January 2020

Project Report No. 616

Integrating Control strategies Against soilborne *Rhizoctonia solani* in OilSeed rape (ICAROS)

Rumiana Ray, Dasuni Jayaweera, Neil Graham, Craig Sturrock and Tahseen Jilani

The University of Nottingham, School of Biosciences, Sutton Bonington Campus, Loughborough,

LE12 5RD

This is the final report of a 39-month project (21140008) that started in July 2016. The work was funded by BBSRC, Innovate UK, Syngenta and a contract for £80,000 from AHDB Cereals & Oilseeds.

CONTENTS

1. Abstract

Rhizoctonia solani anastomosis group (AG) 2-1, which causes pre- and post-emergence damping off, is associated with significant establishment and yield losses in oilseed rape (OSR) worldwide. This project aimed to determine the spatial and temporal development of *R. solani* in relation to its host and the soil environment and develop integrative control strategies against damping off, inclusive of varietal and seed treatment control options.

A genome wide association study of 454 genotypes of the *Brassica* diversity ASSYST panel identified significant genome and transcriptome quantitative loci and candidate genes associated with resistance/susceptibility responses to AG2-1. This information can facilitate the future development of AG2-1 resistant OSR genotypes by identification of genetic markers for use in breeding programmes. Future genetic characterisation will enable understanding of the mechanisms associated with host resistance against *R. solani*. Screening of commercially available varieties identified moderately susceptible and susceptible varieties to AG2-1 infection.

Studies on the effect of soil type and soil water on the development and severity of disease by AG2-1 have shown that the pathogen causes more severe root disease in drier, sandy loam soils. However, under conditions of medium to high soil moisture, the pathogen is able to reach the host by spreading on the surface of the soil. Seed treatment against AG2-1 infection using sedaxane increases root length, volume and lateral root length of OSR seedlings and it is most effective under low to medium soil moisture, limiting AG2-1 surface spread in sandy loam soils.

This project provides the first report of quantification of establishment and yield losses associated with *R. solani* AG2-1. Artificial inoculation of AG2-1 reduced establishment by 60% and yield by 40%. AG2-1 infection also delayed flowering resulting in uneven crop development and fungicidal seed treatment negated this effect by reducing AG2-1 DNA in soil. Higher seed rate can partially compensate for the loss in establishment due to AG2-1 infection and plants treated with sedaxane, metalaxyl-M and fludioxonil (A21748 A) were less damaged by the cabbage stem flea beetle.

Significant yield response under natural infection was associated with moderate cumulative rainfall, an average temperature at GS10 greater than 16^{o}C and good emergence and establishment of the crop. Emergence and establishment were influenced negatively by pathogen DNA of *R. solani* in soil and positively by agronomy factors such as minimum cultivation, soil type, and genotype and seed treatment.

2. Key messages

Rhizoctonia solani AG2-1 reduces establishment, delays plant development and flowering, and causes significant losses in yield of OSR.

Genetic variation for resistance to *R. solani* AG2-1 exists in diverse germplasm and can be used to develop more resistant OSR varieties to *R. solani* in the future. The two commercially available conventional varieties, Campus and Skye, achieved the highest establishment and yield under artificially infected and naturally infected conditions.

Disease development in time and pathogen spread in space are favoured by drier soils with higher soil porosity. Under these conditions, however, seed treatment is also most effective against the pathogen.

The succinate dehydrogenase inhibitor, sedaxane, was demonstrated to be most effective against *R. solani* under low to medium soil water conditions and protected the host against delayed development, root length reductions and lateral root loss. Under water-saturated soils the pathogen may grow preferentially on the soil surface thus escaping fungicide control.

In the field, OSR infection by *R. solani* AG2-1 is associated with greater damage by the cabbage stem flea beetle and the pathogen co-occurs on plant stems with *Leptosphaeria biglobosa*, a causal organism of phoma stem canker. Increasing sowing seed rate or using seed treatment against damping-off, were associated with lower pest damage. The addition of the biostimulant, Epivio^{TM}, containing extracts of algae and Vinasse, amino acids, organic nutrients and micronutrients, to fungicidal seed treatment was associated with reduced treatment effectiveness against *R. solani* and *L. biglobosa*.

Agronomy factors positively influencing emergence and establishment of OSR included previous crop of barley compared to wheat, minimum cultivation compared to ploughing, loamy sand as soil type, and no previous damping off. Yield response was genotype-dependant and associated with good emergence and establishment protected by seed treatment and favoured by moderate cumulative rainfall and average temperature > 16^{o}C by GS10.

3. Summary

3.1. Background

Rhizoctonia solani anastomosis group (AG) 2-1 is an aggressive soil-borne fungal pathogen of oilseed rape (OSR; *Brassica napus*) and canola worldwide and the main causal agent of pre- and

post-emergence damping off, hypocotyl and root rot seedling diseases. Highly virulent isolates of *R. solani* AG2-1 are predominately associated with stem and root rot diseases of OSR seedlings, whilst AG4 isolates are known to infect and cause significant brown stem and root rot diseases in mature plants (Kataria and Verma, 1992). Partial or complete OSR and canola plant stand losses are associated with high seedling disease incidence of 80-100% caused by AG2-1 (Ellis, 1983) whilst yield losses of up to 30% have been estimated due to root rot caused in mature plants (Sippell *et al.*, 1985).

UK Growers typically associate OSR establishment losses with poor seedbed, unfavourable environmental conditions and/or pest damage. However, severe damping off diseases caused by AG2-1 are known to result in similar symptoms of patchy stands, poor seedling emergence or even complete inhibition of germination. The last review by Blake *et al.*, (2004) reported that poor or failed establishment due to patchiness, severely delayed or failure to emerge OSR crops cost the UK industry an excess of £30M in the worst years.

In the UK, the incidence and epidemiology of AG2-1 and other AGs, for example AG4, that are capable of causing disease in commercial OSR remain unknown. Previous reports have shown that AG2-1 can be weakly pathogenic to potatoes within arable rotations, and the pathogen has been reported at incidence of 4%, suggesting that potatoes are unlikely to select for this AG within rotations (Woodhall *et al.*, 2007). However, recent evidence is available on the increased prevalence and geographic spread of *R. solani* in wheat fields. A recent soil survey carried out in 2011/12 consisting of more than 100 winter wheat fields sampled across England identified that *R. solani* AG2-1 was the predominant AG present in 69% of field soils. Also, the main agronomy factor in winter wheat rotations contributing to greater DNA concentrations of AG2-1 in soil was OSR grown as the previous crop (Brown *et al.*, 2014). Soils of fields of winter wheat following OSR contained 1000-fold higher DNA concentrations of AG2-1 than fields of continuous wheat or following other cereal such as maize or oats. This finding strongly suggests that OSR in arable rotations plays an important role in selecting for high accumulation of AG2-1 in soil. Recent work by an AHDB funded PhD studentship 'Soil borne pathogens of oilseed rape: assessing their distribution and potential contribution to yield decline' has confirmed the predominance of AG2-1 in current OSR fields (McCormack, 2018).

Further evidence on the impact of severe damping off disease caused by AG2-1 of *R. solani* on OSR seedlings has been provided by studies using x-ray Computed Tomography (CT) showing that AG2-1 of *R. solani* is capable of impacting significantly on several root architecture parameters of the crop (Sturrock *et al.*, 2015). The pathogen reduced primarily lateral root number, root volume, root surface area and convex hull by more than 50% compared to the disease-free plants within 7 days of infection. Finally, evidence showing that *R. solani* AG2-1 is likely to be a significant

pathogen affecting yield decline of OSR was provided by DNA analysis of samples extracted from soil and OSR rhizosphere from long-term field experiments investigating the impact of rotational sequences on the yield of the OSR crop (Stobart and Bingham, 2013). Results showed that DNA of *R. solani* AG2-1 accumulated significantly in soils where OSR was grown in close rotations or in monoculture. Yield declined by more than 10% in OSR grown as continuous crop compared to the crop grown in rotational 1-year and 2-year break sequences with wheat.

There is already a wealth of information on the distribution and colonisation efficiency of AG4 of *R. solani* in soil, showing that this pathogen is likely to be located in the top 10-15 cm of soil and it spreads faster and further along surfaces than within soil (Budge *et al.*, 2009; Otten and Gilligan, 1998). Colonisation of soil has been shown to be enhanced by increased porosity and bulk density (Otten *et al.*, 2001; Harris et al, 2003), however less is known of the impact of environment and soil parameters on host colonisation efficiency and disease severity.

R. solani produces sclerotia (infection structure of compacted mycelia) capable of surviving in soil for more than 5 years, thus short term rotations and/or cultural practices such as reduced tillage increase its prevalence in soil. Cultural control methods can be ineffective against *R. solani* AG2-1 as minimal cultivation is generally encouraged to use to reduce costs, preserve moisture and soil quality. Rotation can be an effective control method; however, in practice the host crop of OSR will need to be grown in the same field no more than once every 3 or 4 years. At present, the most sustainable method for disease control will be considered the use of resistant varieties. However, a major bottleneck in discovering resistance to soil borne pathogens is the time and resources required to screen large numbers of genotypes.

In the absence of varietal resistance, chemical options for control of the damping off complex are limited to seed coatings with active compounds to protect the developing seedling. In the EU the most used broad-spectrum seed treatment with moderate activity against *R. solani* was thiram, which is no longer registered for use under the EU Pesticide directive 2009/128/EC. The main challenge and opportunity for the protection of OSR seed in the absence of resistance remains the development and use of novel more systemic seed treatments with longer activity against the target, consistent effectiveness against diverse pathogen populations validated under variable environmental conditions, whilst also applied at low doses to avoid environmental pollution.

At present, in the UK, there are no registered commercially available seed treatments against *R. solani* for use in OSR. Furthermore, there is no known varietal resistance against *R. solani* (Babiker *et al.*, 2013) hence commercial OSR varieties are likely to be highly susceptible to Rhizoctonia diseases highlighting the need for the identification of new genes or quantitative trait loci (QTLs) for resistance/tolerance that can be utilised in future OSR breeding programmes.

3.2. Project aim

To develop a sustainable protection strategy against *R. solani* inclusive of varietal resistance and/or novel seed treatment chemistry.

The project objectives addressed through specific work packages were:

1. To identify resistance/tolerance traits and novel loci for resistance to *R. solani.*
2. To determine the spatial and temporal development of *R. solani* in relation to the host, environment and soil characteristics.
3. To quantify yield losses due to damping off disease caused by *R. solani* in OSR.
4. To develop effective integrative control methods against the disease in OSR.

3.3. Anticipated benefits

This project addressed an AHDB research priority for UK growers on improved understanding of field yield loss due to soil-borne pathogens and their diseases, for the development of guidelines to help growers manage Rhizoctonia disease more effectively through the use of novel seed treatments from the class of succinate dehydrogenase inhibitors (SDHIs) and varietal integration.

With changes in the EU regulatory environment through pesticide directives and the EU ban of a range of current fungicides for the control of pests and pathogens, farmers face increased uncertainty. Through this project we thus aimed to evaluate the first generation of novel SDHI seed treatment to effectively control an important OSR disease.

The project also provides information to growers and breeders on the susceptibility of UK and European OSR elite cultivars to *R. solani* and the possibility to exploit candidates with useful disease tolerance traits for future breeding.

The ultimate economic impact of this project was aimed to be the recovery of loss in establishment failure or yield due to the disease through improved control strategies, for example response to variety and seed treatment.

3.4. Work package 1: Identification of *R. solani* resistance traits / novel loci in oilseed rape

3.4.1. Background

Variation in susceptibility of *Brassica napus* genotypes to *R. solani* has been shown to exist (Yang and Verma, 1992; Babiker *et al.*, 2013). However, the progress in identifying useful resistance sources and genes has been hindered by limitations in screening large numbers of genotypes. Through the ICAROS project we developed a high-throughput disease screen to identify and

characterise novel plant phenotypes with tolerance or resistance traits to *R. solani* based on an artificial media system for phenotyping early root traits and seedling disease, first described by Drizou *et al.* (2017).

3.4.2. Methodology

High throughput phenotypic screening of commercial OSR varieties and a large, genotyped germplasm set known as the ASSYST diversity set (454 accessions) was carried out in two different environments, glasshouse and growth room set up at conditions of 20ºC with a 12 hour photoperiod. Genotypes were screened under infected and non-infected conditions, all experiments contained a minimum of three replicates per genotype with three biological replicates per experimental unit. The inoculation method used mycelial plugs from an actively growing culture of *R. solani* AG2-1 on potato dextrose agar, which were positioned at the bottom of plastic trays filled with light expanded clay aggregates. Pre-germinated seeds of OSR were grown under inoculation for 7 days. Plant growing conditions, inoculation method and assessments are described in detail by Drizou *et al.* (2017). Disease was assessed on the hypocotyl and root and scored on a scale of 0-5 (Figure 1). Disease index (%) was calculated as the sum of the number of plants in individual disease rating scales multiplied by the numerical value of their rating then divided by the total number of plants, multiplied by the maximum rating disease scale, and then expressed as a percentage.

Figure 1. Disease severity of *Rhizoctonia solani* AG2-1 on oilseed rape. Examples of genotypic disease responses that can be classified as a) moderately resistant (MR) (0 = no symptoms, 1 = superficial lesions on hypocotyl and taproot); b) moderately susceptible (MS) (2 = lesions taking up to 75% of hypocotyl and 50% of taproot, 3 = lesions >75% on hypocotyl and 50-75% on taproot); and c) susceptible (4 = lesions >75% and necrosis up to 30% on taproot, and 5 = necrosis > 30% on taproot or death). Bar = 1cm.

Genome wide association studies (GWAS) were performed with trait data (hypocotyl and root length, disease severity and plant survival), SNPs and gene expression markers (GEMs) using mixed linear model in GAPIT R package for the SNPs and modelled by a fixed effect linear regression in R with reads per kb per million (RPKM) values for GEMs as described by Havlickova *et al.* (2017).

3.4.3. Results

Fifty five hybrid and conventional OSR varieties were initially phenotyped for disease index of the hypocotyl and root in two separate environments (Figure 2). There was narrow variation in their responses to pathogen infection, quantified as disease index, thus we detected only small differences between them. From these genotypes a smaller number with a range of responses were selected for further testing in field experiments in EU and UK as described in WP3.

Quantitative trait loci (QTL) with significant SNPs and GEMs above a Bonferroni-corrected threshold of $P = 0.05$ ($-\text{Log}_{10} P = 6.7$) were detected across the chromosomes of the A and C pan-genomes of *B. napus* (Table 1).

