AHDB
HORTICULTURE

Grower Summary

FV423a

Onion neck rot: seed infection, pathogens and treatments

Final 2015

Disclaimer

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the AHDB Horticulture office (hort.info.@ahdb.org.uk), quoting your AHDB Horticulture number, alternatively contact AHDB Horticulture at the address below.

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Industry Representative:	Mr Sam Rix
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GROWER SUMMARY

Headline

Around 26% of 2015 commercial bulb onion seed lots were found to be infested with either *B. allii, B. aclada* or both. Both species seem to be equally prevalent.

Background

Neck rot can be a major cause of losses in stored onions in the UK. The extent of losses is variable. Losses of over 50% were reported in the late 60s and early 70s, and more recently losses of up to 40% been reported in individual crops.

The disease can be caused by three different species of *Botrytis*: *B. aclada, B. allii,* and *B. byssoidea. B. byssoidea* is thought to be less important; *B. allii* and *aclada* were previously lumped together as one species, (usually called *B. allii*), hence the vast majority of the literature and reports of the disease during the 20th century refer to neck rot as caused by *B. allii;* we should now interpret these reports as referring to either *B. allii* or *B. aclada* or both. In this project we will refer to *Ba* to represent both/either of the two main neck rot pathogens, *B. allii* and *B. aclada.*

There is no historical information on the relative distribution or significance of these two species, and it is also not known if there are any differences in their biology and epidemiology or sensitivity to fungicides.

The disease is seed-borne but symptoms are not apparent in the field and only develop in store. It is likely that most seed is tested by seed companies, and most seed is treated with fungicides. Nevertheless major losses still occur in some years. These losses could be a result of failure to control seed-borne infection or alternative sources of inoculum.

Until recently, the industry standard seed treatment for neck rot has been HyTL (thiabendazole + thiram) under a Specific Off-Label Approval (SOLA), and emergency approvals, but the registration has now expired. Recent work has shown that Thiram and Maxim and potentially some new products may be effective, but recent studies have so far been limited to direct effects on low levels of apparent seed infection, there have been some contradictory results, and there is no recent information on resistance or on differences between the neck rot pathogens.

It is also possible that some fungicides applied to the growing crop may have an impact by reducing the rate of spread in the field (and so contribute to control of disease in store) but there is little information on this aspect.

The absence of field symptoms means the link between seed-borne infection and storage losses is obscure; seed-to-seedling transmission depends on pathogen loading, and disease in store is further affected by the weather conditions in the growing season and at harvest, therefore there remains some controversy about the importance of seed infection.

There is no formal standardisation of the seed test method used for *Ba* or of the health standard that needs to be achieved. There is also no assessment of inoculum load. Therefore although seed may have been tested/treated what is considered as 'clean' or 'healthy' may differ depending on the source of the seed and the test laboratory, and the methods and standards applied.

Much work on neck rot in the UK was done during the 1970s at Wellesbourne by Robert Maude and colleagues, some MAFF funded work was done at Wellesbourne by the author as part of a project on organic seed production in the early 2000s. More recently there have been some limited HDC studies on seed treatments and a 3 year TSB-funded project on biological seed treatments (Roberts 2013, 2014).

This main aim of the project was to provide an independent assessment of the current prevalence and incidence of the disease in commercially available onion seed in the UK. Specific objectives were to:

1. Determine the prevalence and incidence of *Botrytis* neck rot pathogens on commercially available onion seed.
2. Obtain isolates of *Botrytis* neck rot pathogens from stored bulbs.
3. Identify isolated neck rot Botrytis spp. to species level.
4. Determine the fungicide sensitivity of selected isolates *in vitro.*
5. Determine the efficacy of fungicide seed treatments on apparent seed infection and seed transmission.
6. Determine if there is a difference in seed-to-seedling transmission between the two *Botrytis allii/aclada* species.

Summary

Seed testing

Thirty bulb onion seed lots from six seed companies, and representing all of the most popular varieties were tested for the presence of the neck rot pathogens *Botrytis allii/aclada* (*Ba*) by direct-plating on a selective medium. We detected *Ba* in eight of the thirty seed lots (i.e. 27%). Two of the positive seed lots were untreated. Six of the positive seed lots had been treated with fungicides, and gave negative results when tested 'as received', but were then positive when re-tested after a short (10 min) wash. The percentage infestation levels ranged from 0.5% to 59% (2 and 59% in the untreated lots; 0.5 to 33% in the treated/washed).

In addition we also received and tested ten seed lots pre- and post- physical (steam) treatment. All of the pre-treatment tests were positive and all of the post-treatment tests were negative. As these were specially selected as 'known to be infected' by the seed company concerned they are not included in the above statistics.

