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Reducing the impact of sclerotinia disease on arable rotations, vegetable crops and land use

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CONTENTS

1. Abstract

Sclerotinia disease, caused by the fungus *Sclerotinia sclerotiorum,* has two key phases susceptible to control: long-lived resting bodies (sclerotia) in soil, and airborne spores, produced when sclerotia germinate to produce mushroom-like apothecia, starting in spring. This project investigated control during these key phases, with two main objectives: [1] To improve timing of fungicide applications, based on weather data and/or airborne inoculum detection, and [2] To quantify the effect of soil management and crop rotation on sclerotinia disease.

Two forecasting models were tested for predicting infection by sclerotinia. First, a sclerotial germination model correctly predicted onset of germination by region (SW earliest, NW latest) and by year, but with an error of ± two weeks for individual sites. The model provides a useful forecast guide to the main onset of spore inoculum release by region. Second, a weather-based infection model was tested with sprays applied according to forecast alert dates. The model gave 76–96% control of sclerotinia disease in oilseed rape, equivalent to the best control determined in retrospect with a standard fungicide timing. During experiments, sclerotinia inoculum was measured by petal testing, and high infection (90–100%) in oilseed rape was associated with 15–30% stem rot. Zero or very low petal infection often resulted in low stem rot. Air samples from Burkard spore traps were tested for the presence of sclerotinia DNA using quantitative PCR tests. Peaks of inoculum detected by rooftop spore traps, in the same region as the experimental field sites, generally coincided with the timing of inoculum peaks from air samples from spore traps within-field, suggesting that that regional spore traps can indicate infection risk. The key factors for assessing sclerotinia risk in-field were: infection model alerts, petal infection and forecast temperature and rain.

Optimum crop rotations for sclerotinia control were modelled using dynamic programming, aimed at maximising profits while reducing sclerotinia. The model showed that only one non-susceptible crop in a rotation is needed to prevent long term build-up of sclerotia while also providing the greatest financial benefit. The model confirmed that rotation gives the greatest financial benefits for high sclerotinia pressure, but is also the best financial strategy for low sclerotinia.

In experiments where sclerotial production on different crops was investigated, carrots produced several thousand sclerotia/m^2 (high plant density, small sclerotia), whereas oilseed rape produced a few hundred (lower plant density and large sclerotia). Numbers in lettuce, peas, beans and potatoes were intermediate. It appears that an infected carrot crop may pose the highest risk to following crops in the rotation, but there may be a modifying effect of sclerotial size, i.e. small sclerotia produce fewer apothecia. The amount of sclerotinia spore inoculum produced in spring was similar in oilseed rape crops following ploughing or minimum-tillage, as determined from testing petals for sclerotinia presence.

2. Introduction

Sclerotinia disease caused by the fungus *Sclerotinia sclerotiorum* is a recurring problem in the UK. For example, the disease incidence in 2012 in oilseed rape was 10% of crops and 2% of plants affected; however, much greater levels of disease have been seen in some seasons and an average of 36.5% of crops affected since surveys began in 1986 (www.cropmonitor.co.uk), with yield losses estimated at £20 million per annum. A significant proportion of fungicide use is directed at sclerotinia, e.g. sclerotinia sprays account for 10% of the treated area for oilseed rape and field beans (Garthwaite *et al.*, 2007). The largest area of a sclerotinia-susceptible crop in the UK is occupied by oilseed rape (600,000 ha) in 2007 (https://www.gov.uk/government/organisations/department-for-environment-food-rural-affairs/about/statistics(Defra, 2012)), but other host crops also occupy a significant area; 140,000 ha of potatoes, 161,000 ha of peas and beans, 6,000 ha of lettuce and 10,000 ha of carrots (Defra statistics, 2007). Disease in these crops contributes to the build-up of sclerotial inoculum in soil. All infected plants are a potential source of inoculum for infecting other susceptible crops in adjacent fields or following crops in rotations, so improvements in control in any one crop will have wider benefits. In general, losses relate to the extent of infection, but for some crops such as green beans and vining peas, there is zero tolerance for infection as the entire crop may be rejected if contamination with sclerotinia is found. The risk of sclerotinia infection varies across regions and seasons, and although much is known about the biology of the disease, major outbreaks are still difficult to predict. Foliar fungicides are currently the mainstay of control and can be very effective against sclerotinia, provided they are applied at the correct time, before infection occurs. Soil treatments such as the biological control agent Contans (the fungus *Coniothyrium minitans*) may provide an opportunity to control sclerotia of *S. sclerotiorum* in soil.

Most sclerotinia inoculum originates within-field and is generated from sclerotia produced in infected plants and which can survive in soil for several years. The airborne ascospores released by apothecia from germinated sclerotia are small enough to be dispersed in air over long distances (e.g. >100 km) but disease gradients are rarely seen more than 150 – 200 m from a source (Williams & Stelfox, 1979). Some reports suggest that spore inoculum arrives mainly from adjacent fields and seldom from further afield (Bourdot *et al*, 2001; Hammond *et al.*, 2008); ascospore numbers in air were found by Bourdot *et al.* (2001) to decline to the regional background level only several metres from small plot sources. However, large numbers of sources may collectively result in an important regional background of spores in the air, as described for spores of pathogens such as *Fusarium graminearum* (Schmale *et al.*, 2005). There are also observations that a reservoir of sclerotinia inoculum is maintained on common wild plants, e.g. giant hogweed and cow parsley (Hims, 1979). *S. sclerotiorum* has a host range of more than 400 plant species (Boland and Hall, 1994) and wild hosts such as broad leaved weeds and wild flowers commonly found in meadows, grassland, field margins and in other uncultivated areas can also be infected by the

pathogen. The complete area of wild hosts is difficult to estimate, but in 2008, field corners and margins occupied 25,000 ha, and such areas (http://archive.defra.gov.uk/evidence/statistics/foodfarm/enviro/observatory/research/documents/observatory13.pdf) could be important inoculum sources for infection in nearby fields of susceptible crops.

In recent years, oilseed rape rotations have tended to shorten from one in five years to one in three, or even one in two. Whilst *S. sclerotiorum* can survive between crops as sclerotia for several years, the percentage of viable sclerotia decreases each year, depending on soil conditions; hence, shortening the rotation significantly increases the risk of disease (Koch et al., 2007), whether it is susceptible crops of the same or different species (Twengstrom *et al.*, 1998).

There are a number of key phases in the life cycle of *S. sclerotiorum* where successful control of the pathogen depends on prevention of one or more of these phases over successive years:

- Sclerotia in soil germinate to form apothecia in spring which release airborne ascospores.
- In a flowering crop such as oilseed rape, the ascospores land on and germinate on flower parts which fall and can stick to the leaves and stems. *S. sclerotiorum* uses flower parts as a food source to penetrate and invade the plant.
- In a non-flowering crop, *S. sclerotiorum* spores germinate on leaves and stems with infections initiated at wound sites or where areas become senescent.
- The fungus grows within the plant and forms sclerotia. They fall into the soil at harvest where they can remain viable for several years.

The objectives of this project were to:

1. Improve timing of fungicide applications, based on weather data and/or airborne inoculum detection.
2. Quantify the effect of soil management and crop rotation on sclerotinia disease.
3. Evaluate fungicide timing according to inoculum detection and/or disease forecasting, and quantify the effect of soil management with field experiments using a range of additional crops and UK locations.

A key phase where the industry has particular interest to improve sclerotinia control is the spore production and infection phase, where foliar fungicide applications can be targeted to the time(s) when there is risk of infection by spores. Foliar fungicides are the most widely used method of control for sclerotinia. Ideally, fungicides should be applied just prior to infection, because they have little or no curative activity. In oilseed rape in particular, the high impact on yield of severe crop infection has resulted in the widespread use of fungicides as prophylactic treatments. There are problems with timing, as fungicides give about three weeks protection (ADAS unpublished

data), but the susceptible flowering period typically lasts 6–8 weeks. With current economics and high disease pressure, there is increasing use of two fungicide sprays for sclerotinia in oilseed rape. Growers therefore require a reliable system to target sprays to periods of risk.

One approach to use for timing of fungicide treatments is to use weather-based models to predict when spores will be produced, and/or when conditions are conducive to infection.

There are two models in particular that were of interest to validate for use in oilseed rape and other crops. The first was developed recently in the UK for use in lettuce crops and is based on experiments defining the conditions under which sclerotia germinate to produce apothecia and hence the risk of infection from ascospores (Clarkson *et al*., 2007). This model is based on soil temperature and soil moisture as previously defined in a Defra project (HH3215TFV). A practical forecasting model was developed from this work for lettuce crops using inputs of temperature and rainfall in a two year HDC project. Here it was found that a single spray applied according to this model gave a similar level of sclerotinia control to a two- or three-spray programme (Young, 2008). With modification, this model could be used to predict sclerotial germination in oilseed rape and other crops.

The second forecasting model is based on conditions favourable for ascospore infection once the sclerotia have germinated (Koch *et al*., 2007). This weather-based infection model (using infection conditions defined in the forecasting model 'SkleroPro', developed in Germany for use in oilseed rape) has potential for identifying infection periods in the UK (HGCA Report 433), based on accumulated hours of suitable weather conditions (Koch *et al.*, 2007). It may be applicable to other crops, but its reliability in predicting infection periods required testing and validating. A weather-based forecasting scheme may be simpler and cheaper to run than one based on air sampling and direct detection of *S. sclerotiorum* ascospores by PCR or petal testing on agar, but several approaches may be required to achieve good disease control and to enable a full explanation of disease development to be made at different sites.

Understanding the timing of release of airborne spores, the numbers of spores and their spatial variation is fundamental to targeting fungicide control. A PCR method has been developed (LK0957, HGCA 2949) to detect spores and subsequently refined to be quantitative and more specific (Rogers *et al.,* 2009). This methodology can be used not only to determine the timing and quantity of airborne spores within a crop, but also can used to detect spores at regional and local scales (West *et al.,* 2008, 2009). Growers are keen to have a practical method to determine disease risk and spray timing, but evidence that spore levels are good indicators of infection and final disease incidence at various locations is needed before development of a commercial spore detection method is justified.

Long-term and short-term management decisions such as crop rotation have an impact on plant disease and therefore on farm economics. Reducing sclerotinia disease whilst also maximising profit is more complex than simply lengthening rotations for susceptible crops. Rotation effects are logistically difficult and expensive to investigate through field experimentation; hence a modelling approach was used here, based on experimental and farm data. Bio-economic models provide useful frameworks to investigate the trade-offs between the state of the land, severity of sclerotinia and financial impacts as a result of different cropping decisions. Dynamic programming (DP) is an approach that has been used to study optimum rotations (Stott *et al.*, 1996; Cai *et al.*, 2011). In the work described here, DP was used to explore the impact of different rotation decisions in the short term and long term.

There have been observations that different crops infected with sclerotinia produce different numbers of sclerotia, which may have implications for the risk of infection to following crops. Sclerotial returns to the soil surface have been estimated in combinable peas at approximately two sclerotia/m^2 soil resulting from each 1% disease incidence, with potatoes and peas providing a higher sclerotinia risk than winter oilseed rape (Archer *et al.,* 1992). Knowledge of the variability in sclerotial production in different hosts was required to underpin the modelling approach for sclerotinia decline, and could influence prioritising treatments to crops according to their potential for producing sclerotial inoculum.

The overall purpose of this project was to develop and combine both short-term and long-term strategies to reduce the impact of sclerotinia on UK agriculture across the different commodity sectors. A partnership of researchers and industry enabled a combined approach of field experimentation and modelling work, complemented by a survey of growers views on sclerotinia disease control to ascertain what approaches are the most likely to be taken up in practice.

3. Sclerotial germination model: development work

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3.1. Introduction

In a previous Defra funded project (HH3215TFV), the effect of soil temperature and moisture on the germination of *S. sclerotiorum* sclerotia was examined in controlled environment experiments and in the field. A model was produced from the data which could simulate the germination pattern of *S. sclerotiorum* sclerotia buried at different times of year (Clarkson *et al*., 2007). Significantly, it was shown that the duration of a cold 'conditioning' phase (optimum approx. 4°C) prior to sclerotia being placed in optimum conditions for germination (moist soil, 15–18°C) had a major effect on the germination rate of sclerotia. This germination model was developed further in HDC project FV 294 so that it could be used predictively using simplified weather data inputs of temperature and rainfall. In the revised model, these data are used to calculate when the conditioning phase has ended and subsequently when 10% of sclerotia (T10) are predicted to have germinated to produce apothecia. The aims of this part of the project were:

- To validate the model by observing the germination of sclerotia buried in different locations over three years and comparing with the predicted T10 dates.
- To determine if predicted T10 dates from the model can be used to time fungicide sprays for control of Sclerotinia disease in oilseed rape.

3.2. Methods

3.2.1. Monitoring sclerotial germination

Sclerotia from three different *S. sclerotiorum* isolates (L6, L17, L44) were produced in wheat grain as outlined previously (Clarkson *et al*., 2007) and sent out to research partners (ADAS, BASF, PGRO, Velcourt, Rothamsted) for burial in different locations (Table 3-1). Natural sclerotia collected from infected oilseed rape crops were also buried at the same time when available. Sclerotia were buried at 2 cm depth in oilseed rape fields during October 2009, 2010 and 2011 (4 replicates of 25 sclerotia, ADAS, BASF, Velcourt). Sclerotia were also buried at 30 cm depth in bean/pea fields in October (PGRO) in the same years before being retrieved and buried at 2 cm when beans/peas were planted in the following May/June. Germination of sclerotia to produce stipes/apothecia at all these sclerotial 'depots' was monitored. In 2011, an earlier burial in September was also carried out at selected locations (Table 3-1). Rainfall and soil/air temperature were recorded hourly at all locations using in-field data loggers as input for the model.

3.2.2. Running the germination model

The sclerotial germination model was run for all data sets described above and predicted T10 times were compared with observed T10. The model was modified for use in beans and peas to account for the deep burial such that sclerotia could condition and only progress a certain amount, up to a model score of 0.4 (where 1.0 = 10% sclerotia predicted to have germinated) towards germination. In addition, the model was run for a further 32 sclerotial germination data sets obtained from BASF as part of their historic and ongoing depot monitoring service, from ADAS and from Warwick Crop Centre associated with previous Defra and HDC projects. For many of the historic datasets, there was no logger data associated with the sclerotial germination observations and hence data derived from daily max and minimum air temperature from local ADAS farms was used to run the model. For some datasets the date of sclerotial burial was missing, in this case an estimate was made by looking at the other depot locations buried in the same year.

3.3. Results

3.3.1. Monitoring sclerotial germination

Twenty nine sclerotial germination data sets were obtained for different locations and burial dates over the three years (2009–2011; Table 3-1) and hence represented different environmental conditions. Generally, the germination times of sclerotia from the different *S. sclerotiorum* isolates and the natural sclerotia was synchronised and showed the same pattern; hence for simplicity, germination times are averaged across these data sets. Observed T10 times for burials in OSR ranged from 164 (ADAS Terrington, 2011 burial) to 267 days (Velcourt Hawarden, 2010 burial). The earliest observed T10 during the three years was 29 March 2012 (ADAS Terrington, 2011 burial) and the latest was 29 June 2010 (Boxworth, 2010 burial). Final % germination of sclerotia in oilseed rape ranged from 8% (ADAS High Mowthorpe, OSR 2010 burial) to 95.5% (Rothamsted, OSR 2011 burial). In peas and beans, observed T10 times varied between 245 (PGRO Aylsham, Bean 2010 burial) and 319 days (PGRO Aylsham, Bean 2009 burial). The earliest observed T10 germination was 4th July (PGRO Holbeach, Pea 2011 burial) and the latest 31st July (PGRO Aylsham, Bean 2009 burial). Final % germination of sclerotia in peas and beans ranged from 6% (PGRO Holbeach, Pea 2010 burial) to 54.5% (PGRO Aylsham, Bean 2011 burial). Over the three years, although final germination levels of sclerotia recorded often depended on the number of assessments that were carried out, germination levels were generally lower in 2011 (2010 burials) due to the lack of rainfall, with the exception of the Rothamsted site where sclerotia were watered artificially.

Table 3-1. Burial dates, conditioning times, predicted and observed germination dates and times for sclerotia buried in depots.

Site	Buried[1]	Cond date[2]	Cond time[3]	T10 Pred[4]	T10 Obs[5]	FinalObsGerm[6]	ObsDaysGerm[7]	PredDaysGerm[8]	Diff Pred[9]
ADAS Boxworth OSR	06/11/09	09/11/09	3	29/05/10	14/06/10	14.5%	220	204	16
ADAS High Mowthorpe OSR	20/10/09	07/11/09	18	07/06/10	01/06/10	37.3%	224	230	-6
ADAS Rosemaund OSR	23/10/09	18/11/09	26	08/06/10	27/04/10	48.3%	186	228	-42
ADAS Terrington OSR	12/10/09	08/11/09	27	17/05/10	11/05/10	76.0%	211	217	-6
Velcourt Hawarden OSR	23/10/09	09/11/09	17	17/05/10	25/05/10	64.0%	214	206	8
Velcourt Manor Farm OSR	14/10/09	21/10/09	7	22/05/10	15/05/10	8.3%	213	220	-7
Rothamsted OSR	08/10/09	-	-	-	06/05/10	92.5%	210	-	-
PGRO Aylsham Bean	16/10/09	10/11/09	25	14/08/10	31/08/10	20.8%	319	302	17
PGRO Holbeach Pea	15/10/09	12/11/09	28		not observed				
ADAS Boxworth OSR	07/10/10	17/10/10	10	13/04/11	29/06/11	14.5%	265	188	77
ADAS High Mowthorpe OSR	20/10/10	20/10/10	0	24/05/11	not observed	8.0%	-	216	-
ADAS Rosemaund OSR	06/10/10	19/10/10	13	15/05/11	16/05/11	17.3%	222	221	1
Velcourt Hawarden OSR	07/10/10	18/10/10	11	27/04/11	01/07/11	11.3%	267	202	65
Velcourt Haverholme OSR	06/10/10	16/10/10	10	08/05/11	13/06/11	10.1%	250	214	36
Rothamsted OSR*	04/10/10	18/10/10	14	09/05/11	11/05/11	89.3%	219	217	2
PGRO Aylsham Bean	19/11/10	25/11/10	6	15/07/11	22/07/11	32.2%	245	238	7
PGRO Holbeach Pea	17/11/10	25/11/10	8	29/06/11	28/07/11	6.0%	253	224	29
ADAS Boxworth OSR	18/10/11	20/10/11	2	05/04/12	17/04/12	56.8%	182	170	12
ADAS High Mowthorpe OSR	19/09/11	18/10/11	29	10/04/12	02/05/12	25.5%	226	204	22
ADAS Rosemaund OSR	28/09/11	29/10/11	31	24/03/12	13/05/12	29.5%	228	178	50
ADAS Rosemaund OSR	13/10/11	12/11/11	30	06/04/12	24/04/12	48.5%	194	176	18
ADAS Terrington OSR	27/09/11	20/10/11	23	02/04/12	03/04/12	86.5%	189	188	1
ADAS Terrington OSR	17/10/11	20/10/11	3	02/04/12	29/03/12	65.0%	164	168	-4
Velcourt Martin Lodge OSR	23/08/11	07/10/11	45	26/02/12	01/05/12	42.0%	252	187	65
Velcourt Martin Lodge OSR	05/10/11	19/10/11	14	22/04/12	27/04/12	61.7%	205	200	5
Velcourt Haverholme OSR	04/10/11	15/10/11	11	06/04/12	26/04/12	74.0%	205	185	20
Rothamsted OSR	03/10/11	19/10/11	16	12/04/12	02/05/12	95.5%	212	192	20
PGRO Aylsham Bean	21/10/11	20/11/11	30	21/07/12	17/07/12	54.5%	270	274	-4
PGRO Holbeach Pea	21/10/11	20/11/11	30	23/06/12	04/07/12	27.0%	257	246	11

*sclerotia artificially watered, [1]burial date, [2]predicted conditioning date, [3]conditioning time, [4]prediction date for 10% sclerotial germination, [5]observed date for 10% sclerotial germination, [6]final % germination, [7]observed number of days from burial to 10% germination, [8]predicted number of days from burial to 10% germination, [9]predicted -observed days to 10% germination.

3.3.2. Running the germination model

Over 59 of the 61 sclerotial germination data sets (the two 'early burial' sets were excluded; Velcourt Martin Lodge OSR buried 23/08/11 and ADAS Rosemaund OSR buried 28/09/11), there was a positive correlation between predicted and observed T10 germination times with an R^2 value of 0.62 (Figure 3–1). The model generally predicted the pattern of germination between years and locations (i.e. if germination was going to be early or late). For example, germination of sclerotia buried in oilseed rape fields in the spring of 2010 (sclerotia buried Oct 2009) was predicted and observed to be slower than in 2012 (sclerotia buried Oct 2011) as shown in Figure 3–2, due to lower average temperatures in the period following conditioning (December 2009 onwards). However, the accuracy of the model was variable as T10 predictions ranged from 77 days early to 31 days late compared with the observed germination. The standard deviation of the prediction error (predicted-observed) was 19.7 days and the average prediction error was 2.6 ± 5.4 days over all datasets. Further analysis showed that predictions were less accurate for i) 'early' burials made in September rather than October, ii) when there were extended periods of dry and cool weather in the spring, iii) when germination levels were low (as for 2010 burials in 2011) and iv) when sclerotia were first buried deep and then brought to the surface (peas, beans) if no adjustment was made to allow some progress towards germination at depth.

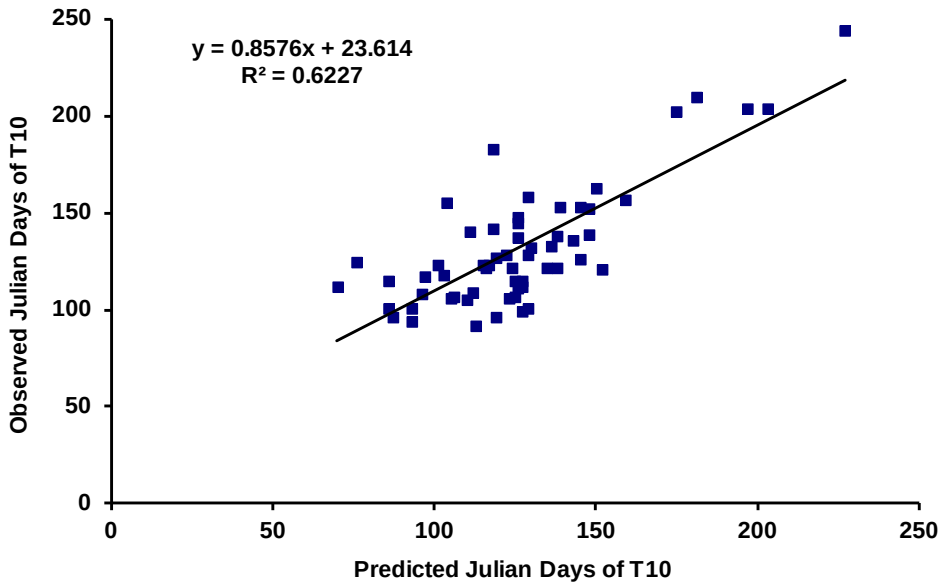

Figure 3–1. Predicted vs observed days to 10% sclerotial germination (T10) for 59 germination datasets representing different locations and years.

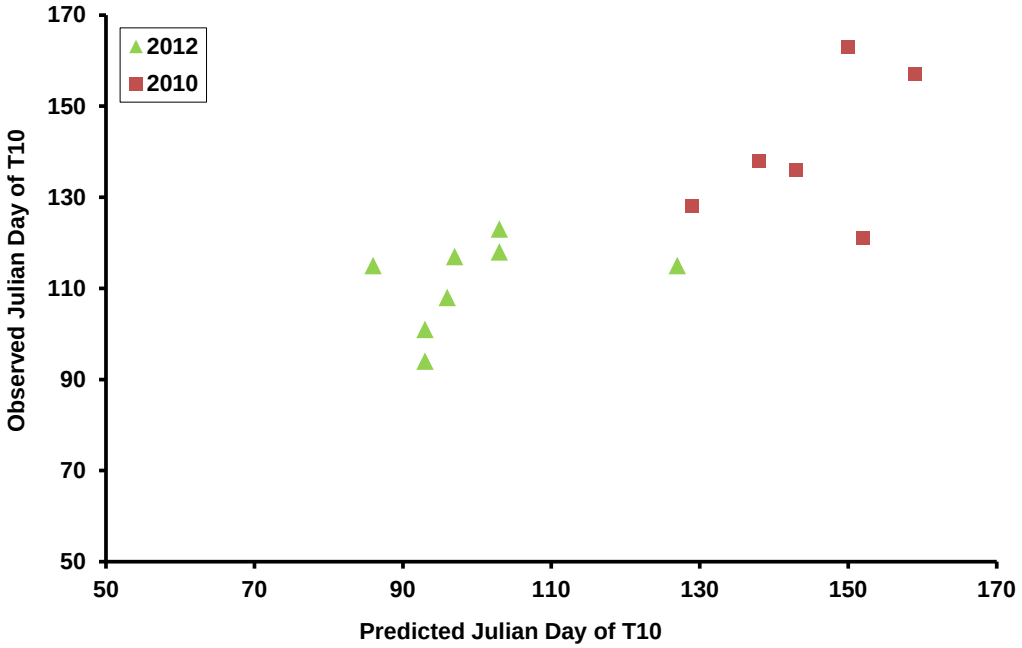

Figure 3–2. Predicted vs observed days to 10% sclerotial germination (T10) for germination datasets (oilseed rape) from 2010 (lower average temperature) and 2012 (higher average temperature).

3.4. Discussion

The results suggest that the sclerotial germination model has potential as a tool for predicting regional and yearly variation in the pattern and timing of apothecial production, but that in its current form, it cannot predict accurately enough for use to time fungicide sprays in OSR or other crops. Furthermore, collection of field specific hourly soil temperature and rainfall data required to run the model on a local scale may not be practical and hence another reason why a regional approach may be more appropriate. However, this project has allowed extensive validation of the model for the first time through the generation of multiple sclerotial germination data sets with associated weather data across different years and locations which will be a valuable resource in the future. Analysis of these data and existing knowledge has indicated where the model could be improved or where there are gaps in our knowledge relating to germination of *S. sclerotiorum* sclerotia as follows:

- The model works best when predicting germination of sclerotia buried at the soil surface in October (which produce apothecia the following spring). This is thought to simulate the behaviour of 'natural' sclerotia in an oilseed rape crop, but has not been shown experimentally. The model generally predicts germination too early if the monitored sclerotia are buried in late August / early September, when most winter oilseed rape is drilled and natural sclerotia would be expected to be brought to the soil surface to start their progress towards germination. This may be because there is some uncertainty in the model relating to the rate of sclerotial germination at temperatures below 10°C due to limited

controlled environment data that was available when the model was constructed. To address this, further controlled environment work would be required.