Table 1. Chromosome locations of top SNPs and GEMs for disease response (susceptibility or resistance) identified above the Bonferroni-corrected significance threshold (trait association is significant above $-\log_{10}P = 6.7$) on chromosomes of *B. rapa* (A1 - A10) and *B. olerecea* (C1 - C9).

$-\log_{10} P$	Chromosome
8.603	A09
7.914	C08
7.382	A02
7.353	A07
7.349	C01
7.326	C07
7.177	A05
7.143	C09
7.052	C05
6.910	C09
6.767	A06

Arabidopsis thaliana mutants were identified based on the annotated functions of *B. napus* orthologues of candidate genes from GWAS with the highest $-\log_{10} P$ (Table 1) to confirm functionality of the genes when infected by *R. solani* AG2-1. Expression of the same candidate genes based on *Brassica* gene orthologues was also confirmed in two commercial OSR varieties, Anastasia and Campus, phenotyped in the field experiments in work package four as moderately susceptible and moderately resistant to AG2-1 infection, respectively.

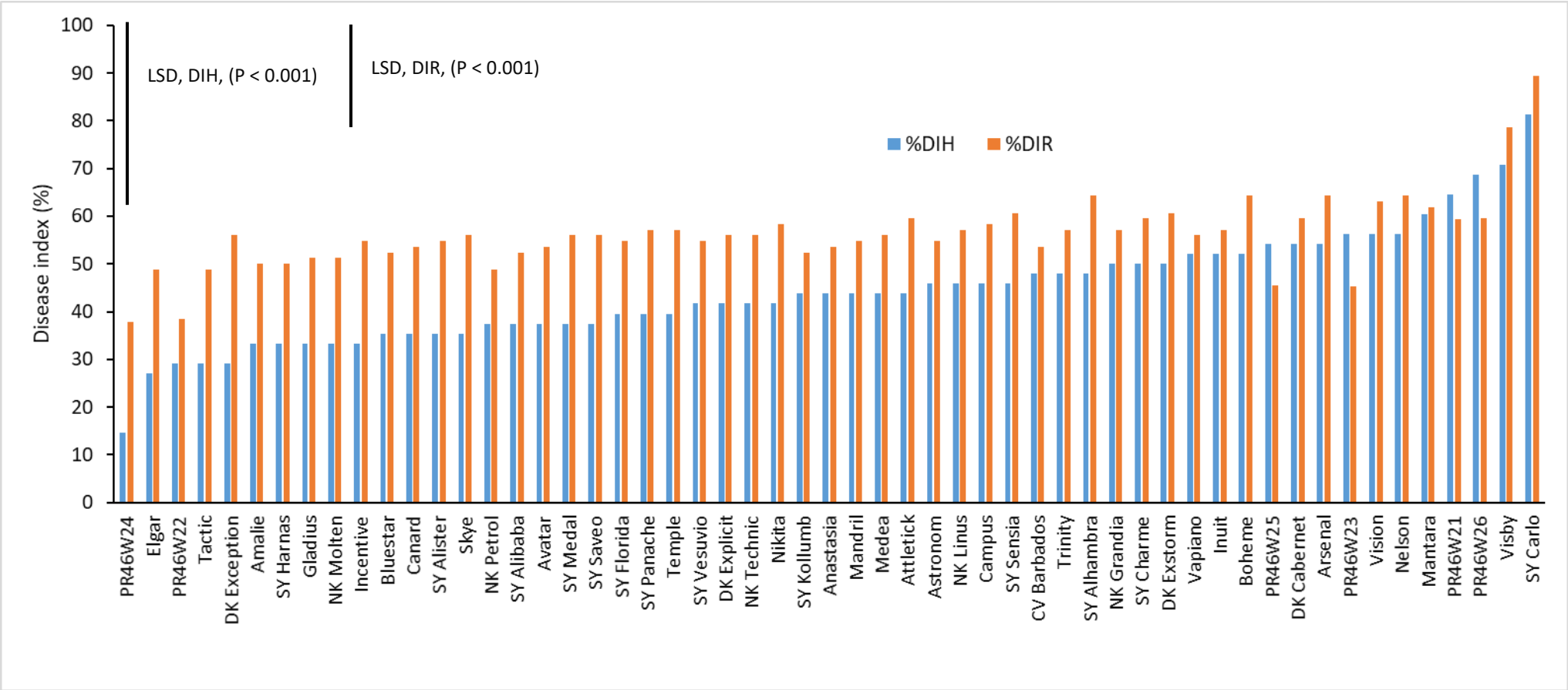

Figure 2. Screening of commercial varieties for disease index of the hypocotyl (%DIH) and the root (%DIR) expressed as percentage as described in methodology. LSD: least significant difference.

3.4.4. Discussion

There is only one published report (Babiker *et al.*, 2013) on the evaluation of US, Canadian and EU germplasm collection containing 85 genotypes from *Brassica*, *Sinapsis* and *Camelina* spp. for susceptibility to *R. solani* AG8 and *R. solani* AG2-1. The authors recorded percent of seedling survival, shoot length and shoot fresh weight reduction as parameters for susceptibility exhibited in inoculated soils and determined that none of the tested genotypes were resistant to AG2-1 although some genotypes exhibited tolerance to AG 8 infection. Moderate resistance to *R. solani* AG2-1 has been previously reported in genotypes of *S. alba* (Yang and Verma, 1992). Here, in controlled environment and under artificial infection, all commercially available genotypes were highly to moderately susceptible to AG2-1 causing severe post-germination damping off in seedlings. There were greater differences between genotypes for hypocotyl rot but very few for root rot. Whilst the developed screen is suitable for phenotypic evaluation of the early host response to pathogen infection, it is less appropriate for the identification of field resistance or tolerance as assessments are only collected for up to 5-7 days and plants are not grown to full maturity. Thus, a small selection of contrasting genotypes from the initial screen were taken for further evaluation in naturally infected and inoculated field experiments the results of which are shown in WP3 and 4.

Through the ICAROS project we were able to screen >450 genotypes of the ASSYST *B. napus* diversity set. Whilst we confirmed that narrow variation of responses existed in commercially available hybrids and conventional lines, more importantly we identified through GWAS QTLs containing significant GEMs and SNPs for resistance and susceptibility responses to AG2-1. This is novel and useful information for breeders as resistance to AG2-1 can thus be introduced either through positive selection using markers associated with resistance or through exclusion of susceptibility using the markers associated with susceptibility. Candidate genes that were top of the GWAS analysis were confirmed for their functionality in Arabidopsis mutants and then further validated by measuring their expression in contrasting OSR varieties. Further characterisation of the molecular and genetics of the newly identified candidate genes will allow us to learn of the mechanisms of resistance and can lead to identification of susceptibility factors to host-specific soil-borne pathogens for which information is lacking at present.

3.5. Work package 2: Spatial and temporal development of *R. solani* in relation to the host, environment and soil characteristics

3.5.1. Background

In the field, *R. solani* AG2-1 is likely to be located in the top 10-15 cm of soil (Budge *et al.*, 2009) as the pathogen has been shown to spread faster and further along surfaces than within soil (Otten and Gilligan, 1998). Rate of soil colonisation is enhanced by increased porosity and low bulk

density (Otten *et al.*, 2001; Harris *et al.*, 2003) however the impact of the host and soil environment on disease incidence and severity remains unknown. Here we carried out controlled experiments to understand the temporal and spatial development of the pathogen and disease in two different soil types, exposed to different moisture regimes. We further evaluated the effect and persistence of seed treatment in protecting seeds under different conditions and were able to visualise root system development using X-ray Computed micro Tomography (µCT) in undisturbed soils.

3.5.2. Methodology

X-ray µCT studies were performed to understand the temporal development of *R. solani* AG2-1. Two soil types, sandy loam and clay, were used for this experiment with cv. Skye (moderately resistant) as the host plant. There were 24 treatment combinations of pathogen inoculation (non-infected or AG2-1 infected), seed treatment (untreated or treated with sedaxane) and watering regime starting at 20% field capacity (to deliver 1 mm, 6 mm and 60 mm simulated rainfall per day) replicated three times. Column preparation and processing of X-ray images were conducted following the method described in Sturrock *et al.* (2015) at 2, 4 and 6 days post inoculation (dpi). Briefly, soil columns (30 mm diameter x 70 mm length) were uniformly packed to a bulk density of 1.1 Mg m^{-3}. Prior to packing, soil was air-dried and sieved to <2 mm. Disease severity was assessed at 6 dpi according to the scoring described in work package one and in detail by Drizou *et al.* (2017).

Spatial development of AG2-1 infection was conducted in treatment combinations described above for the X-ray µCT studies in the presence or absence of the host plant cv. Skye in three replications instead of inoculation. The effect of treatment was determined in combinations where seed was treated or left untreated. The effect of the host was tested in combinations where the untreated host was present or absent. Trays with 1.5L capacity were uniformly packed with soil and toothpicks were added to the soil at a distance of 2 cm by 1.5 cm with a 0.25 mm^2 plug containing actively growing AG2-1 mycelium in the centre of the tray. Toothpicks were removed every two days and plated on *R. solani* selective media as described by Ko and Hora (1971) to isolate AG2-1 within the soil. Surface hyphae spread of AG2-1 was also captured at 6 dpi and analysed using image J software (https://imagej.nih.gov/ij/)

Soil porosity (total and incremental with depth) was quantified as described by in Sturrock *et al.* (2015) in FIJI image analysis software. A resized 16 bit image stack of dimensions 17.1 mm × 17.1 mm × 19 mm (900 × 900 pixels × 1000 images) was first prepared to exclude the area outside of the soil column (i.e. the container and the surrounding air space). Images were binarised to define the air filled pore space with a value of 0 and the 'solid' soil with a value of 1 using the isodata threshold algorithm. Soil porosity for each slice image was calculated based on the percentage of air to the total volume of the resized stack.

Disease severity, soil porosity and surface fungal hyphae spread were analysed using ANOVA and fungal spread within soil was analysed using generalised linear mixed models in Genstat.

3.5.3. Results

Sandy loam soil had significantly lower soil porosity compared to clay soil (Figure 3a and b). Furthermore soil porosity declined sharply in clay soils with increased watering and differences between soils under medium and high watering regimes were small. Disease developed more rapidly under low water regimes (1 mm d^{-1}) in both soil types but was more severe in sandy loam than in clay by 6 dpi (Figure 4). There was an inverse relationship between disease index and water regime, thus water-saturated soils had lower disease compared to dry soils. Sedaxane reduced disease severity under low to medium water regimes in sandy loam in contrast to clay soil. The effect of sedaxane on root system architecture under inoculated sandy loam soil with medium water regime was visualised using X-ray µCT images (Figure 5).

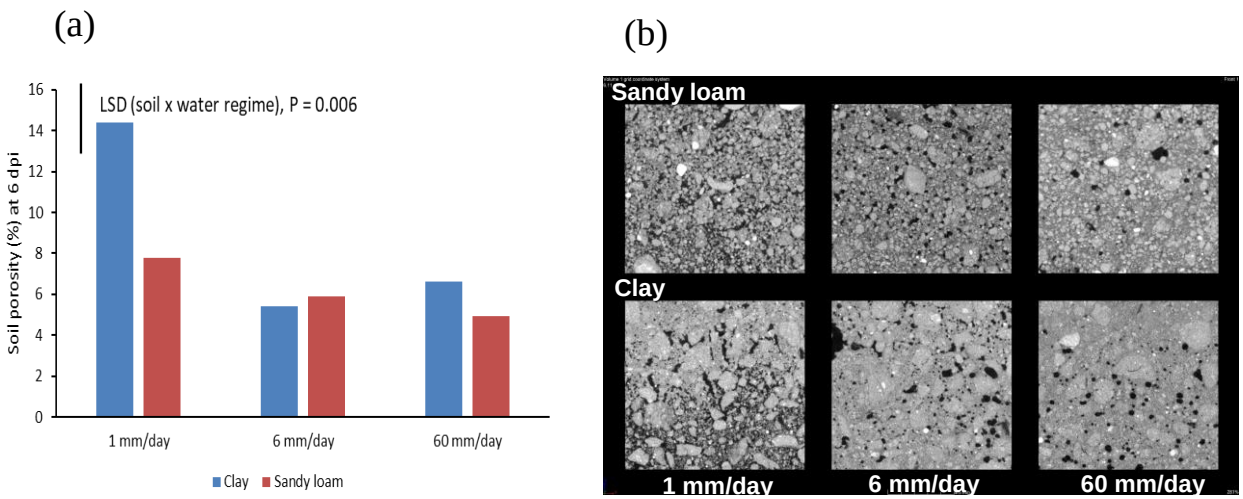

Figure 3. Soil porosity of clay and sandy loam at different moisture content at 6 dpi (a and b). LSD: least significant difference.

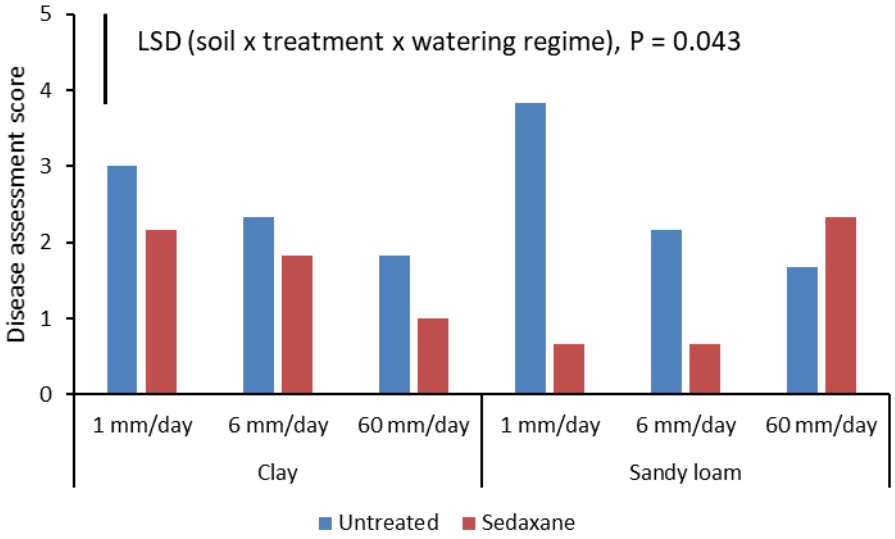

Figure 4. Assessment of *R. solani* AG2-1 disease severity in the two soil types, sandy loam and clay in different moisture content from 1 mm of moisture per day to 60 mm of moisture per day under untreated or sedaxane treated conditions at 6 dpi. Disease severity classified as 0 = no symptoms, 1 = superficial lesions on hypocotyl and taproot, 2 = lesions taking up to 75% of hypocotyl and 50% of taproot, 3 = lesions >75% on hypocotyl and 50-75% on taproot, 4 = lesions >75% and necrosis up to 30% on taproot and 5 = necrosis > 30% on taproot or death. LSD: least significant difference.

