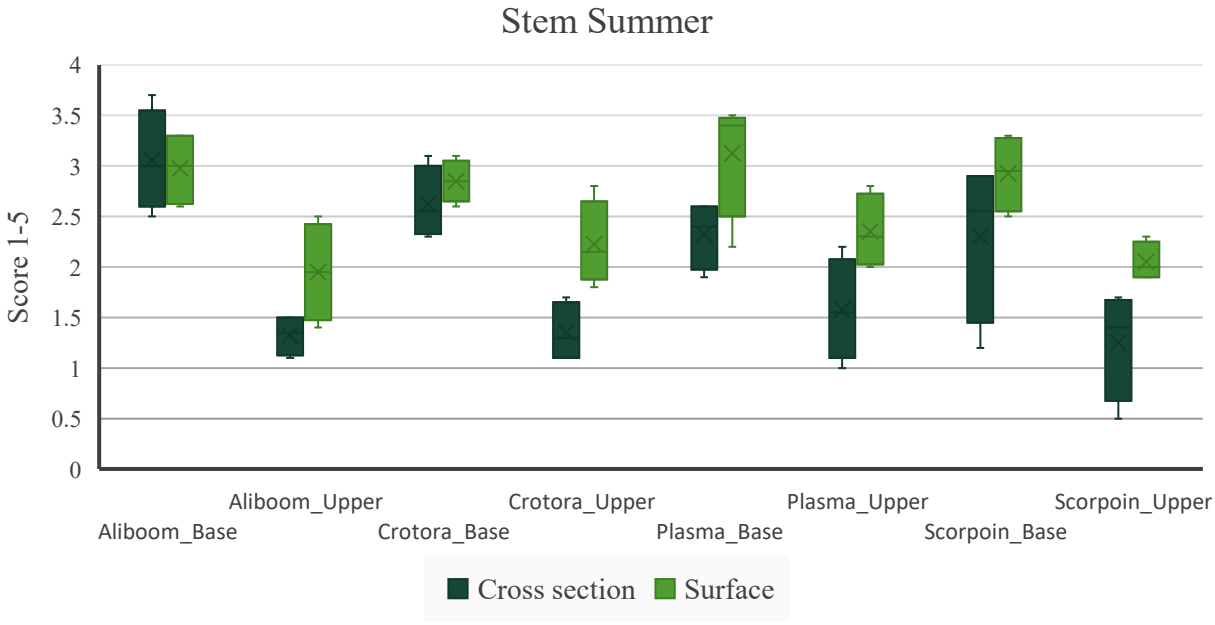

***Figure 7**. Disease score for the samples collected in summer 2023. The y-axis displays the disease score for each variety: Aliboom, Crotora, Plasma, and Scorpion. The average disease score for each variety is based on a total of 40 stems and 40 samples for both the upper and the base parts. Dark green indicating the score for the cross-section part and light green indicating the score for the surface.*

4.2 Molecular detection of *P. biglobosus* and *P. lingam*

The results for the gene copies detection for *P. biglobosus* and *P. lingam* shows that only Aliboom had samples where *P. biglobosus* and *P. lingam* were not detected in leaf samples, DNA from the pathogens were detected in 92 % of the samples (Appenix 1) . In the individual stem samples, all the variety samples were found to have no detection of *P. biglobosus*. Cortora and Alibom had DNA from the pathogens and were detected in 92 % of the samples, while Scorpion had 84 % and Plasma had 96 % of the samples. For *P. lingam* and the individual stem samples, the Scorpion, Plasma, and Aliborn varieties no *P. lingam* was detected. For Scorpion and Alibom, the fungus was dedtected in 92 % of the samples, while for Plasma the funges was detected in 96 % of the samples and 100 % for Cortora.

4.3 *P. biglobosus* and *P. lingam* gene copies per gram

The results of the gene copies of *P. biglobosus* and *P. lingam* of the stem and the leaf samples show that for all four varieties, the value of *P. biglobosus* for the leaf samples collected in the autumn is lower than that for the stem samples collected in the following summer (Figure 8). Overall, Plasma is the variety with the lowest level of *P. lingam* disease detection infection.

