Project title:	Swede: Control of Phoma dry rot
Project number:	FV 431
Project leader:	Dr Faye Ritchie, ADAS UK Ltd
Report:	Final Report, September 2015
Previous report:	n/a
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Date project commenced:	1 May 2014
Date project completed (or expected completion date):	(30 September 2015)

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Grower Summary

Headline

This study has demonstrated that root infection in swede can occur in the presence and absence of leaf symptoms. It was also shown that swede can be infected by the two Phoma species pathogenic to oilseed rape in the UK. Both findings increase our understanding of the disease cycle in swede and can be used to identify suitable control strategies for future testing.

Background

Phoma dry rot caused by the fungal pathogen *Leptosphaeria maculans* (imperfect stage *Phoma lingam*) is a major problem on swedes worldwide. Severe losses can occur in the UK from late autumn onwards in the field and in the stored crop. Most of the recent research on the epidemiology and control of *L. maculans* has been on Phoma stem canker in winter oilseed rape. This provides a substantial background on the disease cycle, infection requirements and variability (races) of this pathogen. In addition to *L. maculans* there is another species *Leptosphaeria biglobosa* that is considered less damaging on oilseed rape but also has *Phoma lingam* as the imperfect stage (known as Phoma B).

The development of Phoma in swedes has been difficult to interpret as leaf symptoms have not always been noticed in crops that developed dry rot problems. Swedes are sown in May which is usually after the period when oilseed rape stubbles release ascospores so young swedes should show little leaf Phoma infection. Swedes could be infected by ascospores during the autumn but passage to the bulb through leaf petioles could take several weeks when leaves are large. The swede bulb is a large structure so there may be direct infection by air-borne ascospores or splash-dispersed pycnidiospores. In oilseed rape, pycnidiospores are not considered to be important under UK conditions. Damage to the leaves or roots could increase the risks of Phoma infection particularly by pycnidiospores.

The aim of the work reported here was to improve control of dry rot in swedes through a better understanding of the disease cycle in this crop. Specific objectives were:

1. Improve understanding of the disease cycle in swedes;
2. Identify key timings for infection and disease development;
3. Compare findings on swede with existing knowledge on oilseed rape.

Summary

Objective 1 – Phoma disease cycle in swede

The progress of Phoma in four swede crops showed differences in disease development depending on geographical location (Table A). At Spalding in Lincolnshire, Phoma leaf spot was first observed on 17 July 2014. Incidence remained low until 22 October when it increased to 18% plants affected. The epidemic continued to develop until 94% of plants were affected on 11 February 2015. In Fife and Perthshire, no Phoma leaf symptoms were reported during the monitoring period, however, fungicides had been applied at these sites.

The Fife site received three fungicide applications, the first on 28 July 2014 [Azoxystrobin 1.0 L/ha (as Amistar: Syngenta Crop Protection)], the second on 12 September [Prothioconazole 0.4 L/ha (as Rudis: Bayer CropScience)] and the third on 30 September [Prothioconazole 0.4 L/ha (as Rudis: Bayer CropScience)]. First symptoms of dry rot on roots in the field were observed on 30 June, prior to first fungicide applications. Dry rot incidence increased to 15% by 4 August. At harvest on 28 November, disease incidence increased to 33%.

In Perth, no phoma leaf spot or dry rot symptoms were reported during crop growth. Dry rot was only reported when the crop was harvested on 20 October and 75% of roots had dry rot on this date. This crop received three fungicide applications, the first on 7 July 2014 [Azoxystrobin 1.0 L/ha (as Amistar: Syngenta Crop Protection)], the second on 4 August [Prothioconazole 0.4 L/ha (as Rudis: Bayer CropScience)] and the third on 27 August [Azoxystrobin 1.0 L/ha (as Amistar: Syngenta Crop Protection)].

This suggests that fungicide timings at these sites were suitable for controlling leaf infection but not for root infection. These observations can be used to target future work and establish optimum fungicide timings for disease control.

Table A. Summary of Phoma leaf spot and dry rot occurrence in four swede crops, cv. Magres, in 2014-15

Site	Date sown	Leaf spot		Dry rot	
		First noted	Maximum incidence (date)	First noted	Maximum incidence (date assess)
1. Lincs	12 May 2014	17 July	94% (11/2/15)	12 Aug	17% (8/12/14)
2. N. Yorks	16 May 2014	3 Dec	44% (3/12/14)	Nil	0
3. Fife	29 May 2014	-	0	30 Jun	30% (28/11/14)
4. Perthshire	15 May 2014	-	0	20 Oct	75% (20/10/14)

Objective 2 – Key timings for infection and disease development

Root and leaf inoculation experiments on swede cv. Magres demonstrated for the first time that both *L. maculans* and *L. biglobosa*, the two species responsible for causing stem canker in oilseed rape are also responsible for causing leaf lesions and dry rot on swede (Table B). In the UK, air-borne ascospores are the predominant source of infection for oilseed rape and first symptoms of naturally occurring leaf lesions in swede pot experiments at ADAS Boxworth on 10 October coincided with first observations in oilseed rape on 22 October 2014 (Figure B).