Sedaxane protected lateral root development, increased root length and volume, which were severely reduced in inoculated seedlings by 6 dpi (Figure 5).

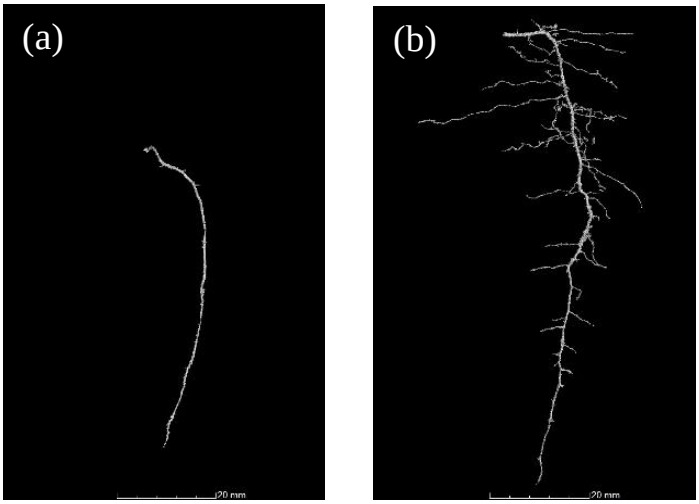

Figure 5. X-ray CT scan of oilseed rape roots in AG2-1 inoculated soil, with (a) untreated and (b) sedaxane treated OSR seed of cv. Skye at 6 dpi. Bar = 20 mm.

There was less surface fungal spread by 6dpi on sandy loam compared to clay soil exposed to 1mm or 60mm water d^{-1}; however, surface spread was favoured most by medium moisture of 6mm d^{-1} for both soil types with no differences detected between soils (Figure 6a). Sedaxane reduced significantly surface spread on sandy loam soils irrespective of water regime but was less effective in constraining the fungus on clay soils (Figure 6b).

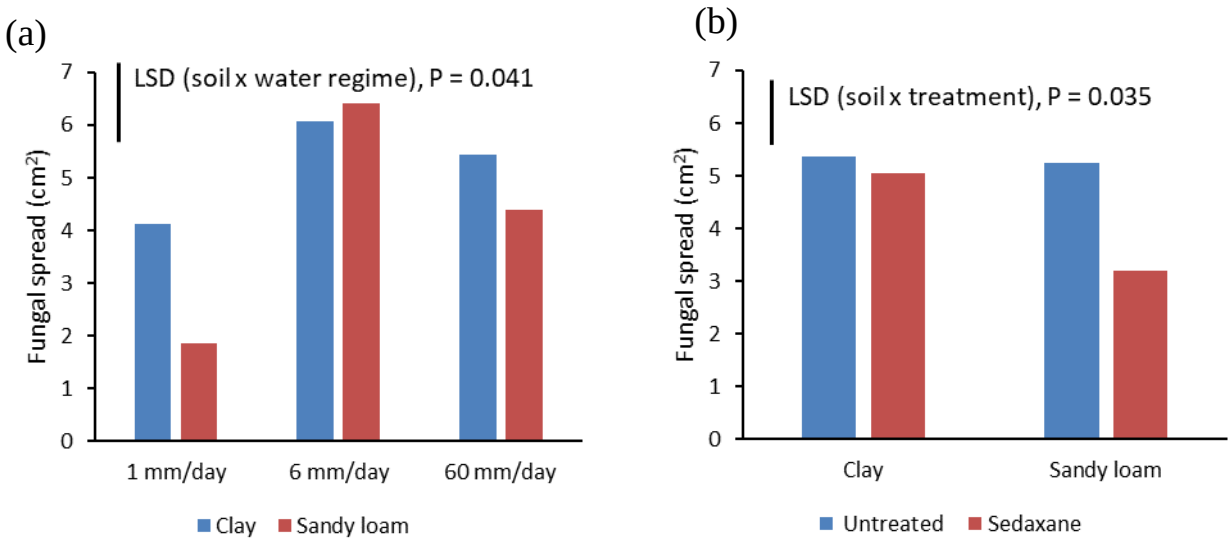

Figure 6. Surface AG2-1 fungal mycelial spread on the two soil types (clay and sandy loam) at different moisture content and the effect of seed treatment (Untreated and sedaxane) at 6 dpi. Significance differences were analysed by ANOVA with (a) between soil type and watering regime (P = 0.041) and (b) between soil type and seed treatment (P = 0.035). LSD: least significant difference.

3.5.4. Discussion

The objective of these studies was to evaluate the effects of soil type, water regime and host presence on the development, spread and disease severity by *R. solani* AG2-1. AG2-1 caused more severe disease on roots in drier soils, however, soil type significantly influenced both disease severity and disease control. Whilst our results agree with previous studies, showing enhanced rate of soil colonisation by *R. solani* AG4 related to increased porosity (Otten *et al.*, 2001; Harris *et al.*, 2003), disease was more severe in sandy loam, which had lower porosity than clay soils at 1mm d^{-1} watering regime. Thus, other factors including soil pH and nutrient content, which affect nutrition and development of both the host and pathogen, are also likely to influence disease outcome. Babiker *et al.* (2013) also previously reported that the virulence of AG8 is favoured in sandy soil compared to silt loam.

OSR develops a single taproot for the extraction of water and nutrients from soil resulting in a major disadvantage in early interactions with highly virulent soil-borne pathogens capable of severely reducing taproot extension during seedling growth. Here we showed that in the absence of seed treatment, root length, volume and lateral roots are significantly reduced under the most favourable conditions for host development (medium water regime, sandy loam soil). Le Cointe *et al.* (2016) demonstrated that pencycuron at 250 g l^{-1} whilst effective in inhibiting the saprophytic spread of AG4 in soil failed to reduce pathogen infectivity. In our studies, fungicide treatment was effective under low to medium moisture regimes, however under high water regime in sandy loam soil there were no differences in disease severity between treated and untreated plants. Thus, it is possible that under high moisture conditions although within soil spread is reduced, the pathogen is still able to reach the host by preferential spread on the soil surface. LC-MS assay developed for this project by the University of Nottingham to quantify sedaxane in the leachate in the two soils showed no trace of the fungicide (not detected, results not shown) indicating that the fungicide does not leach in either soil profile. Indeed, we show here that surface soil spread increased under medium to high moisture regimes and sedaxane was more effective in limiting soil surface spread in sandy loam soils than in clay soils. Previously, faster and further spread of AG4 across surfaces compared to within soil was reported by Otten and Gilligan (1998).

3.6. Work package 3: Yield losses due to damping off disease caused by *R. solani* in OSR

3.6.1. Background

The root system of OSR has been shown to be sub-optimal in the UK resulting in low yields during abiotic stress (drought) and an inefficient use of nitrogen and water (Blake and Spink, 2005). Water and nutrient extraction is usually determined by the ability of the rooting system to explore soil measured in terms of root length density (RLD) (Spink *et al.*, 2005). Even small improvements of RLD have been shown to improve yield of OSR (0.5 t/ha) (Blake and Spink, 2005), whilst reductions in RLD result in significant yield loss. Agronomic practice (sowing rate/plant population) can significantly influence OSR rooting and function. For example, low plant populations can result in improved rooting at depth. However, highly virulent *R solani* AG2-1 has been shown previously to severely reduce lateral root development, root length and volume (Sturrock *et al.*, 2015) thus impacting on early root traits necessary for good crop establishment and thus increasing the risk of yield loss under low seed rates. The aim of this work package was to quantify establishment and yield losses due to damping off disease caused by *R. solani* in field-grown OSR and determine the effect of seed treatment and increasing seed rates to compensate for establishment losses due to damping off disease.

3.6.2. Methodology

Two varieties with contrasting responses to AG2-1 infection, cvs. Skye (moderately resistant) and SY Sensia (moderately susceptible) were grown under artificially inoculated field conditions for two seasons. The experiments were factorial and consisted of 16 treatment combinations of the following factors: variety, artificial pathogen inoculation (non-infected or AG2-1), seed treatment (untreated or treated with A21748 A [sedaxane, metalaxyl-M and fludioxonil (25 ml per 1 million seeds)]) and seed rate (sown at 40 seeds m^{-2} or 80 seeds m^{-2}), replicated in three blocks. All experiments were carried out at the University of Nottingham, Sutton Bonington campus. Inoculum was prepared on millet seed as described by Zeun *et al.* (2013) and was sown at a rate of 20 g m^{-2} of millet seeds per plot alongside the OSR seeds. Seedling establishment was counted in 0.25 m^2 quadrats in two replications per plot. Cabbage stem flea beetle (CSFB) damage was assessed per plot using a score of 1-9 (1 = all plants severely damaged and 9 = no plant damage) at 49dpi. Flowering was scored from 1 to 7 (1 = late flowering and 7 = early flowering). Plots were harvested at maturity to determine total yield per plot in t $ha^{-1.}$

3.6.3. Results

AG2-1 infection reduced establishment by 60% irrespective of seed rate (Figure 7). However increasing the seed rate to 80 seeds m^{-2} compensated for 50% loss due to infection in plots sown at the low seed rate (Figure 7a). Seed treatment provided 95% increase in establishment under AG2-1 infection irrespective of seed rate (Figure 7b). High seed rate (80 seeds m^{-2}) (untreated seed) provided similar establishment to low seed rate (40 seeds m^{-2}) with seed treatment (Figure 7b and Figure 8). There was a significant difference in establishment between varieties ($P < 0.001$) irrespective of inoculation or treatment, with Skye (27 plants m^{-2} on average) always having a higher plant number per m^2 than SY Sensia (23 plants m^{-2} on average).

An increase in CSFB damage was observed in AG2-1 infected plots (Figure 9a). CSFB damage was significantly lower in seed treated plots even in the absence of AG2-1 infection. A high seed rate (80 seeds m^{-2}) also contributed to less CSFB damage (Figure 9b).

Rhizoctonia solani AG2-1 infection delayed flowering whilst seed treatment negated this effect in inoculated plots (Figure 10).

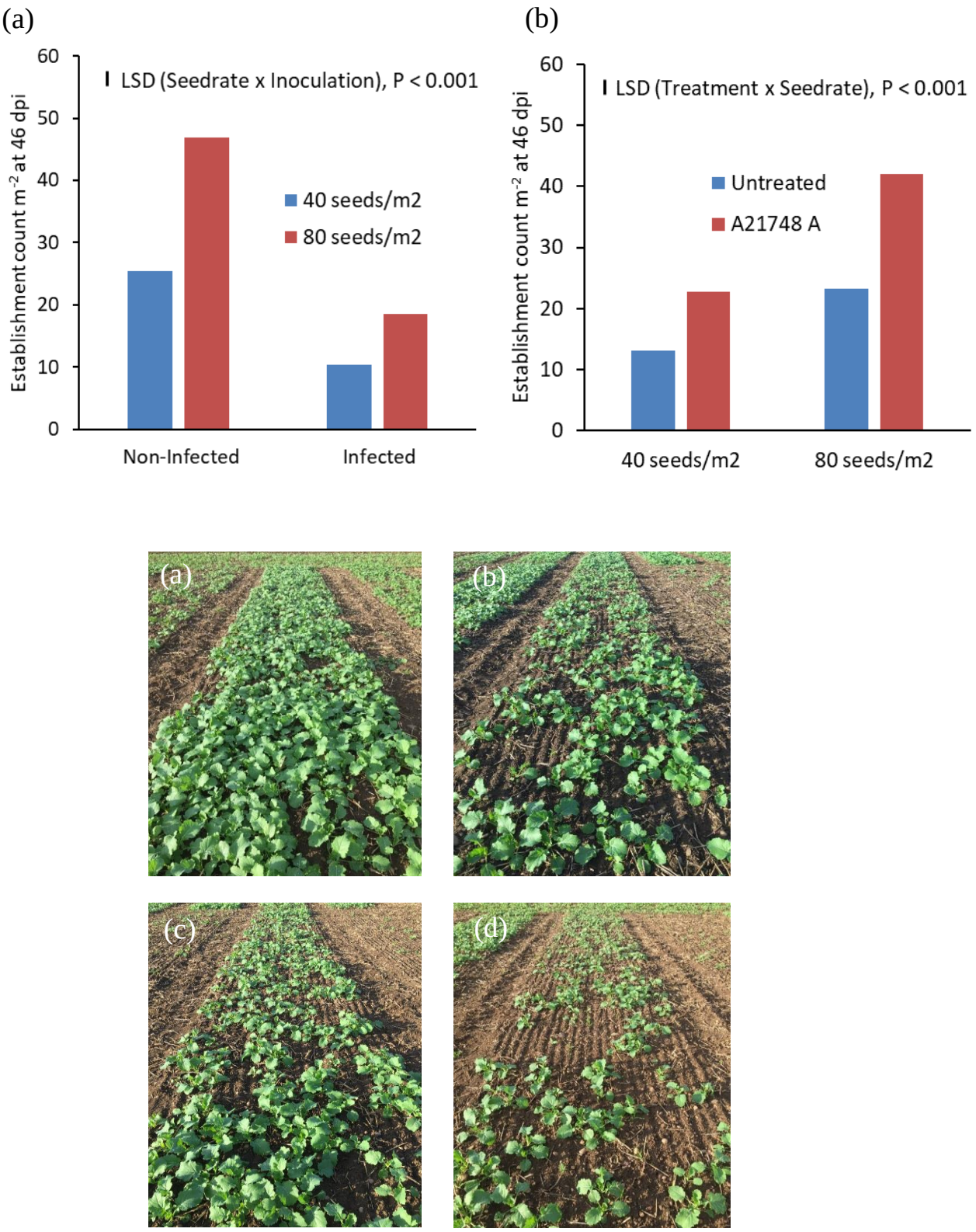

Figure 8. Effect of seed treatment on establishment of oilseed rape plants cv. Skye (a) treated with A21748 A sown at 80 seeds m^{-2}, (b) untreated seed sown at 80 seeds m^{-2}, (c) treated with A21748 A sown at 40 seeds $m^{-2}$and (d) untreated seed sown at 40 seeds m^{-2}.