- As described previously, the model incorporates an estimation of soil moisture based on rainfall and temperature. This was originally derived from a limited number of data sets and assumes the soil contains adequate moisture for germination if temperatures are <12°C and if there has been >4 mm total rainfall in the previous 4 days when temperatures are >12°C but < 20°C in the preceding 24 hours. This may explain why the model did not produce accurate predictions for years with extended cool and dry periods (e.g. the dry spring of 2011 particularly for ADAS Boxworth and Velcourt Harwarden sites) as it assumed the soil contained adequate moisture when in fact it was limiting. The rainfall model could therefore be improved with further analysis of existing data sets.
- The response of deeply buried *S. sclerotiorum* sclerotia in terms of their conditioning and germination is poorly understood and adjustments were made to the model to allow more accurate predictions for this scenario which is associated with spring crops such as peas, beans, lettuce and carrots. There is still limited availability of field datasets and experimental data relating to sclerotia bought to the surface during cultivations for spring crops which needs to be addressed in the future.
- Based on current knowledge, sclerotia which are being monitored in depots to assess risk should be buried in September / October in order to accurately reflect the behaviour of natural populations of sclerotia producing apothecia.
- The sclerotial germination model was derived from controlled environment data where the time to 50% germination was recorded. A distributional assumption was made to allow estimation of the time to 10% germination in the version of the model used in this project. A model could be developed for the overall distribution of germination times, thus allowing the prediction of the time to any appropriate percentage germination level.

4. Weather-based infection model: development work

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4.1. Introduction

The infection criteria from a model developed by Koch *et al.* for infection of oilseed rape by sclerotinia spores ('SkleroPro', 2007) was used as the basis for generating forecast dates for infection. The infection conditions for sclerotinia ascospores were defined as: at least 23 continuous hours with RH% $\geq$ 80% and air temperature $\geq$ 7^{o}C. Inoculum measurements were not included in the original model because inoculum was found to be present throughout flowering in Germany and was therefore assumed not to be a limiting factor. The original 'SkleroPro' model operates using weather data up to the current time, and does not include forecast weather data. The infection criteria from the model were tested on 2007 UK data (Gladders *et al.*, 2008), but inoculum was not measured. The aims of the model development work described here were [1] to test the infection model for predicting infection events and targeting spray dates at UK sites where inoculum could be quantified, and [2] to develop the model to be used with forecasting weather, thus enabling spray dates to be predicted. Spray timing in field experiments, using the model, is described in Chapter 5.

4.2. Methods

In-field data for air temperature and RH % was recorded using Delta-T loggers from March to the end of flowering, under the crop canopy. Hourly forecast weather data for 24 hours and 48 hours ahead for air temperature, rain and RH % was purchased from Netweather.com for March to June. In addition, actual data for air temperature, rain and RH % for the previous 24 hours was purchased for the nearest weather station. Data used for the forecasts was accessed daily from NetWeather for (www.Netweather.tv) each experiment site involved (Table 3-1) and checked for completeness and general validity. Using 48 hour NetWeather forecast data for each site, Excel macros were developed to generate the dates and duration in hours of infection periods. The results were e-mailed 3 times each week during flowering to each site. The decision to spray or not was made by individual site managers, taking into account other factors such as crop stage and weather conditions for spray opportunities. The actual and forecast weather data sets were analysed after flowering to determine the accuracy of the forecast weather, and the number of infection events that were predicted using the actual or the forecast weather. NB. The level of sclerotinia disease control achieved by spraying according to forecast alerts and the standard spray timings is reported in Chapter 5.

4.3. Results

The model alerts (example in Figure 4–1.) were sent as planned throughout flowering, with few interruptions to weather data supply (e.g. interruption to rain data at one site, which was 'patched' in from a nearby site; data supply restored by NetWeather).

All sites
Week beginning 23/04/2012

expected infection condition period

Report generated	site	date	time (24hr clock)	duration (hrs)
Monday	Aylsham	23_4	20:00	23
Monday	Martin Lodge	23_4	12:00	27
Monday	No infection events forecast at Haverholme, High Mowthorpe, Holbeach, Terrington, Whitwich,			
Wednesday	Aylsham	25_4	06:00	28
Wednesday	Haverholme	25_4	13:00	25
Wednesday	Martin Lodge	23_4	11:00	40
Wednesday	Martin Lodge	25_4	05:00	30
Wednesday	Terrington	25_4	07:00	26
Wednesday	No infection events forecast at High Mowthorpe, Holbeach, Whitwich,			

Figure 4–1. Example of weather-based infection report generated and sent by e-mail to all sites three times a week, during oilseed rape flowering.

Actual and forecast NetWeather data were compared by regression analysis. Actual NetWeather and in-field temperature data were similarly compared. Predicted temperature was close to actual (average R^2 = 0.8), but % RH prediction were not a good fit with the actual (average R^2 = 0.3, range 0.2 – 0.5). Example results from one site are shown in Figure 4–2.

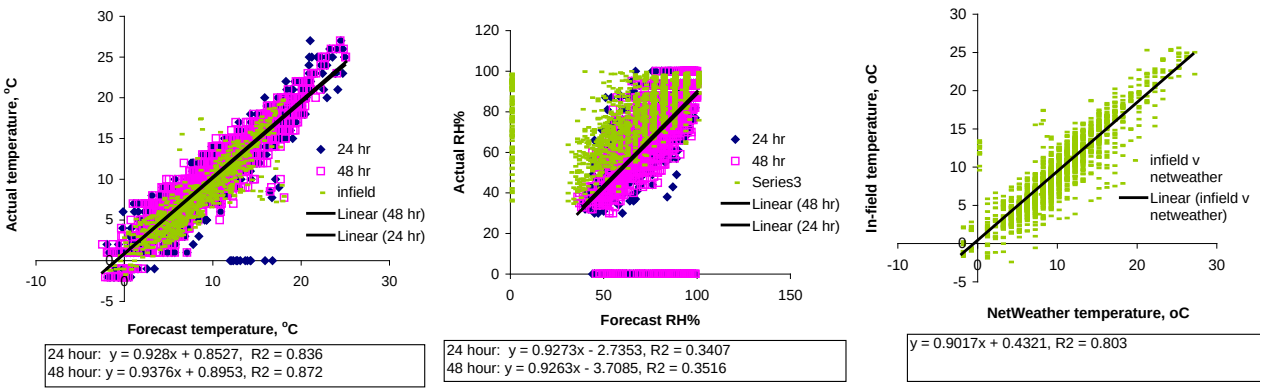

Figure 4–2. Forecast and actual temperature and relative humidity, ADAS Boxworth 2010

The model resulted in obvious differences in the number of forecast infection events between sites in different regions, with more alerts in the south and east sites. The number of alerts per site

during flowering varied from two to ten. The number of forecast infection events was often, but not always, greater with the forecast weather data than with the actual weather data (Table 4-1) i.e., the forecast weather data resulted in more alerts than actually happened in reality. Therefore, the model appears less likely to miss a spray that is needed, and more likely to indicate a spray is needed when it may not be necessary.

Table 4-1. Number of forecast and actual infection events for Sclerotinia disease

			Number of infection events during flowering			
Year	**Site**	**Crop**	**25–48 hr forecast**	**Actual**	**Difference**	**Sclerotinia Incidence Untreated**
2010	ADAS Rosemaund	Oilseed rape	4	1	3	10.8
	ADAS Boxworth	No experiment crop	3	2	1	No crop
	Aylsham, Norfolk	Green beans	3	3	0	0.0
	High Mowthorpe	Oilseed rape	5	4	1	10.9
	Holbeach, Lincs.	Vining peas	5	5	0	0.0
	Manor Farm, Lincs.	No experiment crop	7	5	2	No crop
	Southminster, Essex	Oilseed rape	10	5	5	0.2
	Thanet, Kent	Oilseed rape	10	5	5	29.4
2011	ADAS Rosemaund	Oilseed rape	4	2	2	11.1
	ADAS Boxworth	No experiment crop	1	4	-3	0.0
	Aylsham, Norfolk	Green beans	3	4	-1	4.0
	High Mowthorpe	Oilseed rape	3	0	3	0.0
	Holbeach, Lincs.	Vining peas	2	2	0	0.0
	Haverholme, Lincs.	Oilseed rape	5	2	3	9.9
	Hawarden, Kent	Oilseed rape	8	0	8	17.0
	ADAS Rosemaund	Oilseed rape	4	1	3	35.8
2012	ADAS Boxworth	No experiment crop	1	4	-3	0.8
	Aylsham, Norfolk	Green beans	3	4	-1	6.0
	High Mowthorpe	Oilseed rape	3	2	1	6.0
	Holbeach, Lincs.	Vining peas	2	2	0	52.0
	Haverholme, Lincs.	Oilseed rape	5	2	3	0.0
	Martin Lodge, Kent	Oilseed rape	8	0	8	0.0

4.4. Discussion

The weather-based infection model alerts were generated and sent successfully during flowering, and the three-times-a-week e-mails and 48 hour forecast for alerts provided sufficient time to apply fungicides either before, or by, the alert date, provided weather conditions were suitable. The onset of infection alerts at each site depended almost entirely on warmer night temperatures, because when these temperatures reached 7^{o}C or above, this usually resulted in the 23 continuous hours of RH% $\geq$ 80, as required by the model. The number of alerts therefore tended to be fewer at early- to

mid-flower, increasing towards the end of flowering. The NetWeather data provider was reliable, but data had to be checked to ensure validity because of occasional gaps or obvious data errors. Weather data download and content problems were not common, and were resolved quickly, but their occurrence highlights the need for quality checks on data before using it to run a model.

More frequent alert dates were predicted from the forecast weather than the actual weather for most sites. This results in the model being less likely to miss a spray that is needed, while increasing the risk of applying an unnecessary spray. This is the right bias with the current high value of oilseed rape.

Forecast temperatures were reasonably accurate, but forecast humidity was not, which is to be expected due to highly localised variability in topography, crop stage and rainfall. The use of in-field temperature / RH probes within the crop (rather than standard met station data) would be worth testing, as this may increase the accuracy of the predictions. However, a range of thresholds would need to be examined to ensure that alerts are not missed, or do not become so frequent that they are not useful.

Future work priorities include the following, and would be linked to priorities presented at the end of the following chapter (Sclerotinia disease forecasting and fungicide timing):

1. Determine whether use of in-field humidity sensors would increase the accuracy of forecast dates for infection events, and thereby improve sclerotinia control. The number of alerts generated by the SkleroPro model is very dependent on the RH% threshold, which will change according to crop type or whether the input data is from sensors within the crop canopy (logger) or outside (standard met station). The current work used standard met station data (NetWeather) but the use of met data from in-crop sensors should be evaluated in the future to see if better infection predictions can be made. This may necessitate modification of the RH% threshold through a combination of field and controlled environment experiments
2. Continue to monitor a range of sites for weather-based infection model events and sclerotinia disease development in untreated areas. Where possible, compare spray treatments according to alerts with a set of standard timings. The current work is based on relatively few sites and a larger body of evidence needs to be acquired to demonstrate effectiveness of the forecasting.
3. Develop further the integrated approach using forecasting and risk assessment for practical use, e.g. develop sampling, testing and thresholds for inoculum.

5. Sclerotinia disease forecasting and fungicide timing

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5.1. Introduction

The challenge to growers in the UK is to improve the timing of fungicide applications for sclerotinia disease and reduce unnecessary treatments. Increasingly, oilseed rape crops in the UK are sprayed twice during flowering on a preventative basis, but in hindsight some sprays are not needed and some are ineffective. In the absence of other information, sprays are advised usually at early flower, and then again approximately three weeks later if weather conditions are conducive to infection, i.e. warm and wet. Fungicide treatments timed according to sclerotinia infection events, using alerts from disease prediction models, may give better sclerotinia disease control than fungicides applied at standard timings. Or, a forecasting model may result in the use of a single spray, where a two-spray prophylactic treatment might have been used.

Understanding the timing of release of airborne spores and their numbers could be important for targeting fungicide applications. Predictions would be helpful for sclerotina control because sclerotinia fungicides have low curative activity. A quantitative PCR method has been developed for direct detection of ***S. sclerotiorum*** spores (Rogers *et al.,* 2009), which was tested in field experiments to determine the relationship between spore numbers and disease incidence, and indicate whether spore detection methods can be used in practice for timing fungicides (Chapter 6). However, as an alternative to direct detection of spores, spore production and/or infection risk periods can be predicted indirectly using weather-based approaches.

The aim of this work was to test the accuracy and the targeting of two fungicide timing weather-based forecasting models: [1] the sclerotial germination model (Chapter 3) and [2] the weather-based spore infection risk model (Chapter 4). An additional objective was to determine the relative importance of factors known to influence the risk of sclerotinia infection. In this work, these factors were: onset of sclerotial germination (measured by observed germination and the sclerotial germination timing model), conditions when infection events occur (measured by weather-based infection model), spore inoculum (measured by petal infection detected by plating on agar or qPCR, Chapter 6) and petal adherence to leaves.

5.2. Materials and methods

5.2.1. Sites treatments and sclerotial burial

Oilseed rape experiments were set up at a range of sites, over three years (Table 5-1). At all sites, the experimental treatments were randomised within complete blocks, with three or four replicates. Plot size varied but was always at least 12 x 3 m. Treatment chemicals were applied in 200 litres

water/ha at a pressure range of 200–300 kPa. See Table 5-1 for varieties, drilling dates and dates of application. Herbicides and insecticides were used according to local farm practise to minimise the incidence of weeds and pests. Sclerotia were buried at each site in the autumn (Table 3-1).

A vining pea experiment site at Holbeach, Lincs, and a green bean experiment site at Aylsham, Norfolk, was set up by PGRO in each year. Sclerotia were buried at a depth of 30 cm initially in mid-October in order to condition over winter and reburied to drilling depth at each site, at the time of drilling (vining peas in April, green beans in June, Table 3-1), using the same method as described for oilseed rape sites.

Where possible for each site, sclerotia were collected from a nearby location in the previous year. Unbroken sclerotia approx. 4mm in size were selected. For oilseed rape sites, sclerotia were buried in grids in mid-October, one sclerotium per cell, 1–2 cm depth, 25 cells per grid, four grids per experiment. The soil surface was 'prepared' by gently raking the top 5 cm to break up large clumps and to remove any stones. The grids were then covered with a fine layer of soil, lightly firmed by hand to eradicate any holes made by burying the sclerotia. For pea and bean sites, the sclerotia were placed in mesh bags and buried at 30 cm depth at the field sites, and were then dug up at drilling and reburied in grids as described for oilseed rape. Grids were assessed for the presence of apothecia at 7 day intervals from before flowering to at least the end of flowering. From the time of initial burial a Delta-T logger, sited adjacent to the grids, recorded hourly rainfall, soil temperature and soil moisture at 2 cm.

5.2.2. Fungicide applications

For oilseed rape, scheduled spray applications were made which included single treatments only of yellow bud, early-, mid- or late-flower and additional two-spray programmes using these timings (Table 5-2). All fungicide treatments were Tectura at 1 l/ha (BASF, boscalid + metconazole). Fungicide treatments were also applied as appropriate according to alerts from two different sclerotinia disease risk models (spray dates given in Table 5-1). The germination model predicted dates for appearance of apothecia (fruiting bodies) from sclerotia (Chapter 3), and the weather-based infection model predicted the time and duration of environmental conditions favourable to infection by spores (Chapter 4). For the sclerotial germination model, weather data (soil temperature 2 cm depth, air temperature, soil moisture and rainfall) was downloaded weekly from on-site loggers. The model was run and updated predictions of germination dates were made weekly. The weather based infection model was run on 48 hour forecast RH% and temperature data for each individual site, purchased from NetWeather. This model was run and risk alerts generated three times each week, Monday, Wednesday and Friday.

Table 5-1. Oilseed rape site locations and fungicide application dates, sclerotial germination observation dates, Sclerotinia model prediction dates and % petals infected with Sclerotinia.

	2010 BASF	**2010 BASF**	**2010 ADAS**	**2010 Velcourt**	**2011 BASF**	**2011 BASF**	**2011 ADAS**	**2011 Velcourt**	**2011 Velcourt**	**2012 BASF**	**2012 BASF**	**2012 ADAS**	**2012 Velcourt**	**2012 Velcourt**
	Rose-m'nd	South-minst.	High Mow.	Than-et	Rose-m'nd	Box-worth	High Mow.	Hawar-den	Haver-holme	Rose-m'nd	Terr'tn	High Mow.	Martin Lodge	Haver-holme
Grid reference	SO565 475	TL956 007	SE781 757	TR237 551	SO607 458	TL347 621	SE720 791	TR235 545	TF109 493	SO598 450	TF496 224	SE719 789	TR344 477	TF109 493
Drilling	30-Aug	01-Sep	21-Aug	24-Aug	16-Sep	05-Sep	04-Sep	19-Aug	19-Aug	02-Sep	09-Sep	23-Aug	19-Aug	12-Aug
Variety	Castille	Vision	Castille	Cabernet	DKCab ernet	DO6	Castille	Palmedor	Palmedor	Excalibu r	D06	PR46 W21	PR46W21	Palmedor
Spring plant count UT GS 3.7 - 4.5	35.4	32.0	14.7	29.0	45.0	63.7	13.3	40.0	26.0	41.3	54.1	47.4	37.0	28.0
Spring plant height UT	191.2	162.0	79.9	175.0	50.0	63.7	84.2	165.0	163.0	72.1	52.3	85.4	160.0	170.0
Scler burial date	23-Oct	06-Nov	20-Oct	23-Oct	06-Oct	07-Oct	20-Oct	07-Oct	06-Oct	13-Oct	17-Oct	19-Sep	05-Oct	04-Oct
Yellow bud spray	17-Apr	21-Apr	27-Apr	Not done	14-Apr	12-Apr	11-Apr	none	none	06-Apr	16-Apr	10-Apr	Not done	Not done
Early-flower spray	24-Apr	26-Apr	04-May	29-Apr	22-Apr	18-Apr	20-Apr	15-Apr	13-Apr	13-Apr	23-Apr	23-Apr	16-Apr	26-Apr
Mid-flower spray	10/May	05/May	10/May	13/May	04/May	27/Apr	26/Apr	28/Apr	29/Apr	23/Apr	02/May	10/May	01/May	10/May
Late-flower spray	22-May	20-May	26-May	25-May	17-May	10-May	06-May	11-May	10-May	16-May	16-May	29-May	17-May	21-May
Inf. Model 1st alert during flower	29-Apr	24-Apr	30-Apr	28-Apr	07-May	25-May	07-May	25-Apr	29-May	18-Apr	18-Apr	18-Apr	23-Apr	20-Apr
Inf. Model 1st spray	04-May	26-Apr	05-May	28-Apr	10-May	26-May	17-May	28-Apr	10-May	30-Apr	16-Apr	30-Apr	16-Apr	26-Apr
Inf. Model 2cnd spray	02-Jun	None	26-May	None	None	None	None	None	None	18-May	None	None	None	None
Germ. Model Predict	08-Jun	29-May	07-Jun	22-May	15-May	13-Apr	24-May	27-Apr	08-May	06-Apr	02-Apr	10-Apr	22-Apr	06-Apr
First Obs. germination	19-Apr	24-May	10-May	13-May	11-Apr	21-Jun	11-Apr	01-Jul	13-Apr	16-Apr	19-Mar	30-Apr	26-Apr	19-Apr
10% Obs. germination	27/Apr	14/Jun	01/Jun	15/May	16/May	29/Jun	Not obs.	01/Jul	13/Jun	24/Apr	29/Mar	02/May	27/Apr	26/Apr
Final obs. germination	48.3	14.5	37.3	64.0	17.3	14.5	8.0	10.1	11.3	48.5	56.8	25.5	61.7	74.0
Germ. Model spray	02-Jun	21-Apr	07-Jun	13-May	17-May	12-Apr	25-May	28-Apr	10-May	06-Apr	16-Apr	10-Apr	16-Apr	26-Apr
Petal inf. yellow bud %	54.0	29.0	10.2	71.0	43.8	0.1	3.2	Not done	56.3	35.9	0.0	Not done	Not done	Not done
Petal inf. early-flower%	72.0	12.5	13.4	71.0	39.0	0.0	1.6	93.8	50.0	18.8	31.3	64.1	33.3	6.2
Petal inf. mid-flower %	52.0	33.0	13.5	100.0	17.0	0.0	2.1	18.8	22.9	4.7	9.4	72.0	47.9	12.5
Petal inf. late-flower %	79.0	43.8	7.2	100.0	2.0	0.0	3.3	Not done	52.1	7.8	53.1	56.3	66.6	48.8

For peas and beans, the germination model and the infection model were run and alerts sent in the same way, during flowering for peas (July) and beans (August) which had shorter flowering durations, approximately two weeks.

5.2.3. Disease assessment, weed sampling and yields

For oilseed rape, a sclerotinia stem rot assessment of incidence and severity was made twice, post-flower and pre-harvest on 200 plants per plot. In addition, foliar and stem diseases were assessed for incidence and severity prior to spraying, at each spray date and pre-harvest, using 25 plants per untreated plot. Mean disease severity was noted on pods (alternaria, light leaf spot and powdery mildew in particular), leaves, stems or roots using NIAB whole plot methods. The seed weight and moisture content were measured and the yield at 91% dry matter was calculated. For peas and beans, sclerotinia disease was assessed as % plants affected per plot. Genstat 12 was used to analyse data as a randomised block design.

Weeds and wild plants (e.g. cow parsley and hogweed, known to be hosts) were inspected for symptoms of sclerotinia infection / sclerotia in uncultivated areas surrounding the crop at 10 locations at each ADAS and BASF site, in an area 2 m x 2 m, during site visits from May to September. Samples of potentially infected plants were sent to Warwick Crop Centre where they were incubated in damp conditions to promote mycelial growth and sclerotial formation by *S. sclerotiorum*.

5.2.4. Sclerotinia inoculum: petal tests, petal adherence, sclerotial germination

Petals were sampled at all sites from untreated plots only (12 petals per plot, 4 plot reps), after noon on a fine day, at growth stages 4.2, 4.5, 4.7, 4.9, and placed on agar (potato dextrose agar + 50 µg/L streptomycin sulphate) within two hours of collection. Older, intact flowers (i.e. those nearest the bottom of the open flower zone) were selected. From the same plants, a 1 cm leaf disc from one leaf from the main stem mid-section of each plant was sampled using a cork borer. Petals were taken using sterilised forceps and placed face down onto agar, 4 per plate. Leaf discs were similarly plated. Plates were examined after 8–10 days incubation at room temperature for the presence or absence of sclerotinia. Samples of petals from the same flower, and leaf discs from the same plant, as used for the agar plate test, were sent to Rothamsted Research for PCR tests for sclerotinia (Chapter 6).

At selected oilseed rape sites, twice each week during flowering, counts were made of the number of petals caught in leaf axils and adhered to leaves, on each of 5 randomly selected plants for each of the 4 untreated plots. On each plant, counts were made for one upper leaf, one mid, and one lower.

5.2.5. Weighting of risk factors for sclerotinia infection

Twengstrom *et al.* (1998) explored simple binary models to predict thresholds at which sclerotinia incidence would be high in spring oilseed rape. In their model, June rain and the weather forecast were the most important factors for predicting sclerotinia disease. In the current work, this simple weighted model was used to explore the importance of weather variables and petal infection at each flowering stage. Multiple regression analysis was considered but the data set was too small.

For each site year, the initiating variables, petal infection (V_p), heavy rain (V_h) ($\geq$ 3mm hr^{-1}, as defined by the UK Met Office), light rain (V_l) and sclero pro infection alerts (V_f) were assessed at four flowering stages; yellow bud (YB), early-flower (EF), mid-flower (MF) and late-flower (LF). A binary response (1, 0) was given for each of these variables at each flowering stage. The binary result was multiplied (weighted) by values generated within the range shown below:

AREA	INITIATING VARIABLE	WEIGHTING RANGE EXPLORED
Infection (I)	is there petal infection?	0-40
Rain light (L)	is there light rain?	1-50
Rain heavy (H)	is there heavy rain?	-100 to 0
Forecast (F)	is there a SkleroPro alert at +/- 1 or 2 days of sample?	1-100

The weighted values for each variable were then summed for each flowering period, i.e. for the yellow bud assessment, the score YB_{tot} was: $YB_{tot} = V_p * I + V_h * H + V_l * L + V^f * F$,
where $V_{p=}0$ or 1; $V_{h=}0$ or 1; $V_{r=}0$ or 1; $V_{f=}0$ or 1 and I, L, H, and F fall within the range of the variables above.

The total score (T) for that site season was:
$T = YB_{tot} * YB_w + EF_{tot} * EF_w + MF_{tot} * MF_w + LF_{tot} * LF_w$ where YB_w, EF_w, MF_w, LF_w, lie in the range 0.5 to 2.5 and are the weightings given to each flowering period.

It was assumed that the total score would reflect the risk of sclerotinia infection.
A macro was devised to calculate the correlation between total score and sclerotinia disease incidence for all initiating variables and flowering period weightings within the ranges given above and in steps of 0.1. Sclerotinia infection was plotted against total score, and weighting of each of the variables changed until correlation was maximised, using Excel spin boxes. The weighting scores reflected the relative importance of each factor in the development of stem rot disease in the oilseed rape fields in this project. During this project, only two site years had sclerotinia incidence > 25%, so the dataset was heavily influenced by low disease values.

5.3. Results

5.3.1. Forecasting alerts and sclerotinia control

The germination model gave variable results for % control of sclerotinia stem rot in oilseed rape, compared to the best fungicide timing. When germination was predicted before the start of flower, this triggered the earliest possible spray, the yellow-bud spray. Most of the poor control using this model was associated with germinations predicted late, that did not trigger a spray until the very end of flowering or after, e.g. 32% control at Rosemaund in 2010 (Table 5-1, dates; Table 5-2, sclerotinia incidence and control). For peas and beans, the model was not accurate enough to time a spray for flowering protection, but low disease in most years prevented a valid test of control using the model alerts.

Control of sclerotinia infection in oilseed rape using the weather-based infection model alerts was more successful, as measured by control at sites where there was moderate or higher sclerotinia stem infection. In 2010 the model gave 76.9 – 100 % control compared to the untreated (Table 5-2). In 2011, control was variable, e.g. there was poor control at ADAS Rosemaund where the model forecast did not trigger an alert, but conditions in the crop allowed infection. In 2012, control using the model was good at Rosemaund (92%) but less so at High Mowthorpe (58%). In most cases it was possible to apply the fungicides in advance of an alert, or on the day. From 2010 to 2012, half of the sites had very little or no sclerotinia infection, and control could not be judged at these sites. However, these sites were valuable for analysing the reasons why sclerotinia infection occurred at some sites but not others (see Figure 5–2). Diseases other than sclerotinia (e.g. light leaf spot and Phoma) were generally low.

The weather-based infection model treatment had most of its effect from the first spray, with a second spray applied in some cases at late flower (Table 5-1) if this was within the three week protection window. Many of the infection model treatments involved a single spray, and compare favourably with control achieved with the best fungicide ‘timing’ treatment which was often a two or three-spray treatment (Table 5-2).

An example of the infection alerts, flowering stage, scheduled sprays, rain and temperature data is shown for ADAS Rosemaund 2012 (Figure 5–1). Temperatures were cool and flowering was prolonged; a common occurrence at most oilseed rape sites in 2012. The first infection model alert during flowering did not justify a treatment, as few fallen petals were seen. However, at the second alert, petal fall was observed and a fungicide was applied. This was assumed to give protection for about three weeks, and since flowering continued after this, a second spray was applied at the end of flower. At other sites in other years, the combination of crop stage (i.e. petal fall had started) and an infection model alert were the main factors to trigger the first spray.