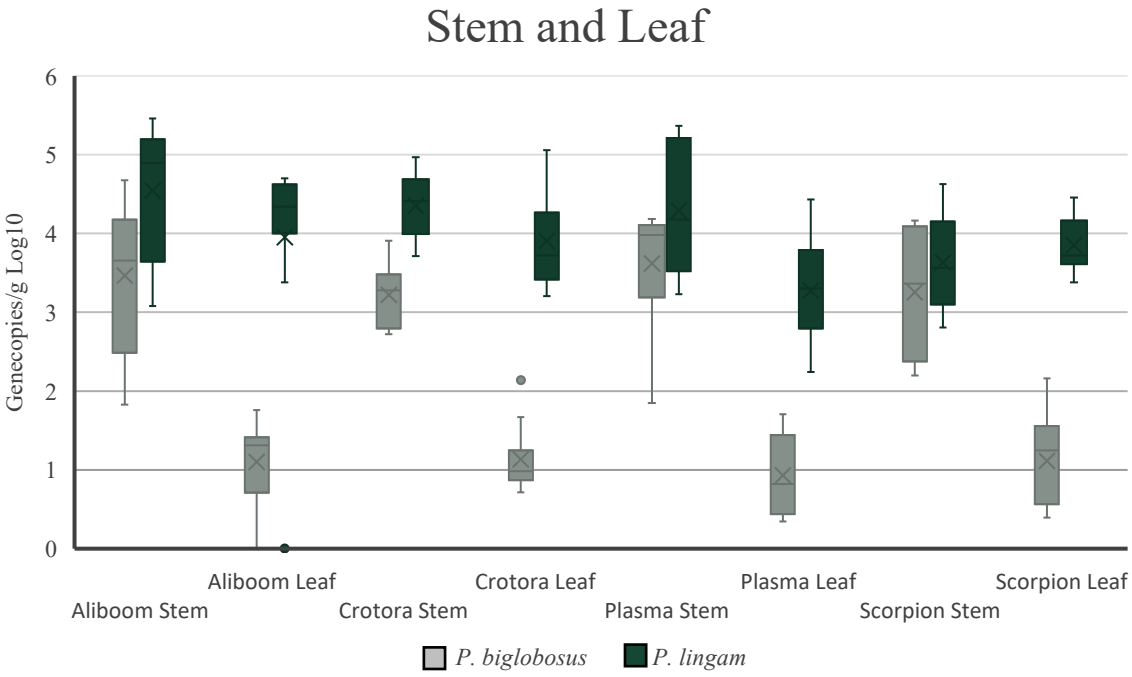

***Figure 8**. Log gene copies per gram OSR tissue for* P. lingam *and* P. biglobosus *for each variety, as well as the leaf sample results collected in autumn 2022 and the values for both the upper and the base parts of the stem samples collected in summer 2023. The stem samples includes all 40 stems, 40 base samples, and 40 upper samples for each varity. Light gray represents the value of* P. biglobosus*, while dark gray represents the result of* P. lingam*.*

The number of gene copies per gram plant tissue of *P. biglobosus* and *P. lingam* for the stem samples for both the upper and the base part dispalys that *P. lingam* dominates over *P. biglobosus* in the whole stem in all four varieties (Figure 9). Additionally, the levels of both *P. lingam* and *P. biglobosus* are higher in the base part compared to the upper part. In both the base part and the upper part, Aliboom,

Crotora, and Plasma have the highest number of *P. lingam*, while Scorpion has the lowest.

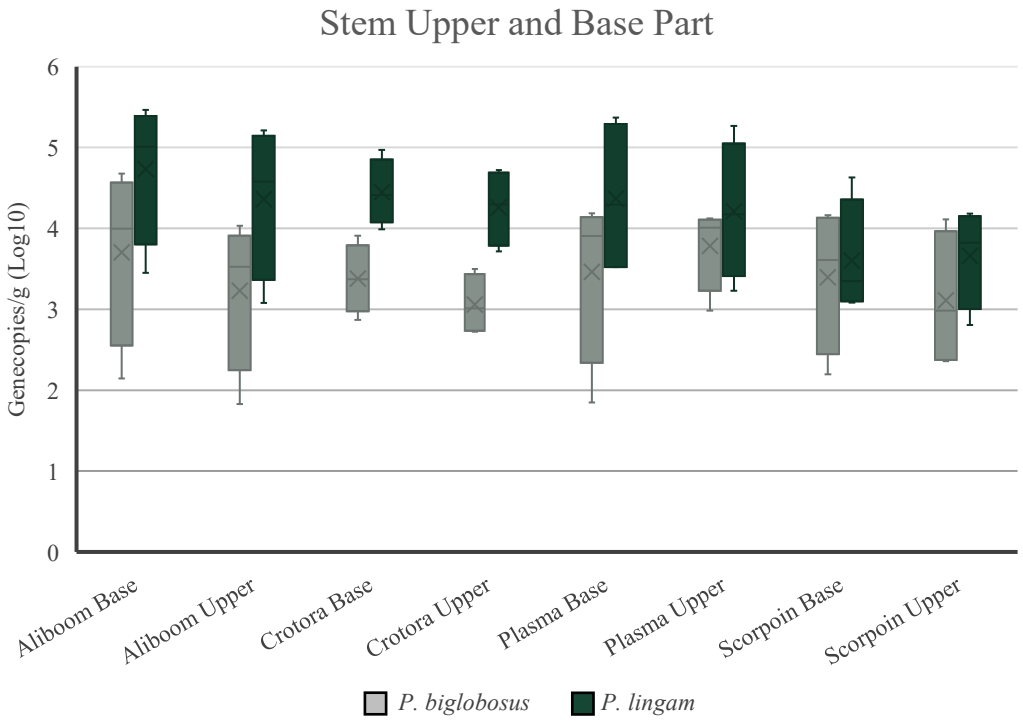

***Figure 9**. Log gene copies per g tissue for* P. lingam *and* P. biglobosus*. The x-axis displays each variety and the average result for the upper and the base of the stem samples collected in summer 2023.The y-axis shows gene copies per gram log10. Light gray represents the value of* P. biglobosus*, and dark gray represents the result of* P. lingam*.*

4.4 Autumn leaf disease score gene copies

The results for *P. biglobosus* gene copies per gram all varieties against "other spots "per plant shows that the R^2-value is 0.38 (Apendix 1). The number of gene copies of *P. lingam* for all varities against spots per plant of *P .lingam* have a R^2-value of 0.68 (Apendix 1).

