Grower Summary

CP 099

Diagnostics: Validation of the lateral flow detection devices for the light leaf spot and powdery mildew vegetable Brassica pathogens and testing of white blister detection test prototypes

Annual 2014

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Project title:	Diagnostics: Validation of the lateral flow detection devices for the light leaf spot and powdery mildew vegetable Brassica pathogens and testing of white blister detection test prototypes
Project number:	CP 099
Project leader:	A Wakeham, National Pollen and Aerobiology Research Unit, University of Worcester, Henwick Grove, Worcester WR2 6AJ
Report:	Annual report, August , 2014
Previous report:	Annual report August 2013
Key staff:	G. Keane S. John G. Petch
Location of project:	University of Worcester
Industry Representative:	A Richardson, Allium and Brassica Centre, Wash Road, Kirton, Lincolnshire.
Date project commenced:	31st July, 2012
Date project completed (or expected completion date):	March ,2015

GROWER SUMMARY

.Headline

- In-field lateral flow tests have been used to measure field bioaerosols for light leaf spot, Brassica powdery mildew and white blister.
- Each has a diagnostic range suitable for use in disease risk forecast studies**.**
- A spore concentration (white blister) in excess of 20 spores per cubic metre of air sampled is thought to be required for disease expression on susceptible plants under suitable environmental conditions. A trial will be repeated in 2015 to confirm these findings.
- The unusually dry and hot conditions experienced during June and July 2013 is likely to have affected Brassica powdery mildew establishment in the trial crops. Spore concentrations during this period were identified (70 spores per cubic metre of air sampled) but no disease establishment was observed. The trial will be repeated in 2015 to establish the biological disease threshold (no of spores) required for disease establishment in the crop.

Background

Background and expected deliverables

In the airborne environment many plant diseases are able to spread between and within cropping systems. In the UK, using either laboratory based analysis or a field based pregnancy style test, HDC funded work has provided the development of systems to monitor field aerosols for target disease inoculum either on a daily or weekly basis. Air sampling systems and tests are available for the following vegetable plant pathogens: *Peronospora destructor* (onion downy mildew), *Mycosphaerella brassicicola* (ringspot), *Alternaria brassicae* (dark leaf spot), *Pyrenopeziza brassicae* (light leaf spot), *Erysiphe cruciferarum* (Brassica powdery mildew) and *Albugo candida* (white blister). By identifying disease (spores) in field air samples growers can time sprays more effectively and make informed decisions on which type of fungicide application to make.

Studies measuring *M. brassicicola* (ringspot) in airborne spore samples has shown that under ideal environmental conditions, high concentrations of spores are required in the air for infection to occur (2000 spores per cubic metre). The current study aims to identify bioaerosol concentrations of light leaf spot, powdery mildew and white blister spores that are required to cause disease symptoms on crops at a commercial scale. The developed diagnostic test formats for each of these diseases will be adjusted to reflect this. Results from previous studies show that light leaf spot ascospores appear in the air in large enough levels to be a problem

only during discrete periods. Light leaf spot inoculum may be present at other times but occurs at too low concentrations to become a problem in developing sprout crops. Fungicides applications can provide good control of Light leaf spot in Brussels sprout crops if applied at times when the disease is in the air. Where routine 'blanket' crop spray programmes have been applied, control can be ineffective. Light leaf spot is endemic in Scotland and becoming common in Brassica production areas of Northern England. Targetted application of effective fungicides in response to spore concentrations can play a vital role in controlling the disease. Inappropriate or unnecessary fungicide applications are not only costly but will increase the pressure for development and selection of pathotypes able to resist previously effective control measures.

Powdery mildew as, light leaf spot can infect any above ground plant part reducing plant growth and yield. Most horticultural brassicas are susceptible to infection and these include Brussels sprouts, cabbage, Chinese cabbage, kohlrabi, broccoli, kale, mustard, collards, cauliflower, radish, and horse radish. Powdery mildew disease is highly airborne and small numbers of conidia (spores) can be wind dispersed over large distances. To date there is little information on the environmental requirements for Brassica powdery mildew development although it appears to be favoured by dry conditions and, in the UK, these usually only occur during early summer. Infection of vegetable Brassica crops is unaffected by the powdery mildew occurring on oilseed rape crops. The occurrence of older tissues where powdery mildew development is more favoured, during autumn and winter, may act as a bridge for the pathogen to occur on Brussels sprouts buttons. Applications of fungicidal sprays (Nativo) are approved for control of the disease however as for light leaf spot, information about the availability of powdery mildew inoculum would be useful in control regimes. The airborne concentration of powdery mildew required for disease development in the crop is unknown but it is thought to play a vital role in the initial development of powdery mildew in brassica crops.