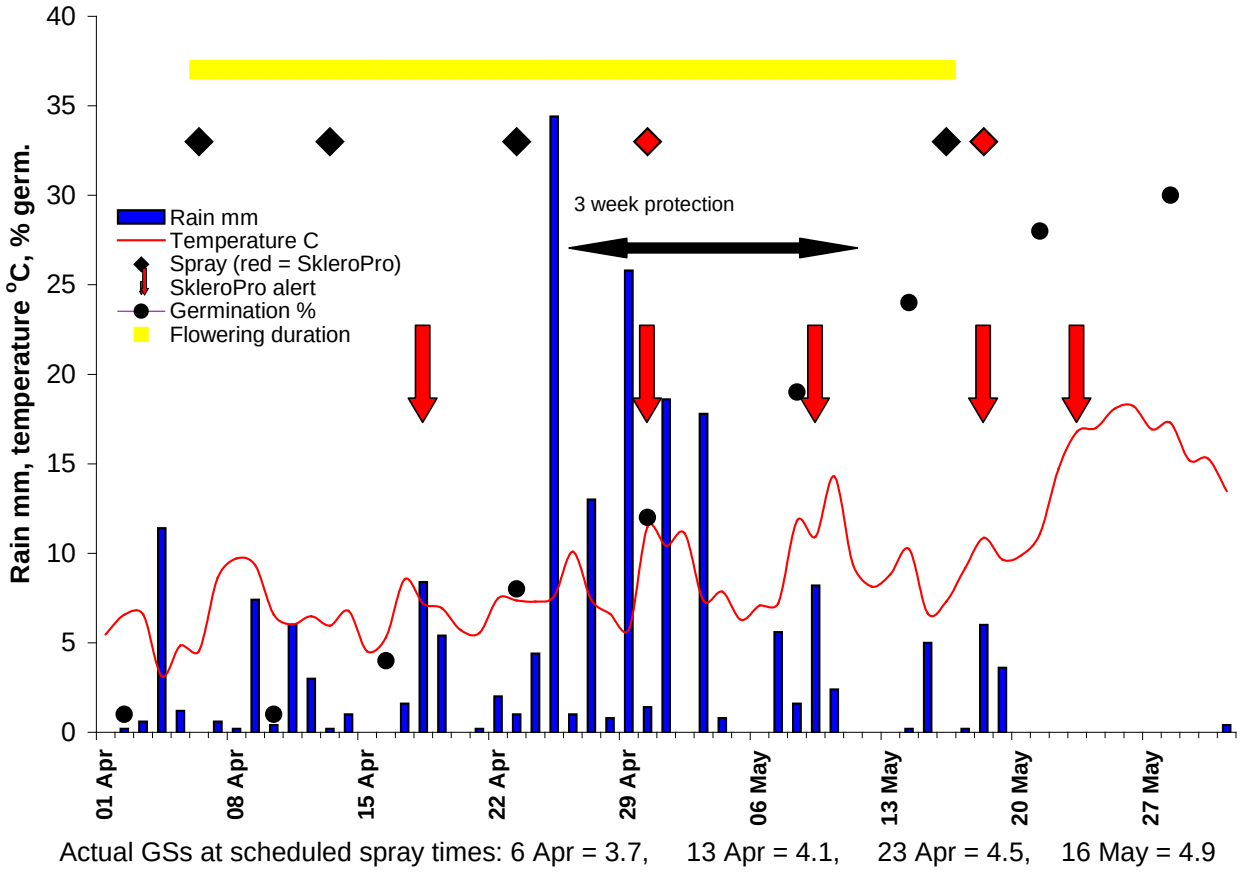

Figure 5–1. ADAS Rosemaund 2012, winter oilseed rape: weather, sclerotial germination and foliar fungicide spray times and SkleroPro alerts.

Sclerotinia spore inoculum test results were not available until after flowering or later, and so were useful in hindsight. The proportion of petals testing positive in agar tests at ADAS Rosemaund 2012 was high at first and then declined (Figure 5–2). Heavy rain after mid-flower is thought to have contributed to this decline. There was inoculum present at late flower, as shown by the results of PCR tests on Burkard spore trap tapes and sclerotial germination observations. The fungicide timing results confirm that disease resulted from a late infection as only those treatments including a late-flower treatment gave good control (Table 5-2).

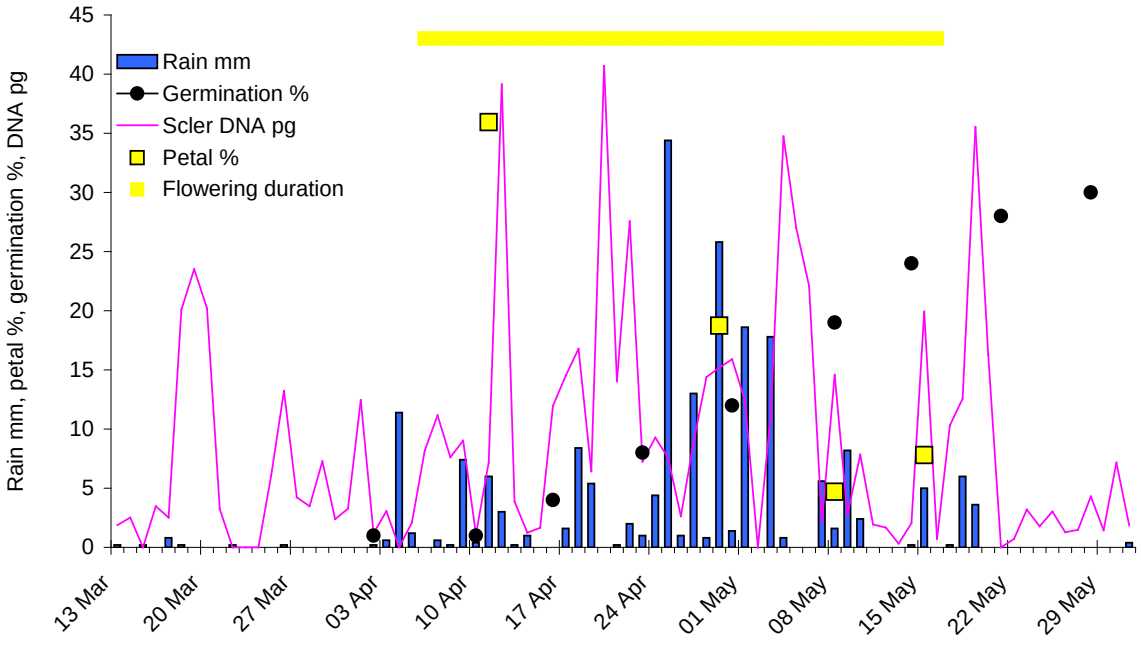

Figure 5–2. Rosemaund 2012, rainfall, sclerotial germination (germination %), and *S. sclerotiorum* inoculum, measured by oilseed rape petal agar tests (petals %) and PCR of Burkard spore trap samples (Sclerotinia DNA pg).

The data for spray dates, weather, alerts and inoculum for ADAS Rosemaund 2012 shows a typical combination of factors leading to sclerotinia infection (Figure 5–2). The charts for the other sites and years suggest that all sites with infection had a similar combination of key risk factors at one or more occasions during flowering. Sclerotinia infection most likely results from key risk factors occurring close together in time, rather than from a very high level of one factor. For example, very high inoculum levels (e.g. 100 % of petals testing positive) alone will indicate risk, but will not necessarily lead to high infection, such as at High Mowthorpe 2012 which had 56–72% petal infection in agar tests but only 6% sclerotinia stem rot in the untreated plots (Table 5-2).

At the pea and bean sites in 2010 and 2011, the weather was extremely dry, and very little sclerotinia disease developed in untreated plots of peas or beans (maximum of 6%). The weather-based infection model did not give alerts during flowering, which in hindsight was correct. In 2012, conditions were wetter. Agar based petal tests on three occasions showed that 38%, 60% and 63% of pea petals were infected, and 52% of untreated pea plants had sclerotinia disease. The best fungicide timing for the 2012 peas was the early flower spray of Switch with 57% control, compared with 42% control from Switch applied according to the infection model alert at very early flower. Switch at late pod gave 50% control. Switch gave better control than Signum at each timing tested in peas. Very little disease developed in beans in 2012.

Table 5-2. Sclerotinia stem rot incidence in oilseed rape and % control using forecasting models.

		2010 BASF Rose-maund Herefs.	**2010 BASF** South-minster Essex	**2010 ADAS** High Mow. Yorks	**2010 Velc't** Thanet, Kent Kent	**2011 BASF** Rose-maund Herefs.	**2011 BASF** Boxworth Cambs	**2011 ADAS** High Mow. Yorks	**2011 Velc't** Hawar-den Kent	**2011 Velc't** Haver-holme Lincs	**2012 BASF** Rose-maund Herefs.	**2012 BASF** Terr'ton Norfolk	**2012 ADAS** High Mow. Yorks.	**2012 Velc't** Martin Lodge Kent	**2012 Velc't** Haver-holme Lincs
Stem rot %	**1. Untreated**	10.8	0.2	10.9	29.4	11.1	0.0	0.0	17.0	9.9	35.8	0.8	6.0	0.0	0.0
	2. Yellow bud1	3.0	0.0	3.5	*	3.4	0.0	0.0	*	*	18.8	0.0	2.1	0.0	0.0
	3. Early-flower	2.1	0.0	2.5	7.1	6.5	0.0	0.0	9.0	10.4	21.5	0.1	2.3	0.0	0.0
	4. Mid-flower	2.8	0.0	0.0	7.3	8.1	0.0	0.0	14.0	9.3	16.5	0.3	1.1	0.0	0.0
	5. Late-flower	4.9	0.1	0.4	*	8.4	0.0	0.0	15.0	14.9	1.8	0.1	1.6	0.0	0.0
	6. Mid- + Late-flower	2.8	0.0	0.3	*	5.5	0.0	0.0	*	*	3.0	0.0	0.3	0.0	0.0
	7. Early- + Mid-flower	1.5	0.1	0.3	2.5	5.4	0.0	0.0	9.0	8.8	21.3	0.0	0.4	0.0	0.0
	8. Early- + Late-flower	2.6	0.0	0.0	*	5.3	0.0	0.0	13.0	12.6	2.5	0.1	0.6	0.0	0.0
	9. Yellow bud + Mid-flower	0.9	0.1	0.9	*	1.6	0.0	0.0	9.0	13.4	18.5	0.1	0.5	*	*
	10. Yellow bud + Mid + Late	1.1	0.0	0.1	6.0	4.1	0.0	0.0	*	*	1.3	0.1	0.0	0.0	0.0
	Germ model or equiv. spray	7.3	0.2	7.9	7.3	10.8	0.0	0.0	14.0	14.9	18.8	0.0	2.5	4.5	3.7
	Infection model or equiv. spray	1.1	0.0	0.0	7.1	9.8	0.0	0.0	14.0	14.9	2.8	0.0	2.5	0.0	0.0
% Control	**Infection model**	89.5	low scler	100.0	76.0	12.4	no Scler	no Scler	17.6	0.0	92.3	low scler	58.3	no Scler	no Scler
	Germination model	32.6	low scler	27.6	75.2	3.4	no Scler	no Scler	17.6	0.0	47.6	low scler	58.3	no Scler	no Scler
	Best fungicide timing	91.8	low scler	91.9	91.5	85.4	no Scler	no Scler	47.1	10.9	95.1	low scler	100.0	no Scler	no Scler
	Worst fungicide timing	54.6	low scler	96.5	75.2	24.7	no Scler	no Scler	11.8	0.0	39.9	low scler	62.5	no Scler	no Scler

* treatment not included

[1] Tectura 1 L/ha at each treatment time

5.3.2. Sclerotinia in weed hosts

No *S. sclerotiorum* sclerotia were found in any of the wild plant samples from the ADAS/BASF sites and no mycelial growth observed after incubation for up to 21 days. However, *S. sclerotiorum* sclerotia were recovered from thistle plants in the margins of an oilseed rape field (Vowchurch, Herefordshire, 2010) as part of a separate Defra project (IF0188). Some of these were shown to have the same genotype as sclerotia recovered from nearby oilseed rape plants suggesting that there is exchange of *S. sclerotiorum* isolates between wild and agricultural hosts.

5.3.3. Key risk factors for sclerotinia infection

There was a trend for increased stem rot incidence in oilseed rape with higher proportions of petals testing positive for sclerotinia as measured by the agar plate test. Petal infection was variable between sites, and between sample times. However, yellow bud and early-flower results were related to stem rot (R^2 = 0.53 and 0.45, respectively), whereas the mid- or late-flower results were not only poorly related (R^2 = 0.31 and 0.16, respectively) but too late to be of practical use.

Most adherence of petals to oilseed rape leaves began around mid-flower, increasing at late flowering (Figure 5–3). Petals were also recorded in axils, but in lower numbers. Petal adherence counts were not quantitatively related to stem rot incidence at the ADAS Rosemaund or ADAS High Mowthorpe sites. However, at the ADAS Boxworth sites where there was no petal adherence observed on the leaves, there was little or no stem rot.

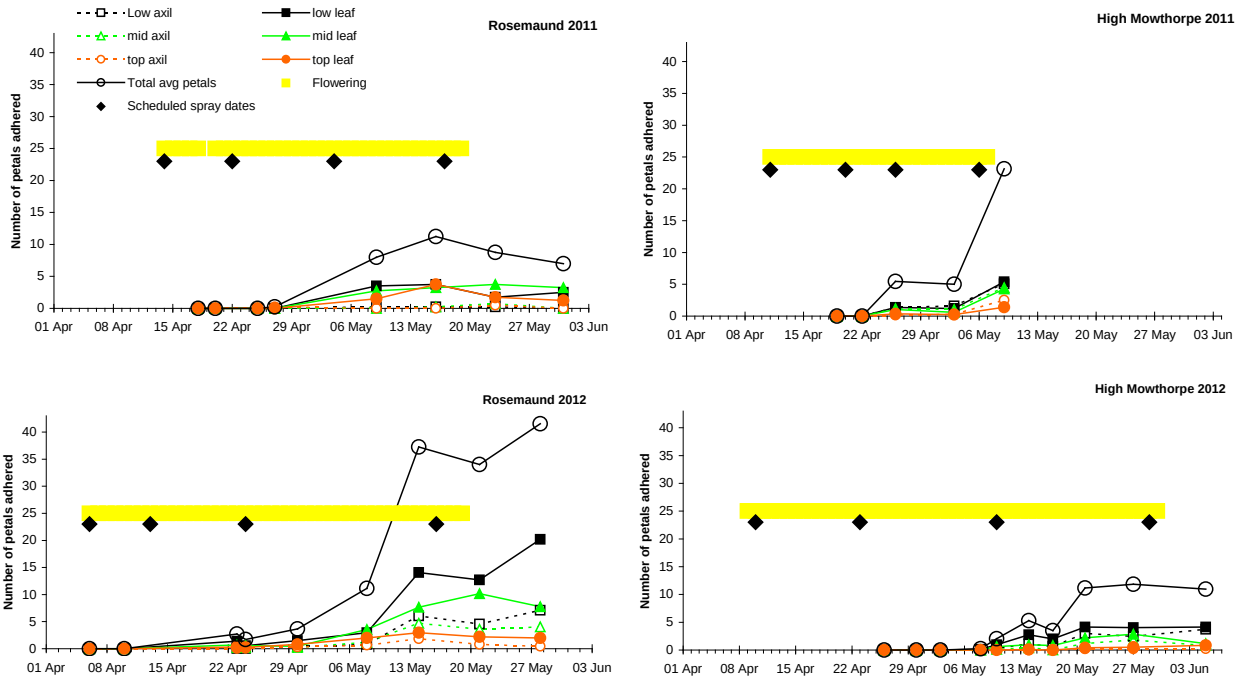

Figure 5–3. Petal adherence to oilseed rape leaves, ADAS Rosemaund and High Mowthorpe, 2011 and 2012. Values are average numbers of petals counted on one leaf or one axil, based on five plants from each of four untreated plots, except for the uppermost line in each chart which is the average totals of the adhered petals on the 3 leaves and axils per plant.

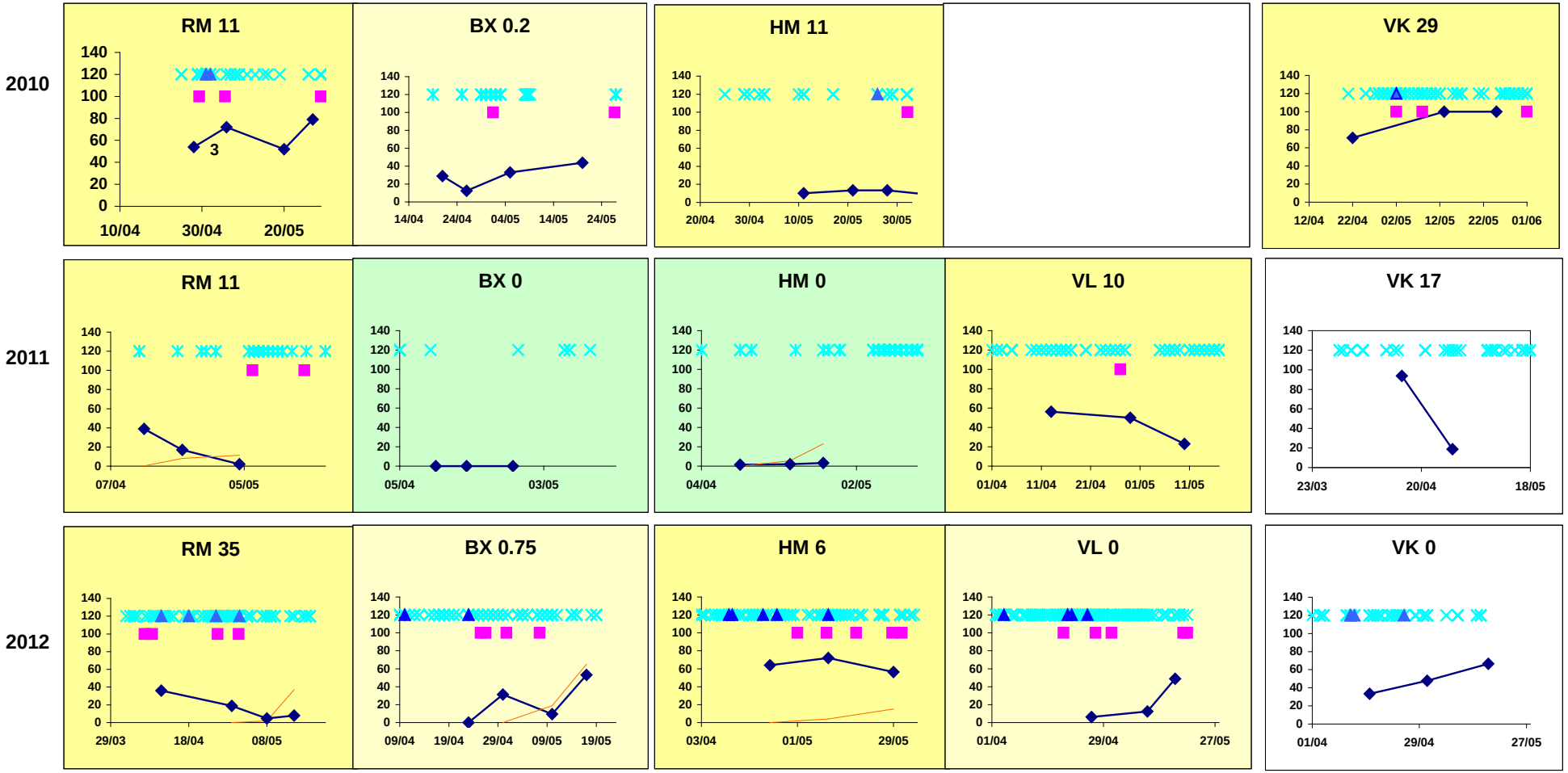

Figure 5–4. Oilseed rape sites: rain, weather-based infection model alerts, petal infection % and petal adherence to crop leaves. Individual figure titles show the site name and % stem rot incidence in untreated plots. RM = ADAS Rosemaund, BX = ADAS Boxworth, HM = ADAS High Mowthorpe, VL = Velcourt Lincs (no data for 2010, Filan overspray in error) and VK = Velcourt Kent. Light blue crosses = rain (darker triangles = heavy rain ≥ 3mm/hr), pink = weather-based infection alert, dark blue diamonds = % petals infected, red line = petal numbers adhering to leaves (not counted in 2010).

Successful infection by sclerotinia appears to be a result of key infection criteria occurring at the same time. Therefore, the frequency of any one factor may not be a guide to risk; for example, a site may have ten weather-based infection alerts during flowering, but infection will not occur if spore inoculum is absent. The frequency of light rain and heavy rain events, petal infection, weather-based infection alerts and petal adherence (where available) for the oilseed rape sites 2010–2012 is shown in Figure 5–4 for different levels of infection at different sites and years.

The contribution of both the initiating variables and the flowering period was analysed. The weighting of each of the initiating variables (alerts, rain and petal infection) was explored to maximise the correlation between disease incidence and total weighted score using an iterative process, with weighting values starting at 1. When the weightings of the initiating variables were determined which maximised the correlation (Table 5-3), the flowering period weightings were explored. The highest correlation between total score (T) at each site season and sclerotinia incidence at each site occurred at the following values:

Flower period weightings: Yellow bud: 0.9, early-flower: 1.7, mid-flower: 1.9, late-flower: 2.1.

Table 5-3. Range of initiating variables for infection which gave maximum correlations (0.724 – 0.725) for sclerotinia disease incidence and total weighted score.

	RISK FACTOR (initiating variable)	**INFLUENCE**	**Variable weights for maximum correlation**
1	Weather-based infection model alert	positive	13 to 14.5
2	Light rain	positive	20 to 24
3	Heavy rain ($\geq$ 3 mm /hour)	negative	-24 to -28
4	Petal infection %	positive	8.5 to 10

The maximum correlation between sclerotinia disease incidence and the total score was 0.725 (Figure 5–5).

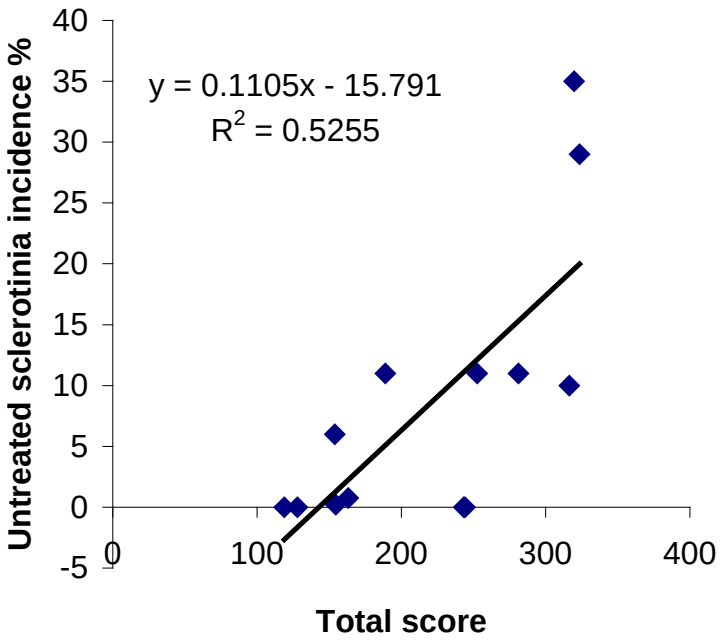

Figure 5–5. Untreated sclerotinia stem rot incidence and total score, based on oilseed rape field experiments 2010–2012. Correlation = 0.725.

The flower period weighting results suggest that the weather events before early flowering were less important than during mid- to late-flower. The variables with the highest weighted scores, i.e., the most influential, were heavy rain which washed spore inoculum out of the air and petals off leaves, and light rain which promoted petal adherance. Heavy and light rain scores were of similar weighting. The weather-based infection model alerts and petal infection were also of similar weighting, but both less important than the rain.

In practice, a weather-based infection model alert only, or petal infection only, could be used to assess the risk of sclerotinia infection. However, the occurrence of several or all of the above risk factors on the same day(s) is a more accurate predictor of a likely sclerotinia infection event, and would provide guidance for the timing of a fungicide application, or would justify a delay. Therefore, even if petal infection is high, petal adherence is noted and a weather based infection event predicted, heavy rain may prevent infection. In practice, heavy rain which causes a spray application to be postponed may also reduce infection.

5.4. Discussion

Control using fungicide applications timed according to the weather-based infection model was good in 2010 and 2012 (Table 5-2), but poor in 2011. However, where stem rot did occur in 2011, no spray schedule was effective, indicating that all the timing treatments tested failed to protect. The weather in 2011 was drier, with low petal infection in general, and low stem rot. There was one obvious miss of an infection event at Rosemaund where conditions were just short of the required

hours at early flower, and no spray was applied, but infection occurred. However, there were no other cases of missed alerts. Heavy rain events appear to have been a likely explanation for the lower stem rot levels in 2012 than expected from the other risk factors measured.

In general, observed germination of sclerotia was already underway by the start of flowering, or started shortly after, but there were some very 'late-germinating' sites. It was not possible to time oilseed rape sprays according to the germination model because of the two-week variability in prediction dates. Also, in some cases, the first alert was before yellow-bud, or after flowering had ended.

The original SkleroPro weather-based infection model has been successful for sclerotinia control in most years in Germany where it was developed (Koch *et al*., 2007) but has had failures in years where infection occurred outside of the times included within the model set up, e.g. at the very end of flower, when lodging promoted plant-plant spread. The SkleroPro model has also been tested in Denmark, with poor results (Jensen *et al.,* 2011). In the current work, the full SkleroPro model was not used; only the infection conditions defined in the model (Koch *et al*., 2007) were used and this allowed for other factors such as inoculum measures to be taken into account, and gave flexibility in the length of time that the model is run and alerts produced. It is probable that UK weather and inoculum conditions are more variable from site to site, and less predictable, than in continental Europe, and an approach which includes local assessments of risk factors such as inoculum and crop stage is required.

The main value of the infection model will be to target fungicide applications to the high-risk infection times, so that those fungicides which are applied will give good control. In practise, the benefit of the model is to give the alert for the first spray. If the first spray can be delayed until required, it is possible that the three week protection window will last until flowering has ended and a second spray will, therefore, not be needed. On the other hand, the model may justify an early spray, and a second spray may then be necessary depending on whether there are more infection alerts while the crop is still flowering.

The agar plate petal infection results from the yellow bud and early-flower samples were better associated with stem rot incidence than the later samples. The yellow bud and early flower samples would be helpful for assessing infection risk, alongside forecasting alerts from the infection model and the general weather outlook. It would be worth developing cheap and quick tests for sclerotinia on petals, as this would provide an in-field assessment of inoculum.

The petal adherence results suggest that counting petals on leaves is not a good indicator of stem rot incidence and therefore not useful. However, a general assessment at a site visit of none, some

or many petals adhering is an additional risk factor to note. A crop with wet leaves plastered with petals is clearly at risk, especially if inoculum is known to be present. But a dry crop with few adhering petals visible should be considered at risk if tests show that inoculum is present. Infection can occur via axils which are almost always moist and hold petals and flower debris.