The results indicate that all four correlations are significantly lower than p-0.05 (Figure 10). The number of gene copies of *P. lingam* is correlated with both the number of *P. lingam* leaf spots and 'other spots', lower then p-0.05 for both correlations. The number of gene copies for P .*biglobosus* have a correlation with both *P. lingam* spots and "other spots", lower then p-0.05 for both correlations.

The results for each variety for *P. biglobosus* gene copies per gram against "other spots" per plant shows that R^2−value for Crotora is 0,79, Scorpion 0,42, Plasma 0,22 and Aliboom 0,03 (Apendix 1). The results for each variety for *P .lingam* gene copies per gram against *P. lingam* spots per plant shows that the R^2-value for

Crotora is 0,96, Plsama 0,87, Scorpion 0,63 and Aliboom 0,23 (Apendix 1). This shows that Crotora hasthe best correlation for both spieces and Aliboom have the lowest correlation.

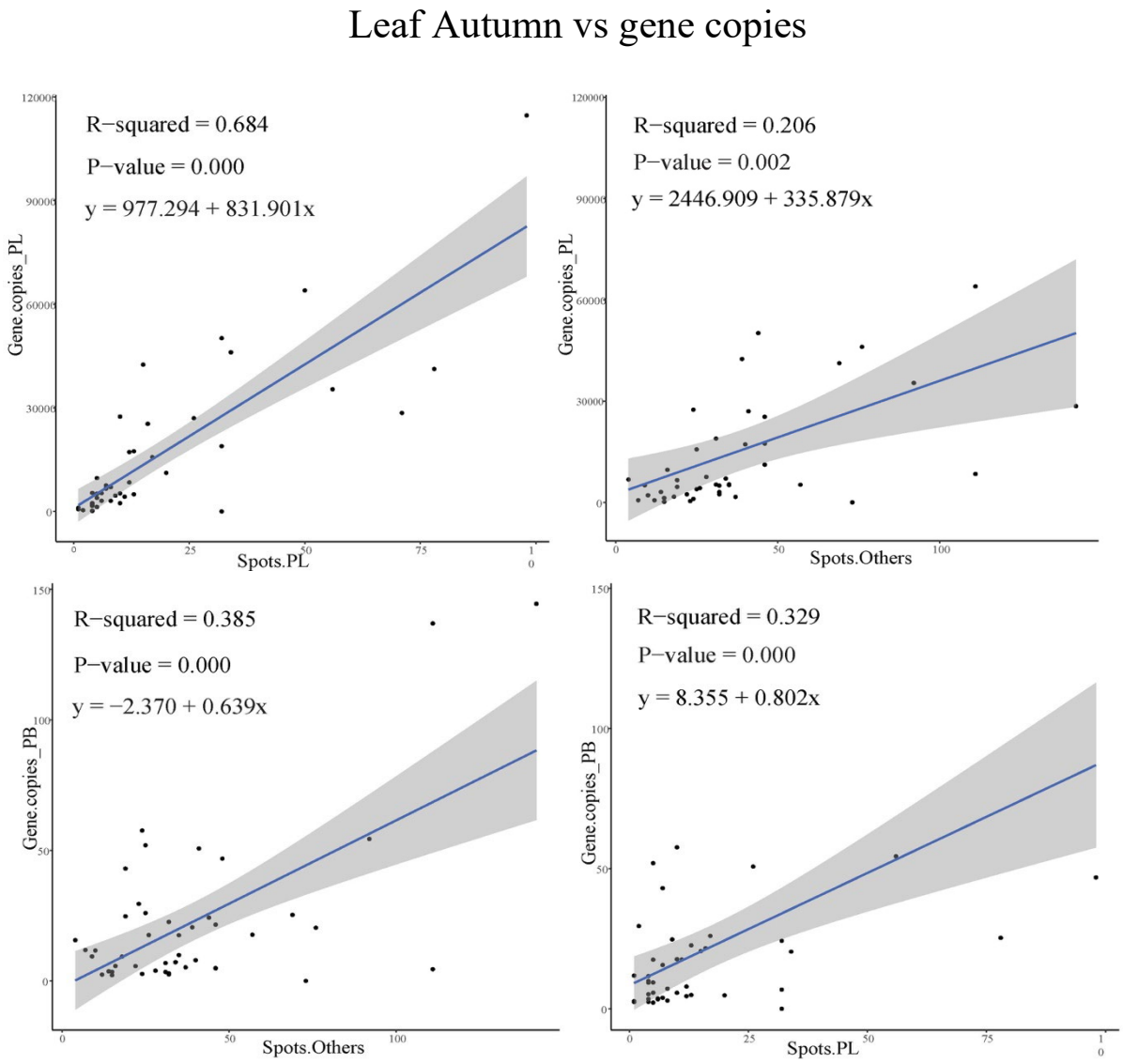

***Figure 10**. Log gene copies per gram plant tissue for* P. biglobosus *and* P. lingam *compared with the disease score of the leaf autumn samples collected in autumn 2022. The y-axis shows the result of gene copies per gram and the x-axis displays the disease score of "other spots or spots of* P. lingam*. The R^2-value, p.value are represented in the digram.*

