Modelling of Likely Sclerotinia Disease Incidence in Oilseed rape Fields in the United Kingdom

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Modelling of Likely *Sclerotinia* Disease Incidence in Oilseed rape Fields in the United Kingdom

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ABSTRACT

Experiments to measure and quantify airborne spores of *Sclerotinia sclerotiorum* in oilseed rape fields was undertaken in a specially sown field at Rothamsted Research (Hertfordshire, UK). Airborne ascospore concentrations were estimated by quantifying the DNA of *Sclerotinia sclerotiorum* present on daily samples (waxed tapes) retrieved from Hirst-type spore trapping devices. The corresponding weather data was taken from a climate station in the field and another 1 kilometre away. This data was used to test an existing model of disease incidence and can be used to inform growers of the right time to spray their crops.

Inoculum, sclerotia of *Sclerotinia sclerotiorum* were deliberately planted in a ring at the centre of the field-site the previous autumn. In the early spring, the trapping devices were placed in the centre of the ring and operated continuously to provide a record of spore presence. Air samples were sent to a laboratory in Rothamsted Research for DNA extraction and qPCR measurement. In parallel, the measured weather data and also information about the Rothamsted field was used to predict the likelihood of spore release according to the "Raiso-Sclero" model.

The qPCR readings of spore presence were successfully recorded and inputted into the various models. The Raiso-Sclero model was successful at identifying the important periods of spore release however it provides a substantial number of false positives. The model gives growers valuable information on when disease incidence is likely to occur and can lead to more efficient crop spraying regimes.

Keywords: Crop protection, Sclerotinia, Oilseed rape, United Kingdom, Modeling, Forecasting, Raiso-Sclero.

1. INTRODUCTION

There is increasing pressure on global food supply as arable land reaches its peak (UNFAO, 2009) meaning that better use must be made of the existing land base if food production is to be increased. If global population trends continue there will be 9 billion people on the planet by 2050 (UNDPI, 2007), thus failure to address this increasing pressure could have catastrophic consequences. Thus options for increasing the production efficiency of the existing land resources must be assessed as over 40% of crops are lost at pre-harvest stage (IAPPS, 2011). An obvious candidate for eliminating inefficiencies is to mitigate crop disease as losses due to disease are close to 20% of total losses (Oerke, 2006).

Crop protection chemicals are preventative not curative; hence the ingress of disease must be anticipated correctly otherwise a treatment will be ineffective (Chaube & Pundhir, 2005). Furthermore the practicalities of applying crop protection chemical mean disease ingress must be anticipated a few days in advance as equipment, labour and chemicals may not be immediately available to the grower.

There is a strong economic case for the protection of Oilseed rape crops due to their high value of approximately €450 per tonne (Farming Online, 2012) as a grower could suffer substantial financial losses due to a disease outbreak. Sclerotinia in particular is a grave threat to oilseed rape crops as its sclerotia can survive in the soil for many years and can release ascospores when the right local conditions are in place. Fortunately the local conditions that lead to ascospore release are measurable and can thus be formed into a model if sufficient calibration and validation data is collected.

Extensive experimental work was performed in France by Syngenta and CETIOM (Varraillon, 2011) to construct a predictive model called “Raiso-Sclero”. This model can predict ascospore release and infected petals. The model was validated by petal kit tests developed by CETIOM (Penaud, 2009). This model was first tried on data from United Kingdom oilseed rape fields in 2011 by Jackman et al. (2012) and there was some good agreement between the days that Raiso-Sclero predicted large numbers of spores would be released and the days that large numbers of spores were measured by DNA extraction.

Hence further experimentation took place to validate whether the model based on French calibration and validation data could be applied to British oilseed rape fields. Further close agreement on the days when large numbers of spores were measured and predicted would be a strong endorsement of the models utility in both the United Kingdom and beyond.

2. MATERIALS AND METHODS

2.1. Experimental Design

To maximise the likelihood that spores would be released and captured during the late spring Sclerotinia sclerotia were deliberately placed in a ring around the area where the spore trapping devices were placed the previous winter. Hence if the correct local

conditions occurred during the spring spores would be released and also irrespective of the wind direction spores would be blown into the path of the spore trapping devices thus ensuring that spore detection took place if spores were released as the spore trapping devices operated continuously.

To collect the site data a met station recorded data every 20 seconds (and logged 30 minute averages) (Vantage Pro2, Davis Instruments, Hayward, CA, USA.) was installed in the area inside the ring of planted sclerotia with two standard Burkard air sampling devices (Rickmansworth, Hertfordshire, United Kingdom, seven-day spore traps, operated from 12v batteries and changed weekly). These were a few metres from the centre of the ring. A platform was used to elevate the traps to maintain them at flower-canopy height throughout the field trial which ensured standard procedures were followed (Lacey & West, 2006).