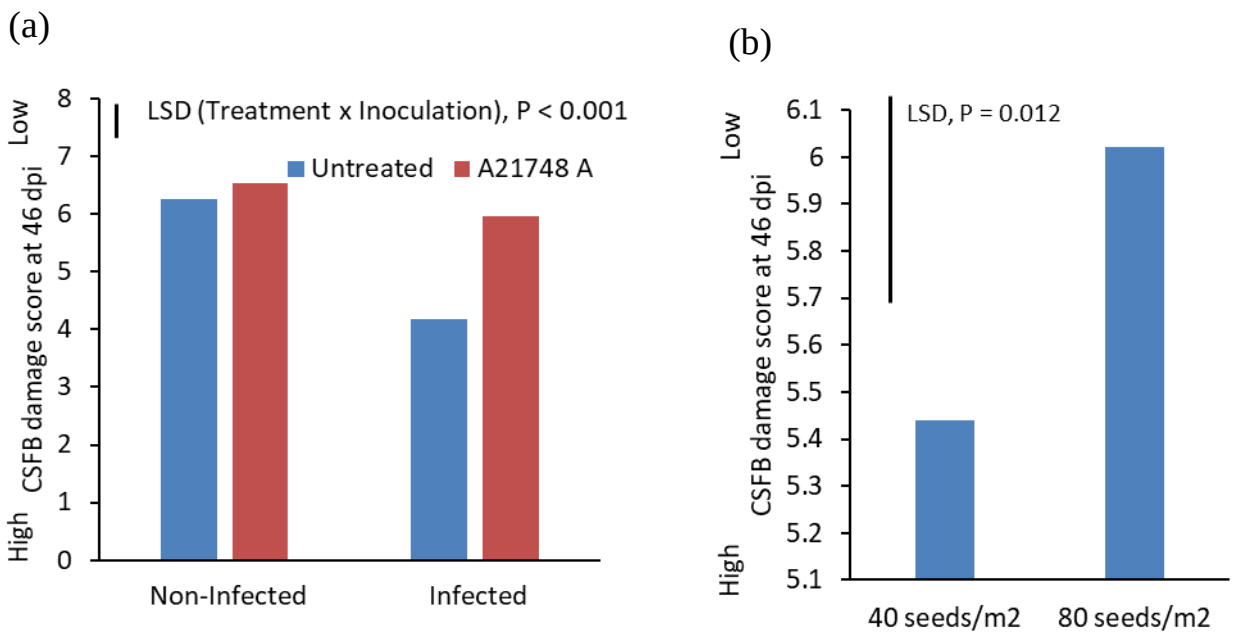

Figure 9. Effect of (a) seed treatment (A21748 A) under *Rhizoctonia solani* AG2-1 infected and non-infected conditions and (b) seed rate, on cabbage stem flea beetle damage for both seasons. There were no interactions with season. LSD: least significant difference.

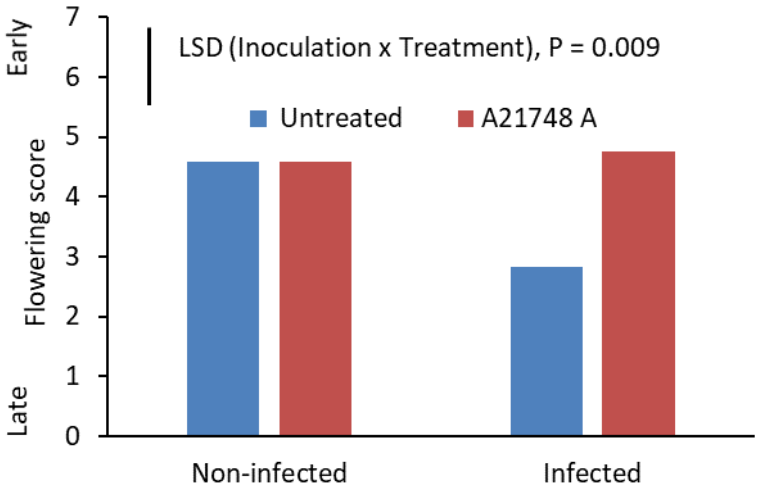

Figure 10. Effect of seed treatment (untreated and A21748 A) and inoculation with *Rhizoctonia solani* AG2-1 on oilseed rape flowering. Flowering score is early (score of 7) to late (score of 1). LSD: least significant difference.

Pathogen infection decreased yield by 40% in both SY Sensia and Skye (Table 2). Seed rate had a small effect on yield loss due to AG2-1 infection however Skye achieved 13% higher yield compared to SY Sensia when the seed rate was increased. Under non-infected conditions the opposite was observed with SY Sensia yielding more in response to seed rate increase than Skye (Table 2).

Table 2. Effect of *Rhizoctonia solani* AG2-1 infection, OSR variety and seed rate on yield (t ha^{-1}).

	SY Sensia		Skye	
Seed rate	40 seeds m^{-2}	80 seeds m^{-2}	40 seeds m^{-2}	80 seeds m^{-2}
Infected	3.49	3.71	3.10	3.85
Non-Infected	5.53	6.57	5.91	5.98

	P-value	LSD
Variety	0.373	0.2573
Seed rate	<.001	0.2573
Inoculation	<.001	0.2573
Variety x Seed rate x Inoculation	0.006	0.5147

LSD – least significant difference of means (5% level).

Seed treatment increased yield by 38% overall whilst higher seed rate and seed treatment achieved greatest yield response in season 2 under high CSFB infestation (Table 3). Response to increased seed rate in untreated plots under infection by AG2-1 was on average 0.5 t ha^{-1}. However, response to increased seed rate under treatment was only seen in season 2 when there was high CSFB damage.

Table 3. Effect of seed treatment with A21748 A and seed rate on yield (t ha^{-1}) in two seasons of experimentation, there were no interactions with inoculation.

	Season 1		Season 2	
Seed rate	40 seeds m^{-2}	80 seedsm^{-2}	40 seeds m^{-2}	80 seeds m^{-2}
Untreated	4.41	4.97	2.84	3.46
A21748 A	6.46	6.13	4.32	5.55

	P-value	LSD
Year	<.001	0.2573
Treatment	<.001	0.2573
Seed rate	<.001	0.2573
Year x Treatment x Seed rate	0.005	0.5147

LSD – least significant difference of means (5% level).

3.6.4. Discussion

This is the first report to quantify establishment and yield loss due to *R. solani* AG2-1 in OSR and determine the effects and response to an increased seed rate and seed treatment. Losses of up to 30% have been attributed to Rhizoctonia diseases in canola when the causal AG was not identified (Verma, 1996). Here, artificially inoculated studies using AG2-1, considered the most virulent AG to OSR, demonstrates that the pathogen is capable of reducing establishment by 60% and yield by 40%. Both the hybrid (SY Sensia) and the conventional variety (Skye) were significantly affected by the infection. AG2-1 infection was associated with greater damage by CSFB and resulted in delayed crop development and flowering of both genotypes.

Goll *et al.* (2014) demonstrated low baseline sensitivity of a broad range of *R. solani* AGs, isolated form diverse UK and EU soils, to sedaxane. Here we show that A21748 A, inclusive of sedaxane, metalaxyl-M and fludioxonil, is highly effective in reducing significantly the effects of infection of AG2-1 in field-grown OSR. Fludioxonil has been previously shown to be effective against AG4, whilst metalaxyl failed to control this pathogen in soybeans (Xue *et al.*, 2007). It is therefore most likely that sedaxane and fludioxonil in A21748 A increased OSR establishment and yield by more than 90% and 38%, respectively. The seed treatment was also effective in negating the effects of the pathogen on crop development and contributed to reduced damage by CSFB on treated plots. The most effective method for disease control and yield response was through integration of low seed rate and seed treatment in the absence of CSFB, however in season 2 when damage was higher, seed treated plots with a higher seed rate achieved more than 1 t ha^{-1} higher yields compared to plots having either higher seed rate or seed treatment alone. This clearly demonstrates that under high CSFB and soil-borne *R. solani* pressure and in the absence of other control methods (cultural or chemical, including insecticide) both the seed treatment and the higher seed rate contribute to protecting yield against combined pest and pathogen damage at the early seedling stage of the crop.

It is worth noting that AHDB-funded research has shown that increasing seed rates can result in higher numbers of CSFB larvae per unit area, resulting in higher pest return for the following season (Annual Project Report 2019, AHDB project 21120049).

3.7. Work package 4: Integrated disease management for *R. solani* in OSR

3.7.1. Background

In the UK, the main causal organism of damping off is considered to be AG2-1 (Brown *et al.*, 2014). However, other AGs, including AG4 and AG8, known to be pathogenic and damaging to yield of OSR have been reported in UK soils (Goll *et al.*, 2014). These AGs cause brown rot disease at an adult stage, thus it is important to consider control methods for future management

scenarios in relation to changes in pathogen populations due to environmental or rotational changes in the UK. The effectiveness of seed treatments against disease caused by diverse AGs under different environments has not previously been determined. Thus, the rationale here was to establish field experiments in UK and Europe to test a range of seed treatments against more than one AG of *R. solani* present under natural field conditions. More importantly, through the second part of this work package we aimed to evaluate the responses of OSR hybrids and test these under European and UK environmental conditions. Whilst hybrids are well established in Europe and have shown benefits to early rooting and yield of OSR, their use in UK remains limited and disease responses to *R. solani* have not been evaluated. Thus, the objective of this WP was to test and compare the performance of conventional lines and hybrids together with seed treatments, to inform UK growers of best management practices for improved disease management and OSR yield. Testing of European adapted genotypes can potentially allow for the early identification of candidates that could be used/adapted under UK conditions in the future. Additional benefits realised through this WP include the evaluation of activity of novel seed treatments against other pathogens causing diseases, for example Phoma, which were also investigated within these experiments.

3.7.2. Methodology

Naturally infected sites were identified by targeted qPCR for AGs of *R. solani* with known pathogenicity to OSR (AG 2-1, 4, 5, 8, 9, 11) in soils of UK, Germany, France and Poland (Table 4). Sites with highest quantities of *R. solani* were selected. AG 2-1 was the predominant group of *R. solani* found in all locations apart from one location in England, where AG8 predominated (Table 4). All trials were designed as randomised blocks and repeated in two seasons (years) in England, France and Germany and in one season in Poland (Table 4).

Effect of genotype and seed treatment on naturally occurring *R. solani* was evaluated in 15 treatment combinations in three replications including seed treatment (untreated, A21748 A and A21748 A in combination with thiamethoxam (TMX)) and a range of EU and UK genotypes (Table 5). Seed treatment effectiveness against naturally occurring *R. solani* was evaluated in 10 treatment combinations consisting of seed treatment (untreated, sedaxane, A21748 A, Thiram 700 SC (28.5 ml per 1 million seeds) and HyproDuet (Thiram + Prochloraz) (45 ml per 1 million seeds)) and two varieties in four replications with TMX or Epivio^{TM} as base treatment in all plots (Table 5). Epivio^{TM} is applied for its properties as biostimulant.

Equivalent trials were carried out at the University of Nottingham at Sutton Bonington Campus but all plots were artificially inoculated with virulent strain of *R. solani* AG2-1 as described in work package three. For all trials (inoculated and naturally infected), seedling establishment was counted in 2 m long sections taken in four rows per plot. Plant samples (15 plants per plot) for

selected treatments and varieties (shown in Figures 13, 14 and 15) were collected at GS19 and soil samples (50 g per plot) were collected from each site prior to sowing as well as at GS32. Plant samples were not collected in artificially inoculated trials due to reduced plant number from the disease and aim to quantify yield effects. For the naturally infected sites, DNA was extracted from plant stems as described by Ray *et al.* (2004) and from soil samples as described by Woodhall *et al.* (2012). The extracted DNA was used to quantify AG2-1, and the causal organisms of phoma stem canker, *Leptosphaeria maculans* (Lmac) and *Leptosphaeria biglobosa* (Lbig), using qPCR conditions as described by Woodhall *et al.* (2017). Primers for AG2-1 are described in Budge *et al.* (2009) and for Lmac and Lbig in Liu *et al.* (2006). Plots were harvested at maturity to determine total yield per plot in t ha^{-1}.

All data analysis were carried out using general ANOVA for artificially inoculated data and unbalanced ANOVA for naturally infected data. Emergence and establishment from naturally infected sites were modelled using multiple linear regression using R statistics. Yield response from naturally infected sites were modelled using decision tree regression model using IBM® SPSS® statistics, which provides visual classification using hierarchal regression model.

Table 4. Sites used for evaluation of naturally occurring *R. solani* AG2-1 in England, France, Germany and Poland in WP4. Further information included is the year of sowing, site soil type, previous crops grown over three years prior to OSR, presence of previous damping off disease symptoms and issue with establishment of the crop, cultivation technique used, date of OSR sowing and soil DNA (AG2-1 pg g^{-1} of soil) quantified prior to sowing.

Year	Country	Site	Soil type	Previous crop prior to OSR	Previous crop year 2	Previous crop year 3	Previous damping off symptoms	Previous issues with establishment	Cultivation technique	Sowing date	Initial site soil AG2-1 DNA (pg g^{-1} of soil)
2017	England	Aspins	Silty Clay loam	Wheat	Potato	Wheat	No	No	Ploughed	08/09	35.40
2017	England	Hilton 2017	Sandy clay loam	Barley	Wheat	OSR	No	No	Min Till	31/08	18.47
2018	France	La Cage	Silty loam	Peas	Sugar beet	Potato	No	No	Ploughed	29/08	8.78
2018	France	Les Grands Bois	Sandy clay loam	Barley	Wheat	OSR	No	Yes	Ploughed	29/08	3.97
2018	Germany	Kribbe	Loamy sand	Wheat	Wheat	Barley	No	No	Min Till	26/08	4.90
2018	Germany	Miesbach	Sandy Loam	Wheat	Wheat	OSR	Yes	No	Ploughed	28/08	4.77
2018	Poland	Toszek	Sandy Loam	Wheat	OSR	Triticale	Yes	No	Min Till	28/08	89.74
2018	England	Ed Banks	Clay/Silty Clay	Barley	Barley	Wheat	No	Yes	Min Till	04/08	34.28
2018	England	Rougham A[a]	Silty sand	Barley	Wheat	OSR	No	Yes	Min Till	06/09	183.11 (AG8)
2019	France	Ransart	Silty loam	Wheat	Peas	Cabbage	No	No	Ploughed	28/08	1.64
2019	France	Simandre	Sandy Loam	Wheat	OSR	Wheat	No	Yes	Min Till	31/08	127.31
2019	Germany	Gallschütz	Sandy Loam	Barley	Wheat	OSR	Yes	No	Min Till	29/08	144.42
2019	England	Huntingdon small	Clay	Wheat	Wheat	OSR	No	No	Direct drilled	28/08	1028.53
2019	England	Fawley	Sandy clay loam	Wheat	Peas	Wheat	No	No	Min Till	31/08	349.24
2019	England	Hilton B	Clay	Barley	Wheat	Wheat	Yes	Yes	Min Till	22/08	13.25

OSR: oilseed rape. Min Till: minimum tillage. [a]: Rougham A had predominantly AG8.

