Protocol 010: INOC 22-26 (Mar 2022)

RECOMMENDEDLISTS

AHDB Recommended Lists (RL) for cereals and oilseeds: Inoculated disease trials (2021–26)

This protocol was believed to comply with relevant agrochemical, environmental and other regulations at the time of writing but it is the responsibility of the contractor to ensure that it continues to comply. In the event of non-compliance the protocol should not be followed but the Field Trials Manager should be notified at once of how the protocol requirements would breach regulations.

Any deviation from this protocol other than under the circumstances described above may result in a breach of contract and should be agreed in advance.

Changes from previous version

Page no.	Section	Details of change

Contents

Part 1: Scope

This protocol covers inoculated disease tests carried out as part of the AHDB Recommended List system for cereals and winter oilseed rape. It covers the growing of the crop in plots, disease inoculation, recording of disease and the submission of data. It does not cover the co-ordination of the testing programme, the analysis of disease data or the calculation of disease ratings.

Part 2: General introduction

Inoculated trials are a very important part of the AHDB RL programme, so it is vital that trials establish evenly, are well-grown and are fully protected from slugs, grazing damage, and insect damage. Trial contractors should be aware that AHDB may make no payment for trials which fail to establish satisfactorily, or are abandoned due to preventable causes (e.g. pest damage).

The definition of satisfactory establishment in this context is that there are sufficient healthy plants to provide a microclimate suitable for disease development and to allow the accurate assessment of disease levels. Irrigation should be available to aid establishment in dry conditions.

The tests should be conducted in such a way that will ensure a high level of disease on susceptible varieties. Contractors who are uncertain that their methods will ensure an adequate level of infection are advised to discuss their methods with the Disease Test Coordinator.

Note that appropriate fungicides may be used to control non-target diseases after consultation with the Disease Test Coordinator.

The occurrence of any non-target diseases must be recorded (according to Appendix 1 and 2) and data submitted alongside that of the target disease.

2.1. Seed supply for tests

The sowing lists (including appropriate comparator varieties) will be determined by AHDB.
The seed has been supplied for trial purposes only and must not be used for further multiplication or any purpose other than that specified by AHDB unless special permission has been obtained. Seed supplied by AHDB must be used only for trials and tests specified by them.

The seed will be treated with a standard seed dressing to give protection against certain seedling and seed-borne diseases. An insecticide dressing may also be applied. Full details of the chemical treatment will accompany the seed. Variety names, codes, thousand seed weight and germination information will be sent to trial managers by e-mail.

Bulks of seed sufficient to sow the specified plots will be sent to trial managers. It is the responsibility of each trial manager to prepare plot packets from the bulk of seed supplied.
Surplus seed may be used for discard and buffer plots or filling in but for no other purpose without the prior consent of the Technical Manager.

2.2.1 Seed supply for spreaders and controls

Seed for spreaders and disease controls will be supplied at the start of the project and if/when these varieties are changed. It is then the responsibility of the contractors to multiply seed for the spreaders and disease controls in order that sufficient is available in subsequent seasons.

2.3 The trial sites

The preferred location of the trial sites are detailed below.

Two trial sites are required (a primary site and a secondary site) for winter oilseed rape phoma/ light leaf spot and verticillium and cereal yellow rust, oat crown rust, winter barley brown rust, eyespot, and fusarium with the two sites being at least 30 miles apart.

Trials shown in Appendix 3 of this protocol as primary sites and those being grown at one site only will be considered the primary site for that disease. As well as managing the inoculated disease trials, these contractors will also be responsible for obtaining and multiplying the inoculum for both primary and secondary sites.

Contractors who are unable to multiply and supply inoculum may tender for the secondary sites as described above.

2.4 Method modifications

Trials contractors must follow the methods described in the protocols. Occasionally, it may be helpful to carry out additional procedures if a trial appears to be at risk of failure. These procedures are not a substitute for the protocol, and if additional methods are used, these must be clearly documented in the workbooks. The most likely circumstances which could generate the need for action are related to weather when extremes of temperature or rainfall may slow down the disease epidemic in the test plots, and result in low assessments which are not discriminatory.

Examples of additional procedures include, but are not limited to; a) the use of mist irrigation b) the use of additional direct inoculation onto test plots rather than spreaders e.g. for rusts and spore suspensions. Additional measures may be discussed with AHDB or the Pathology Trials Coordinator if necessary. Trial contractors may wish to reflect this need for occasional extra procedures to ensure discriminatory disease in their costings.

2.5 Disease assessment schedule

The disease assessment schedule for cereals is as follows:

Growth Stage	Assessment
At or slightly before GS 31	Record foliar disease if moderate infections (around 5%) occur in any plot.
GS 30–45	Winter barley only. Check carefully for rhynchosporium infection and record all plots if any infection is seen.
GS 31–55	An assessment of foliar disease is required if 5% disease develops in any plot. Once infection reaches 5%, assessments should be done at least every two weeks, depending on crop and disease progression.
GS 55–80	Assess all foliar diseases that reach 5% infection in any one plot during this period. Once 5% is reached, aim to assess the trial every two weeks, or frequently enough so that meaningful disease scores can be obtained i.e. the progression of the disease from one assessment to another can be tracked. This may mean visiting the trial more than every 2 weeks, or less than every 2 weeks. It may be appropriate to assess different diseases at different stages within this period (e.g. mildew might be better assessed relatively early and brown rust late).
When assessing diseases, also record the percentage green leaf area (GREEN LEAF AREA%) remaining on the leaves being assessed. If disease levels are too low for an assessment, please record this in the trial diary.	

The disease assessment schedule for oilseed rape is as follows:

	Leaf production	Early stem extension	Yellow bud stage	Late flower	Prior to pod ripening	Swathing
Light leaf spot % - on leaves	X	X	X	X		
- on pods					X	
Downy mildew %	X	X	X			
Stem canker DI					X	
Phoma leaf spot %	X					
Alternaria % - on leaves			X	X		
- on pods					X	
White leaf spot %	X	X		X		
Sclerotinia %				X	X	
Botrytis %				X	X	
Powdery mildew %			X	X	X	
Verticillium (in verticillium trials)	See 13.6.1					
Verticillium stem stripe% (Non verticillium trials)					X	X

For light leaf spot at the disease observation plot sites assessment must start when the symptoms first appear and continue at 28-day intervals unless the disease has failed to move up the plant since the last recording. In that case assessment should start again if the disease starts to move further up the plant. Any disease on the pods should be recorded at pod formation.

Part 3: Yellow Rust of Wheat

Puccinia striiformis f. sp*. Tritici*

3.1 Location of tests*

AW - East of England, SE England, Lincs, Notts or Leicestershire.
WW - East of England, SE England, Lincs, Notts or Leicestershire, the two trials must be located at least 30 miles apart.

3.2 Source of inoculum

Races to use will be available from the United Kingdom Cereal Pathogen Virulence Survey (UKCPVS). There will be two sites (at least 30 miles apart) with site 1 the primary (inoculum multiplication) site as described in 2.3. It is the primary contractor's responsibility to provide suitable inoculum for both tests.