4.5 Summer disease scores correlates with number of gene copies

The results from the disease score for cross section against number of gene copies shows that *P. biglobosus* have a R^2-value of 0,03 while *P. lingam* have a R^2-value of 0,01 (Apendix 1). The results for each variety for *P. biglobosus* shows that R^2-value for Crotora is 0,16, Plasma 0.05, Aliboom 0,02 and Scorpion 0,01. The result for each varity for *P. lingam* in Figure 16 shows that the R^2-value for Scorpion 0,16, Aliboom 0,07, Crotora is 0,05 and Aliboom 0,01. This shows that for *P. biglobosus* Crotora has the best R^2-value while for *P. lingam* Scopion has the best R^2-value.

4.6 Summer disease score cross section gene copies upper part and base part

The results from the disease score of the cross section against gene copies for the upper part and the base part shows that for *P. biglobosus* the R^2-value for the base part collection 0,38, the base part individual 0,05, the upper part individual and the upper part collection 0,048 and 0,044 (Apendix 1). For *P. lingam* the R^2-value for the upper part inividual is 0,09, the upper part collection is 0,03, the base part collection 0,02 and the basepart inividual is 0,0009 .

4.7 Yield result 2023

The yield results from the field trail shows that the variety mix has a relative number on 100 %, Scoprion and Plasma have a relative number on 111 % while Aliboom and Crotora have a relative number on 70 % and 80 % (Figure 11). The results from gene copies of *P. lingam* leaf plotted against the yield, the R^2-value is 0.94 (Figure 12). The results from gene copies of *P. lingam* stem plotted against the yield, the R^2-value is 0.23 (Figure 13). The results from *P. lingam* spots autumn leaf sample plotted against the yield, the R^2-value is 0.61 (Figure 14).

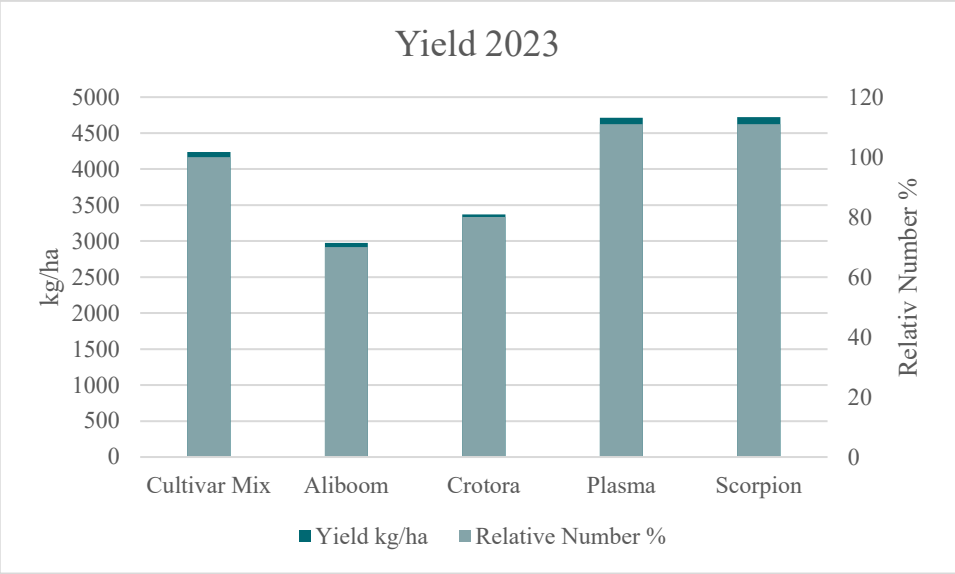

Figure 11. *Yield for 2023 for the field trial Simrishamn. The y-axis to the left shows the yield in kg/ha the y-axis to the right shows the relative number in procent. The x-axis shows each varity Aliboom, Crotora, Plasma and Scorpio and a varietymix as reference.*