Table 5. Varieties and seed treatments used in each country in WP4.

	Variety trial				Treatment trial			
	England	**France**	**Germany**	**Poland**	**England**	**France**	**Germany**	**Poland**
Varieties	Campus (C) SY Saveo (H) Skye (C) SY Sensia (H) Anastasia (C)	Avatar (H) SY Saveo (H) DK Exception (H) Bluestar (2018) (H) Astronom (2018) (H) Architect (2019) (H) Attletick (2019) (H)	Avatar (H) SY Saveo (H) DK Exception (H) NK Linus (H) Gladius (H)	Avatar (H) SY Saveo (H) DK Exception (H) NK Linus (H) Gladius (H)	Mantara (H) SY Sensia (H)	Avatar (H) Bluestar (2018) (H) Attletick (2019) (H)	Avatar (H) NK Linus (H)	Avatar (H) NK Linus (H)
Treatments	Untreated A21748 A A21748 A + TMX	Untreated A21748 A A21748 A + TMX (2018) A21748 A + Epivio™ (2019)	Untreated A21748 A A21748 A + TMX (2018) A21748 A + Epivio™ (2019)	Untreated A21748 A A21748 A + TMX	Untreated Sedaxane A21748 A Thiram 700 SC HyproDuet	Untreated Sedaxane A21748 A Thiram 700 SC HyproDuet	Untreated Sedaxane A21748 A Thiram 700 SC HyproDuet	Untreated Sedaxane A21748 A Thiram 700 SC HyproDuet
Base treatment					TMX	TMX[a] Epivio™ (2019)	TMX (2018)[a] Epivio™ (2019)	TMX

Variety and base treatment used in 2018 and 2019. Sedaxane (5 ml per 1 million seeds), A21748 A sedaxane, metalaxyl-M and fludioxonil (25 ml per 1 million seeds), thiram 700 SC (28.5 ml per 1 million seeds) and HyproDuet (Thiram + Prochloraz) (45 ml per 1 million seeds). TMX: Thiamethoxam (6g $PRkg^{-1}$). Epivio™ : biostimulant compound (extracts from Vinassse, algae, amino acids, organic nutrients and micronutrients). H: restored hybrid variety; C: conventional variety.

3.7.3. Results

At the artificially inoculated site with AG2-1 and under untreated conditions, cv. Campus achieved significantly higher establishment at GS13 compared to the rest of the varieties (Figure 11). All varieties responded to fungicidal seed treatment with Anastasia and Campus establishing best when TMX was also included. Under natural infection, Architect, Avatar, NK Linus grown in sites in Germany, France and Poland, and Anastasia and Campus grown in sites in England, showed an increase in establishment with seed treatment A21748 A, however the greatest increases in establishment for all varieties apart from Campus, Skye and SY Sensia were achieved when insecticide (TMX) was also included in the seed treatment (Figure 12).

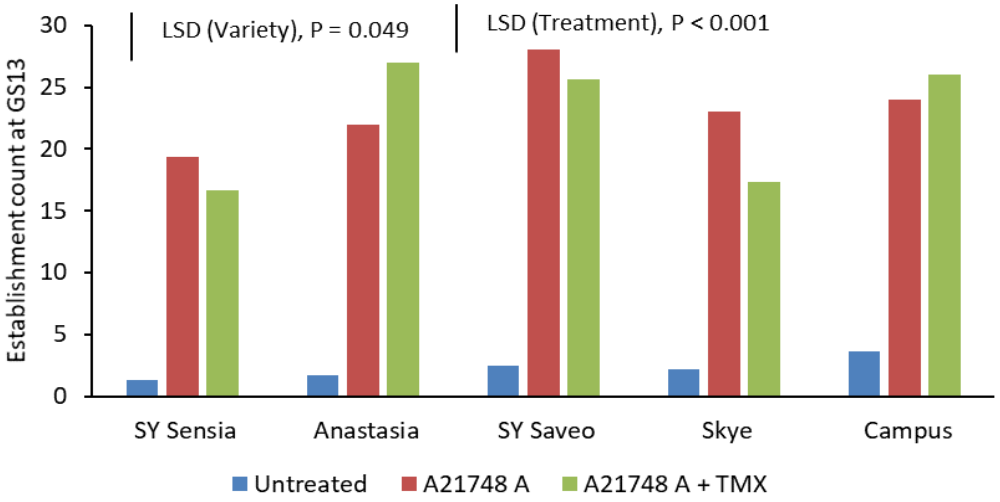

Figure 11. Establishment count at GS13 in two seasons in artificially inoculated sites. LSD: least significant difference.

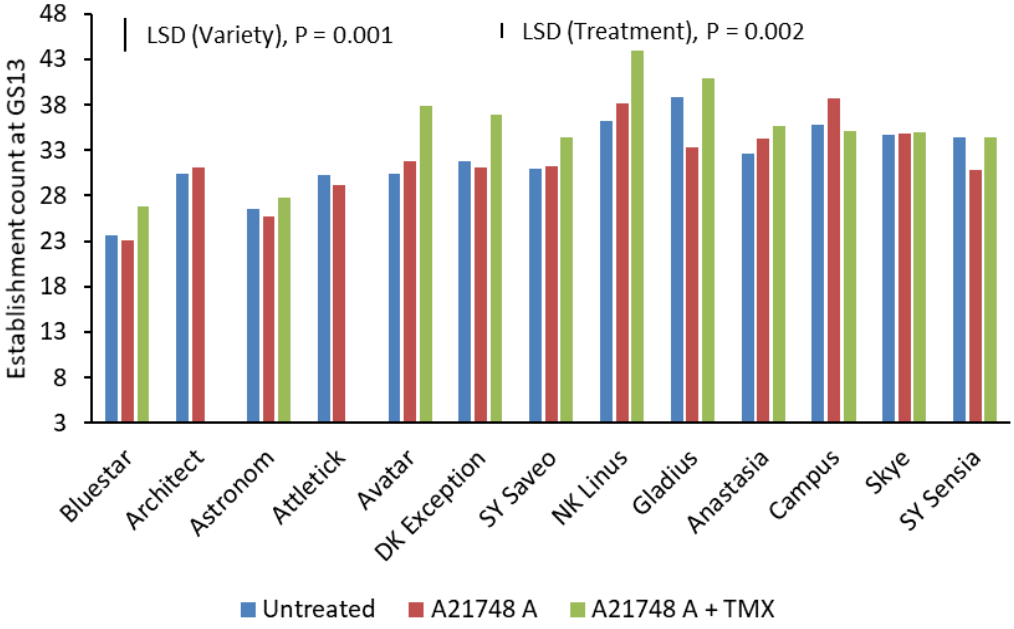

Figure 12. Establishment count at GS13 in naturally infected sites in two seasons. Varieties Architect and Attletick were used in France in 2019 where TMX was no longer registered for use. LSD: least significant difference.

SY Saveo and Avatar and SY Sensia and Skye were the most common varieties grown in EU and UK trials, respectively and were thus selected for pathogen DNA quantification in soil. DNA of *R. solani* AG2-1 in soil at GS32 under artificial inoculation was reduced by 83% by A21748 A compared to untreated plots (Figure 13a). AG2-1 soil DNA in naturally infected sites was reduced with seed treatment A21748 A in SY Sensia and Skye but not in SY Saveo and Avatar (Figure 13b). TMX addition to the fungicidal seed treatment resulted in greater reductions in pathogen DNA in the soil of tested varieties (Figure 13b).

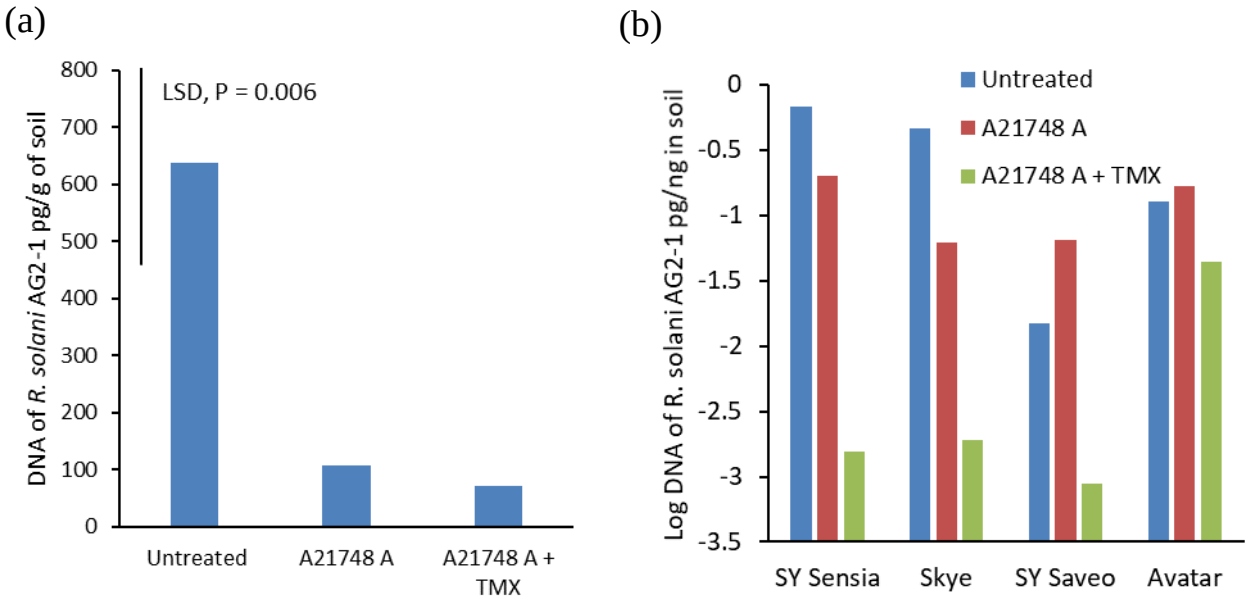

Figure 13. Effect of seed treatment (untreated, A21748 A and A21748 A + TMX) on *R. solani* AG2-1 DNA in soil at GS32 at the (a) artificially inoculated site (pg g^{-1} of soil) and (b) naturally infected sites (log pg ng^{-1} in soil), P>0.05. LSD: least significant difference.

Under natural infection, lower AG2-1 DNA was quantified in seed-treated plants at GS19, with TMX contributing to additional reductions in fungal biomass in SY Saveo and Avatar (Figure 14).

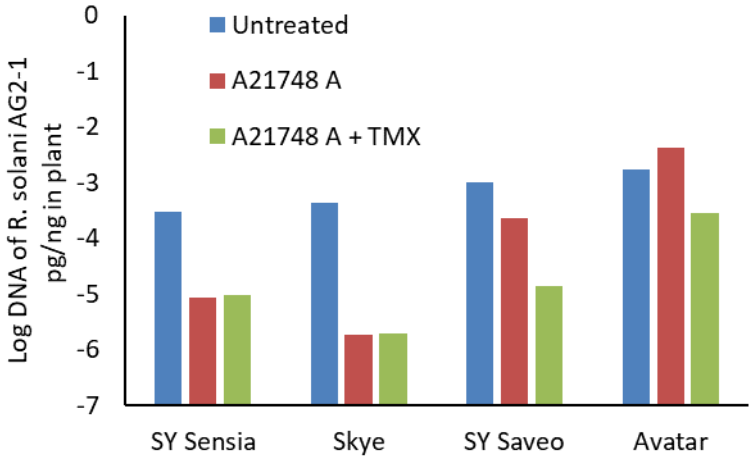

Figure 14. Effect of seed treatment (untreated, A21748 A and A21748 A + TMX) on *R. solani* AG2-1 DNA (log pg ng^{-1}) in plant stems at GS 19 in naturally infected sites (P>0.05).

DNA of the pathogens causing phoma stem canker (*L. maculans* and *L. biglobosa*) in addition to *R. solani* AG2-1 DNA was also quantified in plant stems of OSR collected in naturally infected sites. The greatest reduction in DNA of all three pathogens was seen in plants treated with sedaxane and TMX compared to untreated (Figure 15). Seed treatment A21748 A, reduced pathogen DNA compared to untreated. However, the addition of Epivio™ to fungicidal seed treatment or applying Epivio™ alone resulted in increased DNA of *L. biglobosa* and AG2-1 in plant stems. Results indicate that *L. biglobosa* and *R. solani* AG2-1 are likely to co-exist in mixed infections on plant stems.

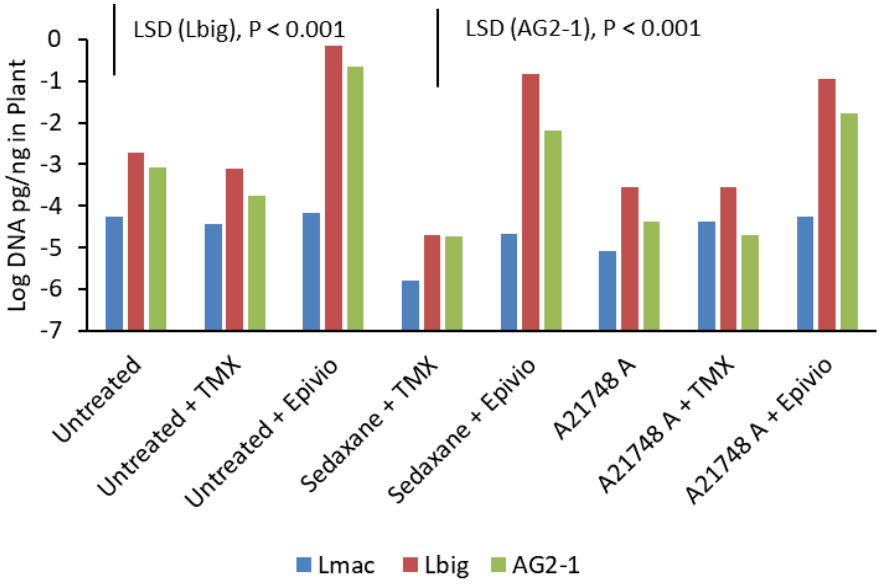

Figure 15. Effect of seed treatment on DNA of *Leptosphaeria maculans* (Lmac), *Leptosphaeria biglobosa* (Lbig) and *R. solani* (AG2-1) in plant stems at GS19 in naturally infected sites. LSD: least significant difference.

The highest yielding varieties under inoculation in the absence of treatment were Campus and Skye (Table 6). However, significant yield response to seed treatment was observed in all varieties, with SY Sensia, SY Saveo and Anastasia showing the greatest increase in yield in response to seed treatment against AG2-1 (Table 6). An additional yield increase of 0.5 tha^{-1} was attributed to TMX for treated Skye and Campus.