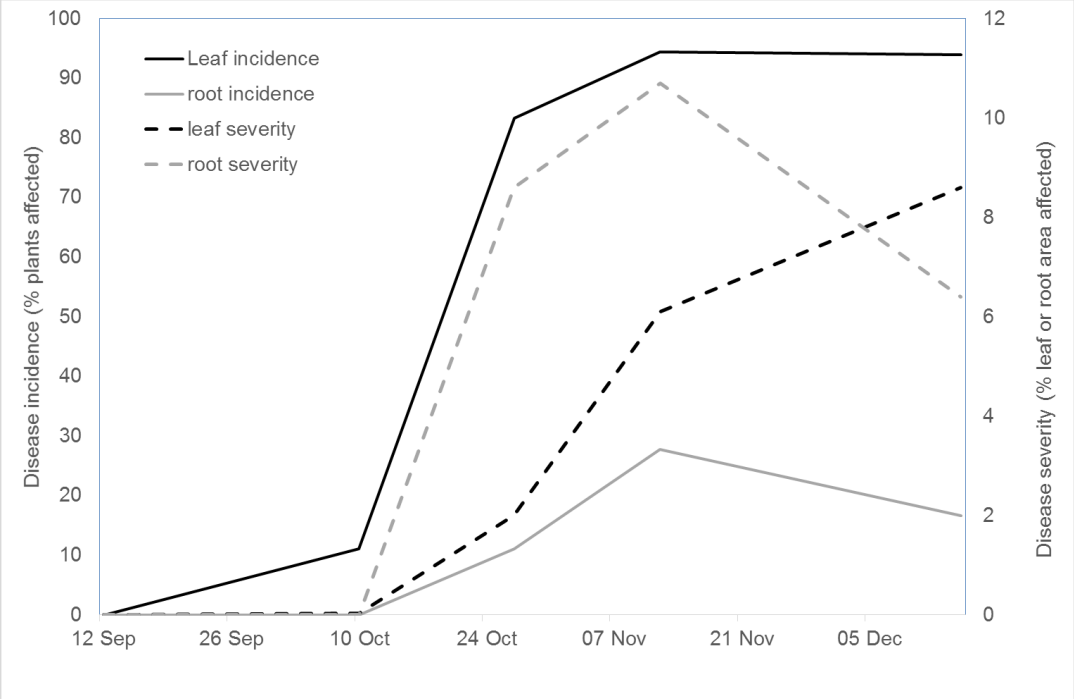


Figure B. Progress of natural Phoma leaf infection and root infection on uninoculated swede in the pot experiment at ADAS Boxworth. Disease was assessed until the final non-destructive root assessment on 12 December 2014. No fungicides were applied.

This study confirmed that both Phoma species (*L. maculans* and *L. biglobosa*) present in the UK infect swede (Table B) and therefore measures to decrease the exposure of crops to ascospores in the autumn would be beneficial to decreasing crop risk e.g. through targeted fungicide applications. Where roots were inoculated, there was a significant effect of wounding on the incidence of roots affected and also the severity of internal dry rot symptoms (Table C), however, whether wounding is a key requirement for dry rot development in field has yet to be proven.

Table B. Infectivity of different spore types of *L. maculans, L. biglobosa* and naturally occurring ascospores on swede leaves on 12 November, 15 days after inoculation.

Trt	Species and spore type	Wounding (Yes/No)	Incidence (% plants with lesion)	Lesion severity (% leaf area affected)
1.	No inoculation	Yes	0.0	0.0
2.	No inoculation	No	0.0	0.0
3.	*L. maculans* (pycnidiospores)	Yes	16.7	0.2
4.	*L. maculans* (pycnidiospores)	No	14.3	0.4
5.	*L. biglobosa* (pycnidiospores)	Yes	11.1	0.7
6.	*L. biglobosa* (pycnidiospores)	No	25.0	0.2
7.	Ascospores	Yes	0.0	0.0
8.	Ascospores	No	40.0	0.6

Table C. Incidence of dry rot on inoculated swede roots, with and without wounding prior to inoculation.

Isolate/spore type	With wounding	Without wounding
No inoculation	11.3 ± 10.6	0.0 ± 0.0
L. maculans	33.7 ± 8.5	8.3 ± 5.1
L. biglobosa	0.0 ± 0.0	11.1 ± 5.8
Ascospores	7.9 ± 5.2	18.1 ± 8.6
FPr	0.017	

A range of symptoms were observed in field and pot experiments and are shown in Figure C.