The key conclusions from testing the sclerotinia forecasting models are:

- Forecasting models can help determine disease risk and hence decisions about fungicide applications, but additional use of key risk assessment factors is important.
- Key regional risk factors are: predicted dates of sclerotial germination, forecast temperature and rain and regional spore trap data (Chapter 6).
- Key in-field risk factors are: spore inoculum, petal infection, observed germination of sclerotia, stage of crop, forecast temperature and rain. Heavy rain reduces the likelihood of infection, whereas light rain promotes infection.
- Forecasting based on the weather-based infection model can be used effectively to time sprays for oilseed rape, up to two days ahead. For crops such as green beans and peas with a short flowering duration, it is useful for decisions to spray or not.
- The sclerotial germination model is useful for predicting regional risk. It cannot target specific spray dates but correctly predicts regional onset of spore inoculum each year.
- Where a sclerotinia spray is needed on oilseed rape, forecasting and risk assessment can inform whether one or more sprays are justified.

Uptake of results by industry:

- Foliar fungicide applications are the most important control method for growers, and therefore an improved risk-assessment scheme or a reliable forecasting model that is easy to use would be likely to have good uptake. Control using fungicide timings according to model alerts, in conjunction with inoculum assessments need to be demonstrated to growers using additional sites and years, to encourage uptake.

Future work priorities are:

1. To demonstrate and evaluate the use of the weather-based infection model in combination with other risk factors, e.g. inoculum for sclerotinia control in a range of susceptible crops. The approach would be modified as appropriate for different crops, and would be based on a network of experiment sites in high-risk sclerotinia areas. Live reporting of results during key crop phases would be important to demonstrate use and benefits of the integrated approach.

2. Develop a delivery method for the forecasting and risk assessment. One scenario is that this is initiated through levy and industry funding as part of future work, and then continued with industry support and reported via levy websites.

3. A cost effective and quick test for Sclerotinia that can be used on-farm needs to be developed to enable reporting of inoculum test results within a few days of sampling.

4. The use of in-field humidity sensors needs to be assessed. This would increase the accuracy of forecast dates for infection events, and thereby improve sclerotinia control. Some modification of the weather-based infection model thresholds might be necessary if in-field sensors are used.

6. Sclerotinia inoculum detection

6.1. Introduction

The work package investigated the relationship between spore detection and sclerotinia disease incidence in oilseed rape crops to assess the potential for DNA-based detection methods (qPCR) to be used to assess disease risk and to guide fungicide timing. This work was an important part of the objective to identify risk periods for infection by airborne spores to improve the timing of fungicide applications, based on airborne inoculum detection and/or weather data. Sclerotinia DNA was assessed by qPCR on air samples and plant samples at multiple sites within a field at ADAS Rosemaund to determine the variability in concentrations of airborne Sclerotinia spores within a field. Daily spore concentrations were recorded at various different sites in the west, east and north of England for validation/comparison with information from sclerotia burial depots and a weather-based prediction model for sclerotial germination (Chapter 3). In addition, airborne spore concentrations were compared to petal infection assessment by qPCR and petal infection assessed by agar plate tests. The work package therefore linked to other parts of the project, particularly to explain disease in fungicide timing experiments and the accuracy of weather-based sclerotial germination models. The work also assessed whether spore production was primarily within-field or from surrounding fields (which has implications for soil management and crop rotations). In different field locations, the numbers of airborne spores trapped over set periods were related to the incidence of petal infection at the same locations both within field and different fields. The aim was to determine whether airborne spore and/or petal infection levels could help explain and predict final sclerotinia disease incidence, in conjunction with weather-based infection models.

6.2. Methods

6.2.1. Air sampling sites

Air sampling took place at the field sites indicated in Figure 6–1. Burkard seven day spore traps were operated throughout the oilseed rape flowering period each year of the project (2010 to 2012). The two sites in the west of England were ADAS Rosemaund (two Burkard spore traps in the field used for fungicide spray timing and petal collection) and NPARU approximately 35 miles away from Rosemaund to the east and on a rooftop approximately 8m above ground level. In the east of England, the two sites used were Rothamsted (field-based spore traps were next to sclerotial burial depots and a rooftop spore trap was approximately 0.5–1km away on a building about 8m above ground level) and ADAS Boxworth (near to sclerotia burial depots approximately 40 miles NE of Rothamsted in years 2010 and 2011) and ADAS Terrington (approximately 80 miles NE of Rothamsted, used in 2012). A site at ADAS High Mowthorpe in North Yorkshire provided a contrasting geographic/meteorological area, the five locations allowing an examination of regional differences in spore timing (for comparison with weather based predictions). Furthermore, the two rooftop sites were designed to allow a comparison of ground-based samplers with rooftop samplers that are thought to represent a smoothed sample representing spore release over larger i.e. regional scales. Agar plate tests were conducted on paired samples of petals and leaves taken for qPCR tests, and on further samples were taken at additional sites where qPCR tests will not be done (Figure 6–1.). The variability of sample results across the field and surrounding fields were analysed with the aim of determining the optimum sampling strategies for spore trapping or petal tests.

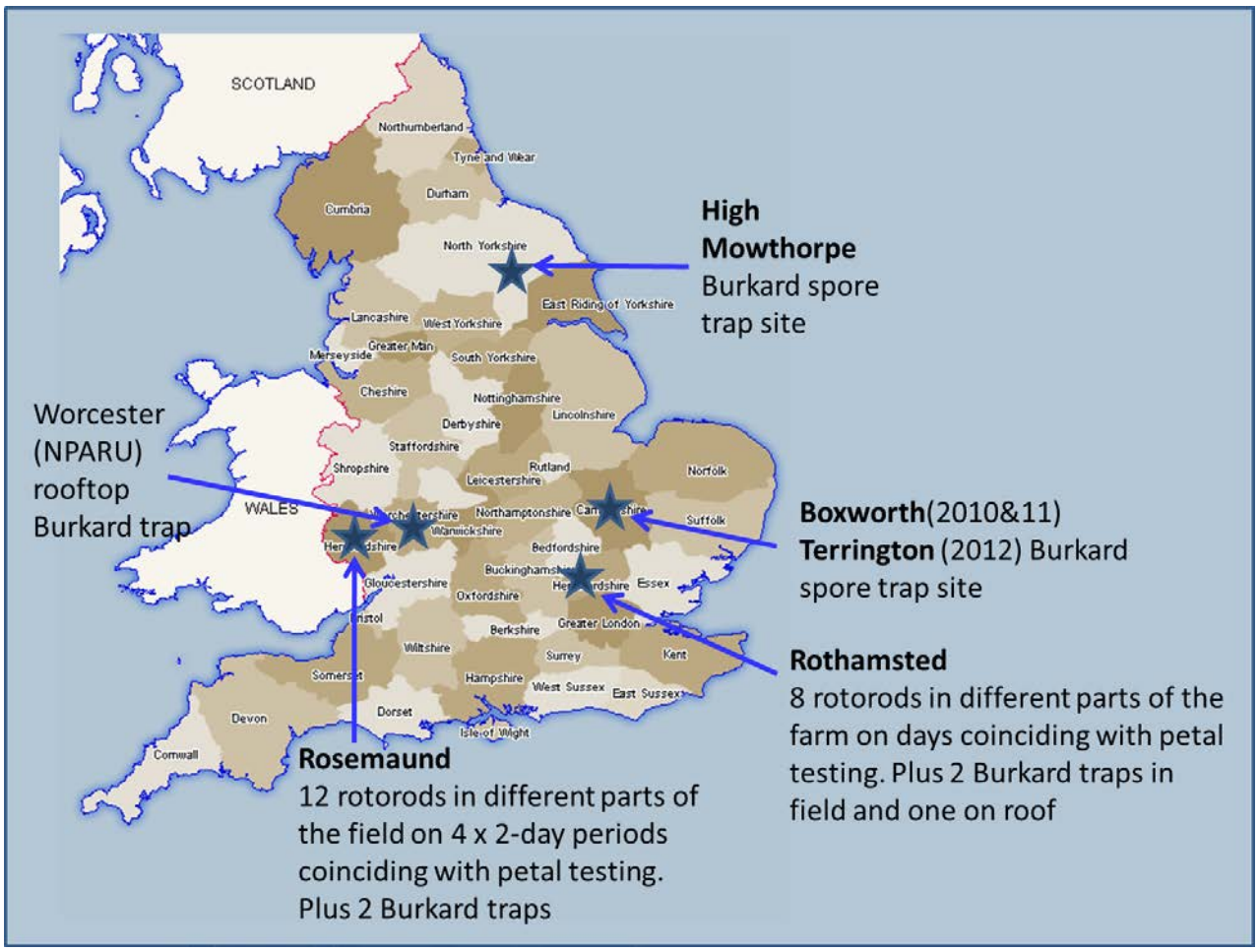

Figure 6–1. Locations of air sampling sites

In addition to air sampling, many sites were used for petal collection, fungicide timing studies and at Rothamsted for measurement of disease gradients form the inoculated point source. Presence of sclerotinia stem rot was measured in transects through the inoculated spore source in June and July.

6.2.2. Air sampling equipment and methods

Fig 1.2 Burkard seven day spore trap operating in an oilseed rape field at Rothamsted

Fig 1.3 Rotating-arm sampler with detachable arms operating in wet conditions in 2012

Figure 6–2 and Figure 6–3.

Burkard seven day spore traps (Figure 6–2) were operated from 12V leisure batteries according to methods described in Lacey and West (2006). New collection drums were sent to each site from Rothamsted and exposed drums were returned to Rothamsted by post/courier. Rotating arm traps used in 2010 were as described in Lacey and West (2006) and were operated for 24 hour periods coinciding with dates of petal sampling. Rotorods were located at four edges of an oilseed rape field at 0.75m height above ground and also at four positions near the centre of the field at 0.75 and 1.5m height. In 2011, a new design of Rotorod (Figure 6-3) was used with detachable perspex arms, which could be placed into Eppendorf tubes and posted after sampling. This was developed by Burkard as part of the project. The devices were also operated on timers for 5 minutes on, 5 minutes off over 48h periods coinciding with petal collection. In 2012, problems with the timers caused by wet weather resulted in the Rotorods being adapted to operate continuously over 24 hour periods, as in 2010.

6.2.3. DNA extraction from air and petal samples

The same DNA extraction method was applied to all air samples. For Burkard seven day spore trap samples, each single-day section of tape (48 x 20mm) was cut in ½ lengthways (two sections 48 x 10mm) and each ½ tape section was spiralled into 1.5ml screw cap tubes for DNA extraction, with the start or earliest spore deposit kept towards the top of the tube. The tubes were labelled and kept at -20°C. Only one ½ section was used with the other duplicate kept in a different freezer. For rotating arm samples, either collection tape on the arm surface was removed or for traps with removable arms, both of the collection arms were placed in a 1.5ml screw cap tube. The DNA extraction procedure for air samples is described in Appendix 2.

Petals were collected from sampling sites at ADAS Rosemaund and ADAS Boxworth (Terrington in 2012). Sclerotinia DNA was extracted from flower petals and leaf discs using MycroLysis Plus (Microzone Ltd), as described in Appendix 3.

A qPCR assay was performed on all DNA extractions using the same method (described in Appendix 4) for air and petal samples and also a set of soil DNA samples from sites across the UK supplied by SRUC.

6.3. Results

6.3.1. Air sampling with Burkard spore traps

Air sampling was conducted on schedule using Burkard spore traps during the oilseed rape flowering season at all sites in 2010, 2011 and 2012.

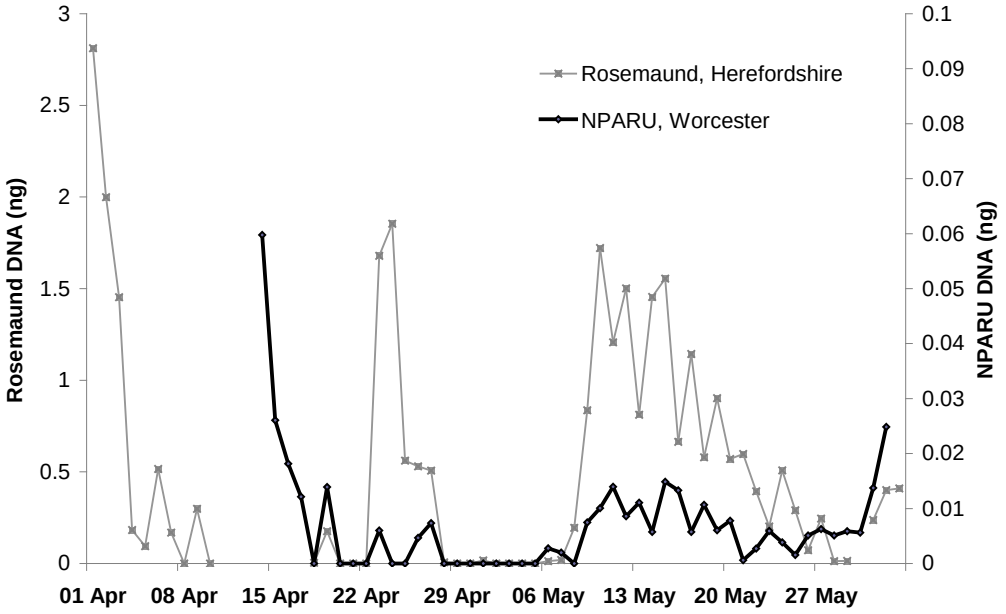

Figure 6–4. *Sclerotinia sclerotiorum* DNA in spore traps at ADAS Rosemaund and a rooftop site at Worcester University (NPARU) 35 miles away.

There was a similar pattern of timing spore presence between the in-field and regional Burkard spore traps. For example, the Rosemaund in-field and Worcester regional spore traps about 35 miles apart showed a similar timing pattern of DNA presence (Figure 6–4), although with differences in the actual DNA [spore] concentration. This suggests that the pathogen population is responding to the same general weather cues in that region to give the same pattern, but the Worcester rooftop site detects fewer spores per m^3 of air due to more dilution in air between the spore source and the rooftop collector. This agreement in timing of DNA presence determined from

local and distant Burkard traps was found in most years for pairs of traps within the same region, but with a few exceptions.

At the western site, ADAS Rosemaund, *Sclerotinia sclerotiorum* DNA was detected as soon as spore trapping began, as early as late March, before apothecia were visible in the sclerotia burial depots (Table 6-1). In contrast, the eastern field sites, Rothamsted and ADAS Boxworth (Terrington in 2012), had very little sclerotinia DNA in air samples until the time apothecia were first seen in the burial depots.

Table 6-1. Timing of sclerotinia apothecia observations and detection of spore DNA in air samples

Site	First Observed	T10 Observed	First spore DNA >0.01ng	First spore DNA >0.1ng
ADAS Boxworth OSR	24/05/10	14/06/10	29/03/10	31/05/10
ADAS Boxworth OSR	21/06/11	29/06/11	12/06/11	not reached
ADAS Boxworth OSR	12/03/12	17/04/12	not used	not used
ADAS Terrington OSR	19/03/12	29/03/12	15/04/12	not reached
ADAS High Mowthorpe OSR	10/05/10	01/06/10	26/04/10	14/05/10
ADAS High Mowthorpe OSR	11/04/11	-	14/05/11	18/05/11
ADAS High Mowthorpe OSR	30/04/12	02/05/12	10/05/12	not reached
ADAS Rosemaund OSR	19/04/10	27/04/10	12/04/10	17/04/10
ADAS Rosemaund OSR	11/04/11	16/05/11	01/04/11	01/04/11
ADAS Rosemaund OSR	16/04/12	24/04/12	18/03/12	not reached
Rothamsted OSR	15/04/10	06/05/10	08/04/10	28/04/10
Rothamsted OSR	11/04/12	02/05/12	07/05/12	23/05/12
Rothamsted OSR*	11/05/11	11/05/11	11/04/11	13/05/11
Rothamsted roof			02/06/10	not reached
Rothamsted roof			11/05/11	16/05/11
Rothamsted roof			09/04/12	not reached

Sclerotinia DNA concentration in air samples appeared to be associated with rainfall. Very wet conditions where rain was prolonged, or there were heavy rain events, reduced the concentration of DNA, i.e. reduced the number of spores in the air. However, short, dry periods following rain appeared to promote spore release. For example, at High Mowthorpe in 2010, sclerotinia DNA increased after each rain event (Figure 6–5). Long dry periods caused a reduction in airborne spores, thought to be due to apothecia drying up. Spore reduction occurred 5–10 days after dry weather depending on soil wetness, air temperature and humidity. Similar spore patterns with rain were recorded for the other field sites.

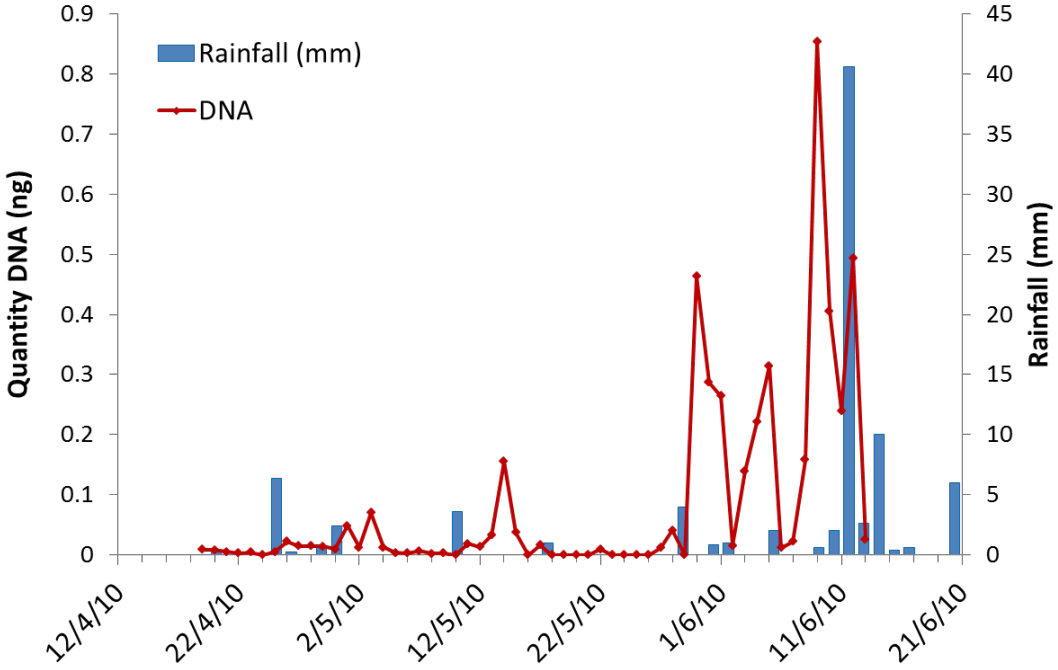

Figure 6–5. *Sclerotinia sclerotiorum* DNA at ADAS High Mowthorpe

Disease gradients from spore point sources

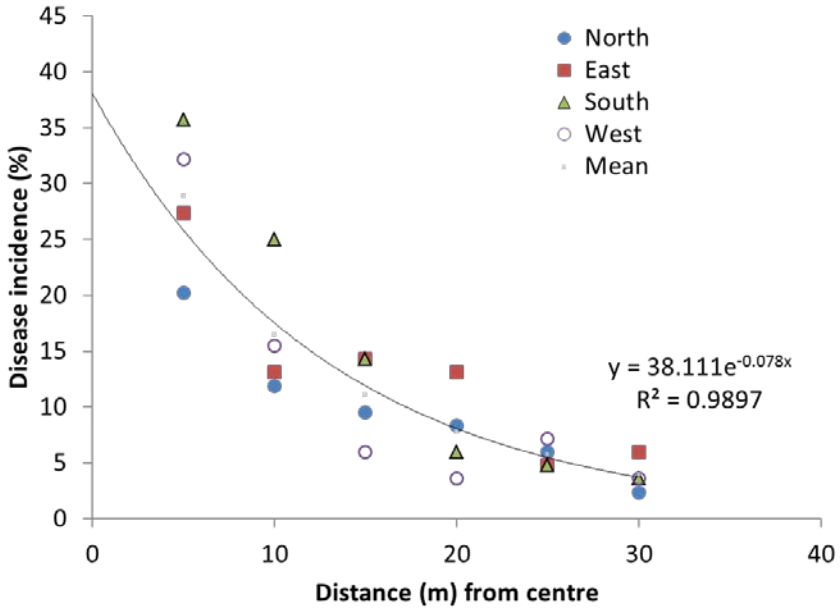

Figure 6–6. Sclerotinia stem rot disease gradient in oilseed rape, 18th July 2012 at Rothamsted.

Sclerotinia stem rot was recorded each year at the Rothamsted site in oilseed rape surrounding the sclerotial germination grids for N, W, E and S transects. Maximum disease was 28% in 2012, and the assessments at a range of distances from the inoculum source in the grids showed a clear gradient of disease in all four directions assessed (Figure 6–6) and a half-distance of approximately 10m. Disease declined to zero by approximately 40 m. This suggests that most

spores travel no further than 30 to 40 m from an inoculum source. However, while many spores deposit in the crop canopy, some are carried into the atmosphere for long-distance dispersal, as recorded by the Burkard spore traps.

6.3.2. Sclerotinia detected with Rotorod traps

The Rotorod traps provided a measure of inoculum (in DNA pg) over a fixed time of 24 – 48 hr, compared to Burkard traps which operated continuously. Rotorod results were therefore analogous to the agar plate tests on petals which were sampled three or four times during flowering. There were some issues with Rotorod traps caused by wet weather in 2012, causing the 'waterproof' timer units to stop, and some data was lost while this was fixed. As with the agar plate tests, the Rotorod DNA results varied between samples as well as between sites. The low and the high Rotorods (paired on the same pole) within the experiment plot area often had very different levels of DNA. Large amounts of DNA on low Rotorods were interpreted as the source of inoculum being mostly from apothecia on the ground, within field. Large amounts of DNA on high Rotorods were interpreted as indicating that inoculum was being carried in the air from a source further away, possibly outside the field. The agar plate test results from petals sampled at the various RotoRod locations indicated a slight gradient of inoculum across fields, e.g. in 2010 the north-east of the field had the highest inoculum scores (Figure 6–7).

Schematic location of RotoRod spore trap locations					
		7 2 Avg of UT plots within trial 4 5		
6	**1**		**3**	**8**

Early-flower % GS 4.2				
		50 50		
42	75	54	33	58
		50 33		

Mid-flower % GS 4.5				
		25 50		
33	50	73	42	33
		42 17		

Mid/late- flower % GS 4.7				
		42 *		
17	*	50	*	17
		* 17		

Late-flower % GS 4.8				
		42 *		
58	*	75	*	67
		* 25		

Figure 6–7. Location of RotoRod spore traps within trial and in surrounding field and % petals testing positive for sclerotinia using agar plate tests, Rosemaund 2010. Uppermost box shows schematic location of spore traps, with % petal infection results in lower boxes.

6.3.3. Agar plate and qPCR tests for sclerotinia on petal and leaf samples

Overall, there were similarities to the pattern of incidence of sclerotinia on petal and leaf disc infection, measured by qPCR and agar plate tests. In general, more samples tested positive using qPCR than agar plate tests. The mean percentage of petal and leaf samples with Sclerotinia as measured by the agar plate test, and extent of colonisation measured by quantification of pathogen DNA (amount of pathogen DNA equivalent to numbers of spores) varied between sites and years (Table 6-2), with a variable relationship between the agar plate and qPCR results (Figure 6–8).

Table 6-2. Incidence and amount of Sclerotinia DNA on petals and leaf discs.

Site	Date	PCR mean petal incidence	**PCR mean petal spore equivalent	PCR mean leaf incidence	PCR mean leaf spore equivalent	Agar plate test mean petal incidence	Agar plate test mean leaf incidence
Rosemaund 2010	28 Apr	100	65	*	*	54	4
Rosemaund 2010	06 May	100	146	77	39	72	33
Rosemaund 2010	20 May	94	34	86	26	52	25
Rosemaund 2010	27 May	100	48	100	74	79	3
Essex 2010	21 Apr	100	115	77	7	29	4
Essex 2010	26 Apr	44	3	25	6	13	0
Essex 2010	05 May	98	13	57	20	33	8
Essex 2010	20 May	94	41	40	4	44	25
Rosemaund 2011	27 Apr	91	25	69	9	39	2
Rosemaund 2011	06 May	89	46	75	31	17	0.1
Rosemaund 2011	26 may	100	60	*	*	25	-
Boxworth 2011	21 Apr	89	11	39	4	<1	0
Boxworth 2011	26 Apr	97	12	64	16	<1	0
Boxworth 2011	10 May	60	3	39	1	<1	0
Boxworth 2011	20 May	52	6	22	1	0	0
Rosemaund 2012	10 Apr	15	4	*	*	13	0
Rosemaund 2012	24 Apr	38	17	*	*	20	0
Rosemaund 2012	08 May	4	1	*	*	19	0
Rosemaund 2012	15 May	0	0	*	*	20	0
Terrington 2012	23 Apr	29	11	0	0	0	0
Terrington 2012	30 Apr	17	5	0	0	31	0
Terrington 2012	09 May	25	2	0	0	9	8
Terrington 2012	17 May	17	5	4.15	1	53	3

** mean spore equivalent = number of spores per petal estimated from PCR test, based on amount of DNA in average sclerotinia spore

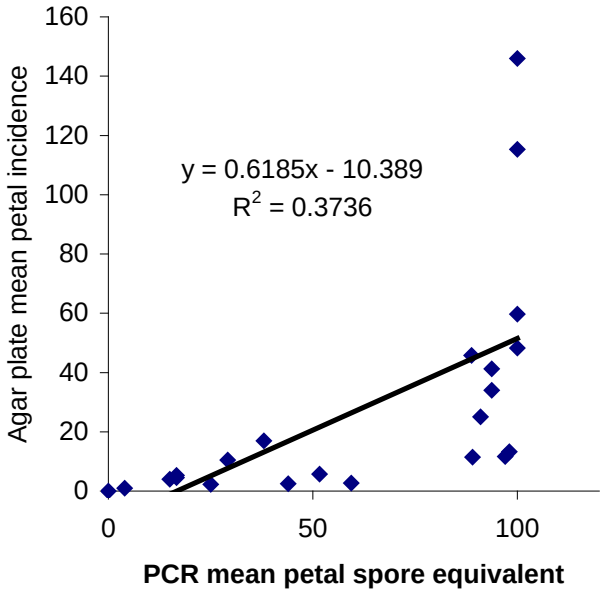

Figure 6–8. Petal test results for agar plate and spore equivalent values based on PCR tests. Data is from four sites, Rosemaund and Essex 2010, Rosemaund and Boxworth 2011 and Rosemaund and Terrington 2012. Values are means of 12 petal samples per plot and four plot reps.

The relationship between agar plate tests and qPCR tests was better using the spore equivalent data (Figure 6–8) which is a measure of the amount of Sclerotinia DNA rather than qPCR incidence data (i.e. % of petals positive for sclerotinia).

6.3.4. Petal DNA tests, petal agar plate tests and air sample DNA tests

There were often differences between the results of agar plate tests on petals, and the corresponding Rotorod air sample for pathogen DNA at the same location and time. For example, at ADAS Rosemaund 2010, at GS 4.2 all plots tested positive for petal infection by agar plate test, with individual plots ranging from 33% to 75% petals infected (Figure 6–9). In contrast, only traces of DNA were collected using Rotorod traps at four out of the 12 plots. At GS 4.5 in 2010, 12/12 plots were positive for Sclerotinia DNA and 11/12 Rotorod locations were positive at the same time.