Table 6. Effect of seed treatment and oilseed rape variety on yield (t ha^{-1}) in *Rhizoctonia solani* AG2-1 artificially inoculated site in England.

Treatment	Untreated	A21748 A	A21748 A + TMX
SY Sensia	0.89	4.52	4.25
SY Saveo	1.37	4.27	4.30
Anastasia	1.45	4.82	4.19
Skye	2.10	4.60	5.07
Campus	2.49	4.87	5.12
	P-value	LSD (d.f 89)	
Variety	0.013	0.6142	
Treatment	<0.001	0.4757	
Variety x Treatment	0.718	1.0638	

LSD – least significant difference of means (5% level). Degrees of freedom are shown in brackets.

Under natural infection, Campus and Skye (4.8 t ha^{-1}) were the highest yielding varieties whilst Astronom (3.53 t ha^{-1}) was the lowest yielding (Table 7). Astronom, Attletick, Avatar, Gladius, SY Saveo, Anastasia and SY Sensia showed a small yield response to seed treatment that was not significant at $P<0.05$ (Table 7). NK Linus and DK Exception showed no response to treatment whilst Campus only responded to seed treatment in the presence of TMX.

Treatment with A21748 A (4.84 t ha^{-1}) and sedaxane (4.49 t ha^{-1}) achieved significantly higher yield of OSR under infection than the untreated (2.6 t ha^{-1}), HyproDuet (2.44 t ha^{-1}) or thiram (1.67 t ha^{-1}) (Table 8). Mantara was more susceptible than SY Sensia to AG2-1 infection and response to treatment in the former was greater.

Under natural infection a small but not significant response of 100-130 kg/ha was only observed when seed was treated with sedaxane and TMX or HyproDuet and TMX (Table 9). On average, an increase in yield of 0.3 t ha^{-1} are achieved with TMX compared to Epivio™. The addition of Epivio™ to fungicidal seed treatment resulted in lower yields.

Table 7. Effect of oilseed rape variety and seed treatment on yield (t ha^{-1}) at sites naturally infected with *Rhizoctonia solani* AG2-1 in UK and EU.

Treatment	Untreated	A21748 A	A21748 A + TMX
Astronom	3.53	3.79	3.66
Bluestar	3.58	3.47	4.07
Attletick	3.89	4.09	-
Avatar	3.96	4.02	4.06
Gladius	3.97	4.09	4.07
SY Saveo	4.01	4.04	4.10
Architect	4.34	4.30	-
NK Linus	4.34	4.11	4.32
DK Exception	4.43	4.37	4.37
Anastasia	4.45	4.60	4.42
SY Sensia	4.63	4.75	4.65
Campus	4.77	4.77	4.86
Skye	4.77	4.79	4.66

	P-value	LSD (d.f. 832)
Variety	<0.001	0.206
Treatment	0.260	0.080
Variety x Treatment	0.546	0.320

LSD – least significant difference of means (5% level). Degrees of freedom are shown in brackets.

Table 8. Efficacy of different seed treatments (untreated, sedaxane, A21748 A, Thiram 700 SC and HyproDuet) on total yield (t ha^{-1}) in artificially inoculated site with TMX as base treatment.

Treatment	Untreated + TMX	Sedaxane + TMX	A21748 A + TMX	Thiram 700 SC + TMX	HyproDuet+ TMX
Mantara	1.74	4.43	4.39	1.68	2.99
SY Sensia	3.45	4.35	5.29	1.65	1.89

	P-value	LSD (d.f 39)
Variety	0.449	0.76
Treatment	<0.001	1.20
Variety x Treatment	0.187	1.69

LSD – least significant difference (5% level). Degrees of freedom are shown in brackets.

Table 9. Effect of fungicidal seed treatments (untreated, sedaxane, A21748 A, thiram 700 SC and HyproDuet) and the additional base treatment (Epivio^{TM}, TMX or Nil) on yield (t ha^{-1}) in sites naturally infected with *Rhizoctonia solani*.

Base treatment	Epivio^{TM}	TMX	Nil
Untreated	4.23	4.23	4.29
Sedaxane	4.19	4.33	-
A21748 A	4.19	4.28	4.32
Thiram 700 SC	4.06	4.26	-
HyproDuet	4.06	4.36	-
	P-value	LSD (d.f. 1488)	
Treatment	0.404	0.173	

LSD – least significant difference of means (5% level). Nil: absence of base treatment. Degrees of freedom are shown in brackets.

Multiple linear regression was used to model the area under the emergence curve (AUEmergence), calculated as cumulative establishment from sowing to GS19, in naturally infected sites only, using all collected agronomy information from our sites (Table 10). The factors in the model were ranked in order of importance determined by the likelihood ratio test and the model explained 59% of the variance. The most important factor was initial site soil AG2-1 DNA (pg g^{-1} of soil), followed by average temperature from sowing till GS10, history of previous damping off disease symptoms, cultivation technique and soil type (Table 10).

Table 10. Multiple linear regression model for area under the emergence curve in sites naturally infected with *Rhizoctonia solani*

Factors/Variables	Overall importance [a]	Levels of factors	N	Effect (+/-)
Initial site soil (AG 2-1 DNA pg/g of soil)	26	< 10 pg /g of soil *	500	
		11 - 1200 pg /g of soil	798	-
Average Temperature (Sowing – GS 10)	24	Low (12 - 16.5 ºC)*	798	
		High (16.5 - 21 ºC)	500	+
Previous damping off symptoms	23	No *	960	
		Yes	338	-
Cultivation technique	16	Ploughed *	400	
		Direct drilled	100	ns
		Minimum cultivation	798	+
Soil type	13	Loamy sand *	140	
		Silty sand	100	-
		Silty Loam	200	-
		Sandy loam	360	-
		Sandy Clay loam	200	-
		Silty Clay	100	-
		Clay	198	-

[a] Likelihood ratio test. ns (not significant). * Factor level used for comparison. $R^2 = 0.59$, $P > 0.001$. Degrees of freedom is 1292. N: number of observations.

Factors associated significantly with reduced emergence were high initial site soil AG2-1 DNA of 11 - 1200 pg g^{-1} of soil, low average temperature from sowing until GS10 of 12-16.5 ºC, previous history of damping-off symptoms, ploughing of the soil rather than minimum cultivation prior to sowing and any other soil type listed in Table 10 compared to loamy sand.

A similar multiple regression approach was applied to model establishment, with the model accounting for 74% of the variance (Table 11). Cumulative rainfall until GS19 was the most important factor impacting on establishment under natural infection, followed by emergence shown as area under emergence curve (AUEmergence), previous crop prior to OSR being grown and soil type.

Table 11. Multiple linear regression model for establishment of oilseed rape at sites naturally infected with *Rhizoctonia solani*.

Factors/Variables	Overall importance [a]	Levels of factors	N	Effect (+/-)
Cumulative rainfall (Sowing - GS 19)	37	Low (0 -120 mm)	698	-
		Medium (120 - 250 mm)*	500	
		High (250 - 350 mm)	100	-
AUEmergence	34	Low (0 - 200)*	1018	
		High (200 - 500)	280	+
Previous crop	21	Wheat *	700	
		Barley	498	+
		Peas	100	-
Soil type	16	Loamy sand *	140	
		Silty sand	100	-
		Silty loam	200	-
		Sandy loam	360	-
		Sandy clay loam	200	-
		Silty clay	100	+
		Clay	198	-

[a] Likelihood ratio test. * Factor level used for comparison. $R^2 = 0.74$, $P < 0.001$. Degrees of freedom is 1293. N: number of observations.

Factors associated significantly with reduced establishment were too low or too high cumulative rainfall up to GS19, low AUEmergence (0-200), previous crop of peas compared to wheat, and any other soil type listed in Table 11, but silty clay, compared to loamy sand.

We used a decision tree based on hierarchical regressions to model yield from our data. Chi-squared Automatic interactive Detection (CHAID) uses a standard algorithm for inducing classification rules (Kadi and Idri, 2015) in the form of a decision tree based on Chi-square test for categorical datasets. In CHAID, the output variable is a categorical variable with a minimum of two classes. Chi-square test for association was applied with output variable (yield) and each of the predictors (environmental variables, pathogen DNA etc., and factors including variety and treatment). The predictor with the highest Chi-square test value ($P < 0.05$) showing most discriminative power among the categories of the output variable is considered first to split the data into a number of categories defined in that specific predictor (Parent Node). The parent node produces one or more child nodes, numbered consecutively. The method divides the data into parent and child nodes until no more splitting is possible. The minimum number of cases to split a parent node was five and five levels were used in the decision tree. CHAID is a robust model and

does not require statistical assumptions such as normality, outlier removal etc. homoscedasticity and collinearity. The primary benefit of using a CHAID is that, by construction, it produces interpretable decision rulesets. The calculated algorithm was validated using a 10-fold cross validation approach to overcome data overfitting (Tanner *et al.*, 2008).

The Decision tree regression model for yield using data from naturally infected sites accounted for 72% of the variance. Further information on number of observations, % split, Bonferroni test, number of nodes and identifiers are shown in Table S1. Yield was best explained by temperature first and influenced by cumulative rainfall, the latter was included as an influencing variable in the model.

The most influential predictor for yield was average temperature splitting into two categories: <16°C or >16°C from sowing to GS10. Data in <16°C node was best categorised by initial site soil AG2-1 DNA which split into three categories: low, medium and high (Figure 16). Low initial site DNA of AG2-1 (<4.77 pg g^{-1} of soil) further split into treatment, with higher yields explained by higher establishment in plots treated with HyproDuet, sedaxane and thiram and lower yields in untreated or A21748 A-treated, also influenced by AG2-1 DNA accumulation in stems. In categorised data with missing values for AG2-1 DNA in plant stems, yields were higher when A21748 A was used compared to untreated.

Medium initial site DNA of AG2-1 (<4.77-127 pg g^{-1} of soil) split into variety, with Avatar, Bluestar and NK Linus having higher yields than Astronom and Gladius, when treated with HyproDuet, sedaxane or A21748 A than when untreated or treated with thiram. Yields of DK Exception and SY Saveo were influenced by their establishment and were lower than yields of Mantara, SY Sensia, Campus, Anastasia or Skye, which were lower when initial site DNA of AG2-1 exceeded 13.25 pg g^{-1} of soil.

Under the high category of initial site DNA, particularly when very high >349.24 pg g^{-1} of soil, varieties were differentiated in descending order for yield: Skye, Campus, SY Sensia, Mantara, Anastasia, SY Saveo. Under these conditions, the difference in yield between the highest yielding variety Skye and lowest yielding variety SY Saveo was 1.42 t ha^{-1} (Figure 16).

Data in > 16°C node was best categorised by pest damage at GS19 and missing values for pest damage (for some sites pest damage was not available and these were classified as missing). The second classifier was initial site soil AG2-1 DNA which split into three categories: low, medium and high. Under both low and medium categories, DK Exception always had a higher yield compared to the rest of the genotypes, whilst Avatar, when exposed to high initial DNA of AG2-1 achieved higher yield when treated with A21748 A compared to the rest of the treatments. SY Saveo had

lowest yield compared to the rest of the tested genotypes. In the category of missing values for pest damage, genotypes ranked with Campus and Skye having higher predicted yields than SY Saveo, Mantara or Anastasia (Figure 17).

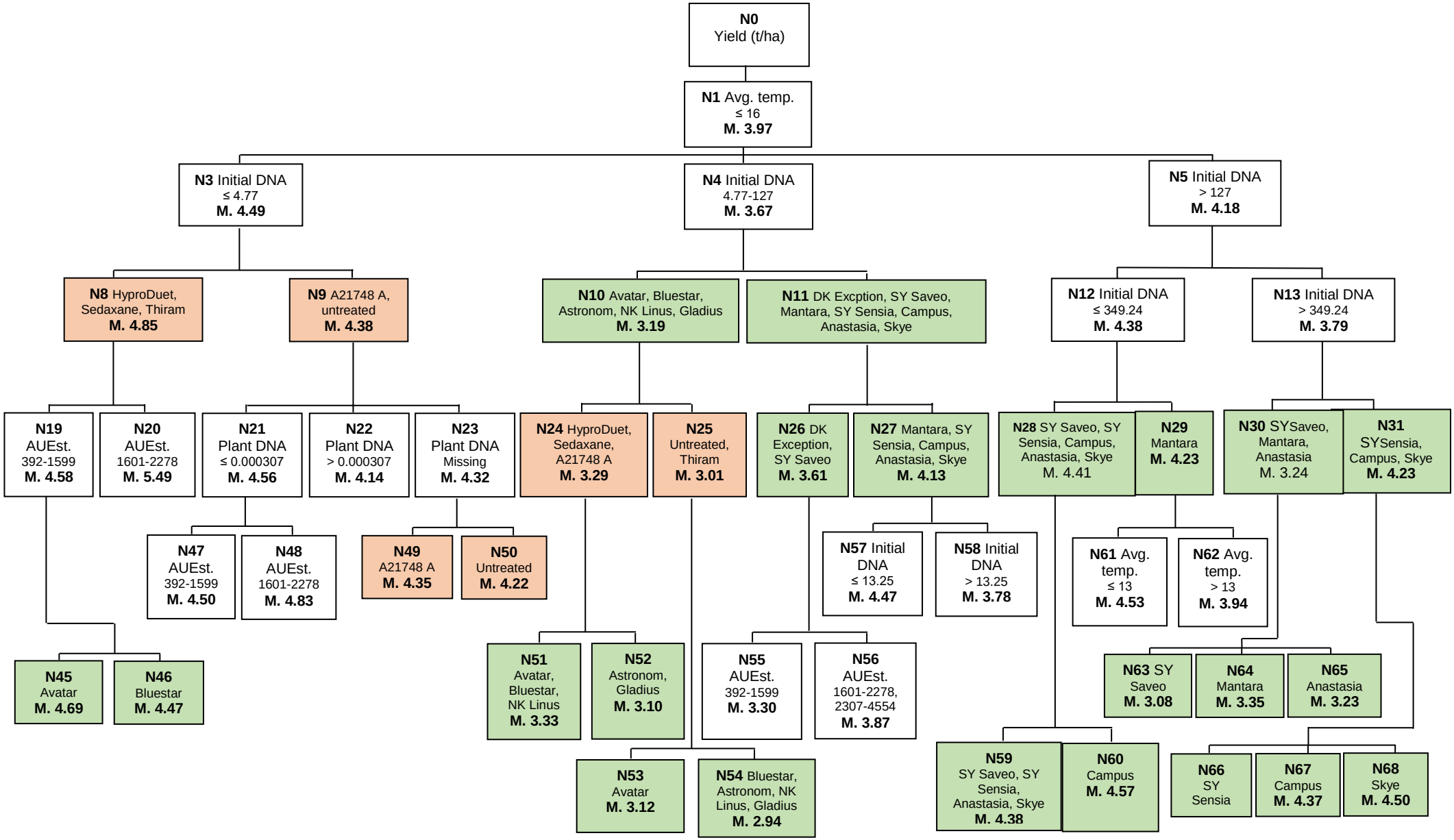

Figure 16. Part 1 of yield model using decision tree (R^2 = 0.719). The data is classified into hierarchal regression models using Chi-square test for categorical datasets. N: Node number. M.: mean yield value. N10-11, N26-31, N45-46, N51-54, N59-60 and N63-68 are varieties (green). N8-9, N24-25 and N49-50 are Treatments (brown). Missing values: data not recorded. Initial DNA is in pg g^{-1} of soil. Plant DNA is in ng pg $^{-1}$.