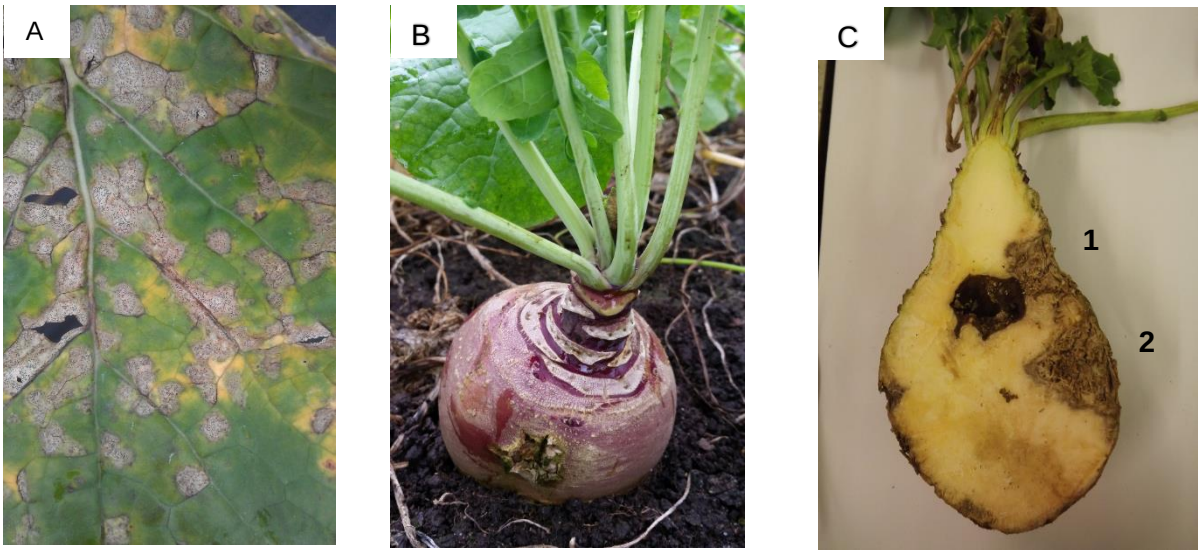


Figure C. Phoma leaf spotting on leaves showing chlorosis of the leaves associated with lesion formation (A) and the extent of leaf damage with the movement of the fungus through the leaf veins on 12 November 2014 in the pot experiment at Boxworth. (B) Dry rot observed on field grown swedes on 27 October 2014 in Scotland (Photo: Tracy Yoxall, SRUC). Cross section of swede (C) showing two lesions originating from different infections: near the upper root following inoculation (1) and the soil surface (2) from the pot experiment at Boxworth.

Objective 3 – Comparison of Phoma on swede and oilseed rape

In oilseed rape, lesions produced on the leaves are responsible for causing stem cankers as the fungus grows through the leaf veins and down through the petiole to the stem. The appearance of symptoms in the stem can take over 6 months and severity is dependent on the timing of the onset of the epidemic in the autumn and subsequent temperatures. In contrast, dry rot on swede was observed within one month of leaf infection or in the absence of leaf infection at monitoring sites. This differed from oilseed rape as there was less time between the appearance of leaf and root symptoms on swede. Rainfall in August and September has been shown to be a key factor in determining ascospore release, with more rain resulting in an earlier release date. Earlier epidemics are known to be more damaging and the Phoma epidemic on oilseed rape in autumn 2014 was late, with first symptoms in oilseed rape observed on 22 October. The most severe Phoma epidemics occur in the south in the UK and this has been attributed to increasing winter/spring temperatures coupled with higher average temperatures. Leaf Phoma and dry rot symptoms observed in swede coincided with when Phoma leaf symptoms were observed in oilseed rape, suggesting that fungicides could be better targeted in the autumn for disease control on swede.

Conclusions

- The two Phoma species pathogenic to oilseed rape present in the UK (*L. maculans* and *L. biglobosa*) can cause both Phoma leaf spotting and dry rot on swede;
- Ascospores can initiate epidemics on swede in the UK;
- Inoculation experiments showed that both ascospores and pycnidiospores can cause Phoma leaf spot and dry rot on swede;
- Leaf infection was not always observed with dry rot on swede;
- Wounding of the root appeared to increase the incidence of roots affected as well as increase the severity of internal dry rot symptoms.

Financial Benefits

This project has identified key parts of the lifecycle at which the casual pathogen of dry rot could be targeted.

In oilseed rape the fungicides to control the disease are targeted in response to a leaf threshold of 10 to 20% plants affected. In the field monitoring part of this project, the presence of leaf infection did not always seem to be necessary for the development of dry rot symptoms at all sites. In the pot experiment, the onset root infection occurred at a similar time in all treatments. Both observations suggest that there may be specific weather criteria that increases the risk of root infection, however, this would require further investigation to confirm.

