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 2013

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Project Number:	CP 099
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
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Further information

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GROWER SUMMARY

Headline

Large batches of lateral flows for field diagnosis of airborne diseases transmission events of light leaf spot, Brassica powdery mildew and white blister spores in collected aerosols have been prepared and, with a diagnostic range suitable for use in field studies. A disease threshold for light leaf spores has provisionally been identified at 200 spores per cubic metre and the on-site test will be supplied to an Agronomist for trials in 2013.

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 field spore concentrations of light leaf spot and powdery mildew 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. Targeted 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. The application of fungicidal sprays (Nativo) is 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 currently 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 a management strategy to control the disease in these crops has included the development of an environmental model (Brassica spot™ – 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 recognition sites to *A. candida* (Race 9) spores and with reactivity to UK commercial isolates will be used in an immunological chromatographic test strip (lateral flow) to provide 'in-field' information on *A. candida* 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:

- Assess target disease spore concentrations in field aerosols and evaluate the effect on infection and symptom development in commercial Brassica cropping systems
- To provide tests which can be used directly by UK growers or consultant to identify presence of light leaf spot, Brassica powdery mildew or white blister spores in the air at concentrations likely to cause disease at a commercial scale.
- Ability to detect white blister, Brassica powdery mildew and light leaf spot in the field 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 been reported).
- Assess the potential to develop a multiplex test to identify risk of multiple pathogens on a single test device

Summary

Batches of lateral flows for field diagnosis of light leaf spot ascospores and Brassica powdery mildew in collected aerosols have been prepared with a diagnostic range suitable for use in field studies. Using a field portable electronic reader, light leaf spot ascospores of between 200 and two million can be estimated. For Brassica white blister, spore concentrations of between 30 and three hundred thousand can be differentiated using an ESE reader. For field trial usage the white blister lateral flow indicates a shelf life of upwards of one year when stored at room temperature. The light leaf spot recorded good test line stability over an 18 month period but the gold antibody conjugate is stable for a three month period only. Studies in Year 2 of the project would look to improve this.

Preliminary investigations in to the development of a multiplex test (several tests on one lateral flow test) have proved promising. At high contaminating spore concentrations, test line sensitivity or specificity for either target pathogen remained unaffected when two spore types were used in one test sample.

The present investigation has assessed the use of monoclonal antibodies, developed previously in an Australian study, to identify a suitable probe that could be used in a lateral flow to quantify airborne spores of *Albugo candida* within UK *Brassica oleracea* cropping systems. A monoclonal cell line (UW 256) showed a level of specificity which was able to discriminate between A. candida (race 4, white blister on Shepherds purse) and *A. candida* (race 9, white blister on B. oleracea). The lateral flow ('in field' test) developed for use in Australian commercial cropping systems and, which also used UW 256 as the specific probe , provided a detection sensitivity of 100 white blister race 4 spores. Using *A. candida* isolates from UK B. oleracea plants the lateral flow prototype has a detection sensitivity of 1000 white blister spores. Depending on the disease threshold (under optimal environmental conditions the number of spores in the air required to initiate uniform disease expression on susceptible plants) the sensitivity of the white blister lateral flow may require alteration for field usage.

At a commercial site in Scotland a crop of Brussels sprout *c.v*. Petrus was monitored for light leaf spot disease from the 13th August 2012 to February 2013. Three types of air sampler were used to monitor light leaf spot disease transmission events. Light leaf spot spore counts, derived from the volumetric spore count and identified by immunofluorescence, indicate that a spore concentration of 200 per cubic metre would under appropriate environmental conditions, lead to infection at low level in semi-resistant B. sprout varieties (*c.v.* Petrus) and significant infection in susceptible cultivars. Throughout this period and, to inform of potential light leaf spot disease risk periods, results derived from weekly laboratory analysis of the field exposed MTIST air samples were made available for use in crop protection decision management strategies. An 'in field' lateral flow was also used in processing weekly collected field aerosols and correlated with the MTIST value. In year 2 of the project, lateral flow batches will be available for use and compared with the weekly laboratory test for monitoring light leaf spot disease transmission events in a commercial crop. The disease threshold of each test will be set at 200 spores per cubic metre of air sampled.

Trials to monitor airborne disease transmission events for Brassica powdery mildew and White blister remain in progress and will continue throughout 2012 to December (Brussels sprout trials) and January 2013 (Cabbage crop). All three air sampling devices are

monitored weekly either by a consultant or University of Worcester staff member and to date little disease has been observed using by air samples. The crops remain visually clean at present. Once disease has established within the crop the fungicide resistance interaction trials should prove useful in determining the disease threshold required to set the MTIST ELISA and in field lateral flow devices to identify disease risk periods.

Financial benefit

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

- The Light leaf spot in field test has a detection 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.
- Tests can also be used in conjunction with disease forecasts

Action Points

- Consultants and Growers can assist validation with the 'in field' test to determine when light leaf spot, Brassica powdery mildew and white blister spores are present in their crop.