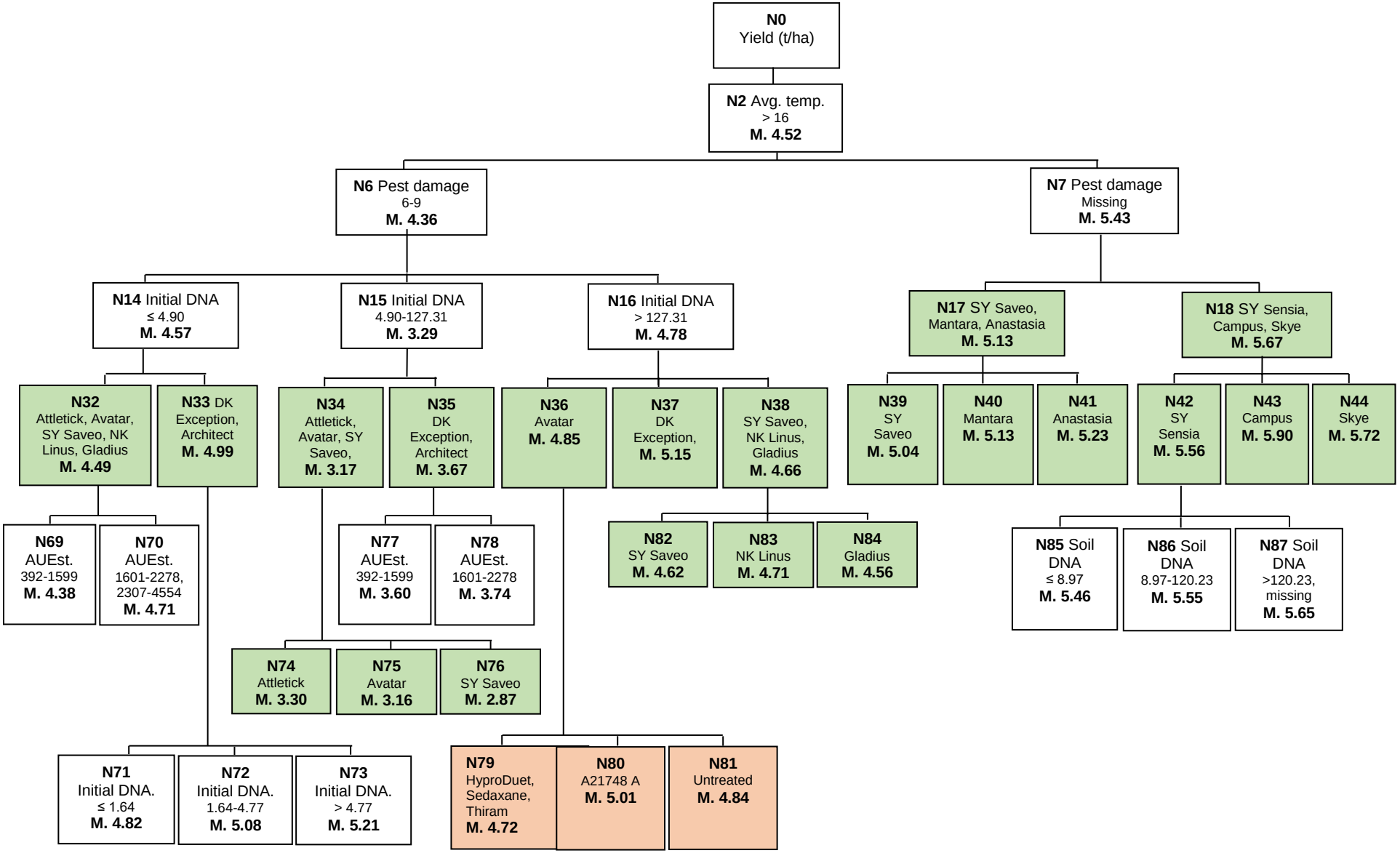

Figure 17. Part 2 of yield model using decision tree (R^2 = 0.719). The data is classified into hierarchal regression models using Chi-square test for categorical datasets. N: Node number. M.: mean yield value. N17-18, N32-44, N74-76 and N82-84 are varieties (green). N79-81 are treatment (brown). Missing values: data not recorded. Initial DNA and soil DNA are in pg g^{-1} of soil.

3.7.4. Discussion

Overall the results on the effect of genotype in establishment and yield were similar in both artificially inoculated and naturally infected trials. Genotypes with higher establishment under natural or artificial infection by *R. solani* AG2-1 achieved generally higher yields. However, yield response to seed treatment under natural infection was small and not significant at 5% LSD. In particular, the hybrids, Bluestar, Architect, NK Linus and DK Exception, yielded less under fungicide treatment, however a note must be made that the former two were tested only in one season. NK Linus and DK Exception also failed to respond in yield to seed treatment inclusive of insecticide despite showing a significant response in establishment to the insecticide addition in treatment.

Under high disease pressure with artificial infection, all genotypes responded in establishment or yield significantly to seed treatment against *R. solani* AG2-1, whilst under natural infection response to the insecticide as part of the fungicidal seed treatment was significantly greater than to fungicide alone. In addition, under natural infection, *R. solani* AG2-1 was found to co-exist on OSR stems with other pathogenic organisms, such as *L. biglobosa* and this relationship was favoured when the biostimulant Epivio™ was included as part of the seed treatment application. Epivio™ claims a soil-priming effect leading to an enhanced rhizosphere microflora activity and improved bioavailability of nutrients (pers. comm. B. Slaats, Syngenta). It is possible that this compound also positively benefits *R. solani* and other pathogenic organisms within the plant rhizosphere in addition to having negative impact on the activity of the fungicide as part of the formulation of treatment. A21748 A and sedaxane were most effective in increasing establishment and yield under artificial inoculation, similarly under natural infection sedaxane and HyproDuet (inclusive of prochloraz and thiram) were also effective.

Following recent changes in EU legislation (Reg. EC 1107/2009) thiram is no longer approved for use in EU countries, thus the options available to growers will include seed treatments inclusive of sedaxane or fludioxonil for treatment against soil-borne pathogens such as *R. solani*. Fludioxonil has been shown in past to be effective against AG 4 (Xue *et al.*, 2007), whilst the SDHIs, sedaxane and penflufen, were highly active against strains of AG2-2, 4, 7 and 11 isolated from soybeans (Ajayi-Oyetunde *et al.*, 2017). Here we show that sedaxane alone or in formulation with metalaxyl-M and fludioxonil (A21748 A) is effective against damping off by AG2-1 in OSR and increased establishment through reductions in pathogen DNA in soil and in plant stems.

The relationship between emergence, establishment and yield under natural infection was highly influenced by the DNA of *R. solani* AG2-1 in soil, agronomy factors (cultivation, soil type, and previous crop), treatment and the environment (temperature and cumulative rainfall). Favourable conditions for emergence included low initial site AG2-1 DNA of <10 pg g^{-1} of soil, the absence of

previous damping off disease symptoms, an average temperature from sowing until GS10 of 16.5 - 21^{o}C, minimum cultivation technique and loamy sand soil type. High emergence (AUEmergence of 200-500), moderate cumulative rainfall of 120-250 mm, previous crop of barley rather than wheat prior to OSR, and sowing in loamy sand or silty clay soil types were most favourable for good establishment.

Yield was influenced by two main environmental factors, average temperature and cumulative rainfall up to GS19. Yield loss was associated with high pathogen DNA found in soils or in plant stems, average temperature <16^{o}C from sowing to GS10. Yield loss was reduced by sedaxane or A21748 A in susceptible genotypes with lower establishment. Yield response of genotypes to seed treatment was an average of 150 kg /ha. However, Campus and Skye were identified as the most tolerant to Rhizoctonia damping-off disease and yielded higher under natural infection or inoculation than the rest of genotypes tested here.

4. Conclusions

R. solani is associated with greater yield loss under low cumulative rainfall by GS19 and average temperature of < 16^{o}C by GS 10. Crop losses result due to reductions in plant establishment, pathogen DNA accumulation in plant stems and delay in crop development and uneven flowering.

R. solani may co-occur with phoma pathogens and infection is associated with increased damage by pests such as the cabbage stem flea beetle. Thus, plant emergence and establishment responses of OSR genotypes were greater when the fungicide seed treatment was applied with insecticide. There is a positive association between *R. solani* and *L. biglobosa* in stems and the biostimulant Epivio^{TM} increased accumulation of these pathogens. Thus, treating against damping off disease protects early seedling establishment, root growth and reduces AG2-1 and *L. biglobosa* in stems. Seed rate increase and seed treatment also contribute to less pest damage in plants.

Under high disease pressure (inoculated trials) sedaxane-based seed treatment increased establishment in all varieties through reductions in pathogen DNA in soil. Without seed treatment, Campus achieved the highest establishment and yield from the conventional genotypes grown in England.

The highest concentrations of the pathogen found in naturally infected sites and in the artificially inoculated, untreated plots were just over 1000 pg g^{-1} of soil and less than 700 pg g^{-1} of soil, respectively. However, for the useful prediction of the disease and its effects on yield other factors including soil type, temperature and rainfall and possibly other microorganisms present in the soil environment are also of importance. As there is a limited information at present on the most

appropriate field sampling strategy for soil-borne pathogens including *R. solani* future work on field sampling strategies paired with molecular test for the quantification of the pathogen in soil will help to accurately identify fields under high disease risk.

5. Acknowledgements

ICAROS (102649) was funded by Innovate UK, BBSRC, AHDB for cereals & oilseeds and Syngenta as the industrial lead. We would like to thank all the participating companies and their staff for their major contributions in completing this project including the trials teams in University of Nottingham, Syngenta UK, France, Germany and Global, Essais-plus FR, BioTek agriculture FR, IPP SOŚNICOWICE Branch Poland ADAS for field organisation and data collection. We would also like to extend our thanks to Dr Lenka Havlickova and Prof Ian Bancroft from the University of York for the support and GWAS analysis. We would also like to thank Mr Michael Tait and Mr Laurent Gobert for their technical advice, trial management and support throughout this project and Dr Jane Wibley for managing the project and Dr Amanda Bennett from AHDB for her support of this project. Finally, we would like to acknowledge the staff and students at University of Nottingham who have contributed to the field and lab experimentations.

Table S1. Yield model using decision tree (R^2 = 0.719) on Statistical Package for the Social Sciences (SPSS). The data is classified into hierarchal regression models using Chi-square test for categorical datasets.

Node	Predicted Mean	Std. Dev.	N	Percent	Parent Node	Variable	Sig.[a]	F	d.f. 1	d.f. 2	Split Values
0	4.15	0.795	1389	100%							
1	3.90	0.694	791	57%	0	Avg temp	< 0.001	56653	1	183008	<= 16
2	4.73	0.804	598	43%	0	Avg temp	< 0.001	56653	1	183008	> 16, <missing>
3	4.49	0.546	100	7%	1	Initial soil AG2-1 DNA	< 0.001	30759	2	127967	<= 4.77
4	3.56	0.662	392	28%	1	Initial soil AG2-1 DNA	< 0.001	30759	2	127967	4.77, 127.31
5	4.23	0.589	299	22%	1	Initial soil AG2-1 DNA	< 0.001	30759	2	127967	> 127.31
6	4.53	0.738	498	36%	2	Pest damage	< 0.001	19265	1	55038	6-9
7	5.43	0.438	100	7%	2	Pest damage	< 0.001	19265	1	55038	<missing>
8	4.85	0.687	24	2%	3	Treatment	< 0.001	2543	1	15867	HyproDuet, Sedaxane, Thiram 700 SC
9	4.38	0.441	76	6%	3	Treatment	< 0.001	2543	1	15867	A21748 A, Untreated
10	3.25	0.381	152	11%	4	Variety	< 0.001	24612	1	69034	Avatar, Bluestar, Astronom, NK Linus, Gladius
11	3.82	0.621	240	17%	4	Variety	< 0.001	24612	1	69034	DK Exception, SY Saveo, Mantara, SY Sensia, Campus, Anastasia, Skye
12	4.36	0.330	199	14%	5	Initial soil AG2-1 DNA	< 0.001	10954	1	43061	<= 349.24
13	3.79	0.776	100	7%	5	Initial soil AG2-1 DNA	< 0.001	10954	1	43061	> 349.24
14	4.62	0.598	298	22%	6	Initial soil AG2-1 DNA	< 0.001	8812	2	42647	<= 4.90
15	3.29	0.340	100	7%	6	Initial soil AG2-1 DNA	< 0.001	8812	2	42647	4.90, 127.31
16	4.78	0.281	100	7%	6	Initial soil AG2-1 DNA	< 0.001	8812	2	42647	> 127.31
17	5.13	0.329	44	3%	7	Variety	< 0.001	7193	1	12388	SY Saveo, Mantara, Anastasia
18	5.67	0.366	56	4%	7	Variety	< 0.001	7193	1	12388	SY Sensia, Campus, Skye
19	4.58	0.479	17	1%	8	AUEstab	< 0.001	2286	1	3806	392-1599
20	5.49	0.720	7	1%	8	AUEstab	< 0.001	2286	1	3806	1601-2278
21	4.56	0.478	24	2%	9	Plant AG2-1 DNA	< 0.001	669	2	12058	<= .0000307
22	4.14	0.322	8	1%	9	Plant AG2-1 DNA	< 0.001	669	2	12058	> .0000307
23	4.32	0.408	44	3%	9	Plant AG2-1 DNA	< 0.001	669	2	12058	<missing>
24	3.35	0.330	96	7%	10	Treatment	< 0.001	4555	1	30793	HyproDuet, Sedaxane, A21748 A
25	3.07	0.401	56	4%	10	Treatment	< 0.001	4555	1	30793	Thiram 700 SC,

