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Collaborative Project
SEVENTH FRAMEWORK PROGRAMME

D11.6
Quantified relationship between reflectance, numbers of spores in air and disease severity

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Concerned workpackage leader: Marleen Riemens

Organisation name of lead contractor: RRES

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PP Restricted to other programme participants (including the Commission Services)	
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CO Confidential, only for members of the consortium (including the Commission Services)	

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1. Summary

In support of the "Emerging Technologies" workpackage (WP11) of the PURE project, a new air sampler has been developed based on the principle of virtual impaction. The sampler operates at an air-flow rate of 20 L/minute and provides an efficient way to sample air for biological particles such as spores of plant pathogens. Air is directed into a chamber where it changes direction towards a suction pump, causing airborne particles to separate from the airflow due to their momentum and fall under gravity to settle passively into a collection vial. Spores, pollen and other airborne particles are deposited either as a dry deposit, or into a liquid. Most other devices that sample into liquids suffer from high evaporation rate which means that the collection liquid has to be replenished or the sampling period restricted to relatively short periods. In contrast, the Miniature Virtual Impactor (MVI) can sample for 24 hours with minimal evaporation, which means spores can be deposited into culture medium to test for viable organisms, or directly into DNA extraction buffer. Viability of collected spores is also unaffected by impaction, which is thought to damage or kill a proportion of spores collected using other methods. The MVI was designed to be compatible with a wide range of downstream diagnostic methods such as microscopy, DNA-based detection, immunological or chemical detection by biosensors, lateral flow devices, dip-sticks or by optical characteristics. As part of other related IPM and precision farming-related projects, the device has been tested at RRES for efficiency of collection of spores of *Sclerotinia sclerotiorum*, which is an important but sporadic pathogen of crops such as oilseed rape, sunflower, soya, peas, carrots and lettuce. Results compared very well with detection of *Sclerotinia* spores sampled simultaneously at the same field-site using traditional Hirst-type air samplers, from which DNA was extracted and spores quantified by qPCR. The MVI is an essential component of an automated air sampling device as it allows airborne particles to be collected into liquid in vials that can be moved along an enclosed track for downstream processing. In collaboration with Burkard Manufacturing Co ltd the new automated air sampler was developed further after the positive first field trials. Five automatic units capable of detecting spores of *Sclerotinia sclerotiorum* using a biosensor were factory tested and although one developed a fault, four were sent to Canada for testing as it was too late in the spring for UK *Sclerotinia* spores to be a problem. The automatic units worked well and gave positive results during a period when conventional spore traps, operated alongside the automatic units, when tested by qPCR at Rothamsted, also tested positive. In a few cases, false negatives occurred, which we think is due to about 5% of biosensor cards being faulty.
Burkard and RRES have adapted the device for DNA-based detection using LAMP assays. LAMP stands for Loop-mediated Isothermal Amplification. LAMP assays facilitate fast DNA-based detection of plant pathogens on site. It is a nucleic acid amplification method. Amplification and the detection of a gene can be accomplished in a single step at a constant temperature (about 65°C). It is highly specific and can therefore quickly identify the presence of a target gene. Further work is planned in new projects that will develop DNA-based detection of spores of target species as reported in the final PURE conference in Poznan in January 2015.

In addition to air sampling developments, novel methods to detect disease in fields have been investigated at RRES and DLO. These methods were under investigation as they have potential not only for phenotyping diseases in breeder nursery plots but also for directing spray equipment to improve disease control in fields.
At RRES and DLO wheat experiments were established in October 2012 and 2013 with several different varieties (with different resistances to yellow rust) and of which half received a fungicide spray regime to generate plots with different disease profiles. These were used in spring 2013 and 2014 for

spectral canopy reflectance images of wheat plots. Results were related to crop growth stage and amounts of yellow rust infection recorded manually. The recorded data were analysed to identify regions within the spectra that are discriminative between healthy and infested wheat plants by yellow rust. Wavebands for identification of yellow rust symptoms were identified manually and results from DLO and RRES were similar. The wavebands identified were as follows: 520 nm was neutral (unaffected by disease and could be used for normalization), 620-630nm was enhanced by yellow rust, 650-675nm was also enhanced by yellow rust, 750, 775 and 860nm were reduced by yellow rust. Using rotating-arm air samplers in and 50cm above the crop canopy, it was found that the concentration of yellow rust spores 50 cm above the canopy declined by dilution to between 1/10th and 1/20th of the amount in the air within the crop canopy when sampled near to a sporulating focus, Whereas the concentration within the canopy was lower than at 50cm above the canopy, when the sampling position was at least 5 m from a visible focus that was upwind. A publication is planned to report these findings.