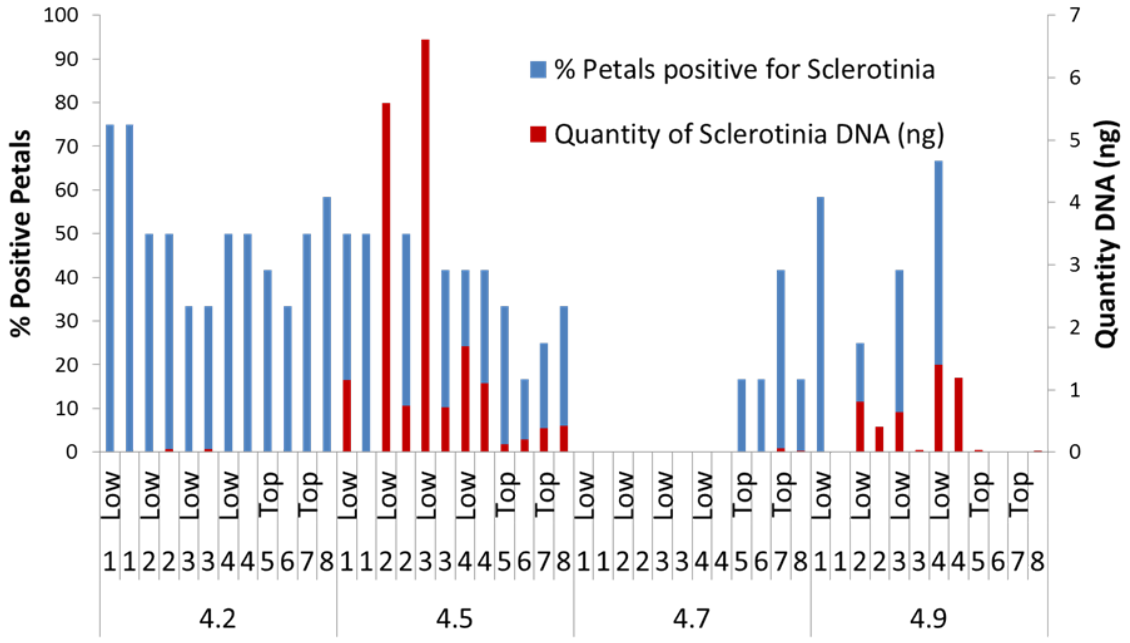

Figure 6–9. Comparison of agar plate (% positive petals) and Ss DNA on Rotorods at ADAS Rosemaund in 2010 at four different growth stages: 4.2, 4.5, 4.7 and 4.9.

In Figure 6–9, positions 1 to 4 were near the centre of the field and included two heights (low and high) and positions 5–8 were near the edge of the field at the high position (above crop canopy). The petal infection incidence measured by agar plate tests did not translate to Rotorod DNA-based tests. For example, at GS 4.2, agar tested petals indicated an infection incidence of 30–75% but DNA tests on air samples that day were mostly zero (four at trace levels were always sites above canopy height). At GS 4.5, agar petal tests indicated 20–50% infection, while DNA tests on air samples were quite high with up to 6.6 ng DNA per sample. Out of the paired locations in the centre (high or low positions), the highest amount of DNA was always at the low position, which suggests that the spores were being produced nearby at ground level. At GS 4.7, agar tests were negative at central locations and 17–42% at the edge of the field, while Rotorod DNA tests were mostly zero except for trace levels at two edge sites (7 and 8). This suggests that spores were blowing in from outside of the field. At GS 4.9, there was excellent agreement between petal and Rotorod tests, with both tests indicating that spores were being produced in the field (low positions at the centre of the field were positive by both methods), while high positions were mostly negative or at trace levels apart from position 4 (field centre) which was also positive. This suggests that at this time, the spores were being produced in the field (at ground level).

6.3.5. qPCR tests on SRUC soil samples

Samples of DNA extracted from soil from fields in Scotland were tested using the developed Taqman qPCR method. All samples tested negative. Selected samples were spiked with pathogen DNA to test for inhibition of the qPCR method but no inhibition was found.

6.4. Discussion

The Rotorod results show high variation in airborne spore concentrations at the field scale, and explain why there were differences in the patterns of DNA detected in samples from the two Burkard traps, which were in different parts of the same field. In 2010 the Rotorod results at Rosemaund broadly agree with the continuous record of spore presence measured by the Burkard spore traps with one exception, which was 20/5/2010, which appears to have reasonable spore levels but the Rotorods tested low. In 2011 there was good agreement between Rotorod and Burkard data for Rosemaund. In 2012 at Rosemaund, the Rotorod results agree with the continuous record of airborne pathogen DNA found from the Burkard spore traps in the same field, which had a high amount on 12th April (39pg) but relatively low amounts on other dates. Rotorod trapping results at Rothamsted may explain the reason for this variability in Sclerotinia spore distribution around crop canopy level at the field scale. Spore releases from small sources could be detected by Rotorod samplers immediately above the source within the canopy, but not above the canopy top. Only 1m away downwind, spores were detected mostly above the canopy and not within it. On some occasions, Rosemaund 2010 at GS 4.9, there was excellent agreement between petal infection (measured by agar plating) and air sampling (assessed by qPCR) but poor agreement at other times, possibly because petals are present for longer than the air sampling period.

A comparison of petal infection by agar plate testing and by qPCR tends to suggest a higher infection incidence by qPCR than agar plate tests, which could be because the qPCR test detects DNA in dead spores. There were also differences in colonisation of petals and leaves measured by DNA. Usually, petals had more pathogen DNA present than leaf samples, but not always. Petals are usually present for only 3–5 days, compared to months for leaves. This suggests that spores are more readily deposited onto petals, and/or more readily germinate on petals (which increases the amount of DNA). Spores already deposited on leaves may grow when petals fall onto them to provide a food-base and humid microclimate. So a persistent fungicide may provide good control by protecting leaves onto which petals will fall.

Spore trapping using the continuous Burkard spore trap validated the sclerotial germination models, and added to the understanding of inoculum timing and distribution. There was a similar general pattern of spore release within a region but (as confirmed by Rotorod trapping) often large differences in airborne spore concentrations between different parts of the same field. Periods of prolonged wet weather reduced spore concentrations. Peak spore releases were in dry periods for up to 3–4 days after rain, but longer dry periods also reduced spore concentrations due to the fruiting bodies drying up. Spores could be detected on rooftop sampling locations at 8m height and 200–2000m from the nearest agricultural fields indicating a regional airborne spore presence. In the East of England (Hertfordshire and Cambridgeshire), ascospores of *Sclerotinia sclerotiorum*

were detected around the same time that apothecia from deliberately buried sclerotia were first visible. However, in the west of England (Herefordshire and Worcester) airborne spores were detected in significant concentrations over three weeks before any apothecia were visible at sclerotial depots. This suggests that there is a greater diversity of microclimates and potential spore sources in the west, with spores blowing into the field from external sources. However, disease gradients from point sources in fields had a half-distance of approximately 10m, which fits with other work which shows that most sclerotinia spores originate within-field, and the average distance travelled is probably no further than an adjacent field.

It would be useful to understand reasons for the difference in spore release between west and eastern England as this could help to identify sclerotinia disease hotspots. Further work is also required to standardise and put thresholds on spore numbers and disease risk, and also determine the optimum sampling strategy for petals or air, and the testing strategy, e.g. pooled samples vs. separate petals. Clearly, a component of petal stick to leaves is also required otherwise disease escape will occur even in the presence of inoculum. This could be modelled or prediction techniques developed, but combined with either a spore prediction model or spore detection method, there is potential to save unnecessary fungicide applications. Results shown here provide further evidence that spore concentrations are linked to disease development (Figure 6–6). New diagnostic tests are becoming available that could allow a rapid and on-site detection to inform growers of imminent infection risk while there is time to make a spray application (West, 2012; West *et al.*, 2013).

Key messages for growers:

- Inoculum is an indicator of infection risk and sclerotinia disease development
- PCR tests for airborne spores indicated that regional sampling could provide useful disease risk information, subject to confirmation in future years.
- Airborne sclerotinia inoculum is usually detected at similar times to the start of local sclerotia germination. The agreement between the timing of regional and local spores detected by trap was closer for the east sites than the west sites

7. Soil management and treatment

7.1. Introduction

A cost effective soil treatment which reduces sclerotinia sclerotial viability, and hence spore inoculum production in spring, would be a valuable control method. If effective, and repeated each year as routine, such treatment would undoubtedly reduce sclerotinia incidence significantly in most crops over a relatively short number of years, and it follows that the need for foliar fungicides would also be reduced. However, there are currently no available soil treatments that are cost

effective or appropriate to apply routinely. There is grower interest in demonstrating the efficacy of soil treatments and inclusion of a trial using a commercially available biocontrol soil treatment. Contans is a commercial formulation of the biocontrol fungus *Coniothyrium minitans,* and there are numerous studies to show that *Coniothyrium* parasitises *S. sclerotiorum* sclerotia and kills them so they are unable to germinate and produce ascospores. There are fewer studies to show that Contans also reduces the subsequent disease development in the crop, and no studies which demonstrate this for large scale UK field crops such as oilseed rape.

Soil tillage methods can influence disease development on crops, largely through their effect on crop debris removal or breakdown which would otherwise harbour fungal growth and spore production. It is not clear what influence tillage will have on sclerotinia disease, nor if there is a difference in disease levels between tillage approaches. A minimum tillage approach may increase sclerotinia disease levels by promoting growth of sclerotinia mycelium and formation of sclerotia on crop debris. However, minimum-tillage will leave sclerotia on the soil surface which then may be exposed to detrimental environmental conditions or be prone to degradation because of close association with crop debris where antagonists may thrive. Ploughing may bury crop debris and sclerotia, but on the other hand could bring to the surface sclerotia from previous high-infection years which can then germinate. Tillage experiments are not possible to set up as randomised block experiments without large resources, due to logistical restrictions when using host farmer sites. However, it is possible to measure sclerotinia inoculum production from adjacent field areas with different tillage, and thus measure the effects indirectly. The need for rotation means that the same field site cannot be used in consecutive years, so a series of spore measurements at different sites and years is needed to build up evidence for the influence of tillage of sclerotinia.

7.2. Methods

7.2.1. Soil treatments: Contans and Perlka

In 2009, the experiment design was a complete randomised block, with 6 replicate plots per treatment and 24 x 24 m plots of winter oilseed rape var. Castille, drilled 4 September at ADAS Rosemaund, GR S0567482 (minimum tillage site). The three treatments were: Contans 2 Kg/ha, Perlka 250 Kg/ha, and untreated. Contans (Belchim UK) was applied immediately pre-drilling, onto dry soil, with weather conditions overcast and cool with showers in the previous 24 hours. The Contans was applied to the appropriate plots by spraying in 200L/ha water and then incorporating to shallow depth of approximately 1 to 2 cm with the drill tines. Fifty sclerotia (collected on-farm from infected crops immediately pre-harvest 2009) were buried at 1–2 cm depth in each plot on 23 October, one sclerotium per cell in a plastic mesh grid placed on the ground and marked with a cane. On 22 February 2010 when the crop cover was 25–30%, 250 Kg/ha Perlka (PP Products) was applied to the appropriate plots, ensuring that there was suitable weather on the application day, i.e. showery weather, to give damp soil. Germination of the sclerotia in grids was monitored

weekly from mid-March until end of flowering. Petals were sampled on 7 May 2010, GS 4.2, prior to the first fungicide spray by the host farmer. Petals were sampled after noon on a dry day, and placed using sterile forceps onto potato dextrose agar with 50 ug/ml streptomycin sulphate within two hours of collection. One fully open intact flower was sampled from each of 12 randomly selected plants per plot, from the main stem. Plates were incubated at room temperature and assessed after 8–10 days for the presence of *S. sclerotiorum*.

In the 2010–2011 experiment there were four treatments: Contans 2 Kg/ha and 6 Kg/ha, Perlka 250 Kg/ha, and untreated. There were 7 replicate plots (24 x 24 m) plots per treatment of winter oilseed rape, drilled 4 September at ADAS Rosemaund, GR SO536460. Sclerotia were buried on 30 September. Perlka was applied on 17 February 2011. Petals were sampled on 18 April at GS 4.3. All other details of the design and methods were the same as in 2010–2011.

7.2.2. Tillage and sclerotinia inoculum

Sites in eastern England were identified where there were adjacent fields with different tillage, such that oilseed rape petal sampling could be done from adjacent minimum tillled and ploughed fields, at the same time. Sclerotinia spore inoculum was compared from both fields using agar plate tests on petals sampled at early flower, before any fungicides were applied for sclerotinia control.

200 petals were tested per field, sampled by collecting 4 petals from each of 5 main racemes, from 10 locations in each field, at approximately 10m intervals.

7.3. Results

7.3.1. Soil treatments: Contans and Perlka

In 2009–2010, there was no difference between the treatments for the percentage of petals testing positive for sclerotinia: untreated, 42%; Contans 2 Kg/ha, 43%; Perlka 44% (NS).
Sclerotial germination was not significantly reduced by Contans or Perlka ($CaCN_2$).

In 2010–11, there was no significant difference between treatments for petals testing positive for sclerotinia: untreated 2.2%; Contans, 2 Kg/ha 2.0%; Contans 6 Kg/ha, 1.6%; Perlka 1.6% (NS).
Sclerotial germination was reduced by Perlka, but not by Contans (Figure 7–1).

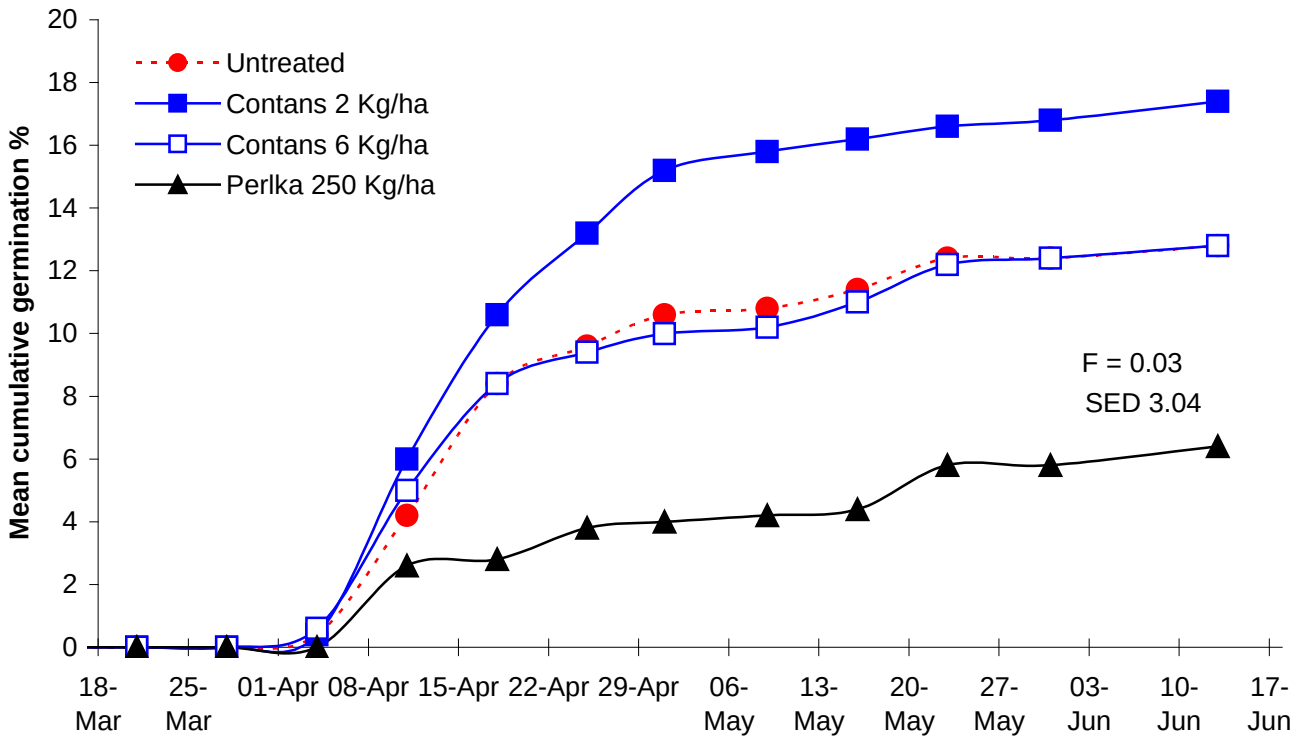

Figure 7–1. Effect of Contans and Perlka on germination of *S. sclerotiorum* sclerotia, ADAS Rosemaund 2011

7.3.2. Tillage and sclerotinia inoculum

For each individual site, there was no difference in inoculum production between the ploughed or minimum tillage field areas, as measured by the percentage of petals testing positive for sclerotinia (Table 7-1). However, there were large differences between years, reflecting the different inoculum levels each year.

Table 7-1. Oilseed rape petal infection following ploughing or mimimum –tillage.

Site	Year	Date sampled	Growth Stage	% Petals infected	
				Ploughed	Min-till
Easton, Norwich	2010	27 April	3.7 – 4.0	1.0	1.0
Horningsea, Cambridge	2010	27 April	4.1	0.0	2.0
Louth, Lincs	2010	10 May	4.7	62.0	52.5
Louth, Lincs	2011	20 April	4.8	9.5	8.5
Louth, Lincs	2012			72.5	64.0

7.4. Discussion

The application methods, soils conditions and weather at the time of the applications were reviewed with Adrian Jackson, Belchim (UK Contans distributor) and also Peter Lueth, Prophyta (producers of Contans). The application methods and sclerotial burial were thought to be appropriate, with no changes advised. Contans has been seen to work better in carrot crops, which tend to have more finely tilled soil which may help growth and survival of Contans, as well as allowing more even incorporation at drilling. The higher value of carrots would also warrant the cost of higher rates of Contans application. If Contans at 6 Kg/ha had worked for oilseed rape, it would be uneconomic to apply.

The similarity between the two different cultivations for sclerotinia inoculum production is in agreement with findings from other studies (e.g. Archer *et al.,* 1992). Minimum tillage may leave sclerotia on the soil surface, but ploughing will bring deeper buried sclerotia to the surface. The current work was based on adjacent half-fields and was not a randomised plot design as this would have necessitated the use of unfeasibly large areas. However, adjacent field areas were a good approach within the limitations of the project resources, and provided robust results. The results were consistent in all years, for high or low inoculum, and indicate that tillage is unlikely to be a major influence on sclerotinia inoculum production.

8. Production of sclerotia in different crops

Additional author: Andy Taylor, Warwick University Crop Centra

8.1. Introduction

The main aim of this part of the project was to determine to number of sclerotia produced by *S. sclerotiorum* and in the field for different crops. These data contributed towards the modelling approach to rotations by allowing estimation of the 'sclerotial returns' made by different infected crops and hence their contribution to disease risk in following years.

8.2. Methods

8.2.1. *S. sclerotiorum* isolates and production of sclerotia on agar

S. sclerotiorum isolates (18) from different hosts (carrot, celery, lettuce, OSR, pea, buttercup) and known to be genetically different were grown on potato dextrose agar (PDA) at 20°C for four weeks (five replicate plates per isolate). The number and weight of sclerotia per plate were then recorded.

8.2.2. Number of sclerotia produced on different crop plants

S. sclerotiorum sclerotia from three selected isolates (L17, L44 and L6 – all from lettuce) stored at 5°C were bisected and each half placed on a PDA plate. Plates were incubated at 20°C for 3 days and then sub-cultured (from the actively growing edge) onto fresh PDA and incubated at 20°C for one day. Sterile wheat grain placed in Petri dishes was inoculated with four 5 x 5mm discs from the actively growing edge of these cultures and incubated at 20°C for 3 days. Colonised wheat grain was then used as inoculum for all the crop plants: oilseed rape (cv. Temple, three experiments of 10 replicate plants), lettuce (cv. Montel, eight experiments of 10 replicate plants), dwarf bean (cv. Tendergreen, six experiments of 10 replicate plants), carrot (cv. Nairobi, four experiments of 10 replicate plants), potato (cv. Estima, three experiments of 10 replicate plants) and pea (cv. Biktop, one experiment of 10 replicate plants). Plants were inoculated at flowering with the exception of lettuce (30cm diameter plant) and carrot (4-5 fully opened leaves). For bean, pea and potato inoculations, wheat grains were placed in stem wounds. Lettuce and carrot plants were inoculated by placing wheat grains on the leaves and oilseed rape (OSR) plants were inoculated by placing wheat grains in the petioles. A high level of humidity was maintained to encourage infection and disease development for all inoculated plants using automatic misting sprays in glasshouses or polytunnels. After plants were fully colonised and necrotic they were allowed to die back completely and dry before sclerotia were collected, weighed and counted.

8.2.3. Sclerotial germination

To investigate the effect of size on germination of *S. sclerotiorum* sclerotia, those collected from plants infected with isolate L6 (all experiments) were pooled by crop type and passed through sieves of increasing diameter to divide them into four size classes: <2mm, 2–4mm, 4–6.7mm and >6.7mm. Sclerotia were then buried in compost within a plastic box (30 per box, 3 replicate boxes per crop/size combination) and conditioned at 5°C for 40 days. Germination to produce apothecia was then recorded over a 12 week period at 15°C. Sclerotia from pea were not included as there were insufficient numbers.

8.3. Results

8.3.1. *S. sclerotiorum* isolates and production of sclerotia on agar

The number of sclerotia ranged from 25–44 per PDA plate over the 18 *S. sclerotiorum* isolates tested. Sclerotial weight (related to size) ranged between 0.0112 and 0.02286g. Three isolates originally from lettuce were selected for the plant inoculations based on differences in sclerotial number per plate and weight. These were L6 (44, 0.0112g), L17 (36, 0.0158g) and L44 (29, 0.0143g).

8.3.2. Number of sclerotia produced on different crops

Inoculated plants all resulted in substantial *S. sclerotiorum* infection for all crop types which were completely colonised, with the exception of potato for isolates L17 and L44. A greater number of sclerotia were produced on OSR (70 sclerotia per plant) and lettuce plants (92 sclerotia per plant) compared to the other crops tested across all experiments, with the smallest number of sclerotia produced on pea (30 sclerotia per plant; Figure 8–1). Isolate L6 produced a greater number of sclerotia on potato (99 sclerotia per plant) than the other isolates as this was the only isolate to completely colonise plants. When isolates were compared, L6 produced more sclerotia (67 sclerotia per plant) than L17 (48 sclerotia per plant), with L44 producing the fewest over all crops except carrot (29 sclerotia per plant); Figure 8–1. However, L44 produced bigger sclerotia on each crop as measured by weight (mean value 0.025g) than L17 (0.018g) or L6 (0.016g;).

These results therefore agreed with ranking in the agar tests. Crop type also had an effect on sclerotial size with the largest produced on oilseed for all isolates (0.044g, Figure 8–2. M). Oilseed rape also supported the highest proportion of larger sclerotia; 17% were in the size category 4–6.7 mm compared to 3.2–7.4% in the other crops (Table 8-1). Taking into account the field density of each crop (OSR 28 plants/m^2, lettuce 8, carrot 150, Bean 40, Potato 3.3 and pea 110) and assuming full plant colonisation, the greatest number of sclerotia per square metre (averaged across the three isolates) would be produced in carrots (3868 m^{-2}, Figure 8–3) and peas (3245 m^{-2}, Figure 8–3) with the smallest in potatoes (176 m^{-2}, Figure 8–3). These results were generally in agreement with the real field data from ADAS.

8.3.3. Sclerotial germination

Larger sclerotia of *S. sclerotiorum* L6 germinated faster, had a greater % germination and also produced more apothecia across all crop types (Table 8-1). The largest sclerotia (>6.7 mm) produced on oilseed rape gave an average of 10.3 apothecia per sclerotium. This compared across all crops to 1.1 apothecia per sclerotium for sclerotia <2mm, 1.6 apothecia per sclerotium for sclerotia 2–4 mm and 4.1 apothecia per sclerotium for sclerotia 4–6.7 mm. Crop type also appeared to have some effect on germination of sclerotia with those derived from carrot having a consistently lower % germination (43–47%) than those derived from other plants across all size grades (mean 50–95.8%, Table 8-1). Data for size of sclerotia, percentage germination, number of apothecia per sclerotium and number of sclerotia per plant were combined to give an overall estimate of number of apothecia that could be produced per square metre in the field. This showed that the most apothecia would be produced by sclerotia from OSR (4055 m^{-2}), closely followed by pea (3498 m^{-2}) and carrot (2793 m^{-2}). The number of apothecia per square metre following a crop of potato, bean or lettuce was predicted to be relatively low in comparison (176–737 m^{-2}, Figure 8–4).

The counts of sclerotia in plants sampled from naturally infected field crops in 2010-2012 were, in general, broadly similar to the results from inoculated plants in polytunnels. Oilseed rape produced fewer, but larger, sclerotia (Figure 8–5) while carrots produced a few thousand (Figure 8–5) small sclerotia. There was variability between sclerotial numbers within each crop for different sites, which is most likely due to sampling areas where incidence and/or plant colonisation was not 100%, as in the polytunnels.

Table 8-1. Germination and number of apothecia produced by sclerotia of different sizes and from different crops. Standard errors of the mean are given in brackets.

Crop	Size class (mm)	Percentage of sclerotia in size class	Percentage germination	Time to 33% germination (days)	Number of apothecia per sclerotium
Potato	<2	41.7	73.3	51.7	1.1
	2–4	53.6	59.6	42.3	1.3
	4–6.7	4.5	84.4	33.0	1.9
	>6.7	0.2	Not done	Not done	Not done
Bean	<2	43.6	50.0	57.7	1.0
	2–4	50.2	66.7	29.7	1.5
	4–6.7	5.1	60.0	25.0	1.7
	>6.7	1.1	Not done	Not done	Not done
OSR	<2	25.0	64.4	56.3	1.1
	2–4	52.7	80.0	41.3	2.1
	4–6.7	16.6	83.3	32.0	3.1
	>6.7	5.7	95.8	22.3	10.3
Lettuce	<2	37.2	59.2	62.3	1.3
	2–4	59.1	71.1	35.3	1.4
	4–6.7	3.2	86.7	26.0	2.6
	>6.7	0.5	Not done	Not done	Not done
Carrot	<2	42.8	43.3	71.3	1.1
	2–4	48.6	45.6	69.0	1.6
	4–6.7	7.4	46.7	53.0	1.7
	>6.7	1.1	Not done	Not done	Not done
Mean of all crops	*<2*	*38.1*	*57.1 (±4.3)*	*59.9 (±3.1)*	*1.1 (±0.02)*
	2-4	*52.9*	*64.6 (±4.0)*	*43.5 (±4.1)*	*1.6 (±0.12)*
	4-6.7	*7.4*	*83.6 (±3.5)*	*31.9 (±2.4)*	*4.1 (±0.95)*
	>6.7	*1.7*	*95.8 (±2.1)*	*22.3 (±1.3)*	*10.3 (±1.41)*

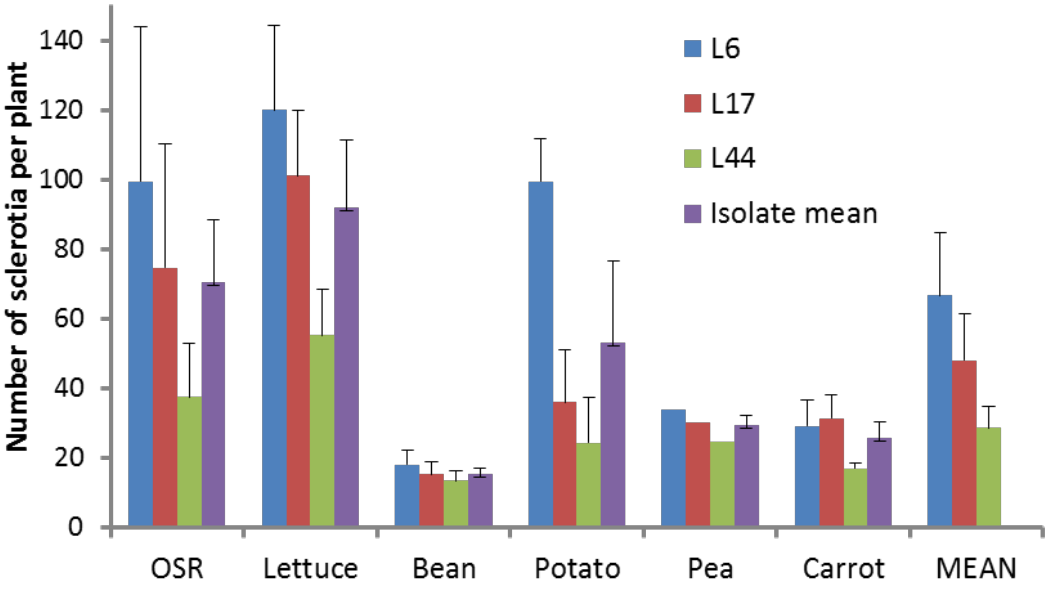

Figure 8–1. Mean number of sclerotia produced per plant by three S. *sclerotiorum* isolates on different crop plants. MEAN=averaged over all crop plant types.