											Untreated
26	3.58	0.495	71	5%	11	Variety	< 0.001	4211	1	38238	DK Exception, SY Saveo
27	3.94	0.605	169	12%	11	Variety	< 0.001	4211	1	38238	Mantara, SY Sensia, Campus, Anastasia, Skye
28	4.40	0.305	159	11%	12	Variety	< 0.001	2700	1	33272	SY Saveo, SY Sensia, Campus, Anastasia, Skye
29	4.18	0.389	40	3%	12	Variety	< 0.001	2700	1	33272	Mantara
30	3.24	0.575	44	3%	13	Variety	< 0.001	6505	1	9788	SY Saveo, Mantara, Anastasia
31	4.23	0.626	56	4%	13	Variety	< 0.001	6505	1	9788	SY Sensia, Campus, Skye
32	4.55	0.581	250	18%	14	Variety	< 0.001	2890	1	34438	Attletick, Avatar, SY Saveo, NK Linus, Gladius
33	5.03	0.516	48	4%	14	Variety	< 0.001	2890	1	34438	DK Exception, Architect
34	3.17	0.284	76	6%	15	Variety	< 0.001	2200	1	3448	Attletick, Avatar, SY Saveo
35	3.67	0.201	24	2%	15	Variety	< 0.001	2200	1	3448	DK Exception, Architect
36	4.85	0.230	32	2%	16	Variety	< 0.001	1236	2	4757	Avatar
37	5.15	0.271	12	1%	16	Variety	< 0.001	1236	2	4757	DK Exception
38	4.66	0.221	56	4%	16	Variety	< 0.001	1236	2	4757	SY Saveo, NK Linus, Gladius
39	5.04	0.297	12	1%	17	Variety	< 0.001	134	2	5448	SY Saveo
40	5.13	0.389	20	1%	17	Variety	< 0.001	134	2	5448	Mantara
41	5.23	0.231	12	1%	17	Variety	< 0.001	134	2	5448	Anastasia
42	5.56	0.394	32	2%	18	Variety	< 0.001	569	2	6935	SY Sensia
43	5.90	0.185	12	1%	18	Variety	< 0.001	569	2	6935	Campus
44	5.72	0.323	12	1%	18	Variety	< 0.001	569	2	6935	Skye
45	4.69	0.343	9	1%	19	Variety	< 0.001	163	1	2695	Avatar
46	4.47	0.601	8	1%	19	Variety	< 0.001	163	1	2695	Bluestar
47	4.49	0.396	19	1%	21	AUEstab	< 0.001	347	1	3806	392-1599
48	4.83	0.708	5	0%	21	AUEstab	< 0.001	347	1	3806	1601-2278
49	4.35	0.421	32	2%	23	Treatment	< 0.001	142	1	6980	A21748 A
50	4.22	0.372	12	1%	23	Treatment	< 0.001	142	1	6980	Untreated
51	3.40	0.333	80	6%	24	Variety	< 0.001	3178	1	19447	Avatar, Bluestar, NK Linus
52	3.08	0.250	16	1%	24	Variety	< 0.001	3178	1	19447	Astronom, Gladius
53	3.27	0.438	24	2%	25	Variety	< 0.001	2562	1	11343	Avatar
54	2.92	0.358	32	2%	25	Variety	< 0.001	2562	1	11343	Bluestar, Astronom, NK Linus, Gladius
55	3.34	0.362	33	2%	26	AUEstab	< 0.001	3926	1	13095	392-1599
56	3.74	0.439	38	3%	26	AUEstab	< 0.001	3926	1	13095	1601-2278.3, 2307-4554
57	4.46	0.545	86	6%	27	Initial soil AG2-1 DNA	< 0.001	8886	1	25141	<= 13.25
58	3.78	0.458	83	6%	27	Initial soil AG2-1 DNA	< 0.001	8886	1	25141	> 13.25
59	4.37	0.293	135	10%	28	Variety	< 0.001	1871	1	26590	SY Saveo, SY Sensia, Anastasia, Skye
60	4.59	0.328	24	2%	28	Variety	< 0.001	1871	1	26590	Campus

61	4.53	0.306	20	1%	29	Avg temp	< 0.001	10750	1	6679	<= 13
62	3.93	0.173	20	1%	29	Avg temp	< 0.001	10750	1	6679	> 13
63	3.08	0.419	12	1%	30	Variety	< 0.001	87	2	4304	SY Saveo
64	3.35	0.657	20	1%	30	Variety	< 0.001	87	2	4304	Mantara
65	3.23	0.566	12	1%	30	Variety	< 0.001	87	2	4304	Anastasia
66	4.07	0.600	32	2%	31	Variety	< 0.001	277	2	5479	SY Sensia
67	4.37	0.706	12	1%	31	Variety	< 0.001	277	2	5479	Campus
68	4.50	0.519	12	1%	31	Variety	< 0.001	277	2	5479	Skye
69	4.37	0.473	162	12%	32	AUEstab	< 0.001	2471	1	29284	392-599
70	4.71	0.690	88	6%	32	AUEstab	< 0.001	2471	1	29284	1601-2278, 2307- 4554
71	4.82	0.504	24	2%	33	Initial soil AG2-1 DNA	< 0.001	299	2	5151	<= 1.6386115
72	5.08	0.270	12	1%	33	Initial soil AG2-1 DNA	< 0.001	299	2	5151	1.64, 4.77
73	5.21	0.642	12	1%	33	Initial soil AG2-1 DNA	< 0.001	299	2	5151	> 4.77
74	3.30	0.239	32	2%	34	Variety	< 0.001	488	2	2619	Attletick
75	3.16	0.261	32	2%	34	Variety	< 0.001	488	2	2619	Avatar
76	2.87	0.215	12	1%	34	Variety	< 0.001	488	2	2619	SY Saveo
77	3.60	0.189	12	1%	35	AUEstab	< 0.001	123	1	826	392-1599
78	3.74	0.195	12	1%	35	AUEstab	< 0.001	123	1	826	1601-2278
79	4.72	0.078	12	1%	36	Treatment	< 0.001	338	2	1520	HyproDuet, Sedaxane, Thiram 700 SC
80	5.00	0.248	12	1%	36	Treatment	< 0.001	338	2	1520	A21748 A
81	4.84	0.238	8	1%	36	Treatment	< 0.001	338	2	1520	Untreated
82	4.62	0.198	12	1%	38	Variety	< 0.001	104	2	2662	SY Saveo
83	4.71	0.203	32	2%	38	Variety	< 0.001	104	2	2662	NK Linus
84	4.56	0.266	12	1%	38	Variety	< 0.001	104	2	2662	Gladius
85	5.46	0.492	11	1%	42	Soil AG2-1 DNA	< 0.001	85	2	3961	<= 8.96
86	5.55	0.376	8	1%	42	Soil AG2-1 DNA	< 0.001	85	2	3961	8.96, 120.23
87	5.65	0.318	13	1%	42	Soil AG2-1 DNA	< 0.001	85	2	3961	> 120.23, <missing>

Parent node: categories where the data is split by the most discriminative power and predictor with the highest Chi-square test value ($P < 0.05$). Growing Method: EXHAUSTIVE CHAID. Dependent Variable: Yield (t/ha). Sig[a]: Bonferroni adjusted significance. Cumulative rainfall was used as an influence variable. Initial soil AG2-1 DNA: pg/g pf soil. Avg temp: average temperature. Soil AG2-1 DNA: pg/g of soil. Plant AG2-1 DNA: pg/ng. AUEst.: Area under establishment curve.

6. References

Ajayi-Oyetunde O.O., Butts-Wilmsmeyer C.J., Bradley C.A. (2017) Sensitivity of *Rhizoctonia solani* to Succinate Dehydrogenase Inhibitor and Demethylation Inhibitor Fungicides. *Plant Disease*, 101, 487-495.

Babiker E. M., Hulbert S. H., Schroeder K. L., Paulitz T. C. (2013) Evaluation of Brassica species for resistance to *Rhizoctonia solani* and binucleate Rhizoctonia (*Ceratobasidum* spp.) under controlled environment conditions. *European Journal of Plant Pathology,* **136**, 763-772.

Blake J., Spink J. (2005) Variability of rooting in OSR. *Aspects of Applied Biology,* **73**, 195-198.

Blake J., Spink J., Bullard M. (2004) Successful establishment of oilseed rape.

Brown M., Woodhall J., Mooney S., Ray R. (2014) The occurrence and population dynamics of *Rhizoctonia solani* in soil of winter wheat. *In*: The Dundee Conference. Crop Protection in Northern Britain 2014, Dundee, UK, 25-26 February 2014, 2014. The Association for Crop Protection in Northern Britain, pp. 107-112.

Budge G. E., Shaw M. W., Colyer A., Pietravalle S., Boonham N. (2009) Molecular tools to investigate *Rhizoctonia solani* distribution in soil. *Plant Pathology,* **58**, 1071-1080.

Drizou F., Graham N. S., Bruce T. J. A., Ray R. V. (2017) Development of high-throughput methods to screen disease caused by *Rhizoctonia solani* AG 2-1 in oilseed rape. *Plant methods,* **13**, 45-45.

Ellis P. J. (1983) Diseases of rapeseed in the Peace River region of Alberta*. M.P.M. Thesis. Simon Fraser University, Burnaby, B.C., Canada,* p 89.

Harris K., Young I. M., Gilligan C. A., Otten W., Ritz K. (2003) Effect of bulk density on the spatial organisation of the fungus *Rhizoctonia solani* in soil. *FEMS Microbiology Ecology,* **44**, 45-56.

Havlickova L., He Z., Wang L., Langer S., Harper A. L., Kaur H., Broadley M. R., Gegas V., Bancroft I. (2017) Validation of an updated Associative Transcriptomics platform for the polyploid crop species *Brassica napus* by dissection of the genetic architecture of erucic acid and tocopherol isoform variation in seeds. *The Plant Journal,* **93**, 181-192.

Goll M. B., Schade-Schuetze A., Swart G., Oostendorp M., Schott J. J., Jaser B., Felsenstein F.G. (2014) Survey on the prevalence of *Rhizoctonia* spp. in European soils and determination of the baseline sensitivity towards sedaxane. *Plant Pathology*, **63**, 148–154.

Kadi I., Idri A. (2015) A Decision Tree-Based Approach for Cardiovascular Dysautonomias Diagnosis: A Case Study. *IEEE Symposium Series on Computational Intelligence*, 816-823.

Kataria H. R., Verma P. R. (1992) *Rhizoctonia solani* damping-off and root rot in oilseed rape and canola. *Crop Protection,* **11**, 8-13.

Ko W., Hora F.K. (1971) A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. *Phytopathology*, **61**, 707-710.

Le Cointe R., Simon T.E., Delarue P., Hervé M., Leclerc M., Poggi S. (2016). Reducing the Use of Pesticides with Site- Specific Application: The Chemical Control of *Rhizoctonia solani* as a Case of Study for the Management of Soil-Borne Diseases. *PLoS ONE,* **11**,

Liu S. Y., Liu Z., Fitt B. D. L., Evans N., Foster S. J., Huang Y. J., Latunde-Dada A. O., Lucas J. A. (2006) Resistance to *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape) induced by *L. biglobosa* and chemical defence activators in field and controlled environments. *Plant Pathology,* **55**, 401-412.

Otten W., Gilligan C. (1998) Effect of the physical condition on the spatial and temporal dynamics of the soil bourne pathogen *Rhizoctonia solani*. *New Phytologist,* **138**, 629-637.

Otten W., Hall D., Harris K., Ritz K., Young I. M., Gilligan C. A. (2001) Soil physics, fungal epidemiology and the spread of *Rhizoctonia solani*. *New Phytologist,* **151**, 459-468.

Ray R. V., Jenkinson P., Edwards S. G. (2004) Effects of fungicides on eyespot, caused predominantly by *Oculimacula acuformis*, and yield of early-drilled winter wheat. *Crop Protection,* **23**, 1199-1207.

Sippell D. W., Davidson J. G. N., Sadasivaiah R. S. (1985) Rhizoctonia Root Rot of Rapeseed in the Peace River Region of Alberta. *Canadian Journal of Plant Pathology*, 184-186.

Spink J., Street P., Sylvester-Bradley R., Berry P. (2005) The potential to increase productivity of wheat and oilseed *Report to the Government Chief Scientific Adviser, Professor John Beddington*, 71-78.

Sturrock C. J., Woodhall J., Brown M., Walker C., Mooney S. J., Ray R. V. (2015) Effects of damping-off caused by *Rhizoctonia solani* anastomosis group 2-1 on roots of wheat and oil seed rape quantified using X-ray Computed Tomography and real-time PCR. *Frontiers in Plant Science,* **6**.

Tanner L, Schreiber M, Low JGH, Ong A, Tolfvenstam T, *et al.* (2008) Decision Tree Algorithms Predict the Diagnosis and Outcome of Dengue Fever in the Early Phase of Illness. *PLOS Neglected Tropical Diseases*, **2**, e196.

Verma, P. R. (1996) Biology and control of *Rhizoctonia solani* on rapeseed: a review. *Phytoprotection*, **77**, 99–111.

Woodhall J., Laurenson L., Peters J. (2012) First report of *Rhizoctonia solani* anastomosis group 5 (AG5) in wheat in the UK. *New Disease Reports,* **26**.

Woodhall J. W., Brown M. J., Perkins K., Valdeolmillos E. S., Boonham N., Ray R. V. (2017) A TaqMan real-time PCR assay for *Rhizoctonia cerealis* and its use in wheat and soil. *European Journal of Plant Pathology,* **148**, 237-245.

Woodhall J. W., Lees A. K., Edwards S. G., Jenkinson P. (2007) Characterization of *Rhizoctonia solani* from potato in Great Britain. *Plant Pathology,* **56**, 286-295.

Yang J., Verma P.R. (1992) Screening genotypes for resistance to pre-emergence damping-off and postemergence seedling root rot of oilseed rape and canola caused by *Rhizoctonia solani* AG-2-1, *Crop Protection*, **11**, 443-448.

Xue A., Cober E., Morrison M., Voldeng H., Ma B. (2007) Effect of seed treatments on emergence, yield, and root rot severity of soybean under *Rhizoctonia solani* inoculated field conditions in Ontario. *Canadian Journal of Plant Science*, **87**, 167-174.

Zeun R., Scalliet G., Oostendorp M. (2013) Biological activity of sedaxane – a novel broad-spectrum fungicide for seed treatment. *Pest Management Science,* **69**, 527-534.