3.3. Multiplication of inoculum

It is the contractor's responsibility to provide inoculum for the test from recent outbreaks of the disease, and to ensure that the isolate mixture is updated as much as possible. The multiplication of each isolate of the fungus must be done in isolation from other races of the pathogen or on varieties known to be resistant to other races, from which they are not isolated.

During the multiplication of each isolate, at least one generation each year must normally be on a variety with the corresponding specific resistance. However, this may not always be possible for adult plant resistances in the year of use.

Inoculation of spreader seedlings must be done in isolation from other races of the pathogen.

3.3 Varieties in test

Both winter wheat and spring wheat varieties will be sown in October. All RL varieties plus appropriate controls will be included. The sowing list will be supplied by the AHDB RL Team.

3.4 Number of tests each year

Winter wheat Two trials with 2 to 4 isolates. The trial must contain 2 replications and should be isolated from trials inoculated with other races by a 20 metre (minimum) fungicide treated barrier crop.

Spring wheat – will be grown in a trial adjacent to the winter wheat.

3.5 Method

Plots with a minimum size of 2 x 1m rows are drilled at a seed rate of 250-275 seeds/m^2 and a row spacing of 17-20 cm. Irrigation must be provided if required for establishment and maintaining plant growth in very dry conditions. Susceptible spreader variety(ies) will be nominated by the Disease Test Coordinator which are to be sown adjacent to every plot. Plots are inoculated at GS 30-31 either by transplanting infected seedlings of each isolate used into the spreader, or by direct inoculation of the spreaders by a spore / talc mixture. For mixed isolate trials each component of the mixture must be applied separately to discrete 'spots' within each spreader plot.

Irrigation may be used to keep transplants growing but mist irrigation to encourage disease should be avoided as mist washes off spores. A good crop, inoculum and natural dews are usually adequate to establish rust epidemics.
Percent leaf area infection is assessed using the appropriate disease assessment key from the AHDB RL trials protocol.

Part 4: Brown Rust of Wheat

Puccinia triticina f.sp. *tritici*

4.1. Location of tests*

AW – East of England, Southeast England
WW – East of England, Southeast England

4.2. Source of inoculum

As for yellow rust (one trial only).

4.3. Multiplication of inoculum

As for yellow rust (one trial only).

4.4. Varieties in test

All RL varieties plus appropriate controls will be included. The sowing list will be supplied by the AHDB RL Team.

4.5. Number of tests each year

As for yellow rust.

4.6. Method

As for yellow rust of wheat. To minimise the risk of the spread of other diseases into the brown rust trial it should be isolated from other inoculated plots by a 20 metre (minimum) fungicide treated barrier crop.

Part 5: Eyespot of wheat

Oculimacula yallundae (W-type), *Oculimacula acuformis* (R-type)

5.1. Location of tests*

Not specified, though the two trials must be at least 30 miles apart.

5.2. Collection, storage and multiplication of inoculum

It is the primary contractor's responsibility to provide suitable inoculum for both tests. New isolates should be collected from the field each year to replace some of those in storage. Isolates are sub-cultured a maximum of three times each year and may only be kept in culture for 3 years to ensure that they have retained their pathogenicity.

5.3. Varieties in test

Winter wheat varieties only; spring wheat varieties are not tested for this disease. All RL varieties plus appropriate controls will be included. The sowing list will be supplied by the AHDB RL Team.

5.4. Method

Trials must be sown by the end of September at the latest in 4 fully randomised, replicated blocks. Tenders should be to conduct a single test at a single site.

A plot size of approximately 1m x 1m should be used. Plots are drilled with a maximum seed rate of 200/m^2. Plots are inoculated at around the 1-2 leaf stage but before end October, with 10g/m^2 infected oat grain spread onto the ground evenly within each plot.

Trials must be fungicide treated as required to control non-target diseases.

Samples of 20 shoots, taken from a minimum of 10 assessment points in the plot and avoiding edge rows should be assessed for eyespot symptoms once at around GS 75 (depending on disease development) using the eyespot index key (see Appendix 1).

Lodging and whiteheads should also be recorded as soon as they appear. A complete replicate must be sampled in a single day and assessed in a single day not later than one day after sampling.

Part 6: Fusarium head blight in wheat

Fusarium graminearum, Fusarium culmorum

6.1. Location*

East of England, Southeast England, Lincs, Notts and Leicestershire.

6.2. Collection, storage and multiplication of inoculum

It is the primary contractor's responsibility to provide suitable inoculum for all tests. Isolates should be of known chemotype and proven to be pathogenic. These should be maintained and used across years. Sub-culturing should be limited to three occasions while isolates are in storage. Additional isolates may be added to the inoculum when their chemotype and pathogenicity is known.

6.3. Varieties in test

Winter wheat and spring wheat varieties will be sown together and sown in October in three adjacent trials each with three replications. All RL varieties plus controls which will include early and late maturing varieties and resistant and susceptible varieties. The sowing list will be supplied by the AHDB RL Team.

6.4. Method

The trials are blanket inoculated with 3 spray inoculation timings for each early, mid, and late trial (so 9 timings in total). The three trials are inoculated as follows:

1) The early trial will be inoculated when 25% of the varieties have reached mid-anthesis. It will then be inoculated 2 and 4 days post the initial inoculation.

2) The mid-anthesis trial will be inoculated when 50% of the varieties have reached mid anthesis and again at 2 and 4 days thereafter.

3) The late trial will be inoculated when all of the varieties have reached mid-anthesis and again 2 and 4 days thereafter.

Inoculum is a mixture of 2 isolates each of *F. graminearum* and *F. culmorum* at a total conidial concentration of 250 – 400K spores/mL in between 200 – 600 L/ha.

Assessments will be based on thermal time after inoculation. Thermal time will start from the date of the first inoculation and will be 280DD, 340DD, and 400DD after the first inoculation (400DD may not be possible if senescence has begun). Assessments will be done on a plot basis by randomly selecting 25 ears per plot and taking an average percent disease.

Only disease assessments that have an average of 20% infection can be used in the ratings. Therefore, thorough misting/irrigation is extremely important to promote disease establishment and development.

6.5. Control of non-target diseases

To control non-target disease in fusarium trials, a T0 and T1 fungicide application may be made. Please apply a T2 and T3 only if absolutely necessary to control disease. An example programme is shown below.

Timing	**Application**
T0	Folicur 0.5 L/ha (add Talius 0.15 L/ha if mildew present at either T0 or T1)
T1	Phoenix 1.5 L/ha + Comet 0.5 L/ha + Moddus at 0.2 L/ha (add Ignite 0.75 L/ha if yellow rust present at time of application)
T2 Only if necessary to control non-target disease	Phoenix 1.5 L/ha + Comet 0.5 L/ha + Terpal at 0.5 L/ha (add Ignite 0.75 L/ha if yellow rust present at time of application + add Cyflamid 0.25 L/ha if mildew present)
T3 Only if necessary to control non-target disease	Apply Comet 0.5 L/ha (post flowering) if rusts are present

Part 7: Brown rust of barley

Puccinia hordei

7.1 Location of tests*

One trial to be located in NE or NW England or Yorkshire and the Humber or Scotland.
One trial to be located in the East of England, SE or SW England.