2.2. Spore DNA Measurement

The airborne particles which include the spores of interest and other material such as pollen were trapped onto waxed tapes which were first cut axially in half to create an A-sample and a B-sample and then divided up into 24 hour sections. Each of the 24 hour sections was put into a small screw cap tube (2ml). Each tube was cold stored at -20°C before processing. Samples collection was for a 61 day period from the 8th of April to the 7th of June.

Each sample was first bead heated with ballotini beads (0.5mg & 400micron beads) in an SDS-based buffer. The resulting DNA was captured by centrifugation, ethanol washed, dried and then re-suspended into 100 micro litres of water. The Sclerotinia sclerotium DNA was quantified by a Taqman method whose specificity was confirmed by tests against more than 20 Sclerotinia sclerotium isolates and also against over 20 other species such as Brassica napus and Botrytis cinerea.

2.3. Raiso-Sclero, ascospore release prediction

Raiso-sclero predictions are based on daily minimum and maximum temperatures, precipitation and humidity data. This data allows soil microclimate to be estimated based on the soil texture which will be known from the soilmap of the United Kingdom. Important parameters such as soil water potential can thus be predicted with a modified Green-Ampt model and soil temperature can be predicted using the STICS model (Brisson et al., 1998). The Clarkson (2004) model of fungus cycle was used with some assumptions about soil water potential (>= -100kPa) and soil temperature (15-20°C). The ripening of the apothecia was modeled at a soil horizon of 5 centimeters and they were assumed to live for 8 to 20 days. The actual release of the ascospores by the apothecia was predicted to occur upon a sudden drop in local humidity (Clarkson et al., 2003). These humidity changes go in tandem with local turbulence which spreads the ascospores around the canopy. Hence a relative value can be computed for the daily local intensity of spores. The model settings that were based on the outcome of the French tests were retained.

3. RESULTS AND DISCUSSION

The amount of measured spore DNA was considered significant if more than 0.01ng of DNA was extracted from the samples. The results for the crop flowering season are shown in Figure 1 for both of the spore trapping devices over the 61 day period. The vertical black bars represent a large Raiso-Sclero prediction (0.3 or greater) and the coloured area represents a substantial detection of spore DNA.