Stored bulbs

Three bulb samples with typical neck rot symptoms were received. *Ba* was successfully isolated in each case.

Identification of species from seed and bulbs

Over eighty isolates were sub-cultured from seed-test plates or bulbs for further characterisation. Based on colony characteristics, sporophore and spore morphology, etc. these were reduced to about forty for testing by PCR. Isolates were initially tested using neck rot specific primers that give a positive result with *B. allii, B. aclada* and *B. byssoidea*. The initial PCR results generally confirmed the expectations based on colony morphology.

A further PCR with *Botrytis* specific primers followed by digestion (cutting the DNA at a specific place) was used to separate the *Ba* isolates into *B. allii* and *B. aclada*. The results indicated that both *B. allii* and *B. aclada* are present in UK commercial onion seed. Some seed lots contained only one or the other species, but some seed lots contained both species. Both species were also found in stored bulbs with neck rot; in two samples all isolates were *B. allii*, in one all isolates were *B. aclada*.

DNA extracts were also sent to Wellesbourne for sequencing of an IGS region, the sequencing data confirmed the identification based on PCR/digestion, but has also provided tentative indications of two distinct types within the *B. allii* isolates.

Note that the occurrence of *B. aclada* is not new. Before 2002, both *B. allii* and *B. aclada* were lumped together as one species: *B. allii*, and many strains reported as *B. allii* in the pre-2003 literature have since been re-identified as *B. aclada*. *B. allii* is probably a hybrid of *B. aclada* and *B. byssoidea.*

Fungicide sensitivity

A selection of eleven isolates (six *B. allii* and five *B. aclada*), from different seed lots and from bulbs, were tested for sensitivity to selected seed treatment fungicides. The fungicides were incorporated into the agar medium at the same rate as used for seed treatment. The fungal isolates were inoculated onto the plates as 5 mm diameter agar plugs, and then growth was assessed by measuring the diameter of the resulting growth, if any.

Apron XL (metalaxyl-M) had little effect on any of the isolates. This was expected as metalaxyl-M is not considered to have activity against *Botrytis* spp.

Maxim 480FS (fludioxonil) gave variable results: although the growth and sporulation of all isolates was inhibited compared to the controls, some isolates were completely inhibited, but other isolates eventually grew to the edge of the plates. All of the most fludioxonil-resistant isolates were *B. aclada*.

Thiram gave similar results with all isolates: very limited growth, but not completely inhibited.

Table 1. Effect of chemical fungicides incorporated into the agar medium on the growth rate of neck rot isolates.

Isolate	Year	Source	Lot	Species	Radial growth rate (mm/day)			
					Control	Apron	Maxim	Thiram
8336	2003	seed	n/a	*B. aclada*	8.0	5.0	3.1	0.6
9736	2015	seed	2054W	*B. aclada*	8.0	5.0	0.4	0.5
9738	2015	seed	2056W	*B. aclada*	8.0	5.4	2.4	0.4
9752	2015	bulb	2105	*B. aclada*	8.5	5.0	1.0	0.5
9744a	2015	seed	2058W	*B. aclada*	8.5	4.6	1.1	0.4
9737	2015	seed	2054W	*B. allii*	8.0	5.0	0.5	0.5
9745	2015	seed	2058W	*B. allii*	7.0	5.0	0.4	0.5
9749	2015	seed	2099	*B. allii*	7.5	5.0	0.4	0.5
9754	2015	bulb	2016	*B. allii*	8.0	5.0	0.4	0.5
9757	<1972	bulb	n/a	*B. allii*	7.8	5.3	0.4	0.6
9722B	2015	bulb	2037	*B. allii*	8.0	5.0	0.6	0.5

Seed treatments

Four isolates, two *B. allii* and two *B. aclada* were used to inoculate untreated bulb onion seed. Seed was then treated with either fungicides or biological treatments at recommended rates, or hot water. The hot-water and fungicide treated seed was then tested by direct-plating on selective medium. [Note there is no value in direct-plating of seed treated with biologicals as the applied microbes are either inhibited by the medium (bacteria) or overgrow the plates (fungi)]. The inoculated-treated seed was also sown in modules in the glasshouse to examine seed-to-seedling transmission. Seedlings were then harvested 3-4 weeks after sowing to check for transmission.

The results indicated:

Apron XL is ineffective against *B. allii/aclada* (as expected).

Maxim 480 FS is inhibitory but not eradicating and gave significant reductions with all four isolates, but notably was less effective with one of the *B. allii* isolates (9737).