We conclude that it is feasible to use reflectance measurements to identify yellow rust infected wheat. The identification of wavelengths that are distinctive to detect yellow rust in winter wheat with reflectance measurements was possible at both DLO and RRES. However, the correct identification of the disease severity was still rather low. Therefore, we do not recommend to use this technique to quantify the disease severity with reflectance measurements at this moment. Further work is needed to enhance the specificity of the technique and capability to detect early infection stages.
The relationship between airborne spores and reflectance of the crop, was successfully quantified in the same experiment at RRES. The highest number of spores was collected from the air above the spots with the wavebands ranging from 620-630nm and 650-675 nm (highest infection profile in winter wheat), an intermediate number of spores from air collected further away from intermediate infected areas and the lowest number of spores from air above winter wheat with wavebands in the range of 520nm. The MVI air sampler was built and tested in a wind-tunnel. It has been patented and is able to sample air for viable *Sclerotinia* spores, incubate them and make a detection using a biosensor, with the result sent by SMS text along with hourly weather data. The device is being adapted for DNA-based detection using LAMP assays.

2. Objectives

Through air sampling and optical sensing analysis, the amount and type of pathogens present can be determined. With reflectance measurements in the field, deviations from a healthy crop can be identified. Potentially large geographical regions and relatively small positions (<1 m2) in crop fields can be identified where special attention at specific moments is required. These spatial data will allow improving decision making algorithms and forecasting models. Accordingly, task 1 of the 'Emerging Technologies' workpackage of the PURE project aimed at developing optical sensing and airborne inoculum sampling methods for macro scale mapping and monitoring of crops to identify unhealthy regions in cropped fields. This deliverable reports on the possibilities to quantify the relationship between the reflectance, number of spores in air with disease severity, which is part of the task 11.1 on airborne sampling and optical sensing methods for macro scale mapping.

3. Approach

To quantify the relationship between reflectance measurements and the disease severity, reflectance measurements in winter wheat were performed by DLO in the Netherlands. Fields with yellow rust resistant cultivars and sensitive cultivars were sown. Four stress levels were created through mixing the two cultivars: resistant winter wheat, mix 1:2 (resistant: susceptible), mix 2:1 (resistant: susceptible), and susceptible.
The measurements were carried out with two identical spectrometers (Q-wave – RGB Lasersystems GmbH), with a spectral range from 380 till 950 nm and a resolution of 0.5 nm. One of the spectrometers was used to measure the crop reflection (measure spectrometer in Figure 1) and the other to measure the solar radiation (reference spectrometer in Figure 1). On four days, spectra were recorded in the range from 350-900 nm reflection wavelengths. The recorded data were analysed to identify regions within the spectra that are discriminative between healthy and infested wheat plants by yellow rust. Correct classification of the infested plots based on the spectra used was 50%.

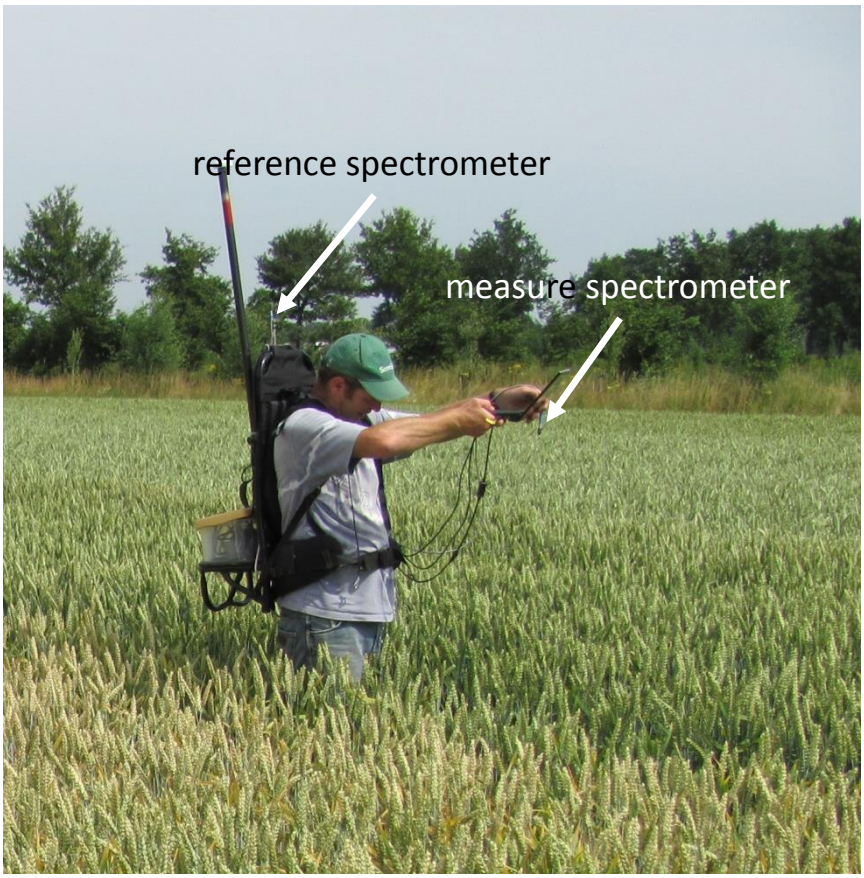

Figure 1. Spectrometers used to measure crop reflectance and solar radiation.