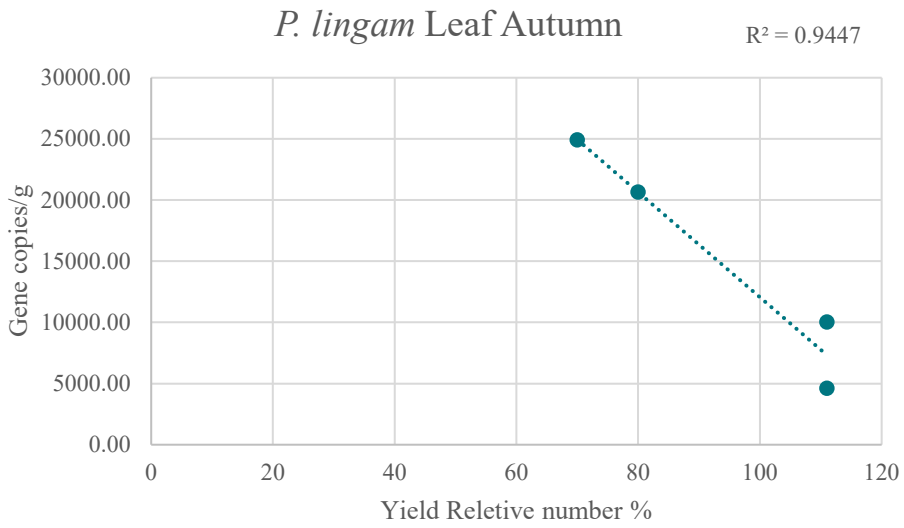

Figure 12. *Correlation between yield for 2023 for the field trial Simrishamn and the number of gene copies per gram of* P. lingam *leaf samples collected in autumn 2022.*

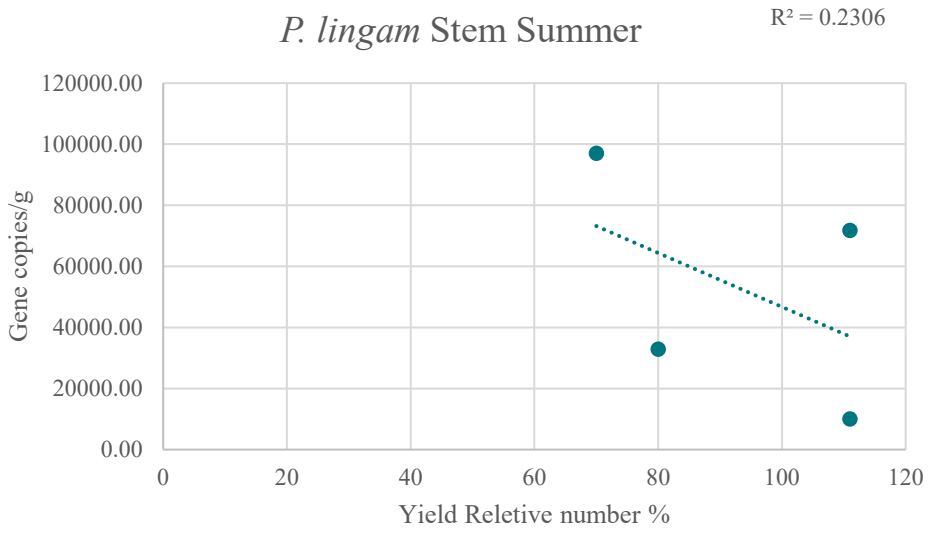

***Figure 13**. Correlation between yield for 2023 for the field trial Simrishamn and number of gene copies per gram of* P. lingam *stem samples. The y-axis shows the average value of the gencopies per gram of* P. lingam *for the four varities of the stem sample. The x-axis shows the yield 2023 in relative number % for the four varities. In the right upper corner the R^2-value is represented and there is also a correlation line in the diagram.*

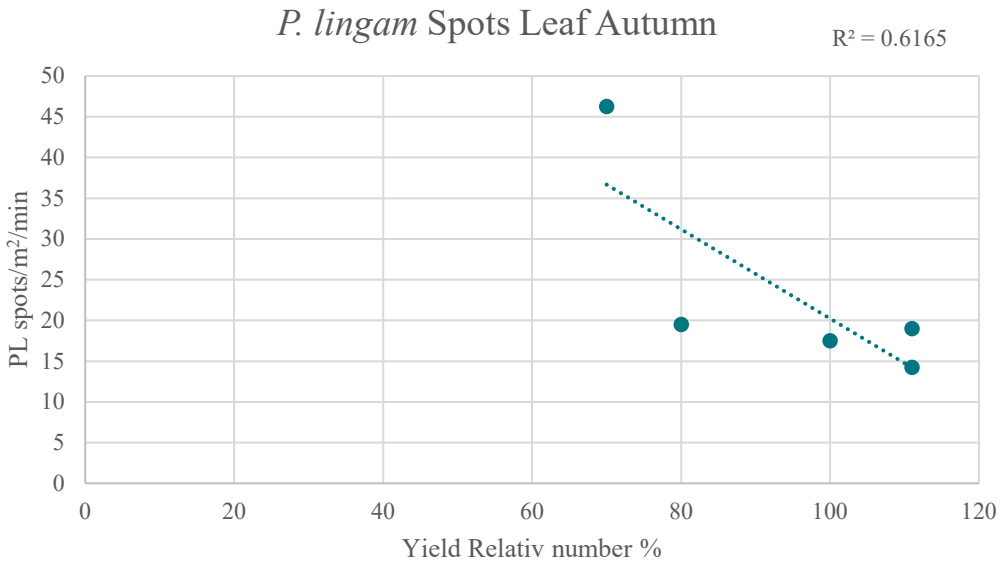

***Figure 14**. Correlation between yield for 2023 for the field trial Simrishamn and spots of* P. lingam *(spots/m^2/min) on leaf in autumn 2022 graded by C. Blackert.*