Methods to follow those described for brown rust of wheat above.

Part 8: Net blotch of winter and spring barley

Pyrenophora teres f. *teres*

8.1. Location of tests

A specific area is not specified but in high net blotch risk area. The location should be justified in the tender. Having the capacity to be irrigated in the event of really dry years would be advantageous.

8.2. Multiplication of inoculum

It is the contractor's responsibility to provide inoculum for the test from recent outbreaks of the disease, and to ensure that the isolate mixture is updated as much as possible. Infected straw is collected in the field each year and each "isolate" is the debris from a single site. Debris may be collected from the test site each year for use the following year but some "topping up" from other sites is desirable each year.

8.3. Varieties in test

All RL varieties plus appropriate controls will be included. The sowing list will be supplied by the AHDB RL Team.

8.4. Method

Trial(s) will be inoculated with debris collected from different sites containing 2 replications. The trial(s) must be located at a site suited to the development of the disease and where other diseases are unlikely to interfere.

Plots are drilled with a minimum area of $1m^2$. For inoculation, infected straw is spread onto plots at GS 30 and 32 for both winter and spring barley.

Trials must be treated with fungicide to control non-target disease.

Infection is assessed at 5–7-day intervals starting when there is 5% infection on susceptible varieties (usually 2–3 assessments), using the assessment key Appendix 1.
Green leaf area must also be recorded if there is appreciable loss of green leaf area that is not attributable to the target disease.

Part 9: Rhynchosporium of winter and spring barley

9.1. Location of tests*

SW England, NW England, Shropshire and Herefordshire, Wales or Scotland. For the SB trial irrigation should be available.

9.2. Multiplication of inoculum

It is the contractor's responsibility to provide inoculum for the test from recent outbreaks of the disease, and to ensure that the isolate mixture is updated as much as possible.
Seedling leaves of a susceptible variety are to be inoculated with a spore suspension of *R. commune* (10^6 spores mL^{-1}), harvested from cultures on Lima Bean Agar. It is important to increase the humidity for 48 hours after inoculation.

Leaves developing lesions are then cut and dried at room temperature before freezing at -20°C for storage longer than 3 months, or at 5°C for shorter periods.

When required, the leaves are incubated for 48 h and spores washed off.

Adult plants leaves collected from naturally occurring outbreaks or from trials may also be harvested and dried down for storage.

As *R. commune* is known to have a race structure, material from different outbreaks should be used as far as possible. Additions should be made each year from outbreaks where higher levels occur on varieties previously thought to be resistant.

9.3. Varieties in test

All RL varieties plus appropriate controls will be included. The sowing list will be supplied by the AHDB RL Team.

9.4. Method

The trial should be inoculated twice, towards the end of April and end of May for winter barley, and around mid-May and early June for spring barley. Inoculum should contain 10^6 spores mL^{-1} and be applied at a rate of approximately 100 mL per m^2 of test plot. Minimum plot size should be 1 m^2. It is essential that irrigation is used to pre-wet the plots in the absence of rainfall. Further cycles of irrigation, or a location with reliable rainfall, are also essential.

Part 10: Crown rust of winter and spring oats

10.1. Location of test*

One trial in East of England or Southeast England and one in the Southwest of England each for spring and winter oats.

10.2. Multiplication of inoculum

It is the contractor's responsibility to provide inoculum for the test from recent outbreaks of the disease, and to ensure that the isolate mixture is updated as much as possible. It is the primary contractor's responsibility to provide suitable inoculum for both tests.

10.3. Varieties in test

All RL varieties plus appropriate controls will be included. The sowing list will be supplied by the AHDB RL Team.

10.4. Method

Multiplication of inoculum and inoculation of tests is the same as for wheat yellow rust and brown rust.

Part 11: Stem canker of oilseed rape

11.1. Location of tests*

Inoculated tests should be carried out at two sites, one in East of England and a second in an oilseed rape growing area of higher rainfall in the West Midlands or South West England. Both sites must be appropriate for the severe development of the disease. Four replicates should be grown at each site.

11.2. Inoculum

It is the primary contractor's responsibility to provide suitable inoculum for both tests. Infected plant residues are collected from trials and crops from a minimum of 3 different areas of the UK where the disease is prevalent (e.g. Eastern, Central and Western) after harvest and stored in well ventilated plastic bags or net sacks outdoors under shelter. The inoculum should reflect the diversity of the fungal population found in the UK and should be discussed with the Disease Test Coordinator.

6.2 Inoculation

Infected stem pieces are placed in small plots (at least 3 rows of approx. 4m) at a rate of 3 pieces per m^2 at the 1-2 true-leaf stage. Irrigation is applied to disperse inoculum and encourage infection if required, during dry weather. It may be possible to achieve a sufficient level of irrigation using a slurry-tanker, but care must be taken not to damage the plants. Methods of irrigation available should be discussed with the Disease Test Coordinator before use.

6.3 Assessment

Phoma leaf spot is observed during the autumn and winter as an indicator of successful infection. Stem canker is assessed in the latter half of June according to Appendix 2 of this protocol on either 30 or 50 stems per plot, ignoring the outside row of each plot. Fifty plants should only be assessed per plot if infection levels are low. If this happens the contractor should seek advice from the Disease Test Coordinator before the test is complete.

The Disease Test Coordinator will nominate about 8 varieties to be re-examined between 7 and 10 days after the main scores are taken. The initial and second scores for these varieties must be emailed immediately to the Disease Test Coordinator. If the scores for these varieties have changed significantly it may be necessary to re-score the whole trial. The Disease Test Coordinator will advise if this is necessary.

Part 12: Light leaf spot of oilseed rape

12.1. Location of tests*

Inoculation will be done at the two stem canker sites above. Detailed recording of natural infection using the methods described below will also be undertaken at the RL disease observation plot sites.

12.2. Inoculation

The stem debris inoculum spread for stem canker can sometimes be sufficient to cause infection with light leaf spot, but the infection produced may be in “foci” which fail to spread disease evenly across the trial area. It is therefore usually necessary to inoculate plots with a suspension of conidia. Irrigation is applied to disperse inoculum and encourage infection if required during dry weather. In addition, a light leaf spot susceptible variety is sown down the centre of each of the trial plot rows and inoculated to maintain disease pressure. Conidial inoculum may be washed with minimal amounts of water from infected leaves collected from field outbreaks and stored frozen at -20°C until ready for use. Inoculum should be derived from recent disease outbreaks in different parts of England and Scotland (e.g. North, West). Inoculum from the current season’s trial should also be collected. Alternatively, isolates may be cultured on agar and used to generate conidial inoculum which can again be stored frozen until needed. It is the primary contractor’s responsibility to provide suitable inoculum for both tests.

12.3. Assessment

Infection levels are recorded using the key in the AHDB RL protocol.
Assessment must start when the symptoms first appear and continue at 28-day intervals unless the disease has failed to move up the plant since the last recording. In that case, assessment should start again if the disease starts to move further up the plant. Any disease on the pods or stems should be recorded at the end of flowering/pod formation.