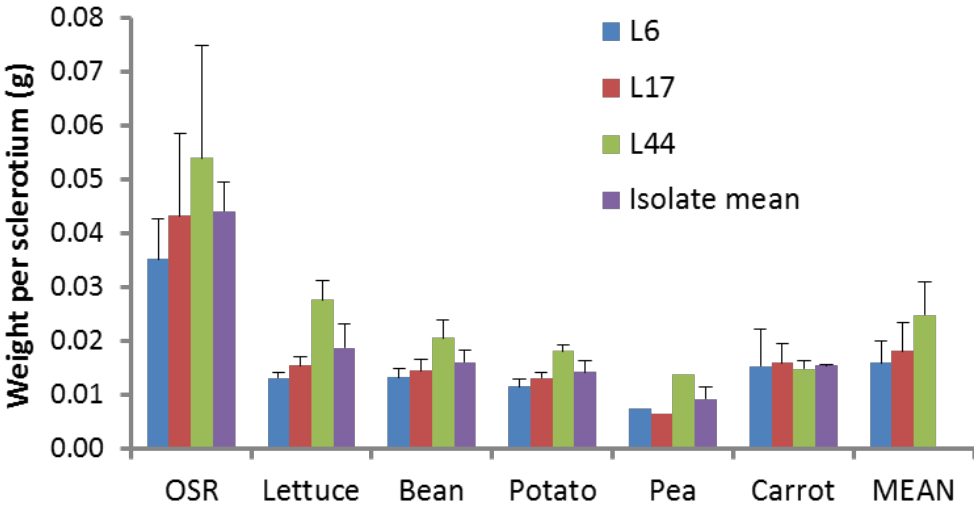

Figure 8–2. Mean weight per sclerotium produced by three S. *sclerotiorum* isolates on different crop plants. MEAN=averaged over all crop plant types.

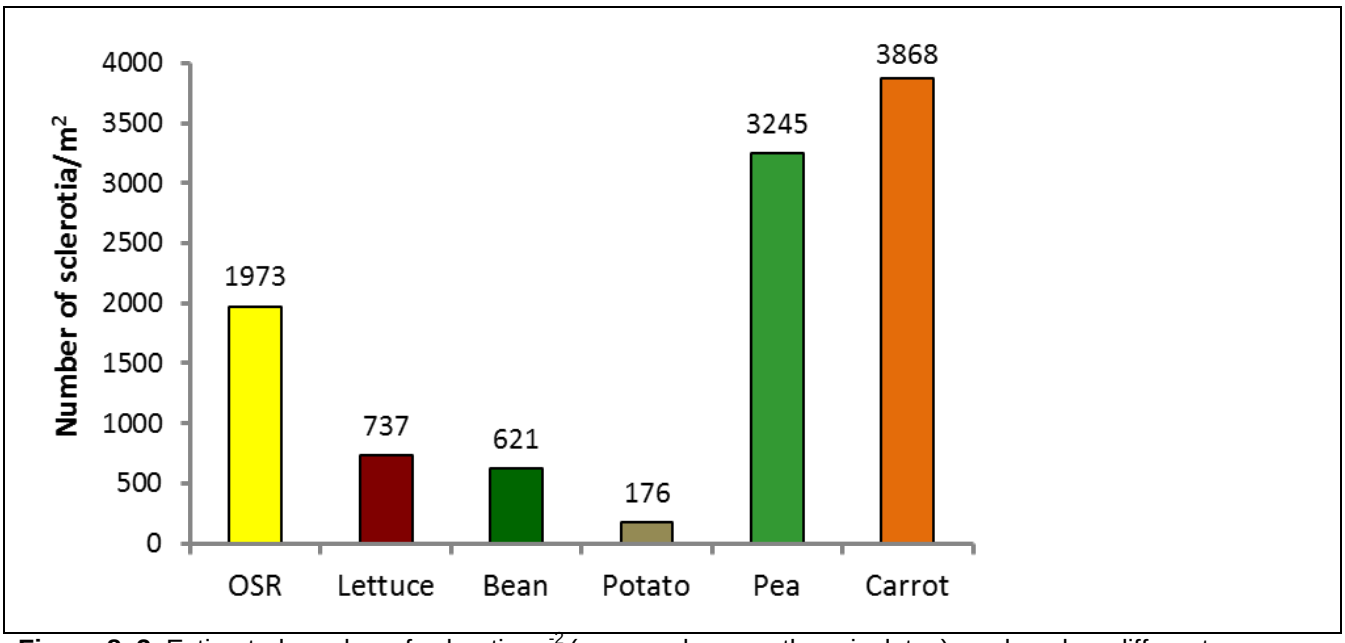

Figure 8–3. Estimated number of sclerotia m^{-2} (averaged across three isolates) produced on different crops in the field. Values are based on sclerotial production in polytunnel experiments, and average plant densities relevant to each field crop.

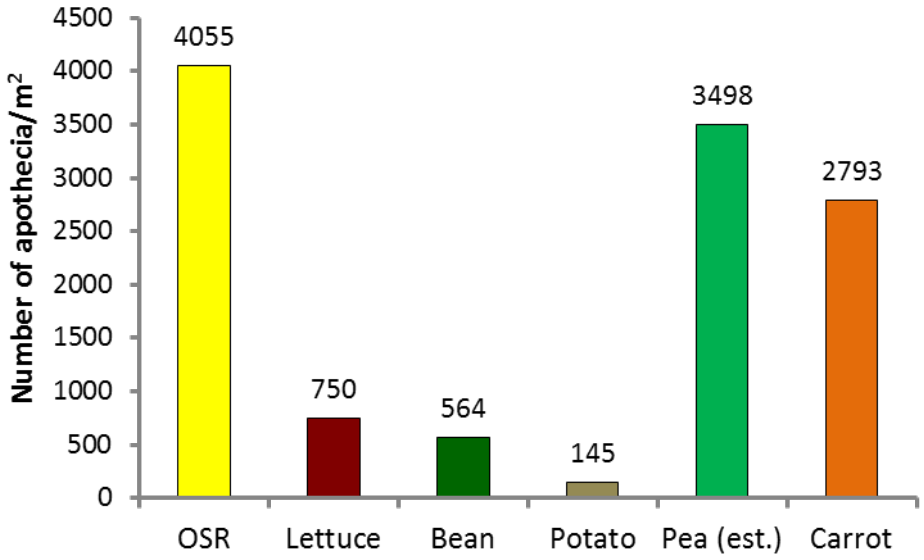

Figure 8–4. Estimated number of *S. sclerotiorum* apothecia per sclerotium produced in the field. This data compares sclerotia produced on different crop types. The value for pea was estimated (see text for details) using mean values for all other crops (est.= estimated).

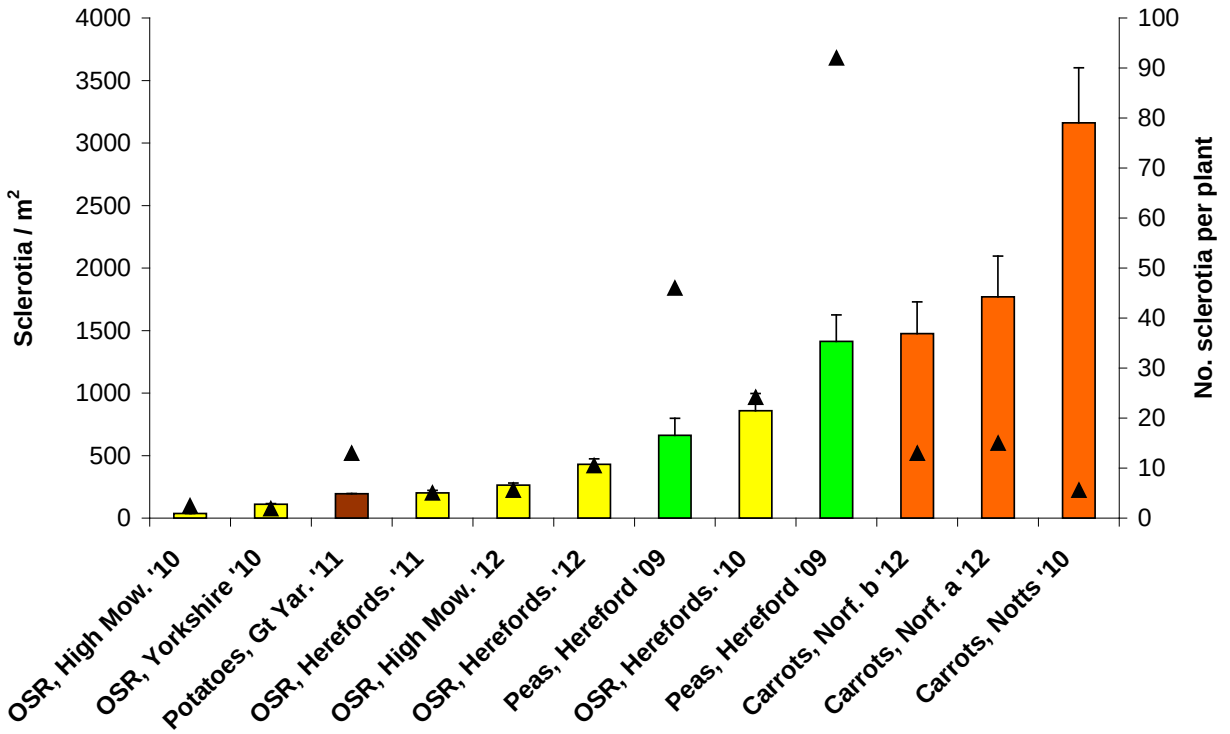

Figure 8–5. Estimated number of sclerotia m^{-2} and sclerotia per plant produced on different crops, in field sites 2010–2009. Values are based on plant density records and counts of sclerotia per plant (for carrots, plant + soil surface), in 10 plants per site sampled from sclerotinia-infected areas (untreated).

8.4. Discussion

The number and size of sclerotia varied between different *S. sclerotiorum* isolates and different crop species. The *S. sclerotiorum* isolates tested had consistent strategies for sclerotial production across different crop plants; L6 produced large numbers of small sclerotia whereas L44 produced small numbers of large sclerotia with L17 intermediate between the two Crop plant type also moderated production of sclerotia; oilseed rape supported a higher proportion of larger sclerotia for all isolates compared to the other crop types. The data on sclerotial production allowed estimation of sclerotial returns in the field based on crop plant densities and also the potential for apothecial production. This showed that infected carrots and peas (assuming complete plant colonisation by *S. sclerotiorum*) potentially support the highest returns of up to approx. 3000–4000 sclerotia m^{-2} to the soil. When size and germination ability of sclerotia is taken into account, then sclerotia derived from oilseed rape, pea and carrot have the greatest inoculum potential with up to 4000 apothecia m^{-2} potentially produced. It must be stressed that this is a hypothetical figure as it assumes that all the sclerotia returns are near the soil surface and have optimum conditions for germination. In reality this figure will be much lower as many sclerotia will be deeply buried and hence will not germinate while others will be killed by microorganisms or adverse environmental conditions. However, the ranking of crops in terms of inoculum potential is likely to hold true and hence is informative for modelling and determining the optimum crop rotations for minimising Sclerotinia risk.

9. Determining optimum crop rotations by dynamic programming

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9.1. Introduction

Sclerotinia survives in the soil as sclerotia (resting bodies) for up to 10 years, so a high level of inoculum built up in the soil in one crop can have a significant impact on subsequent susceptible crops in a rotation. Crop rotation can be used to minimise the impact of the disease. Crop rotation gives many other benefits, including maintaining soil structure and fertility, reducing agricultural chemical usage, reducing flood losses and avoiding build-up of pathogens and pests, but here our interest is restricted to the effect of rotation on the temporal dynamics of disease.

Long-term and short-term management decisions, such as crop rotation, have an impact on the epidemiology of plant disease and therefore on farm economics. Reducing sclerotinia disease while maximising profit is more complicated than simply lengthening rotations for susceptible crops; hence this study. Bio-economic models provide useful frameworks to investigate the trade-offs between the state of the land, severity of sclerotinia and financial impacts as a result of different cropping decisions. We therefore developed a dynamic programming (DP) model of the crop rotation decision problem to study these trade-offs. The objective was to find the cropping decision sequence that maximises the net present value of cropping on a unit of land over both the long- and short-term time horizons. By changing key parameters in the DP and re-optimising, the impact of alternative assumptions and crop rotations was explored.

9.2. Materials and methods

9.2.1. Structure of the DP model

DP (Bellman, 1957) is a mathematical technique which is especially of value in a situation where a sequence of inter-dependent decisions has to be made, e.g. livestock replacement, forest management and crop rotations. The basic principles of DP were fully explained by Kennedy (1986) and their use in determining optimum crop rotations has been described by several authors (Onstad and Rabbinge, 1985; Stott *et al*., 1996; Trengove and Manson, 2003; Cai *et al.*, 2011). In this study a DP model was developed using Microsoft Excel and Visual Basic version 6.5 for Windows (Microsoft Corporation, 2007). The model was run separately using the general purpose dynamic programming (GPDP) software (Kennedy, 1986). The objective of the DP was to find the cropping decision sequence that maximises the net present value (i.e. current value of current and future net returns from one hectare of farming land expressed as an annuity) of cropping on that land over the short-term and long-term time horizons. Land was represented by 25 states including 5 sclerotinia states (S1–S5, based on numbers of sclerotia in the soil) and 5 non-susceptible crop states (G1–G5) representing the number of years since last non-susceptible crop decision. In total

a maximum of 6 cropping decision options (i.e. a combination of susceptible crops, non-susceptible crops and treated susceptible crops) could be included in each run of the model. The DP calculated which combination and sequence of crops was required to be included in the optimal solution to reduce sclerotinia to the extent that maximised profit. Susceptible crops considered were: carrots, oilseed rape, spring beans, spring peas, lettuce and potatoes. It was assumed that growing susceptible crops raises the number of sclerotia in soil, but subsequent non-susceptible crop (non-susceptible) decisions reduce it at differential rates. Figure 9–1 illustrates the event time line and the decision tree structure of the DP model.

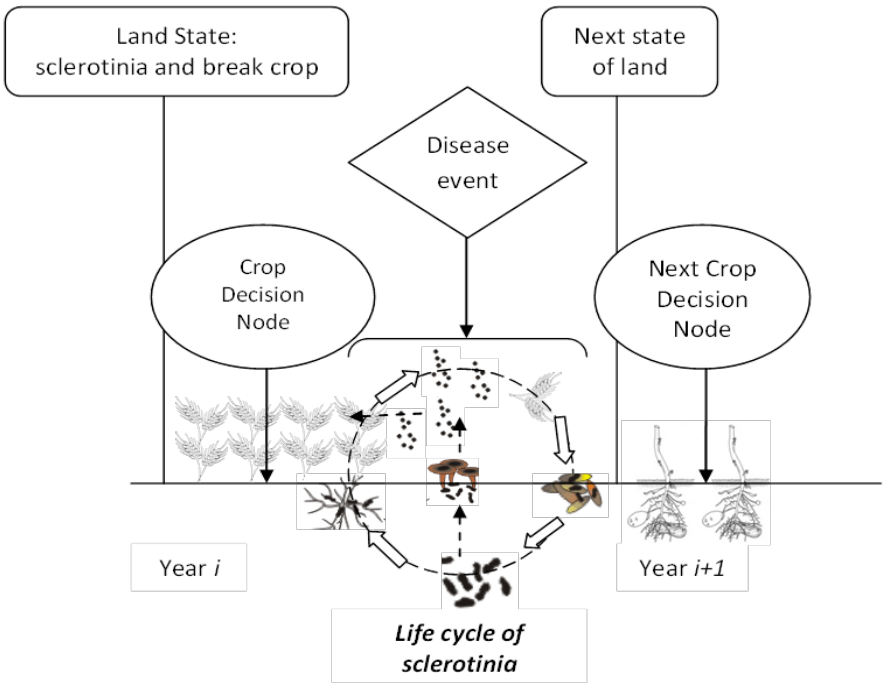

Figure 9–1. Crop decision and sclerotinia disease event time line that represents the Decision Tree structure used in the DP model. In this figure, *i* equals 1–4 for short-term runs of the model (year 1 to year 5) and it equals 1 to >20 for long-term runs of the model.

9.2.2. Model inputs and assumptions

Stage return

Various assumptions and values were used (Table 9-1) to determine the gross output for each possible state. All the yields and prices of each susceptible and non-susceptible crop are based on figures reported in the farm management handbook (SAC, 2011/12) except figures for lettuce.

Table 9-1. Yields and output prices used in the model.

Crop	Yield[1] (t/ha)		Price[1] (£/ha)		Reference
	Produce	Straw	Produce	Straw	
Carrots (C)	64.0	8.0	120	15	SAC 2008/09
Winter wheat (WW)	8.0	4.2	155	28	SAC 2011/12
Spring wheat (SW)	6.5	3.6	175	28	SAC 2011/12
Winter barley (WB)	7.5	4.1	145	40	SAC 2011/12
Spring barley (SB)	5.5	2.9	145	40	SAC 2011/12
Winter oilseed rape (WOSR)	4.0	-	350	-	SAC 2011/12
Spring oilseed rape (SOSR)	2.5	-	350	-	SAC 2011/12
Spring beans (SB)	5.0	-	200	-	SAC 2011/12
Spring peas (SP)	4.0	-	200	-	SAC 2011/12
Potato -early ware (P)	39.2	-	175	-	SAC 2011/12
Lettuce (L)[1]	48750	-	0.21	-	Young *et al.*, 2007

[1] Lettuce yield and price are head/ha and £/head respectively.

The variable costs associated with each state were based on figures presented in Table 9-2 (SAC, 2011). By subtracting variable costs from gross outputs, the gross margins were calculated. The stage returns were calculated based on the gross margin of the current cropping decision but with a yield and variable cost adjustment function dependent on the state of the land at the current stage.

Table 9-2. Variable costs of included crops (£/ha).

	C	WW	SW	WB	SB	WOSR	SOSR	SB	SP	P	L
Seed	520	91	87	84	72	63	80	130	130	840	2,010
Fertiliser and salt	406	294	243	274	186	276	137	64	73	410	720
Polythene		-	-	-	-	-	-	-	-	800	-
Topper, harvest, tractor	358	-	-	-	-	-	-	-	-	-	-
Labour & tractor	115	-	-	-	-	-	-	-	-	-	-
Pesticides (sprays)	853	128	88	88	58	142	49	98	109	75	980
Other crop expenses		-	-	-	-	-	-	-	-	41	675
Straw	2000	-	-	-	-	-	-	-	-	-	-
Market commission	768	-	-	-	-	-	-	-	-	-	-
TOTAL	5,020	513	418	446	316	481	266	292	312	2,166	4,385

9.2.3. Yield loss and assumptions

The build up and decline curves of sclerotinia in soil as a result of cropping decisions and their impacts on marketable yields were obtained from previous experiments and from expert opinion mentioned below. It was assumed that the yields of susceptible crops are lowered at a rate inversely proportional to sclerotinia level (i.e. S1–S5 states, S1 being worst and S5 best states) and raised by growing a non-susceptible crop (i.e. G1–G5 states) in a similar manner. An estimated function (Equation 1) of marketable yield loss (t/ha) and sclerotinia root disease incidence for carrots (McRoberts *et al.*, 2007) was used to estimate the proportion of disease-free yield lost to the disease in successive years of susceptible cropping:

$$Yld = 120.96 - 0.927 * srr\% \quad \text{Equation (1)}$$

where *Yld* represents annual marketable yield of susceptible crops (t/ha) and *srr* denotes sclerotinia disease incidence (%). It was assumed that sclerotinia survives in the soil as sclerotia for up to 5 years. The build up rate of sclerotia in land (that was assumed to be equal to *srr*), as a result of continuous susceptible cropping, was estimated by all the project leaders to be 0%, 10%, 30%, 90%, 100% for year 1 to year 5, respectively. Decline rates were similarly estimated by expert opinion. By replacing these rates for s*rr* rates in Equation 1, the yield losses for years 1–5 were calculated and the proportion of yield loss determined as: 0%, 7.7%, 23%, 69% and 76% for years 1–5 respectively. The yield figures presented in Table 9-3 are the outcome of multiplying the yields from healthy crops (Table 9-1) by the annual yield loss rate calculated above. The annual yield loss of conterminously cropping the crops (i.e. winter wheat, spring wheat, winter barley and spring barley) were estimated by the experts and used in the model. These were: 0%, 2.25%, 5.25%, 11.25% and 22.50% for years 1–5, respectively (Table 9-4). Spraying was considered as a possible treatment option. For all the susceptible crops, an annual effectiveness rate of 18% (20% effectiveness (McRoberts, 2007) minus 1.5% wheeling loss) of improving marketable yield was assumed. Therefore an annual extra variable cost of £76 (two extra sprays at £38 each) for treatment was considered in the scenarios that treatment options made available for the DP model.

Table 9-3. Assumed yield loss due to the impact of sclerotinia on susceptible crops.

Crop	Yield loss (t or *****head/ha) based on time span of continuous growing susceptible crops (Year 1 to Year 5)				
	Y1	Y2	Y3	Y4	Y5
Carrots	0.0	4.9	14.7	44.1	49.0
Winter oilseed rape	0.0	0.3	0.9	2.8	3.1
Spring oilseed rape	0.0	0.2	0.6	1.7	1.9
Spring beans	0.0	0.4	1.1	3.4	3.8
Spring peas	0.0	0.3	0.9	2.8	3.1
Potato -early ware	0.0	3.0	9.0	27.0	30.0
Lettuce*	0.0	3736	11208	33624	37361

Table 9-4. Assumed yield loss due to continuous growing of non-susceptible crops.

Crop	Yield loss (t/ha) based on time elapsed since last non-susceptible crop for Year 1 to Year 5				
	Y1	Y2	Y3	Y4	Y5
Winter wheat	0.0	0.2	0.4	0.9	1.8
Spring wheat	0.0	0.1	0.3	0.7	1.5
Winter barley	0.0	0.2	0.4	0.8	1.7
Spring barley	0.0	0.1	0.3	0.6	1.2

9.2.4. Transition probabilities

Two transition probability matrices were used, one for the susceptible crops and one for the non-susceptible crops. These matrices define the probabilities of moving from a current state of land, in terms of infestation and the time elapsed since the last non-susceptible crop, to the next state by

deciding to grow a certain crop from the decision set. They also regulate the transitions from one state to another by preventing or allowing certain movements. In other words, they reflect the life-cycle of the disease in the format of transition probabilities based on cropping decisions. The probabilities used are based on the authors' assumptions. Table 1 of Appendix 12.5 presents the probability of next state given the current states for the susceptible crops (i.e. carrots, winter oilseed rape, spring oilseed rape, spring beans, spring peas, potatoes and lettuce). Table 12.2 of Appendix 12.5 presents the probability of next state given the current states for the non-susceptible crops (i.e. winter wheat, spring wheat, winter barley and spring barley).

9.2.5. DP model runs

The data described in the previous sections provided the input required for the GPDP software (Kennedy, 1986) that was used separately to run the model. Three main scenarios were examined and the DP runs were undertaken. The scenarios examined were:
Scenario 1: Only susceptible crops (i.e. carrots, lettuce, potatoes, winter oilseed rape, spring peas and spring beans) provided to the DP as decision choice set.
Scenario 2: Including a non-susceptible crop to susceptible crops in the decision choice set.
Scenario 3: Including a treatment option for susceptible crops and a non-susceptible crop to susceptible crops in the decision choice set. The above-mentioned susceptible crops and a non-susceptible crop plus a treatment option for carrots, as an example of this scenario, is presented in this report.

For each of the three scenarios a long-term (>20 years) time horizon and a short-term (five-year) time horizon were considered and investigated. In scenario 1–3 for a long-term time horizon, only one susceptible crop in each run was added to the decision choice set (i.e. continuous cropping). The DP model was run for a long-term time horizon using a discounting factor of 5%, and expected net present values (ENPV) expressed as annuities were estimated by the model. In DP runs considering a short-term time horizon six susceptible crops (i.e. carrots, lettuce, potatoes, winter oilseed rape, spring peas and spring beans) were added to the decision choice set and the DP decided on which crops to be included in the optimal decision.

9.3. Results

Results of long-term model runs are presented in Figure 9–2 and the results of short-term model runs are presented in Table 9-5 and Figure 9–4, Figure 9–5 and Figure 9–6.

9.3.1. Long-term model runs

In scenario 1, continuous susceptible cropping led to financial losses in the long term (results for four susceptible crops are presented in Figure 9–2). Carrots and lettuce made higher losses than

winter oilseed rape and potatoes in this run of the model (scenario 1). However, one non-susceptible crop (i.e. winter wheat) in the rotation in scenario 2 mitigated long-term build up of sclerotia in land and major financial losses (Figure 9–2). Adding a treatment option for the susceptible crops to the rotation of susceptible and non-susceptible crop in scenario 3 further enhanced the financial returns and reduced the adverse effect of sclerotinia on outputs.