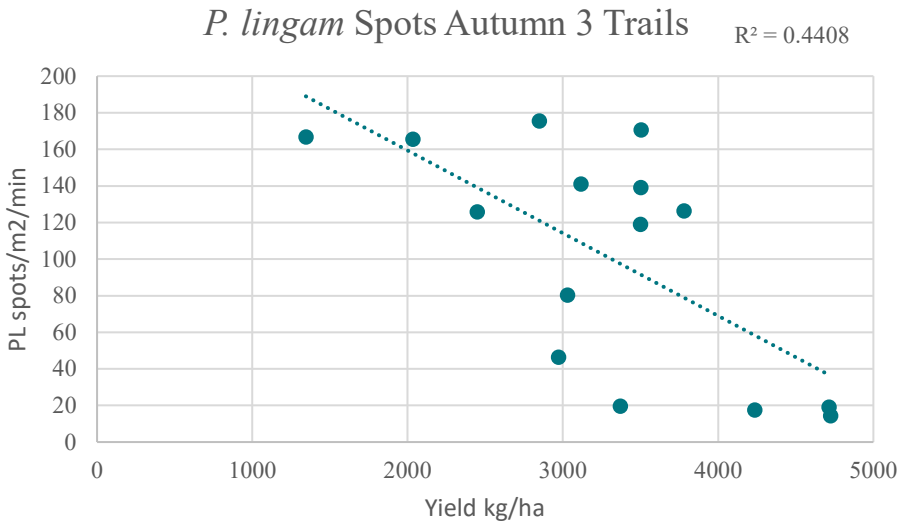

Figure 15. *Correlation between yield for 2023 for the field trial Simrishamn, Rosenhäll and Nyhem and spots of* P. lingam (*spots/m²/min) on leaf in autumn 2022 graded by C. Blackert.*

5. Discussion

This study found that both *P. lingam* and *P. biglobosus* are present in Swedish OSR fields, with *P. lingam* dominating in ´both the base and the upper part of the stem, contradicting expectations. Both species coexist, with *P. lingam* consistently more prevalent in both autumn and summer. High blackleg incidence in the autumn seems to affect yield negatively, but the storm Hans' impact on harvest results adds uncertainty to this conclusion.

During this work, various methods were employed. During the initial stage, the disease score was easier to perform on the cross section compared to the stem. The symptoms on the stem were unclear and diffuse, making the disease score less reliable. The stem samples were ground to a powder using a grinder until they were 2-5 mm in size, which resulted in varying outcomes. The primary aim was to achieve a fine powder with minimal larger pieces. The outcome was dependent on the quantity of material used and the thickness of the stem.The primary advantage is that digital droplet PCR (ddPCR) was used instead of polymerase chain reaction (PCR). Both methods were used to detect specific DNA sequences, but they differ in several ways. ddPCR is a quantitative method that divides the sample into small droplets and performs PCR in each individual droplet, providing absolute quantification and determining the exact concentration of target DNA in the sample. In contrast, PCR is a method used to exponentially amplify a specific DNA sequence. This provides a relative quantification, allowing for comparison between different samples, but not an exact value for the target DNA. ddPCR is even more sensitive and can detect lower levels of DNA with higher precision compared to PCR (*Comparison of digital PCR methods* u.å.).

The first hypothesis was that both *P. biglobosus* and *P. lingam* are present in Swedish winter OSR fields, and their prevalence differs between varieties. Several results support this hypothesis; (Omer & Wallenhammar 2020, Kuusk et al. 2002) the ddPCR (Figure 8) shows the existence of both *P. biglobosus* and *P. lingam* in Sweden during both autumn and summer. This indicates that both spieces are present in the autumn in Sweden. For the summer samples, both *P. biglobosus* and *P. lingam* where detected in all varities. This indicates that both spieces are present during the summer as well. It is expected that pathogens will become more prevalent and spread further north in Sweden in the future. This is due to the theory that pathogen pressure will increase as the number of pathogens and their spread in the Northern Hemisphere, particularly in Northern Europe, increases. According to Chaloner et al. (2021), the most significant changes in pathogen composition are predicted to occur in spring and autumn. The expansion of OSR in Sweden may result in a broader range of hosts for pathogens and increased pressure. It is

important to consider the potential consequences of this development (*Jordbruksverket Jordbruksmarkens användning 2022,* Zheng et al. 2020).