Part 13: Verticillium stem stripe of oilseed rape

13.1. Location of test

Not specified.

13.2. Source of inoculum

The trial site can be naturally or artificially infected. There should be uniform disease distribution across the trial. Inoculation of sites, where required, should be done as follows:

- Soil should be inoculated 1-2 weeks after sowing.
- Inoculum should consist of a maize meal and vermiculite substrate which has previously been infected with UK isolates of *V. longisporum* and incubated at room temperature until abundant microsclerotia have formed.
- The mixture should be air dried before spreading on the test plots, using an amount calculated to give between 40-100 colony forming units cm^2 of soil.

13.3. Trial type

Either small (3-6 m long), or larger (e.g. 12 m long) plots may be used. There will be 4 replicates per variety. The trial should not go to yield. The trial should be established and maintained in line with the WR22-26 AHDB Oilseeds winter oilseed rape trials Protocol, with the exception of fungicide treatments, where one autumn treatment to control stem canker should be used as needed.

13.4. Number of tests each year

There will be two trials per year.

13.5. Varieties in test

All RL recommended and candidate winter oilseed rape varieties, approximately 40. The sowing list will be supplied by the RL team.

13.6. Assessment

13.6.1. Timing of assessment

The timing of assessment is crucial. Yellowing of half a leaf (Appendix 2) is considered to be an early symptom of verticillium stripe and can be observed at the base of the canopy from the end of April onwards. From mid-June onwards plots should be inspected regularly. First symptoms of verticillium stripe are usually yellow streaks running the length of the stem or a large branch. Often, this is a single streak per stem, and it will eventually become more obvious and turn brown (Appendix 2). Symptoms should be assessed weekly during late June or early to mid-July. Symptoms can develop rapidly over a period of 7 to 14 days and the best assessment date can vary between years. Scoring too early could mean under-estimation of symptom incidence and severity, even with scraping to reveal sub-epidermal symptoms. Equally, scoring too late could mean difficulty in seeing verticillium on stems which are rapidly

drying out. In crops that remain very green at the end of the season, symptoms may not be fully expressed until a few days after desiccation.

13.6.2. Disease assessment method

Disease assessments should be done on stems that have been removed from the plot so the entire circumference of the stem can be assessed easily. Pull up 30 stems per plot and score the middle to upper thirds of each stem for the presence of stem shredding and black microsclerotia. Stems should always be scraped to confirm whether or not infection is present. Some varieties, particularly those slower to mature, will only exhibit internal symptoms. Assign stems to infection classes defined in Table 1. Calculate an index (0-100) by multiplying the number of stems in each category by the numerical value of the category, dividing by the total number of stems, and multiplying by 100/5.

Table 1. Infection classes for severity of verticillium symptoms on oilseed rape stems.

Scale point	Description
0	no symptoms
1	1–25% circumference affected
2	26–50% circumference affected
3	51–75% circumference affected
4	>75% circumference affected
5	severely affected and plant dead

If no microsclerotia are visible, scrape back a section of stem so that any sub-epidermal grey discolouration typical of verticillium infection can be seen. If this is present, revise scores to take it into account.

13.6.3. Assessment of other diseases

If other disease such as phoma stem canker are observed, this should be noted in the trial diary, but it is not a requirement to assess diseases other than verticillium.

Part 14: Completion of records

The trial workbook should be used to record all data. A detailed user-guide for the workbooks is available separately.

Completed data should be returned to trials@ahdb.org.uk as follows:

Report	Deadline	Sheets to be returned (return only these sheets: do not return the whole workbook)
1. Early data	Autumn sown trials: within 2 months of sowing. Spring sown trials: as soon as possible and within one month of sowing.	Site data. Map of area. Field layout sketch. Trial layout. Trial diary.
2. Disease data	11th July.	Disease data recording sheet. Trial diary update. Agchem details.

Plots data sheets contain columns for records likely to be undertaken during the recording period but some may not be needed; any additional recording columns will be supplied in a separate file: these additional recording columns should be pasted whole into the relevant data sheet using the 'Copy/paste' function (i.e. select, copy and paste the whole column).

14.1. Early data (Site data, location, and plan details)

This information should be returned shortly after full establishment and within two months of sowing. This report should include:

- Completed site data sheet.
- Map of area. Site location details i.e. how to get to the field by road.
- Field layout sketch. Sketch showing the layout of the trial(s) in the field, in relation to other trials and showing access roads, gates etc.
- Trial layout. Trial sketch showing plot numbers and variety IDs.
- A short post-establishment report of the condition of the trial in the diary sheet.
- Confirmation of sowing to plan or full details of any changes to plan.

This should be done by clearly highlighting the changes in the sheet 'Trial plan 2' contained in the workbook. On receipt of the returned workbook the Disease Test Coordinator will ensure that the plan has been modified correctly and that the de-randomising features of the workbook are working. If necessary, a modified workbook will then be returned for use by the trial manager.

14.2. Disease data

To be submitted as recorded, with the final submission no later than 11th July. Wheat yellow rust data must be submitted within three days of the assessment. The final submission must contain all disease data recorded to date, and details of trial treatments and agrochemical inputs.

Disease records must be returned in this report to allow their inclusion in the database for new ratings, which are calculated shortly after this date. Any disease records taken after this date should be returned as soon as possible after the assessment: if it is not possible to include in the current season rating calculations they will be included in the database for future use.
Any notes made since the first submission of data should be shown on the trial diary sheet, which should also be returned.

Part 15: Reporting problems

The tests should be conducted in such a way that will ensure a high level of disease on susceptible varieties.

The procedures described above outline when or by what growth stage certain actions should be undertaken. In addition, it is expected that the test contractor will have the ability to ensure a good level of infection in the inoculated tests.

AHDB recognises that the tests are with biological systems and are subject to the vagaries of the weather**.** However, the test contractor must inform the Disease Test Coordinator and RL Technical Manager immediately by phone or e-mail if actions are not possible within the windows suggested above or if the speed or degree of disease development appears unsatisfactory. The test contractor must also explain to the Disease Test Coordinator and RL Technical Manager the reason(s) and circumstances of the problem or missed action. The trial diary within the trial workbook should be used to document problems and agreed actions.

15.1. Trial inspection

All trials will be inspected by an authorised Trial Inspector.
For inoculated disease trials two inspections will take place. A first inspection after full establishment is to ensure that adequate measures are being taken to prevent damage by pests (e.g. birds, rabbits). The second inspection will be during the spring/summer around the time that disease is to be recorded.

The requirements for Trials Operators in respect of inspections are as follows:

1. To give reasonable access to trials to inspectors and provide full location and site details (if not already given with site data).
2. To supply the inspector with information (for example sprays applied etc.) within seven days of a request.
3. To co-operate with the inspector in making any non-routine assessments required to establish the validity of the trial (for example population counts).
4. To carry out any action agreed in consultation with the inspector. In particular it is important that any requirement to shorten plots is undertaken and that missing values are returned on any plots which have been dropped from the trial.

The trials inspection also provides an opportunity for feed-back to AHDB about any problems with the trials protocol and Trials Operators are encouraged to make any points to the inspector so that these can be considered for future revisions to the protocol.