White blister is caused by the oomycete pathogen *Albugo candida* and is a common disease of many economically important cruciferous vegetables and oilseed crops. Significant yield losses from this disease have been reported on the oilseeds *B. rapa* and *B. juncea* and, to a lesser extent, on susceptible lines of *B. napus.* Affected vegetables include broccoli, Brussels sprouts, cauliflower, radish, mustard, Chinese cabbage and turnip. The impact of disease in these crops is of a cosmetic nature and can render crops unmarketable. To date, more than 10 distinct biological races of *A. candida* have been identified and classified based on host specificity. Race 9 infects *B. oleracea* and management strategies to control the disease in these crops has included the development of an environmental model (Brassica spot^{TM} – White

Blister model). The present study aims to improve the white blister disease risk forecast by including information on availability of *A. candida* airborne disease. Monoclonal antibodies with reactivity to *A. candida* (Race 9) spores will be used in an immunological chromatographic test strip format (lateral flow) to provide information on *A. candida* concentration in collected field air samples. Similarly, lateral flow tests and laboratory diagnostic tests developed in HDC FV33 for identification of airborne disease of light leaf spot and Brassica powdery mildew will be adjusted for commercial field usage. Enabling provision of information for requirement of fungicide spray applications in response to peaks in airborne spore numbers.

The expected deliverables from this project are:

- Measure disease in field aerosols for light leaf spot, powdery mildew and white blister. Evaluate the effect on infection and symptom development in commercial Brassica cropping systems
- Provide tests which can be used directly by UK growers or consultants to identify presence of these three diseases in the air. Identify the spore concentrations likely to cause disease at a commercial scale.
- Ability to detect white blister, Brassica powdery mildew and light leaf spot in field bioaerosols before disease is visible in the crop.
- Improved use of fungicide applications within vegetable Brassica production systems and the reduced likelihood of tebuconazole resistance within light leaf spot populations (already reported).
- Assess the potential to develop a multiplex test to identify risk of multiple pathogens on a single test device
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Summary

Diagnostic Tests

Batches of lateral flows (rapid on-site tests) have been used successfully to measure field bioaerosols for light leaf spot, Brassica powdery mildew and white blister. Each has a diagnostic range suitable for use in disease risk forecast studies. The development and use of the tests has the advantage of detecting the very earliest possibility of disease occurrence. Each test should provide a shelf life of between one and two years at room temperature. A multiplex text has been developed for the measurement of light leaf spot and white blister spores from a single bioaerosol sample. These tests will be used in the field and their use reported on in the final report (2015).

Field Trials

Light leaf spot

It is probable that the regional harvest of oil seed rape provided the source of the light leaf spot disease plumes recorded in field trials from July through to September 2013. In conjunction with environmental data, the development of regional oilseed rape harvest source maps could provide a useful tool towards forecasting light leaf spot disease risk on Brussels sprout crops in the UK. During at risk periods the on-site tests could be used to confirm and measure the spore (disease) concentration. This would provide a targeted and inexpensive (approx £7 / test) way of determining disease risk.

Brassica Powdery Mildew

Brassica powdery mildew spores were identified in field bioaerosols during the trial period. In July 2013, disease pressure for powdery mildew intensified. More than 70 spores per cubic metre of air were identified during this period in the weekly collected crop bioaerosols. However no powdery mildew disease was recorded on any of the plants in the trial. This is likely to have been a result of the environmental conditions. The UK Met Office recorded 2013 as the seventh sunniest summer since records began in 1929. A prolonged heatwave remained in place to the middle of July, when temperatures regularly passed 30ºC (86F). The south east of the country, to include the location of the trial, recorded the lowest amount of rainfall since 1995. The unusually dry and hot conditions may have prevented establishment of the disease in the crop. The trial will be repeated in 2014 to confirm the concentration of spores required to initiate powdery mildew in the crop. The results of which will be reported in HDC report CP99 July, 2015.

White blister

Conversely, white blister disease became established in the trial crop during September 2013. A spore concentration of >20 spores per cubic metre was estimated to have provided risk of disease development. Using these criteria the in-field lateral flow test was able to detect the disease in weekly collected bioaerosols ahead of symptom development on the crop. Improved management of the disease and, reduced applications with effectiveness of the fungicides applied, should be achieved by including information on availability of spore concentration (i.e. using an on-site test) with the white blister forecast model (MORPH Brassica spot). An extension of HDC CP99 will look to confirm this in Year 3 of the project (six month extension). The results of which will be reported on in July 2015.

Financial benefit

The specific action points for growers at this stage in the project are:

- The light leaf spot in field test has a disease threshold set at 200 spores per cubic metre air sampled for provisional timing for application of Signum to vegetable Brassica crops. This is likely to improve the efficacy of this chemical especially in production of vegetable brassicas in Northern Britain.
- The white blister in field test has a disease threshold set at >20 spores per cubic metre air sampled. The tests should be used in conjunction with the MORPH (Brassica Spot) white blister disease forecast.

Action Points

- The light leaf spot in field test kit will be available to the UK horticultural industry from 2015. The Brassica powdery mildew and white blister field test will be available from 2016. Alison Wakeham at NPARU can be contacted for further information.