The ddPCR results, especially in Figure 8, indicate that Plasma has a lower level of *P. lingam* infection in the leaf in the autumn, whereas the value in the stem is at a high level. The results also show that Scorpion has a lower number of *P. lingam* gene copies in both the upper part and the base part (Figure 8). The disease score results (Figure 4) from the autumn leaf sample indicate that there are fewer spots of *P. lingam* on the Plasma and Scorpion varieties. The reason to the lower value for Plsama and Scorpion could be due to the presence of the *Rlm7* resistance gene. For Aliboom and Crotora, the two varieties lacking *Rlm7*, the value for *P.lingam* gene copies was at a higher level for both stem and leaf samples and also in the upper part and the base part. This indicates that there is a difference in susceptibility between varieties and the resistance is not overcomed, which it could be after a few growing seasons due to changes in the population of *P. lingam*, which can render the resistance gene ineffective (Huang et al., 2009). The incidence of *P. lingam* has been low in Sweden (*Phoma – växande sjukdom i höstraps* 2012). This could be a reason for the resistance to be remain.

The second hypothesis was that *P. lingam* is primarily present in the base of the stem, whereas *P. biglobosus* is mainly found in the upper part of the stem. The results from the ddPCR and Figure 9, show that there are more number of gene copies per gram of *P. lingam* in the upper part and base parts for all varieties compared to gencopies of *P. biglobosus*. This result that there is more of *P. lingam* in the upper part is not consistent with the hypothesis or with theory. The theory says that in the summer, stem base canker is mainly caused by the more damaging *P. lingam*, while upper stem lesions are mostly connected with the less damaging species *P. biglobosus* (Salam et al. 2007; Stonard et al., 2010).

The varieties that do carry the *Rlm7* Plasma and Scorpion do not differ in the number of gene copies of *P. biglobosus* compared to the varieties that don´t carry this gene, which is inconsistent with other studies. Field trials show that a greater quantity of *P. biglobosus* was found in both upper and basal stem cranking for varieties possessing the effective *Rlm7* gene (Mitrousia et al. 2018).

The results also show that there is a high amount of *P. biglobosus* in the base, which is not consistent with earlier studies from Sweden (Kuusk et al. 2002). However, newer studies from Sweden are more consistent with the results from this study, indicating that *P. biglobosus* is present in the base part of the stem (Omer & Wallenhammar 2023). This finding is also consistent with results from other European countries. A field trial in England has shown the presence of *P. biglobosus* in the base part of the stem, which can cause stem base canker (Huang et al., 2022). Field trials have shown that a greater quantity of *P. biglobosus* was found in both the upper and basal stem cranking for varieties possessing the

effective *Rlm7* gene against *P. lingam* (Mitrousia et al. 2018). This could be a reason for the presence of *P. biglobosus* in the base part. However, if this were true, the incidence of *P. lingam* would be lower in both the upper and lower parts.

The third hypothesis is that the proportion of the two species changes between autumn and summer. The result of gene copies per gram in autumn leaf samples and summer stem samples shows that there is variation in the presence of *P. biglobosus* and *P. lingam* between autumn and summer (Figure 8). The amount of *P. lingam* is consistently high in both autumn and summer, while for *P. biglobosus*, there is a low amount of gene copies per gram in autumn, and the amount increases in summer but not to the same level as *P. lingam*. The hypothesis is supported for *P. biglobosus*, as the proportion changes from autumn to summer. However, it is not supported for *P. lingam*, as it remains at the same level in both seasons.

Studies from 2002 showed that *P. lingam* was the dominant species during both autumn and summer samples in Sweden (Kuusk et al. 2002). Studies from 2023 show that *P. lingam* was the dominant species during the autumn, and *P. biglobosus* was the dominant species during the summer. There was a clear change between the species in the autumn and in the summer in Sweden (Omer & Wallenhammar 2023). A reason why there are fewer *P. biglobosus* gene copies per gram in the leaf samples compared to the stem samples in this study could may be due to the later release of *P. biglobosus* ascospores from the rapeseed stubble in the season, resulting in less inoculum in the autumn compared to *P. lingam*, which releases its ascospores earlier in the autumn (Stonard et al., 2010). This also indicates that in Europe, stem cranker is more closley assosiated with ascospores that are spread in the autumn then pycnidospores (Huang et al. 2005). According to Frąc et al. (2022), the increase in value from low to high for *P. biglobosus* from autumn to summer may be due to the more rapid proliferation of *P. biglobosus* mycelium within the plant compared to that of *P. lingam*.

The fourth hypothesis is that a high incidence and severity of fall infections of blackleg negatively affect the yield. The yield result shows that the yield for the varieties with the *Rlm7* resistance gene, Plasma and Scorpion, are higher than the yield of the varieties without the *Rlm7* gene, Aliboom and Crotora (Figure 11). This suggests that the resistance gene has a positive impact on yield, but other factors such as climate adaptation and resistance to other pathogens may also play a role. For example, this field trial is a clubroot field trial, the field is infected with clubroot and the varieties are resistant against clubroot. This leads to that it is difficult to disentangle if it is the resistance against clubroot or the *Rlm7* resitsance gene agaisnt *P. lingam* that have the biggest impact on the yield for Scorpion and Plasma. The varities in the variety mix does not carry any known resistance against clubroot and two of the varities four varities in the mix carry the *Rlm7* gene (Gunnarssom 2021). The yield result for Scorpion and Plasma was11 % higher then the variety mix. This