Appendix 1 - Assessment keys for cereal diseases

Foliar disease assessment

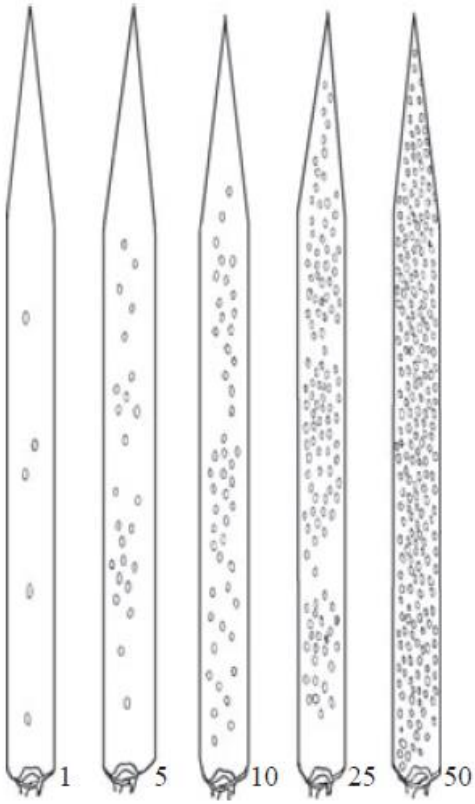
1) Examine top 4 leaves. If top leaf has been fully expanded for less than 14 days, refer to 2nd leaf as 'top leaf'.
2) Ignore all naturally senescent leaf tissue.
3) Include all chlorosis and necrosis attributable to disease.
4) Record % infection; use interpolated values (e.g. 3%) if necessary.
5) If foci present, record average over the plot as a whole.

% Infection	Mildew	Yellow rust	Brown rust
0		No infection observed	
0.1	3 pustules per tiller	1 stripe per tiller	25 pustules per tiller
1	5 pustules per leaf	2 stripes per leaf	100 pustules per leaf
5	2 lower leaves appear ¼ infected	Most tillers infected but some top leaves uninfected	Top leaf - numerous pustules but leaves appear green overall
10	2 lower leaves appear ½ infected	All leaves infected but leaves appear green overall	Top leaf - pustules dense enough to give brown appearance in patches
25	Leaves appear ½ infected ½ green		
50	Leaves appear more infected than green		
75	Very little green leaf tissue left		
100	Leaves dead - no green tissue left		

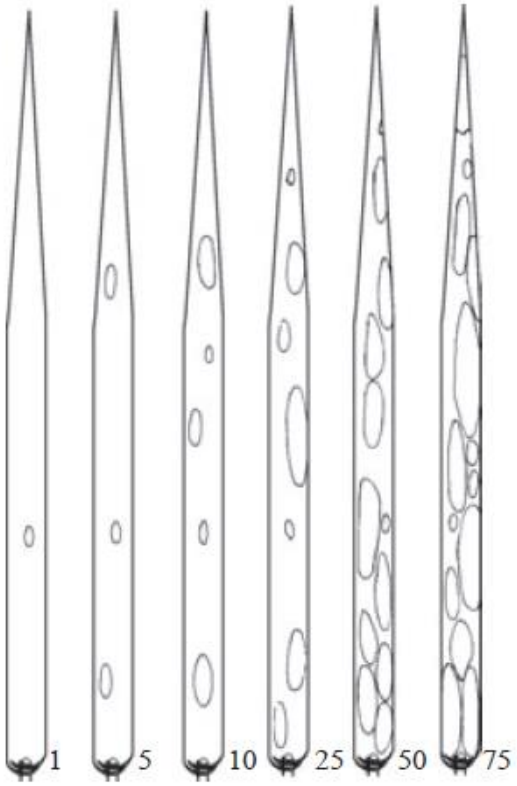
% Infection	Septoria	Rhynchosporium	Net blotch
0		No infection observed	
0.1	1 lesion per 10 tillers	1 lesion per 10 tillers	1 small lesion per 10 tillers
1	2 small lesions per tiller	1 lesion per tiller	1 small lesion per tiller
5	Small lesions beginning to form areas of dead tissue across width of leaf	Discrete lesions on most tillers, about 2 per leaf	2 lower leaves appear ¼ infected. Other leaves - few lesions
10	2 lower leaves – large areas of diseased tissue some covering $^1/_3$ of leaf	Lesions coalescing but leaves appear green overall	2 lower leaves appear ½ infected. Other leaves - numerous lesions
25	Leaves appear ½ infected ½ green		
50	Leaves appear more infected than green		
75	Very little green leaf tissue left		
100	Leaves dead - no green tissue left		

Mildew

Wheat



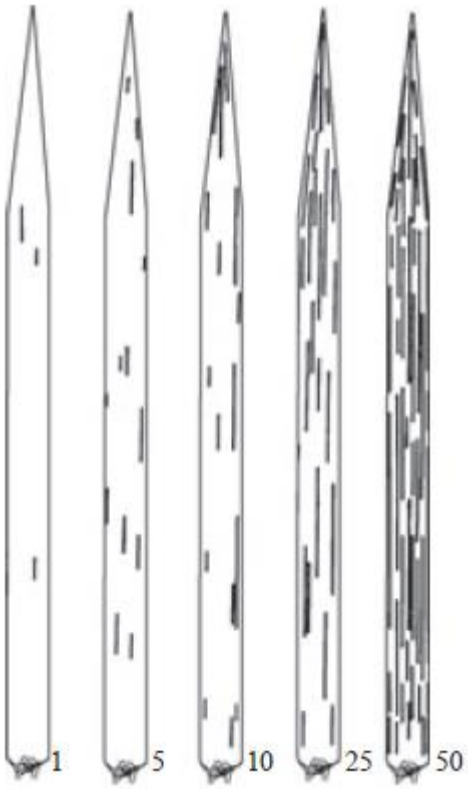
Barley



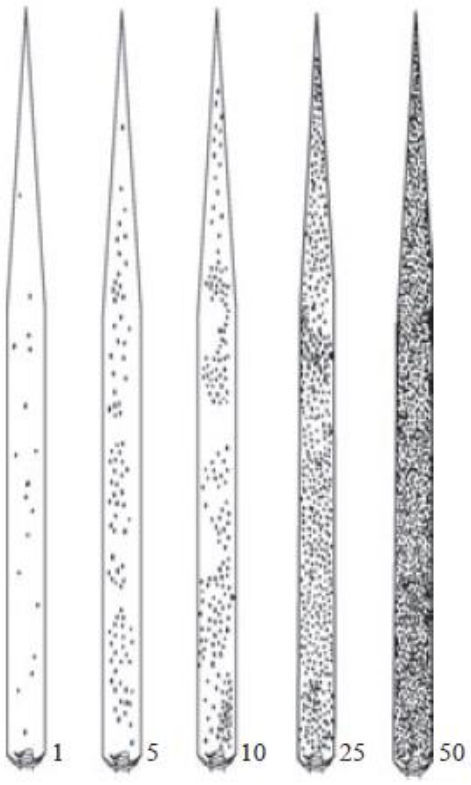
Erysiphe graminis on wheat and barley: percentage of leaf area affected.

OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 42, 419–425

Yellow rust on wheat



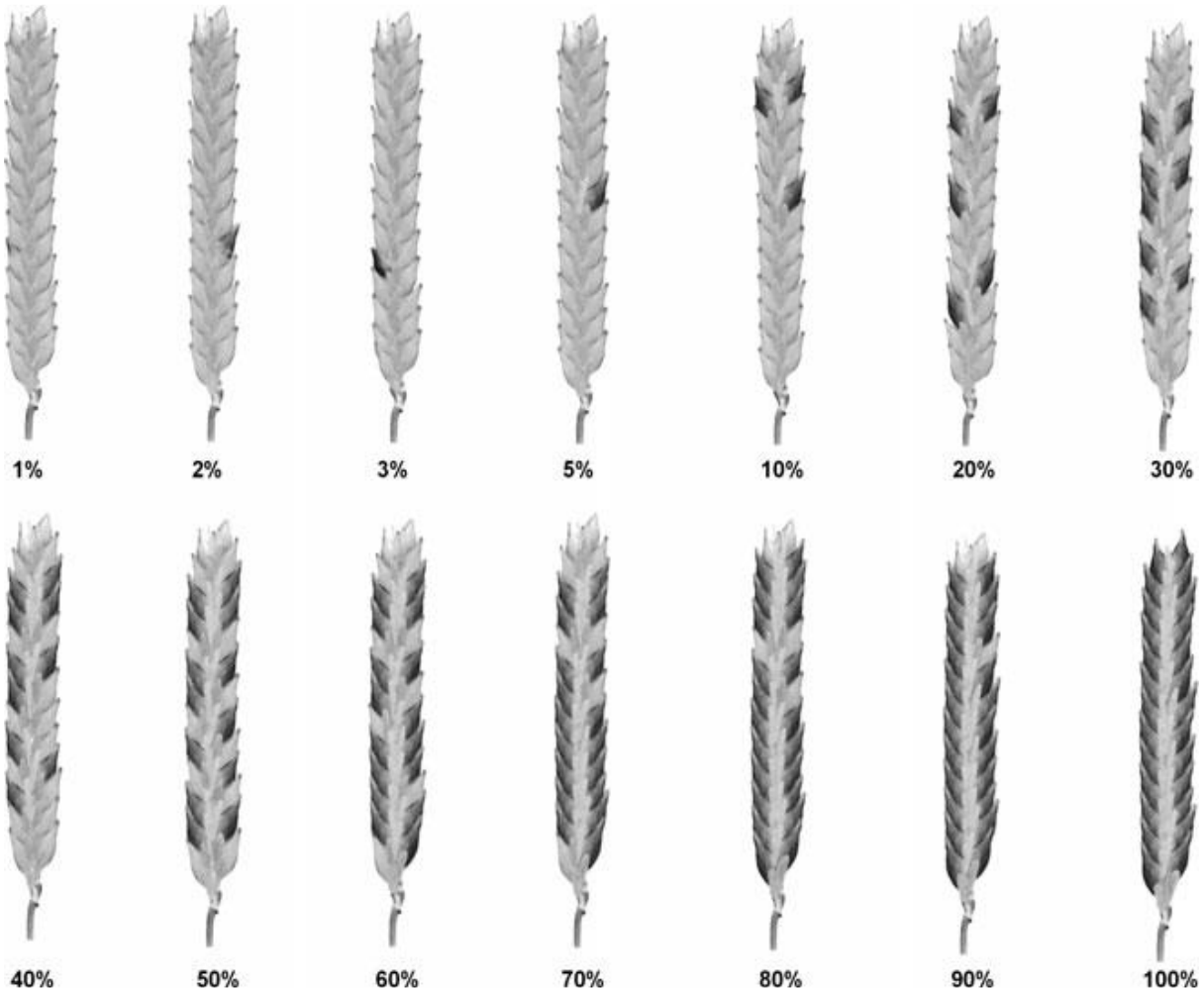
Brown rust on barley



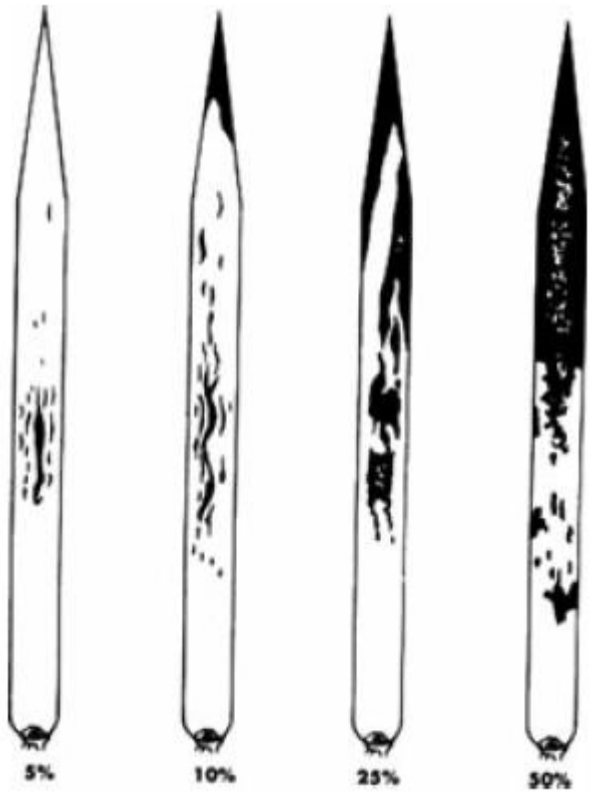
Puccinia striiformis on wheat: percentage of leaf area affected.

Puccinia hordei on barley; Puccinia triticina on wheat: percentage of leaf area affected.

Ear Blight of wheat (*Fusarium* spp.)



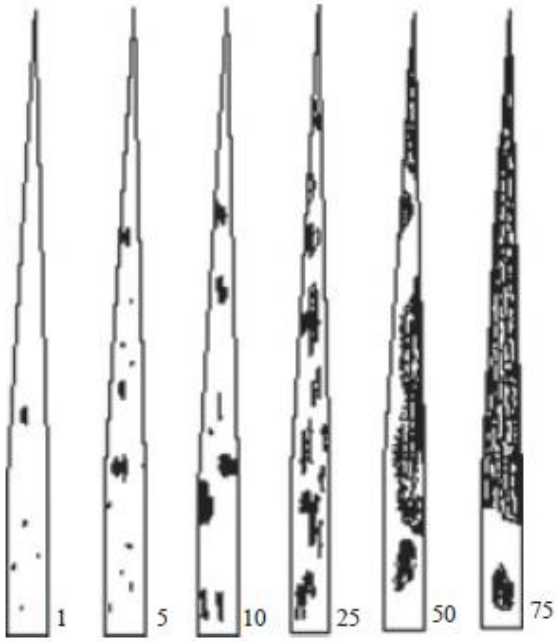
Septoria tritici



Septoria tritici on wheat; percentage of leaf area affected.