At the same time, field experiments with high levels of yellow rust and *Septoria* leaf blotch were performed in the UK at RRES. Three different varieties with different susceptibilities to *Puccinia striiformis* and *Mycosphaerela graminicola* were used both untreated and with a two-spray fungicide program.
The fungicide program successfully controlled both diseases although traces of yellow rust returned towards the end of the season due to spread from the untreated plots. A B & W Tek Exemplar smart CCD spectrometer was used to measure leaf reflectance under different severities of yellow rust from 350-1050nm, using a 25µm inlet slit and a spectral resolution of 1.5nm. Wavebands for identification of yellow rust symptoms were identified manually that are similar to findings at DLO. The wavebands identified were as follows: 520 nm was neutral (unaffected by disease and could be used for normalization), 620-630nm was enhanced by yellow rust, 650-675nm was also enhanced by yellow rust, 750, 775 and 860nm were reduced by yellow rust.
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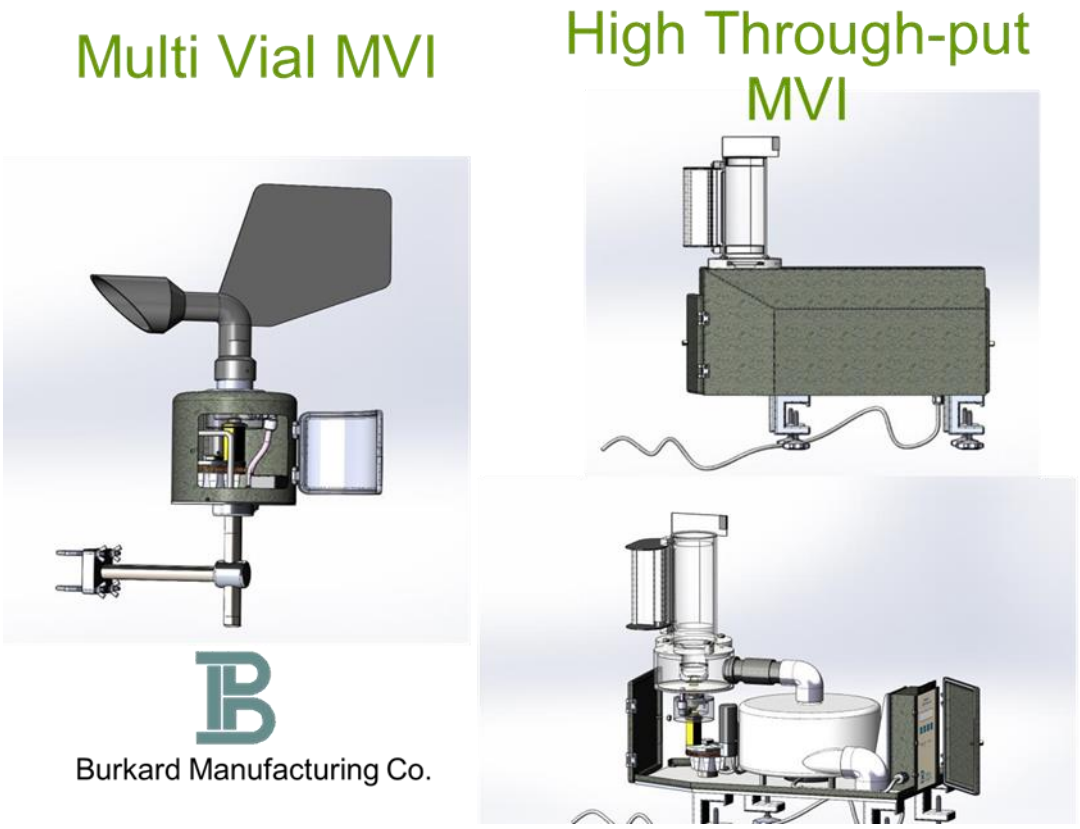

Figure 2. Multi Vial MVI and High Through-put MVI.

4. Conclusions

We conclude that it is feasible to use reflectance measurements to identify yellow rust infected wheat. The identification of wavelengths that are distinctive to detect yellow rust in winter wheat with reflectance measurements was possible at both DLO and RRES. However, the correct identification of the disease severity was still rather low. Therefore, we do not recommend to use this technique to quantify the disease severity with reflectance measurements at this moment. Further work is needed to enhance the specificity of the technique and capability to detect early infection stages.

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