indicates that the *P. lingam* has a higher impact on the yield compared to clubroot infection in this particular field trial due to the result of the variety mix. The field trial was harvested 2023-08-12 (*Nordic Field Trial System - Försöksdokumentation: OS7-025-2023-003. Höstraps. Sortförsök resis. mot klumprotsjuka* u.å.). This leads to that the field trial being harvested after the storm Hans which took place in 7-8 August (Eklund & MSB 2023). This indicates that the extreme wheather conditions can greatly impact the resistance to pod shattering, which is particularly relevant for the Plasma and Scorpion varieties that have resistance against it. On the other hand, Crotora and Aliboom varieties do not have resistance against pod shattering. In the variety mix, two out of four varieties carry resistance against pod shattering, following the same pattern as *Rlm7*. This could also be a contributing factor to the yield result. The correlation between *P. lingam* gene copies for the leaf autumn samples and the yield for the four varieties is high (Figure 12). This suggests that fall infections play an important role in yield reduction. However, the impact of the storm Hans and the resistance to pod shattering on the result is likely significant. The correlation between *P. lingam* gencopies in stem summer samples and yield is lower than that of fall infections, indicating a greater impact on yield. This could be an evidence that the resistance gene *Rlm7* functions in both Scorpion and Aliboom, supporting the theory that qualitative resistance occurs in cotyledons and leaves after penetration by hyphae from ascospores (Huang et al., 2009). Additionally, the results demonstrate a strong correlation between *P. lingam* spots in the autumn and yield (Figure 14). In the future, farmers can conduct a disease score on their own fields in the autumn to investigate the presence of *P. lingam* and make a decision on fungicide treatment. However, the results need to be strengthened with new field trials where extreme weather does not affect harvest conditions, as happened in this case.

5.1 Implications for practice

In Sweden, many farmers use Caryx (Metconazole and Mepiquat chloride) as a growth regulator with a side effect of fungicide. This should have an effect on Phoma. According to Huang et al. (2022), Triazole fungicides are more effective against *P. lingam* than against *P. biglobosus*. Therefore, it would be more effective to use Folicur Xpert (Tebuconazole & Protioconazole) and Orius (Tebuconazole) as they are registered as fungicides and also have a growth regulatory effect. Therefore, a good way to manage *P. lingam* would be to use Folicur Xpert or Orius and sow the OSR at the right time, so the need for regulatory effects is not necessary. However, according to Brachaczek et al. (2021), *P. biglobosus* requires a higher dose of this fungicide to achieve comparable results to *P. lingam*. Therefore, using a product specific to *P. lingam* may promote the growth of *P. biglobosus*. In Sweden, this would not be a problem due to the lower importance of

P. biglobosus but higher tempartures in the future can lead to significant yield losses caused by *P. biglobosus* which had happened in hot summers in Poland (Liu et al. 2014).

Spraying in autumn is particularly recommened, because that the earlier the pathogens reach the stem, the more severe the stem canker will be at harvest. By spraying in autumn, the fungies can be prevented from reaching the stem, according to Huang et al. (2022).

5.2 Future studies

For future studies, a field trial could be set up to test the presence of *P. biglobosa* and *P. lingam* without the presence of other diseases, as in this case where the field trial was organised in a clubroot infested field. This would provide a clearer understanding of the impact of *P. lingam* and *P. biglobosa* on the yield. It would also be interesting to take samples from several different fields that differ geographically and where the distance to previous years' OSR fields are clarified. This would give an indication of how far the ascospores can spread in the autumn and what impact this has on the incidence. It would also give an indication of how widespread geographically the occurrence of *P. lingam* is in Sweden. This would help farmers in Sweden and advice could be given to grow resistant varieties or spray with fungicides against *P. lingam*. An advice that we can give to farmers after this report is that probably the autumn occurrence of *P. lingam* plays a big role on the yield and if there are spots on the leaves in the autumn it is worth doing a fungicide control in the autumn.

5.3 Conclusion

The key findings from this study is that both *P. lingam* and *P. biglobosus* are present in Swedish OSR fields and the prevalence differ between varieties. The second finding is that *P. lingam* is the dominating the species complex in both the base and the upper part of the stem. For *P. lingam* there is an equal number of gene copies in the upper and the base part, and the same applies to *P. biglobosus*. This contradicts the hypothesis that, *P. biglobosus* mainly is found in the upper part of the stem. Further, the two species co-exist within OSR fields and the proportion of the *P. lingam* does not change between the autumn and the summer, at the same time, *P. biglobosus* is more prevalent in the summer than in the autumn samples. Both in the autumn and in the summer, *P. lingam* was more prevalent than *P.bigobosus*. The last finding is that high incidence and severity of blackleg in the

autumn seems to negatively affects the yield, however, the storm Hans has impacted the harvest results, which means that confirmation cannot be fully guaranteed.

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Apendix 1

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