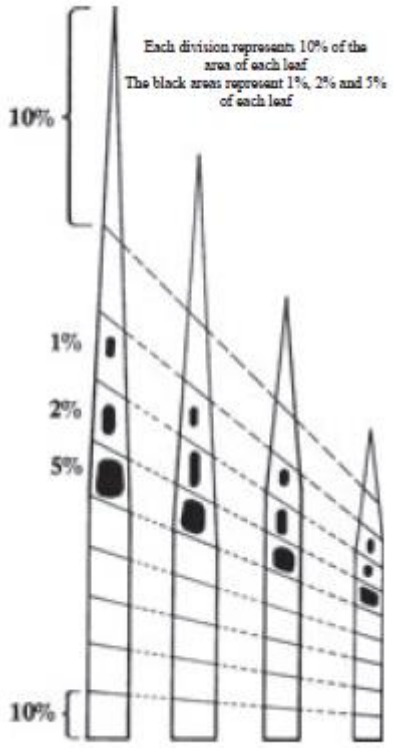
OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 42, 419–425

Net blotch on barley



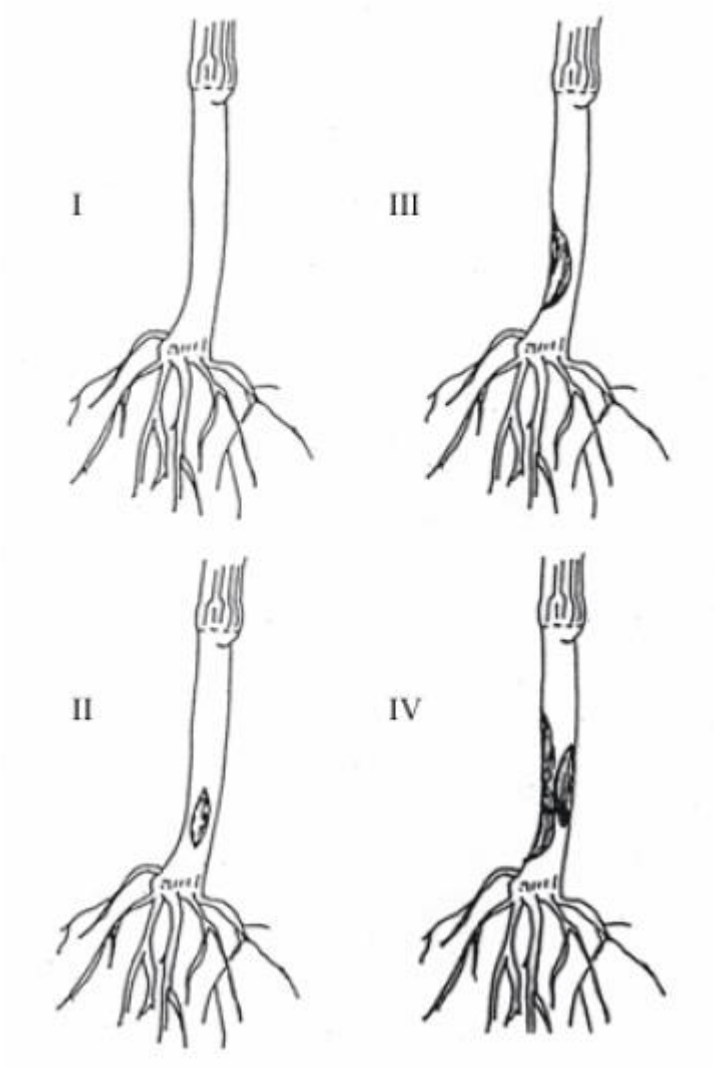
Pyrenophora teres on barley: percentage of leaf area affected.
OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 42, 419–425

Rhynchosporium on barley



Rhynchosporium secalis on barley, percentage of leaf area affected.

Eyespot of wheat (*Pseudocercosporella herpotrichoides*)



EPPO PP 1/28 (3)

Infection category	Disease severity description
1	Uninfected.
2	Slight eyespot (one or more small lesions occupying less than half the circumference of the stem).
3	Moderate eyespot (one or more lesions occupying at least half the circumference of the stem)
4	Severe eyespot (stem completely girdled with lesions; tissue softened so that lodging would readily occur).

Notes of assessment

1) Examine 20 tillers per plot.

2) Assign each tiller to one of the infection categories above.

3) Write the number of tillers in each category on the record sheet.

4) An index will be calculated from the data as follows:

$$\text{Disease index} = \frac{(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d)}{(a + b + c + d)} \times \frac{100}{3}$$

Where a, b, c, and d are the number of tillers examined which fall into the categories 1, 2, 3 and 4 respectively.

For further details, see Scott, P R and Hollins, T W (1975). Effects of eyespot on the yield of winter wheat. Annals of Applied Biology 78, 269-279.

Appendix 2 - Assessment keys for oilseed rape diseases

Light leaf spot, Alternaria, downy mildew, Phoma and white leaf spot ON LEAVES AND PODS

1) Examine all leaves and pods in 3 areas of each plot.
2) Ignore all naturally senescent tissue.
3) Include all necrosis and chlorosis attributable to disease.
4) Estimate % infection using the descriptions below. Record the average % infection from the 3 areas. Interpolate values if necessary.
5) Where stem are affected by of light leaf spot, record the % stem area with symptoms

% Infection	Leaves	Pods
0	No infection observable	
0.1	Trace of infection	
1	Diseased leaves with 1 small lesion; plants with a few scattered lesions	Terminal raceme with a few scattered lesions
5	Leaves appear 1/10 infected; diseased leaves with 2 lesions	Terminal raceme appears 1/10 infected. diseased pods with 1 or 2 lesions
10	Leaves appear ¼ infected, diseased leaves with few large or many small lesions	Terminal raceme appears ¼ infected. Diseased pods with 2 or more lesions
25	Area appears ½ infected ½ green	
50	Area appears more infected than green	
75	Very little green tissue left	
100	Leaves/pods dead - no green tissue left	

These descriptions are guides for specific levels; interpolate between these points as necessary e.g.15%, 27%, 60% etc.

Stem canker

Stem canker should be assessed from about the middle of June onwards, depending on season. External and internal symptoms should be assessed. Normally, 30 stems per plot from the central part of the middle row should be pulled up, and a length of about 30 cm cut off. Stem canker symptoms visible on the lower (10cm or so) external part of the stem should be scored according to the external scale column below. Using secateurs, each stem should then be cut transversely through the central part of the leaf scar region at the stem base, and the cross-sectional area of them stem affected by canker symptoms assessed according to the internal column

	External symptoms	Internal symptoms
0	No infection observable	No infection visible
1	<25% girdling of the stem	Superficial - 1-5% surface area
2	26-50% girdling	6-25% surface area
3	51-75% girdling	26-50% surface area
4	76 -100% girdling	51-75% surface area
5	100% girdling + stem weakness	76-100% surface area
6	100% girdling + stem death	100% area and stem death

A disease index for external and internal scores should be calculated using the following formula:

$$\frac{(0xa + 1xb + 2xc \text{ etc.})}{(a+b+c + \text{etc.})} \times 100/6$$

where a, b, c etc. are the number of plants in each disease category

A mean of the external and internal index must be submitted in an RL workbook, but records of external and internal indices should be retained.