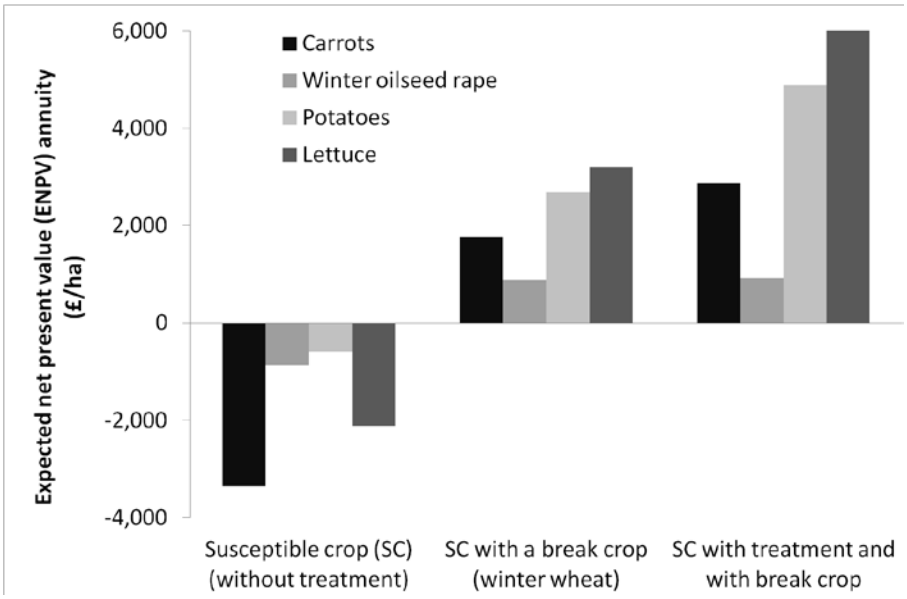

Figure 9–2. Effect of management decisions of three different scenarios: i) continuously growing only susceptible crops (SC), ii) susceptible and non-susceptible crops and, iii) susceptible crop and applying treatment on financial outcomes of carrots, winter oilseed rape, potatoes and lettuce in long run (infinite time horizon).

For oilseed rape the results of long-term runs showed that continuously growing oilseed rape generated a financial loss (ENPV of -£866/ha). Including winter wheat as a non-susceptible crop to the decision set, featured both oilseed rape and winter wheat in the optimal decision that generated financial profit (ENPV) of £882/ha. By adding a treatment option to the decision set, the optimal decision predicted by the DP included all the three crop decisions oilseed rape, treated oilseed rape and winter wheat that generated an ENPV of £919/ha that was equal to the gross margin of a healthy oilseed rape crop. The long-run state probabilities of the optimal decision in this case were 45% for oilseed rape in S5, 53% for treated oilseed rape in S4, and 2% of winter wheat in S3. Sclerotinia states S1 to S2 did not featured in the optimal decision (i.e. long-run probabilities of 0.0) indicating that the DP limits the land infestation by including a non-susceptible crop in the rotation.

9.3.2. Short-term model runs

Results of the DP short-term run for scenario 1 showed that the optimal decision consisted of spring peas (20% of the states) for the highest sclerotinia states (i.e. S1–S2), potatoes in moderate

sclerotinia state (S2, 20% of the states), and lettuce for low sclerotina states (S3–S5, 60% of the states) (Table 9-5). The model minimised the impact of the disease and therefore avoided great financial losses in highly- and moderately-infested states (S1 and S2) by including spring peas and potatoes (ENPV of -£29 per ha and -£9 per ha for S1 and S2, respectively) in the optimal decision (Figure 9–3). Results showed no difference in financial returns in year 1 to year 5 for the high- and moderately-infested land states (i.e. states 1–15). However, for the low-infested land states (i.e. states 16-25) lower financial returns were predicted for years 1 and 2 compared to the last three years 3–5 (Figure 9–3).

By the inclusion of winter wheat as a non-susceptible crop in the decision choice set in scenario 2, the DP's optimal decision crops were lettuce and winter wheat. The optimal decision in year 1 consisted of winter wheat for the states S1 to S2 (40%) and lettuce for S3 to S5 (60% of the states). In year 2, winter wheat was the optimal decision for S1 to S3 (60%) and lettuce for S4 to S5 (40%). For year 3 to year 5, winter wheat accounted for 80% of the optimal decision in S1 to S4 and lettuce was the best decision for S5 (20%) (Table 9-5 and Figure 9–4).

In scenario 3, where a treatment option for carrots was added to the decision choice set of crops, the optimal decision in year 1 remained similar to scenario 2 with winter wheat and lettuce as the best options (Table 9-5). In years 2–5, lettuce in moderate- and low-infested states was replaced by treated carrots to improve the state of sclerotinia. The optimal decision in year 1 consisted of winter wheat in sclerotinia states of S1 to S2 (40%), and lettuce for S3 to S5 (60%). In year 2, winter wheat remained the best decision for S1 to S2 states. For S2 to S3 and one state in S4 (24% of all the states) treated carrots provides the highest benefit, and lettuce was the optimal decision for the lowest sclerotinia state S5 (36%). In year 3 to year 5, winter wheat remained the best decision for the highest sclerotinia states of S1 and S2 (40%). Treated carrots chosen as the best decision in S3 and S4 states (40%) and lettuce was the optimum decision for the lowest sclerotinia state S5 (20%).

Table 9-5. Optimal rotations and proportion of cropping decisions in each state for year 1 to year 5 of a short-term time horizon.

Scenarios	Proportion of decisions and state numbers for year 1 to year 5					
Susceptible crop only						
	Years 1–5					
Crop	Proportion	State				
Spring peas	0.20	1–5				
Potatoes	0.20	6–10				
Lettuce	0.60	11–25				
Susceptible and non-susceptible crop						
	Year 1		Year 2		Year 3-5	
Crop	Proportion	State	Proportion	State	Proportion	State
Winter wheat	0.40	1-10	0.60	1-15	0.80	1-20
Lettuce	0.60	11-25	0.40	16-25	0.20	21-25
Susceptible crop and treatment and non-susceptible crop						
	Year 1		Year 2		Year 3-5	
Crop	Proportion	State	Proportion	State	Proportion	State
Winter wheat	0.40	1-10	0.40	1-10	0.40	1-10
Treated carrots	0.00	-	0.24	11-16	0.40	11-20
Lettuce	0.60	11-25	0.36	17-25	0.20	21-25

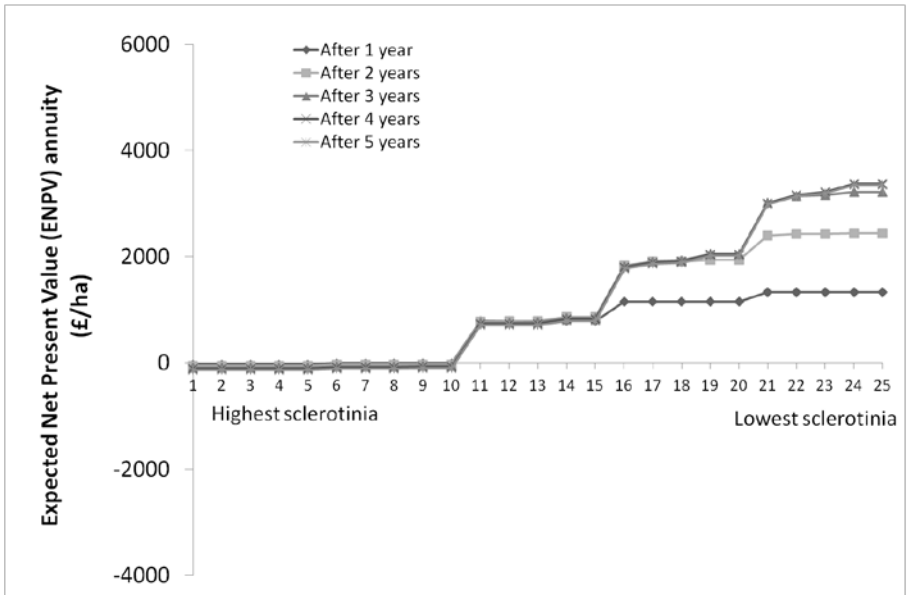

Figure 9–3. Financial outcomes of the optimal solution of growing only susceptible crops (scenario 1) in a five-year time horizon. The x-axis represents the 25 modelled states (refer to text for details).

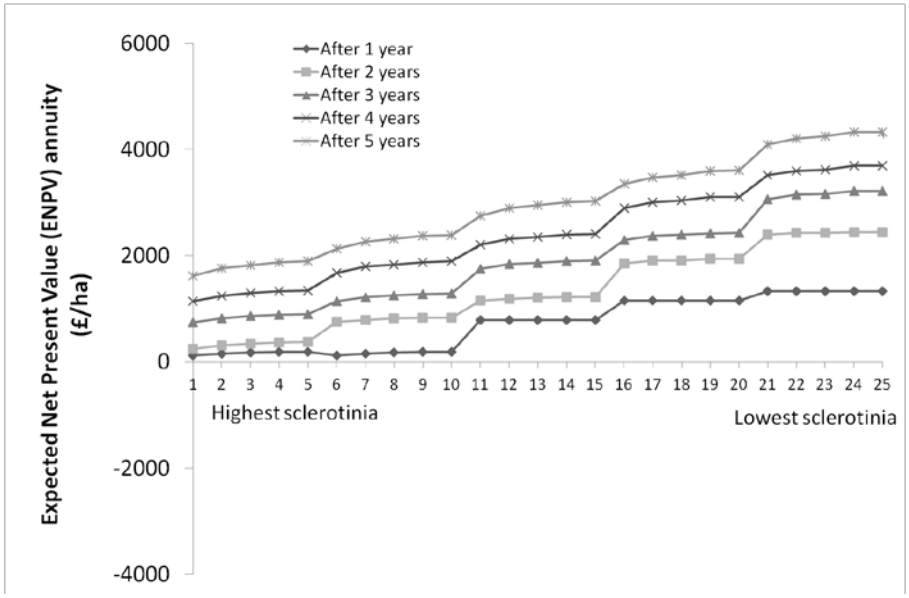

Figure 9–4. Financial outcomes of the optimal solution of growing susceptible crops and a non-susceptible crop (i.e. winter wheat) (scenario 2) in a five-year time horizon.

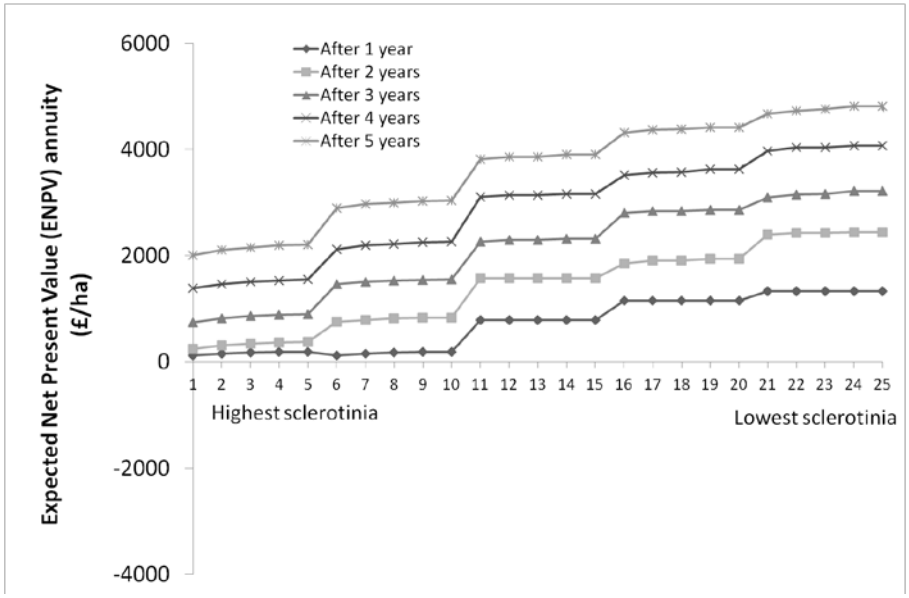

Figure 9–5. Financial outcomes of the optimal solution of growing susceptible crops and providing a treatment option (scenarios 3) in a five-year time horizon.

Results showed that when sclerotinia is at the highest level (i.e. S1), inclusion of a non-susceptible crop in rotation and/or treatment did not improve the average financial returns of the all 25 states in year 1 (Figure 9–6). However, including a non-susceptible crop in rotation and adding treatment improved the returns in year 2–5.

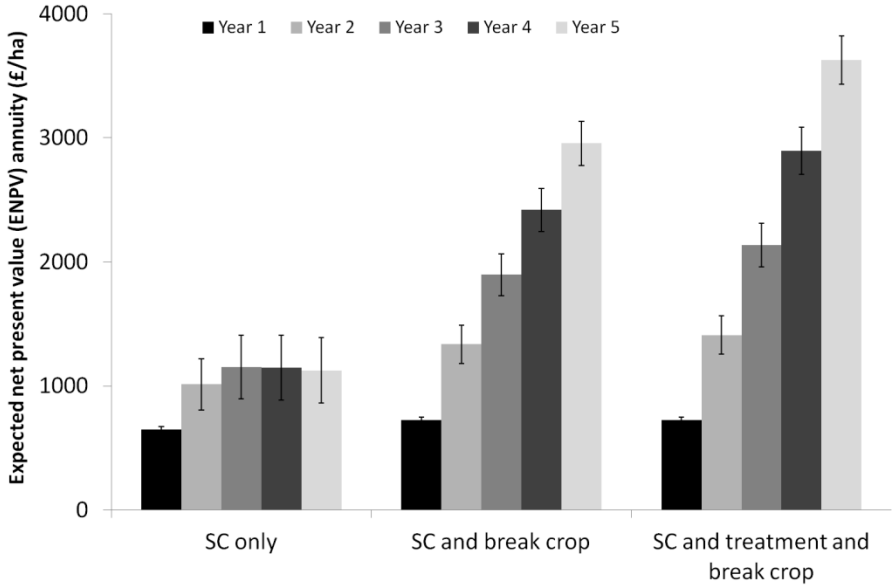

Figure 9–6. Financial outcome of optimal solutions of three scenarios calculated by DP for year 1 to year 5. Presented figures are Mean±SE (error bars) of 25 modelled states (sample size of 25 in one run of the model).

For oilseed rape including winter wheat as a non-susceptible crop to the decision set in short-term runs showed that in year 1, in worst- and moderately infested land states (S1 to S3 and 3 states in S4), winter wheat was the best decision (72% of all states). In 2 states of S4 and all states of S5 (i.e. minimum infestation) oilseed rape featured as best decision (28% of the states). However, in year 2 to year 5 the optimal decision consisted of winter wheat for sclerotinia states S1 to S4 (80% of states) and oilseed rape featured only in sclerotinia states S5 (20% of states). The average ENPVs of all the states for year 1, year 2, year 3, year 4 and year 5 were predicted at: £180, £517, £678, and £834 respectively. These results showed that in short-term oilseed rape could be the best choice only if the land infestation with sclerotia is its minimum. In moderate to high level of land infestation the optimum decision is to grow a non-susceptible crop.

9.4. Discussion

Continuous susceptible cropping in the long-term resulted not only in substantial financial losses but also a great accumulation and build-up of sclerotia in land over time, a build-up that poses a great risk to susceptible future crops. Despite a profitable outcome for continuously growing susceptible crops in some land states (moderate to low sclerotia infestation) in the early years of a short-term time horizon (5 years), the model confirms that major losses would be expected for the majority of land states in the last years (year 4 and 5). Including one non-susceptible crop in the rotation in the long-term reduced build-up of sclerotia in soil and improved the financial returns that were reflected in positive probability-adjusted ENPVs. The financial improvement calculated by the model were 64%, 96%, 57% and 55% of the gross margins of healthy crops for carrots, winter oilseed rape, potatoes and lettuce respectively. Combination of rotation with a non-susceptible

crop and treatment showed the highest effectiveness in minimising the impact of the disease. It should be noted that although the DP can handle the stochastic nature of the disease and the long-term cyclical cropping decision, calibrating the model to mimic certain (short-term) rotations is difficult if not impossible.

Running the model with the six susceptible crops in the decision choice set, the optimum rotation in the short-term featured a high proportion of lettuce (60%) in the low infested states and an equal proportion of spring peas and potatoes (20% each) in high- and moderately-infested states of land. With no non-susceptible crop in the rotation, highly-infested land states could not be improved and therefore generated a loss that was minimised by choosing spring peas, potatoes and lettuce. However, it was still possible to make positive financial return for the land with moderate to low levels of infestation (i.e. state 10 to state 25). Adding winter wheat to the decision choice altered the optimum crop rotation in that winter wheat and lettuce featured in the optimal rotation. The proportion of winter wheat decisions predicted by the model increased from 40% in year 1 to 80% in year 5 aiming at minimising accumulation of the sclerotia in land by the end of the short-term time horizon (year 5). The financial returns in year 1 were the lowest and in those year 5 were the highest predicted. Despite the low financial returns, particularly in year 1 and year 2, in the highly-infested land states, the inclusion of a non-susceptible crop in the rotation mitigated losses in these states. Minor improvements (10%) observed in the average financial figures of the all states in year 1 were achieved by adding a non-susceptible crop or a non-susceptible crop and treatment. The improvement in average figures was higher in subsequent years and in year 5 reached to its maximum of 62% and 69% for scenario 2 and scenario 3, respectively. In practice, the ability to grow high value horticultural crops, such as lettuce, is dependent on other factors, such as soil type. This was not accounted for in the analysis.

In the current model, two transition probability matrices were used, one for the susceptible crops and one for the non-susceptible crops. Ideally crop-specific transition probability matrices are needed to capture the differences between the crops. These matrices regulate the transitions from one state to another by preventing or allowing certain transitions and therefore play a crucial role in characterising the optimal decisions of the DP. In the absence of field data that could inform these matrices, we used our best assumptions in reflecting the transitions of land state based on the disease status and type of the crop (susceptible or non-susceptible crop). We considered it as one of the limitations of the current model that needs further attention and improvement. The modelling work helped with identifying these data gaps and the areas that more research is needed. Another limitation of the current model was the relationship of the level of disease (sclerotia) and the potential yield loss. In the absence of crop-specific data, a disease-yield loss relationship from a carrot experiment was used for all the 6 crops included in the model. Therefore, the relationships between the number, size and frequency of sclerotia and the yield loss as well as the build-up and

decay curves of sclerotia by continuously growing susceptible and/or non-susceptible crop could usefully be investigated in future research projects.

Conclusions and key messages:

Under the assumptions made in the presented DP model we showed that rotation and treatment against sclerotia build up was not only financially justified but also permitted intensive yet sustainable production of susceptible food crops in the long run. Allocation of even a small proportion of cropping decisions to non-susceptible crops, coupled with treatments in the rotation, will mitigate long-term build-up of sclerotia in land, reduce the financial losses and keep the land at a low level of sclerotia infestation. This provides the opportunity of gaining higher benefits by growing susceptible crops in less infested land while avoiding susceptible cropping in highly-infested land or the need for long periods of non-susceptible cropping. However, in the short-run, high proportions and high frequencies of cropping decisions need to be either allocated to non-susceptible crops or treated-susceptible crops in order to avoid accumulation of the disease and to generate profit. In the examined scenarios, rotation gave the greatest financial benefits when sclerotinia pressure was higher, but it was also the best financial strategy for land with low sclerotinia. For oilseed rape, results showed that in the short-term, continuous oilseed rape could be the best cropping choice only if the land infestation with sclerotia is very low. In moderate to high levels of land infestation, the optimum decision is to grow non-susceptible crops. The examples presented show that DP methodology provides a useful framework to explore the trade-offs between long- and short-term gains of crop rotation in relation to plant diseases in arable agriculture that are at the heart of sustainable food production and land use.

Messages:

- Rotation and treatment against sclerotia build up is not only financially justified but also permits intensive yet sustainable production of susceptible food crops in the long-run.
- Inclusion of at least one non-susceptible crop even with a low frequency in the rotation will mitigate the long-term build-up of sclerotia in land, reduce the financial losses and keep the land at a low level of sclerotia infestation.
- Inclusion of non-susceptible crops in the rotation in the long-term (ten years or more) will reduce the level of sclerotinia inoculum in soil and make it possible to grow the crops which provide the most financial benefit, i.e. susceptible crops.
- Inclusion of non-susceptible crops in the rotation in the long-term reduces the land infestation that provides the opportunity of gaining higher financial benefits by growing susceptible crops.
- In a five-year time horizon (short-term), high proportions and high frequencies of cropping decisions need to be either allocated to non-susceptible crops or treated-susceptible crops in order to avoid accumulation of the disease and to generate profit.

- Rotation gave the greatest financial benefits when sclerotinia pressure was higher, but it was also the best financial strategy for land with low sclerotinia.
- For oilseed rape, in the short-term, a low frequency of use of non-susceptible crops is the best decision only if the land infestation with sclerotia is at its minimum (one in four years is recommended). At moderate to high levels of land infestation, the optimum decision is to grow non-susceptible crops more frequently.
- The model indicates that in the long-term, the inclusion of a non-susceptible crop to the rotation of oilseed rape almost doubles the average profitability of a hectare of land of all sclerotinia levels.
- Treatment for sclerotinia, although beneficial to profitability compared to not treating, was not as profitable as the inclusion of non-susceptible crops.

10. Growers' views on approaches to sclerotinia control

Additional author: C Hall, Land Economy and Environment Research Group, SRUC

10.1. Introduction

This part of the link project aimed to uncover growers' views of a range of issues of relevance to the wider project, including the extent to which growers might consider a range of different approaches for sclerotinia control and might be willing to engage in co-operative activity.

Q methodology is a well-established approach for investigating subjectivity (Brown, 1993), where the target population is defined, the issues complex and views likely to be diverse (Barry and Proops, 1999). Combining qualitative and quantitative methodological stages (Webler *et al*., 2009) it provides in-depth understanding of the attitudes of a particular sector of society to a subject of interest to them. Used widely across a range of social science disciplines (see for example Hall, 2008; Webler *et al*., 2003; Sweden, 2006; Wolsink, 2004; Byrd, 2002; Steelman and Maguire, 1999; Walter, 1997; Davies and Hodge, 2007; Robbins, 2000), the output from a Q methodology study is a detailed description of the different positions held by different groups of respondents. One of the key strengths of the approach is the fact that it is self-referent (McKeown and Thomas, 1988), minimising researcher bias and allowing respondents to frame the issue themselves. This latter position is obtained by conducting an initial step within the study whereby comments are collected directly from participants using open-ended questions, comments that are subsequently utilised in a ranking exercise called 'Q sorting' that lies at the heart of Q methodology.

10.2. Materials and methods

A short survey was circulated to growers across England and Scotland who attended workshops and grower meetings during 2010 and 2011. The survey asked growers about different options for

sclerotinia control and included a series of open-ended questions designed to elicit statements of opinion on a range of relevant issues. Forty five responses were received. Details of the survey respondents are shown in table 10-1. All respondents were male, and the majority had been farming for between 21–30 years. Fifty nine percent had taken over the farm from a previous generation of the family and 38% were farm owners. The average farm size was between 201–300 hectares, and only 6% had any land certified organic.

Table 10-1. Survey respondents

Variable	(Valid) Percentage
Sex	
Male	100
Female	0
Years in farming	
11-20	24
21-30	42
31-40	33
Did you take over the farm from previous generation of your family?	
Yes	59
No	41
Will you pass on the farm to the next generation of your family?	
Yes	34
No	44
Don’t know	22
Ownership status	
Farm owner	38
Tenant	11
Both owner and tenant	8
Other	43
Farm size	
<100ha	17
101-200ha	23
201-300ha	27
301-500ha	13
501-900ha	17
1000ha	3

Any organic certified land?	
Yes	6
No	88
Not at present but may do in the future	6

Respondents were farming in 15 UK counties, as listed below.

- Aberdeenshire
- Herefordshire
- Fife
- Angus
- Oxfordshire
- Perth
- Buckinghamshire
- Cumbria
- East Lothian
- East Yorkshire
- Gloucestershire
- Inverness
- Lancashire
- Lincolnshire
- Moray

Growers were presented with a list of eight potential options that could be used for sclerotinia control and asked to indicate whether they would consider using them.

The one option that received a positive response rate of 100% was “treat crops with available fungicides”. The two options that the lowest percentage of growers would be willing to consider were “treat soil with a non-chemical biological control agent” and “co-operate with neighbouring farms to treat soil and reduce disease pressure”. However, more than 80% would consider co-operating with neighbouring farms to spray oilseed rape crops to reduce disease pressure, but this may indicate simply that growers are happy to co-operate so long as this means that they continue to do what they normally do, and no more.

10.3. Results

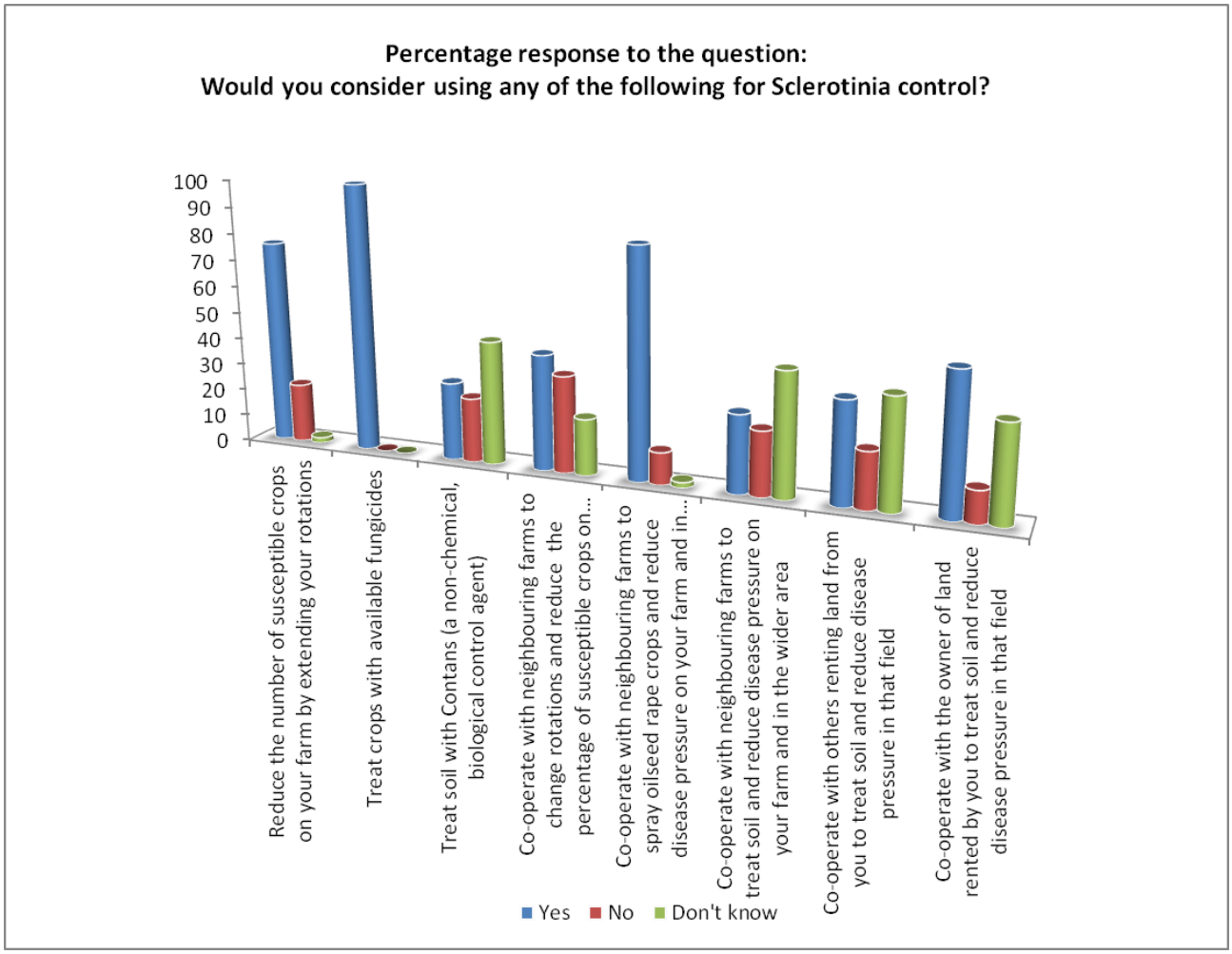

Figure 10–1. Percentage of growers who would consider different sclerotinia control options

The open-ended questions that were asked in the survey are as follows:

- What do you think are the main problems relating to sclerotinia control?
- What influences the decisions you make about how to control sclerotinia?
- What would encourage you to co-operate with neighbouring farms in order to reduce sclerotinia pressure?
- What would discourage you from co-operating with neighbouring farms in order to reduce sclerotinia pressure?