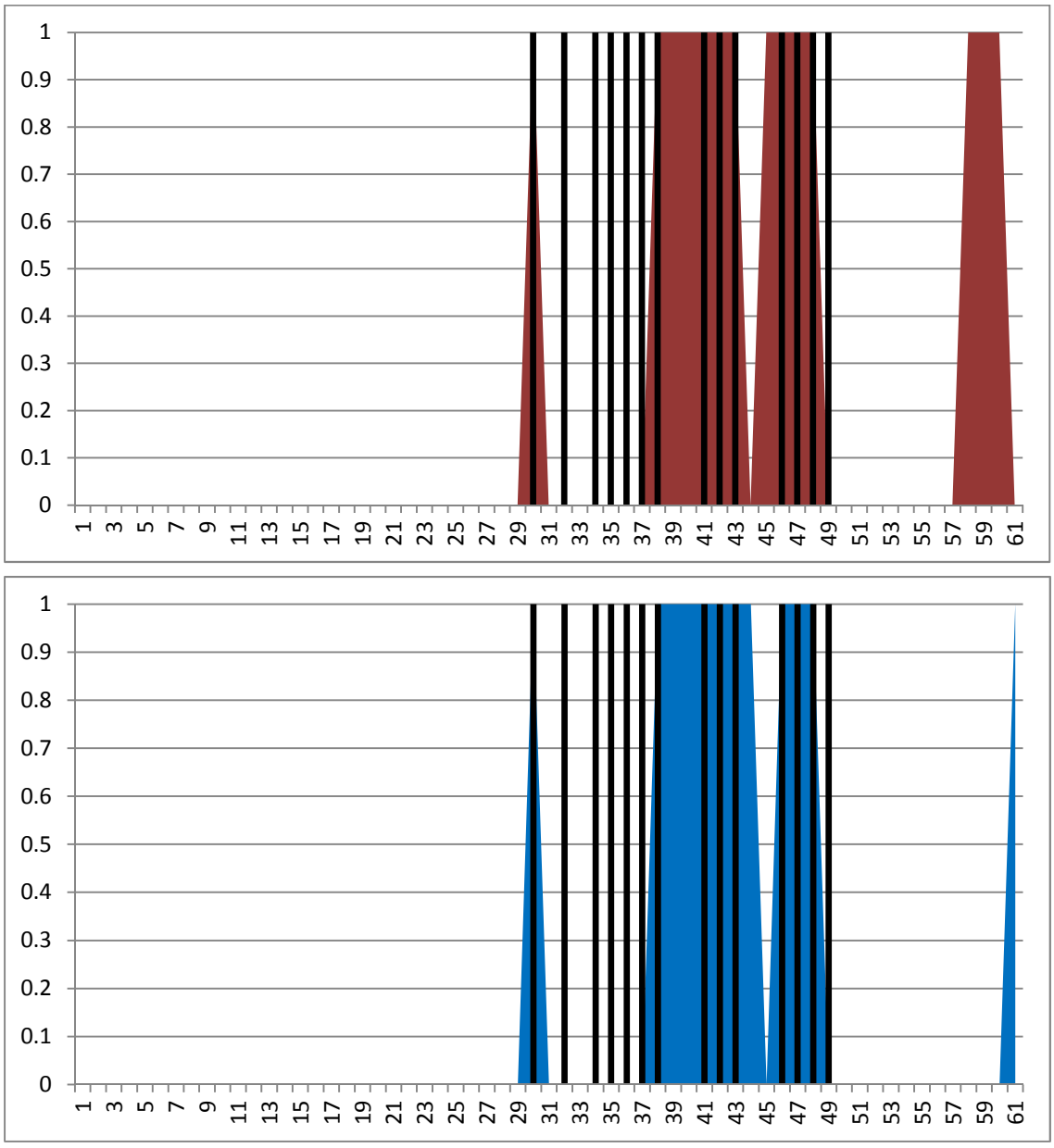

Figure 1. Comparison of the days when substantial spore release was predicted and measured: Black bars = predicted by Raiso-Sclero, Red area = measured by sensor A, Blue area = measured by sensor B.

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Observation of Figure 1 shows that the Raiso-Sclero model was successful at identifying the first spore release on day 30 (the 7th of May) which is a vitally important date as crop chemical is normally effective for 14 days and thus subsequent false negatives within 14 days would not lead to infection.

The model was also successful at identifying the period of longest release between days 38 and 48 (the 15th of May to the 25th of May) although there were some false negatives and false positives during this period. As the false positives were during a long period of spore release they would not have led to unnecessary spraying. Similarly as the false negatives were during a long period of positive predictions and were never more than two consecutive days it is unlikely that the application of crop protection was critically delayed.

The model failed to predict the spore release at the very end of the 61 day season however at this late point the prospect of disease infection is not as important as petal fall will normally have been completed by this stage of the year meaning large-scale infection would not take hold as petal stick is no longer possible.

The model was largely successful and this was using model settings fine tuned in another country. If the model was recalibrated with more United Kingdom data its predictive power could be improved leading to fewer false positives and false negatives and thus even stronger confidence in its application. This would require considerable experimental work using collection methods such as the French “petal kits” (Varraillon et al., 2011) aswell as the DNA extraction techniques employed at Rothamsted Research. British oilseed rape fields are well suited to predictive models of this type as pre-flowering infection is not likely due to the cold climate although post flowering infection of the type described by Clarkson et al. (2007) for lettuce cannot be ruled out.

4. FUTURE WORK

A good indication of the risk of airborne inoculum of *S. sclerotiorum* can be determined from this predictive model although some model predictions were not always correct which closely matches the overall results from the 2011 experiment (Jackman et al., 2012). The results from further experimentation that took place this year will help confirm this, as will repeat experiments in future years.

Future experimentation on how far spores travel from the crop canopy would be useful. This could be achieved by measuring spores at a variety of heights and distances from the spore source and then examine the subsequent patterns of disease in the surrounding area. Models of infection such as that of Koch et al. (2007) can then be measured up against the observed infection patterns.

The Raiso-Sclero model is currently being extended from accommodating three soil types (predominantly clay, predominantly sand and predominantly loam) to thirteen soil types. Additional validation data on soil moisture has been recorded throughout France to also improve the performance of the model.

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5. CONCLUSIONS

The French Raiso-Sclero model of *Sclerotinia* spore release has once again proven to be a mostly reliable estimator of the release of large numbers of ascospores in the crop canopy air. There were relatively few false negatives and when they did occur they would not have led to any major delay in the application of crop protection chemicals. Some false positives did occur during an extended period of spore release but again this would not have led to any major misapplication of disease mitigation.

It is reasonable to postulate that with the collection of further experimental data in the UK the model can be fine-tuned for typical UK environmental conditions and that model predictions should improve. Similarly additional data on observed crop infection will allow an overall damage model to be constructed that will permit a robust economic analysis of the decision to spray or not that can support or supersede existing infection models.

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