Typical Verticillium stem stripe symptoms

Figure 1. Early leaf symptoms of verticillium stripe on a susceptible variety in mid-May **yellowing of half a leaf**.

Figure 2. Characteristic striping along the length of the stems observed in early July.

Figure 3. Close up of verticillium stripe showing an affected branch and the distinction between the brown stripe and green plant tissue.

Figure 4. Scraped stem reveals vascular staining not visible on the outside of the stem.

Figure 5. Microsclerotia, exposed due to the stem surface becoming dried out, paper-like and splitting, on winter oilseed rape in mid-July.

Other diseases

Club root
Any suspected club root in trials should be confirmed by sampling between 10 and 30 plants within the suspected area, and its presence notified to the Technical Manager.

Sclerotinia %
Should be assessed as the % of stems with complete girdling leading to 'whiteheads' within a plot.

Botrytis%
Should be assessed as the % of stems affected within a plot.

Verticillium (non verticillium trials) %
Should be recorded as the % of stems affected within a plot. If Verticillium stem stripe is observed, please inform the Disease Trials Coordinator at the earliest possible opportunity. You may be asked to do a more in-depth assessment following the separate verticillium stem stripe protocol which assesses verticillium index (for extra payment).

Appendix 3 Inoculated disease tests 2022-2027

Crop	Trial no.	Trial type	Preferred location	Time of sowing	Notes	Reps
AW	855	*Yellow rust	East (south of a line Bristol to the Wash)	Late autumn	Includes supply of inoculum	2
AW	856	*Brown rust	South of England	Late autumn	Includes supply of inoculum	2
SB	935	Net blotch	In high net bloch risk area - justify in tender	Spring	Includes supply of inoculum	2
SB	937	Net blotch	In high net bloch risk area - justify in tender	Spring		2
SB	936	*Brown rust	South of England	Spring	Includes supply of inoculum	2
SB	940	Brown rust	North of England	Spring		2
SB	941	Rhynchosporoium	West/SW/Wales or W. Scotland - Irrigation must be available	Spring		2
SO	938	*Crown rust	East of England	Spring	Includes supply of inoculum	2
SO	939	Crown rust	Southwest of England	Spring		2
WO	901	*Crown Rust	East of England	Autumn	Includes supply of inoculum	2
WO	902	Crown Rust	Southwest of England	Autumn	Includes supply of inoculum	2

WB	880	*Brown rust	South of England	Autumn		2
WB	881	Brown rust	North of England	Autumn	Includes supply of inoculum	2
WB	882	*Net blotch	In high net bloch risk area - justify in tender	Autumn	Includes supply of inoculum	2
WB	883	Net Blotch	In high net bloch risk area - justify in tender	Autumn		2
WB	884	Rhynchosporoium	West/SW/ of England, Wales or West Scotland	Autumn		2
WR	886	*Phoma & light leaf spot 1	East of England and at least 30 miles from Phoma & LLS site 2	Autumn	Includes supply of inoculum	4
WR	887	Phoma & light leaf spot 2	West of England and at least 30 miles from Phoma & LLS site 1	Autumn		4
WR	888	Verticillium		Autumn	Natural infection or inoculation	4
WR	889	Verticillium		Autumn	Natural infection or inoculation	4
WW	823	*Eyespot site 1	At least 30 miles from WW eyespot site 2	Autumn	Includes supply of inoculum	4
WW	824	Eyespot site 2	At least 30 miles from WW eyespot site 1	Autumn		4
WW	825	*Yellow rust trial 1	East of England and at least 30 miles away from WW YR site 2	Autumn	Includes supply of inoculum	2
WW	826	Yellow rust trial 2	East of England and at least 30 miles away from WW YR site 1	Autumn		2

WW	827	*Brown rust	South of the Wash	Autumn	Includes supply of inoculum	2
WW	829	*Fusarium site 1 trial 1	East/south of England	Autumn	Includes supply of inoculum.	3
	830	*Fusarium site 1 trial 2				3
	831	*Fusarium site 1 trial 3				3
WW	832	Fusarium site 2 trial 1	East/south of England	Autumn		3
	833	Fusarium site 2 trial 2				3
	834	Fusarium site 2 trial 3				3

Key: WW=winter wheat, AW=late-sown winter/spring wheat, SW=spring wheat, WB=winter barley, SB=spring barley, WO=winter oats, SO=spring oats, WR=winter oilseed rape.

Primary sites. Contractors bidding for trials marked must be equipped to produce disease inoculum as directed by the protocol and provide this inoculum to non-primary sites.

Appendix 4 – Payments for abandoned or rejected trials

AHDB Recommended List inoculated disease trials			Payment Proportion of:	
Problem	**Reason for failure or loss of data**		**Trial**	**Replicate**
Trials abandoned before disease assessments				
Trial not drilled	Factors outside of the trial operator's control (e.g. weather).		25%	N/a
Trial not drilled	Controllable factors (e.g. missed opportunity to drill)		0%	N/a
Failed to establish	Factors outside of the trial operator's control (e.g. waterlogging where there is no history of any problem).		50%	50%
Failed to establish	Controllable factors (e.g. bird damage).	See note 1	0%	0%
Abandoned mid-season	Excessive OSR volunteers in OSR trial if in an unsuitable rotational position (<5 yrs since OSR crop).	See note 2	25%	25%
Abandoned mid-season	Excessive OSR volunteers in OSR trial if in a suitable rotational position (>5 yrs since OSR crop).		50%	50%
Abandoned mid-season	Excessive cereal volunteers	-	25%	25%
Abandoned mid-season	Factors outside of the trial operator's control (e.g. drought or waterlogging where there is no history of any problem).		50%	50%
Abandoned mid-season	Controllable factors (e.g. excessive weeds or pest damage).	See note 3	25%	25%

Trial completed but no results obtained				
Disease did not establish	Factors outside of the trial operator's control (e.g. drought or high temperatures)		25%	25%
Disease did not establish	Controllable factors (e.g. protocol not followed).	See Note 4	50%	50%
Data unavailable or loss of accuracy	Incorrect treatment mid-season (trial operator or host farm error).		50%	50%
Data unavailable or loss of accuracy	Trial drilled with errors or by not following the plan correctly.	See note 5	TBC	TBC
Data omitted	Factors outside of the trial operator's control (e.g. take all or drought)		100%	100%
Data omitted	Factors under the control of the operator		50%	50%

Note 1 Controllable factors: This assumes that it has been possible to take corrective action, but it has not been done. If the operator has been unable to take action due to (e.g.) weather the payment will be 50%.
Note 2 Unless there has been written prior agreement to place trial on a site that has grown OSR <5 years ago.
Note 3 This includes trials sown in a location that increases the risk of damage (e.g. too close to a wood or other potential sources of animal activity such as a badger sett).
Note 4 If irrigation or misting was specified for this trial in the protocol and this was not applied
Note 5 A decision on payment for this problem can only be determined on a case by case basis, depending on the amount of data or accuracy lost.