In addition, in order to put responses in a wider context, a further two questions were asked, namely:

- What do you think are the main risks to the future of crop production in the UK?
- What do you think are the main 'positives' for the future of crop production in the UK?

Responses to some of these questions are included in Figures 10–2 to 10–5. In terms of what growers thought were the main problems relating to sclerotinia control, the most popular category

to which answers could be assigned was “rotations” (Figure 3–1). Primarily these statements were about the shortening of rotations, thus growers believed that this was one of the main causes of increased problems of sclerotinia. There were a range of other categories to which answers could be assigned, including (in order of popularity) timing of spraying (i.e. farmers were not getting timing right), the withdrawal of chemical control methods and products, the inability to predict disease occurrence, a lack of knowledge and awareness of the disease, a range of practical problems, and the weather.

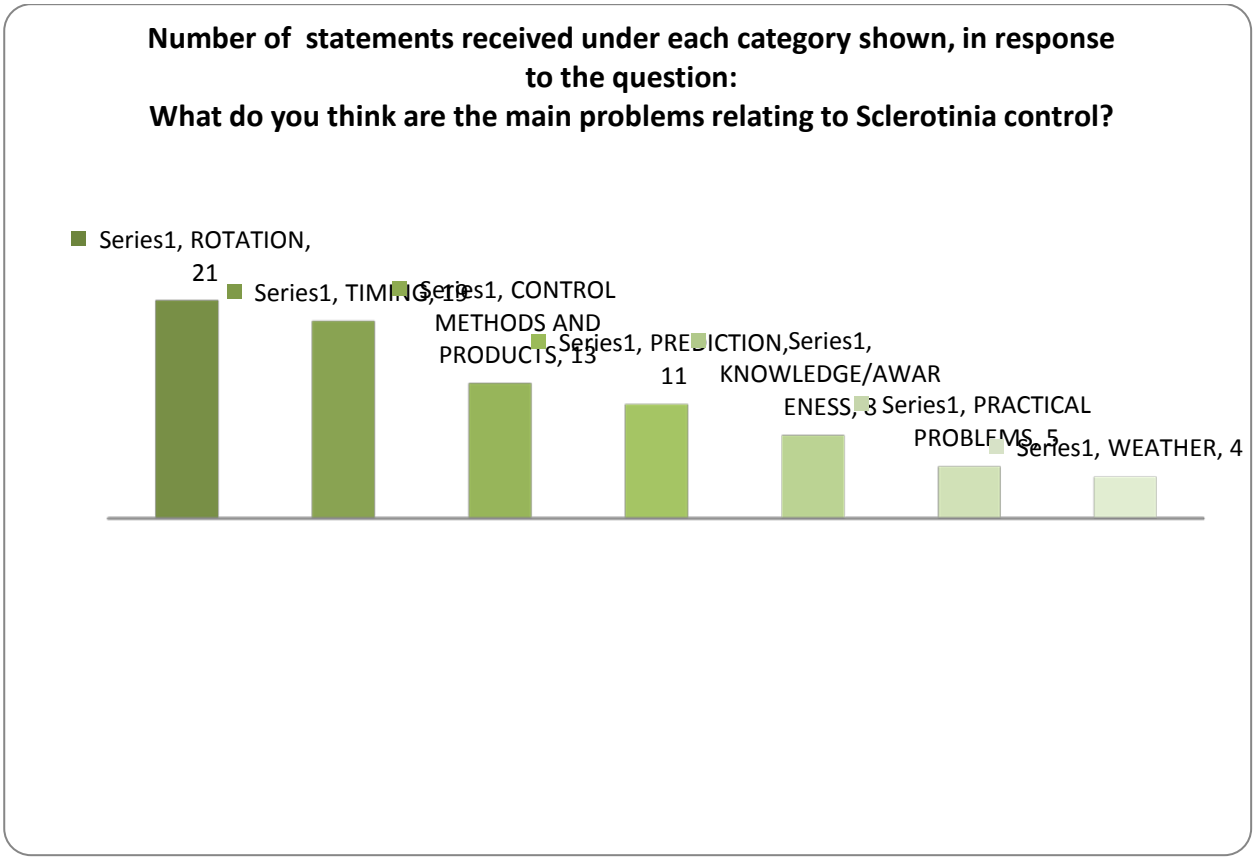

Figure 10–2. Main problems relating to sclerotinia control

Farmers were asked what influenced their decisions about how to control sclerotinia. Most common responses related to the weather and the stage of the rotation (Figure 10–3). Other responses indicated that perceived or predicted disease risk was a factor. In addition, issues such as available finances, advice received, crop growth stage and availability and efficacy of control products were also influencing factors.

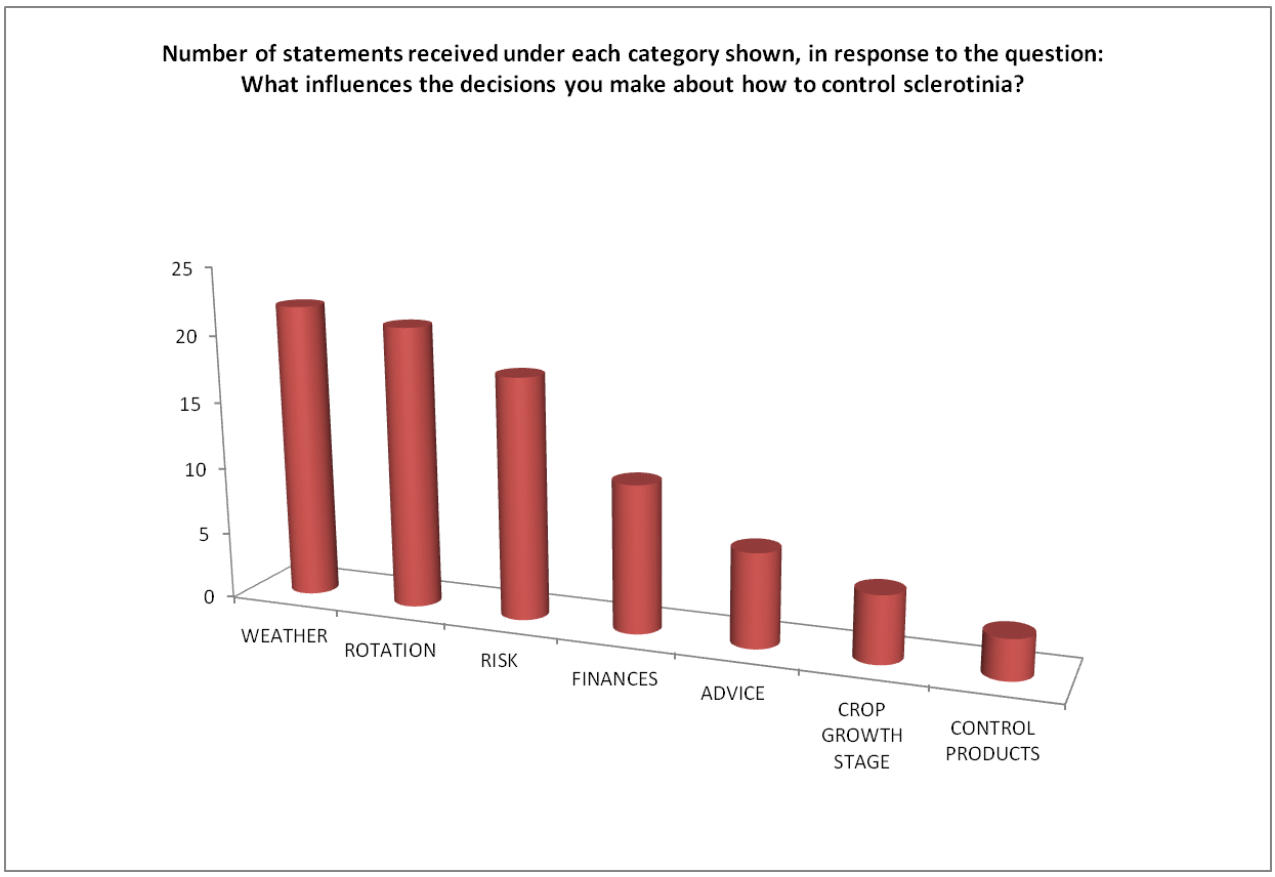

Figure 10–3. Influences on decisions about sclerotinia control

There were a number of important issues that respondents indicated would encourage them to co-operate with neighbouring farms in order to reduce sclerotinia pressure. Evidence of benefit was important, as was information and financial support (Figure 10–4). However, many growers indicated that it would be important to know more about the nature of the programme before committing to co-operation. Thus there is scope for facilitating co-operation but farmers would want a lot of information about what that would entail before being willing to sign up.

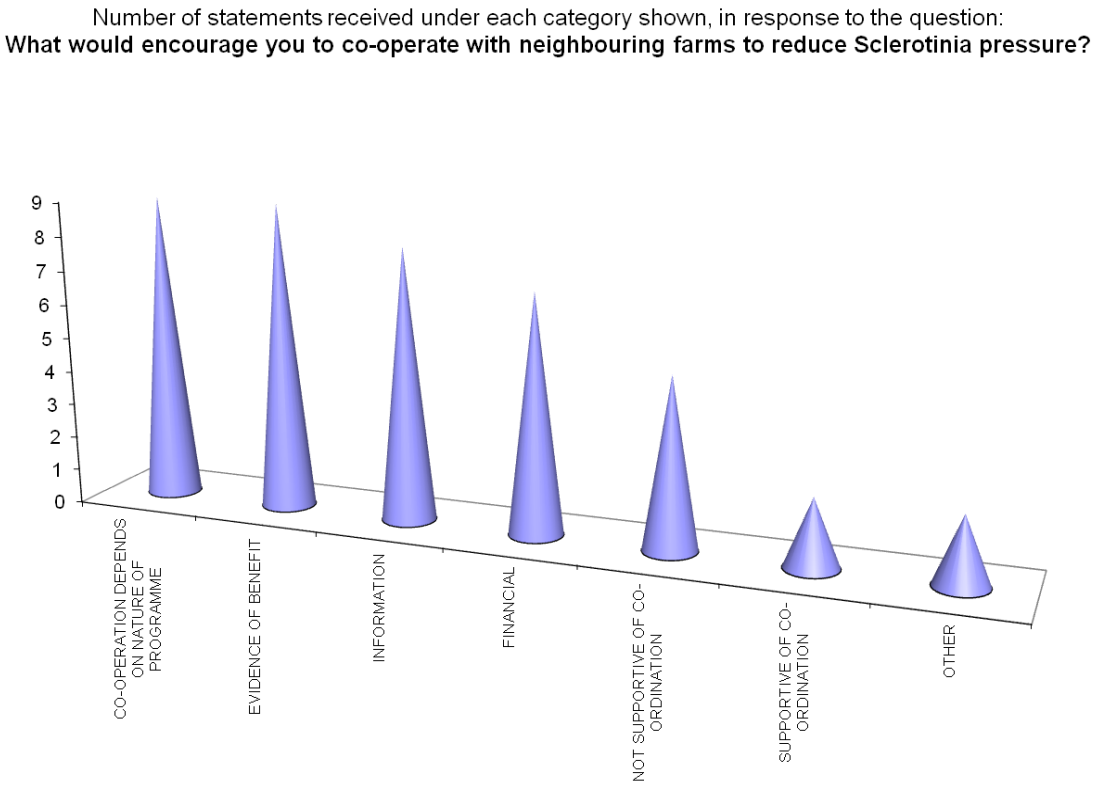

Figure 10–4. What would encourage co-operation with neighbouring farms?

The growers were also asked what would discourage them from co-operating. The category to which the largest number of responses could be assigned was ‘people’ (Figure 10–5). The farmers had a range of comments about the challenges of working with different personalities. This suggests that the issue of trust between different individuals is expected to be a problem. However, there were also thought to be many problematic practicalities that would discourage co-operation, including the challenge of co-ordinating action throughout the growth cycle, and the need to have an overall co-ordinator and verifier. Finances were also thought to be a barrier to co-operation.

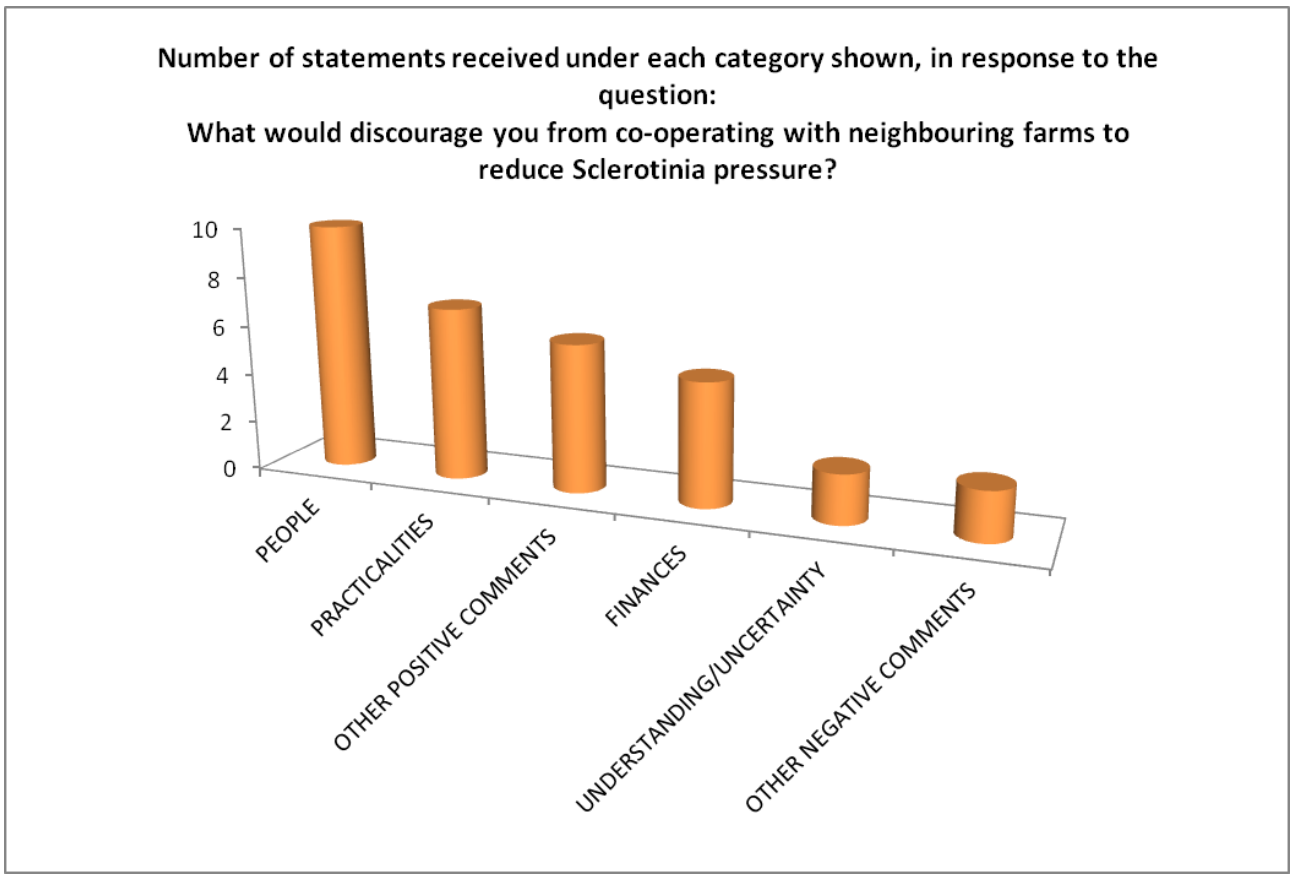

Figure 10–5. What would discourage co-operation with neighbouring farms?

10.4. Discussion

Overall, the responses to this survey raise a wide range of important points in relation to the control of sclerotinia, the actions that farmers might be willing to undertake and the potential barriers and motivations that exist. In order to probe these issues in more depth, the qualitative data from this first stage survey could be used in a second stage of research in an exploratory study with a small number of farmers.

The survey revealed that growers had a good awareness of why they had problems with sclerotinia, with short rotations identified most commonly as a factor. Modifying rotations was a control option they would consider but their preference was for control with fungicides. It would be interesting to further probe these perceptions as information about the long term financial benefits of better rotations arising for the dynamic programming undertaken as part of this project might provide them with information that would change this preference.

Key messages

- Growers rated problems with rotation length as the number one reason for problems with sclerotinia

- Growers said that conducive weather was their number one reason for applying treatment, closely followed by growing in a tight rotation as a reason.
- In terms of managing sclerotinia, growers were most comfortable with the use of fungicides as a control strategy, followed by extending rotation lengths. Although generally supportive of the idea of cooperating with others, they were less comfortable with this than with the first two.
- Grower concerns about cooperation were focused on issues with people but lack of information about benefits was a factor in these reservations.

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APPENDICES

Appendix 1, Oilseed rape yields in field experiments 2010-2012

Oilseed rape sites 2010-2012, yields

		2010 BASF Rose-maund Herefords.	2010 BASF South-minster Essex	2010 ADAS High Mow. Yorks	2010 Velcourt Thanet, Kent Kent	2011 BASF Rose-maund Herefords.	2011 BASF Boxworth Cambs	2011 ADAS High Mow. Yorks	2011 Velcourt Hawarden Kent	2011 Velcourt Haver-holme Lincs	2012 BASF Rose-maund Herefords.	2012 BASF Terr'ton Norfolk	2012 ADAS High Mow. Yorks.	2012 Velcourt Martin Lodge Kent	2012 Velcourt Haver-holme Lincs
Yield, t/ha	**1. Untreated**	4.68	4.82	3.28	5.52	4.69	2.97	4.63	3.68	5.60	2.71	3.74	2.97	4.40	3.34
	2. Yellow bud[1]	5.27	4.87	3.56	*	4.99	3.13	4.40	*	*	3.24	3.96	2.97	*	*
	3. Early-flower	4.91	4.74	3.51	5.30	4.97	2.85	4.26	3.77	5.57	3.26	3.99	3.01	4.52	3.65
	4. Mid-flower	4.52	4.93	3.53	5.44	4.99	2.90	4.67	3.83	5.84	3.13	4.03	3.11	4.58	3.44
	5. Late-flower	4.85	5.08	3.70	*	4.92	2.97	4.68	3.73	5.84	3.46	4.04	3.06	4.8	3.72
	6. Mid- + Late-flower	4.95	5.12	3.67	*	5.05	2.69	4.57	*	*	3.65	4.10	3.15	4.73	3.61
	7. Early- + Mid-flower	4.94	4.72	3.54	5.90	5.04	2.97	4.55	3.8	5.84	3.31	4.15	3.09	4.68	3.70
	8. Early- + Late-flower	4.88	4.99	3.68	*	4.93	3.12	4.97	3.88	5.79	3.67	4.16	3.16	4.53	3.91
	9. Yellow bud + Mid-flower	5.59	4.94	3.56		4.98	2.95	4.59	*	*	3.42	3.96	3.08	*	*
	10. Yellow bud + Mid + Late	5.28	5.06	3.56	5.95	4.99	3.11	4.51	3.76	5.97	3.55	4.16	3.17	4.84	3.86
	Germ model or equiv, yield	4.12	4.82	3.62	5.44	4.97	3.04	4.70	3.83	5.84	*	3.96	2.70	4.52	3.65
	SkleroPro or equiv, yield	4.62	4.93	3.77	5.30	4.98	3.15	4.65	3.83	5.84	3.65	3.96	2.93	4.52	3.65

[1] Tectura 1 L/ha at each treatment time

Appendix 2. DNA extraction procedure for air samples

A single scoop of ballotini beads (0.5 g x 400-455µm diameter) was added to each 1.5 ml screw cap tubes containing a spore trap tape section, and, in a fume cupboard, 440µl of extraction buffer added using a new pipette tip for each sample (Buffer formula- 2XTEN [500mM NaCl, 400mM Tris-HCl, 50mM EDTA, pH8]; 0.95% SDS; 2% polyvinylpyrrolidone; 5mM 1,10-phenanthroline monohydrate). This was made up into a master mix and then 0.1%β-mercaptoethanol added just before use. Tubes were then placed into a FastPrep machine and processed 3 times at 6.0m/s, 40sec, with 2 minutes cooling on ice between cycles. Using a new tip each time, 400µl 2% SDS (sodium dodecyl sulphate) was added and mixed by inversion and a brief shake. These were incubated at 65°C in a water bath for 30mins. In a fume cupboard, 800µl of the bottom phase of phenol:chloroform (1:1) was added to each tube and vortexed briefly. This was then centrifuged at 13,000rpm for 10mins using a refrigerated centrifuge (4°C). An additional set of pre-autoclaved 1.5ml flip-top Eppendorf tubes was prepared with 30µl of 7.5M ammonium acetate + 480µl of isopropanol (both of which kept at -20°C). In a fume cupboard, the supernatant was pipetted from the original tubes into the new tubes using a new tip each time, leaving the beads and any solid residue in the tube. After gentle mixing, this was stored at -20°C overnight. The following day, the tubes were centrifuged at 13,000rpm for 30mins, again at 4°C, noting the orientation of the tubes in the centrifuge (a pen mark was made uppermost) as the DNA pellet was not always visible. In a fume cupboard, the supernatant was poured off carefully, leaving the DNA pellet which was washed with 200µl of 70% ethanol (kept at -20°C), centrifuge (pen mark uppermost) at 13,000rmp for 15mins. The ethanol was carefully removed using a new pipette tip each time and the DNA pellet left to dry in a sterile flow cabinet (approx 1 hour). The pellet was resuspended in 100µl sterile deionised water (30µl used in 2012). Tubes were placed in a water bath at 65°C for 5mins and then tapped or shaken a little to aid DNA resuspension. DNA suspensions were stored at -20°C.

Appendix 3. DNA extraction procedure for petals and leaves

A petal or leaf disc was placed into a 0.2ml PCR tube and heated for 5mins at 95ºC. Then 40µl of MicroLYSIS Plus (Microzone) was added and the following cycle run twice with vortex/spin between (Cycle: Step 1: 65ºC for 15mins, Step 2: 96ºC for 2mins, Step 3: 65ºC for 4mins, Step 4: 96ºC for 1mins, Step 5: 65ºC for 1mins, Step 6: 96ºC for 30secs, Step 7: 20ºC hold). To each tube was added 2mg PVPP (Polyvinylpyrrolidone- Sigma Cat No. P-6755) and 40µl of TE buffer (pH8.0), and tubes were vortexed then spun at 13,000rpm for 15mins. The supernatant was removed to a new 0.2ml tube, 2.5x ethanol and 10µl 7.5M ammonium acetate added and vortexed. Tubes were spun at 13,000rpm for 15mins, the supernatant discarded, the pellet allowed to air dry in a sterile flow cabinet and then re-suspended in 10µl water.

Appendix 4. qPCR Method

The qPCR assay was performed using 4µl of sample DNA in a total reaction volume of 20µl. The forward and reverse primers and the Taqman probe used in this qPCR were the same as described in Calderon et al (submitted). The reaction mix contained 10µl of 2 x FastStart universal probe ROX Master mix (Roche Diagnostics), 2µM Taqman probe, 3.75µM forward primer and 1.25µM reverse primer. The ratio of forward to reverse primer was optimised to account for their variable binding specificities. The amplification conditions consisted of an initial denaturation step at 95ºC for 10mins followed by 40 cycles at 95ºC for 15secs, 56ºC for 45 secs and 72ºC for 45 secs. The samples were tested in duplicate. Six serial log10 dilutions of purified *S. sclerotiorum* DNA ranging from 20ng to 2 x 10-4ng per reaction were used to prepare a standard curve. The quantity of fungal DNA present in each reaction was calculated from the standard curve.

In order to determine the amount of *S. sclerotiorum* DNA per ascospore the qPCR assay was performed on known concentrations of ascospores.

Appendix 5. Transition probability matrices, susceptible & non-susceptible crops

Probability of Next States given Current States for susceptible crops (i.e. carrots, winter oilseed rape, spring oilseed rape, green beans, vining peas, potatoes and lettuce)

State No.	Land States		Next State																						
	G[a]	S[b]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1	1	0.10	0.50	0.30	0.10																					
2	2	1	0.10	0.50	0.30	0.10																					
3	3	1	0.10	0.50	0.30	0.10																					
4	4	1	0.10	0.50	0.30	0.10																					
5	5	1	0.10	0.50	0.30	0.10																					
6	1	2	0.10	0.50	0.30	0.10																					
7	2	2		0.10	0.50	0.30	0.10																				
8	3	2			0.10	0.50	0.30	0.10																			
9	4	2				0.10	0.50	0.30	0.10																		
10	5	2				0.10	0.50	0.30	0.10																		
11	1	3					0.10	0.50	0.30	0.10																	
12	2	3						0.10	0.50	0.30	0.10																
13	3	3							0.10	0.50	0.30	0.10															
14	4	3								0.10	0.50	0.30	0.10														
15	5	3								0.10	0.50	0.30	0.10														
16	1	4										0.10	0.50	0.30	0.10												
17	2	4											0.10	0.50	0.30	0.10											
18	3	4												0.10	0.50	0.30	0.10										
19	4	4													0.10	0.50	0.30	0.10									
20	5	4													0.10	0.50	0.30	0.10									
21	1	5															0.10	0.50	0.30	0.10							
22	2	5																0.10	0.50	0.30	0.10						
23	3	5																	0.10	0.50	0.30	0.10					
24	4	5																		0.10	0.50	0.30	0.10				
25	5	5																		0.10	0.50	0.30	0.10				

[a]G: represents the time (number of years) elapsed since the last grain crop.
[b]S: represents the sclerotinia state

Probability of Next States given Current States for non-susceptible crops (i.e. winter wheat, spring wheat, winter barley and spring barley).

State No.	Land States		Next State																						
	G[a]	S[b]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1	1	0.20					0.80																			
2	2	1		0.20					0.80																		
3	3	1			0.20					0.80																	
4	4	1				0.20					0.80																
5	5	1					0.20					0.80															
6	1	2						0.20					0.80														
7	2	2							0.20					0.80													
8	3	2								0.20					0.80												
9	4	2									0.20					0.80											
10	5	2										0.20					0.80										
11	1	3											0.20					0.80									
12	2	3												0.20					0.80								
13	3	3													0.20					0.80							
14	4	3														0.20					0.80						
15	5	3															0.20					0.80					
16	1	4																0.20					0.80				
17	2	4																	0.20					0.80			
18	3	4																		0.20					0.80		
19	4	4																			0.20					0.80	
20	5	4																				0.20					0.80
21	1	5																					1.00				
22	2	5																						1.00			
23	3	5																							1.00		
24	4	5																								1.00	
25	5	5																									1.00

[a]G: represents the time (number of years) elapsed since the last grain crop.
[b]S: represents sclerotinia.