Thiram is inhibitory but not eradicating, and results were less consistent than Maxim. It was less effective than Maxim in direct plating tests and similar for three out of the four isolates in transmission tests. One of the *B. allii* isolates (9737) was not controlled.

Hot water was the most effective treatment for three of the four isolates in the direct plating assays and although it appeared to fail for one isolate, inoculum loading was visually reduced. Hot water gave significant reduction for all isolates in the transmission tests.

The two bacterial BCAs (HDC195, HDC196) were overall less effective and varied depending on the *Ba* isolate. The fungal BCA (HDC194) was almost as effective as the chemical fungicides.

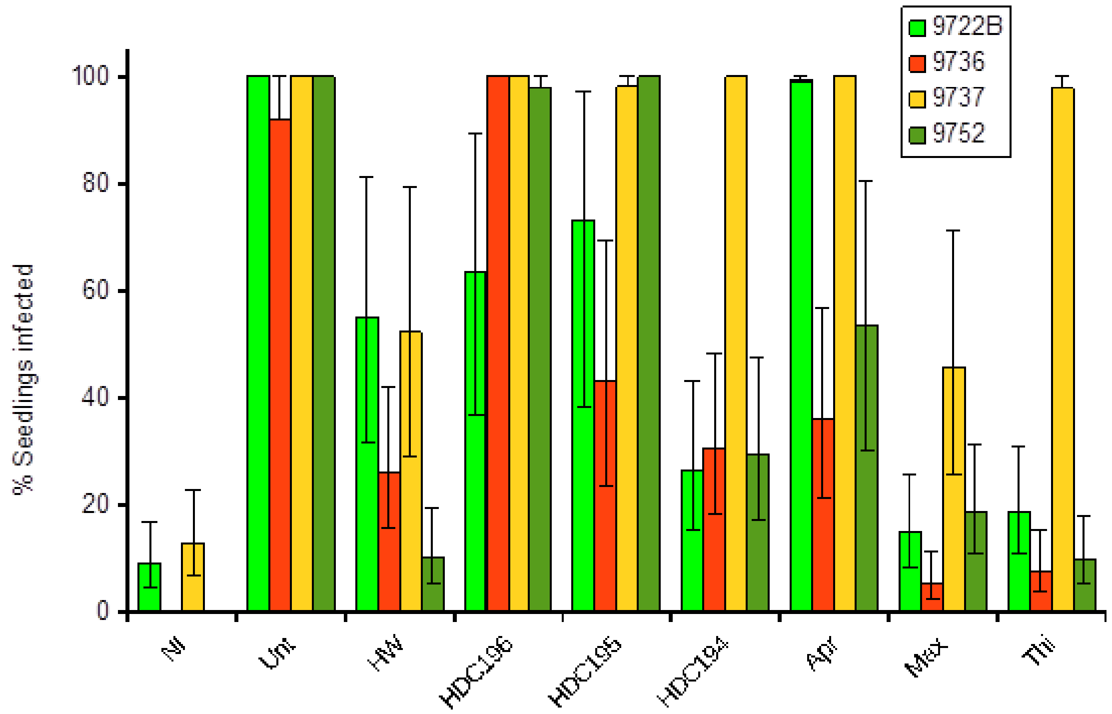

Figure 1. Effect of seed treatments on the % onion seedlings infected with four different neck rot isolates: *B. allii* (9722B, 9737); *B. aclada* (9737, 9752). Error bars represent the 95% confidence limits.

Dose-response assays

Unfortunately the dose response assays to compare transmission between the two species provided little information, as the range of doses used was insufficient. However, at the time of sampling (3-4 weeks after sowing) there was evidence that secondary spread had already occurred with both *B. allii* isolates but not with the *B. aclada* isolates.

Financial Benefits

There are no clear direct financial benefits from this project as it sought to provide background data to explain variability in control and disease outbreaks. Based on average loses of around 10% p.a. direct financial losses from neck rot are estimated at around £7.5 million p.a.

Action Points

- Do not assume that fungicide-treated onion seed is free from neck rot pathogens.
- Do not assume that chemical fungicide treatments will be fully effective against neck rot.
- Request information from seed suppliers on their policies and steps taken to minimise the risk from neck rot: e.g. do they test all seed lots; do they reject seed lots with high levels; do they apply a physical treatment; etc.
- Further work is needed to understand possible differences in the epidemiology of the two pathogens and the impact on control options.
- Further work is needed to understand field spread and the impact, timing and efficacy of field-applied fungicides.
- The manufacturer/supplier of the experimental BCA HDC 194 should be encouraged to bring the product to market as a seed treatment.