It has also highlighted that both Phoma species present on oilseed rape in the UK (*L. biglobosa* and *L. maculans*) can infect swede roots and leaves. This has significance for disease control as *L. biglobosa* is often associated with Phoma development later in the season than *L. maculans* and it is critical that strategies to monitor and control the disease are effective on both species. The next step will be to test whether fungicides can be better targeted, either in response to weather criteria or a leaf threshold, to reduce future losses.

Action Points

- Avoid growing swedes in close proximity to fields where oilseed rape was grown the previous season.
- Bury crop debris from previous oilseed and swede crops to minimise production of air-borne spores.

Recommendations for future work

This project has identified three areas to investigate to improve the control of dry rot in swede:

1. Identify weather variables affecting dry rot development, whether those criteria are related to timing of spore release and can be used to predict crop risk;
2. Improve the targeting of fungicide programmes for dry rot control using weather based risk criteria or fungicide application in relation to a leaf disease threshold;
3. Evaluate the efficacy of fungicides currently used to control Phoma in oilseed rape against dry rot on swede.

Science Section

Introduction

Phoma dry rot caused by the fungal pathogen *Leptosphaeria maculans* (imperfect stage *Phoma lingam*) is a major problem on swedes worldwide (Koike *et al*., 2007). Severe losses can occur in the UK from late autumn onwards in the field and in the stored crop.

Most of the recent research on the epidemiology and control of *L. maculans* has been on Phoma stem canker in winter oilseed rape. This provides a substantial background on the disease cycle, infection requirements and variability (races) of this pathogen. In addition to *L. maculans* there is another species *Leptosphaeria biglobosa* that is considered less damaging but also has *Phoma lingam* as the imperfect stage (known as Phoma B) (Stonard *et al*., 2009). These developments make it timely to re-examine and evaluate the historic references to swede dry rot (e.g. Butler and Jones, 1949).

The Phoma cycle in winter oilseed rape is initiated by air-borne ascospores produced on winter oilseed rape stubbles that produce Phoma leaf spots. The fungus then grows symptomlessly within the leaf petiole to reach the stem where stem cankers appear about six months later. The onset and duration of air-borne spore production has been investigated and is dependent on rainfall after the oilseed rape has been harvested. Early epidemics occur in seasons with high rainfall in August and September and late epidemics occur in dry autumns (Huang *et al*., 2005; Salam *et al*., 2007). Winter temperatures influence the development and appearance of cankers as they develop slowly when temperatures are low (Evans *et al*., 2008).

The development of Phoma in swedes has been difficult to interpret as leaf symptoms have not always been noticed in crops that developed dry rot problems. Swedes are sown in May which is usually after the period when oilseed rape stubbles release ascospores so young swedes should show little leaf Phoma infection (assuming that seed is healthy). Swedes could be infected by ascospores during the autumn but passage to the bulb through leaf petioles could take several weeks when leaves are large. The swede bulb is a large structure so there may be direct infection by air-borne ascospores or splash-dispersed pycnidiospores from Phoma leaf spots on swede. In oilseed rape, pycnidiospores are not considered to be important under UK conditions. Damage to the leaves or roots could increase the risks of Phoma infection particularly by pycnidiospores.

The expansion of oilseed rape during the last 40 years provides a new source of inoculum for swede crops. In Scotland, Phoma canker is a minor disease in winter oilseed rape though leaf symptoms can be commonly found. Low temperatures prevent the leaf spots forming

cankers although this risk may increase in future if temperatures rise (Evans *et al*., 2008). The disease cycle on swedes may therefore differ between oilseed rape and non-oilseed rape growing areas and between England and Scotland.

Control of swede dry rot relies on the use of healthy seed, rotations with non-brassica crops, burial or incorporation of previous crop residues and avoiding areas close to oilseed rape (HDC Factsheet 17/12, Phoma on vegetable brassicas). There are differences in varietal susceptibility, though production is centred on the susceptible cv. Magres. Growers have developed their own fungicide spray regimes using products based on tebuconazole, flusilazole and prothioconazole. There are now fewer fungicide options as flusilazole was withdrawn from sale from 12 October 2013 and could no longer be used after 12 October 2014. Fungicide performance data are available for Phoma canker control in winter oilseed rape and could be exploited to identify new products for use on swedes (Gladders *et al*., 2009).

The aim of the project was to improve understanding and control of dry rot in swedes with the objectives as follows:

1. To improve understanding of the disease cycle in swedes;
2. To use artificial inoculation to identify key timings for infection and disease development;
3. To prepare a final report that includes comparison with existing knowledge from oilseed rape.

To address these objectives, the importance of plant damage, the contribution of pycnidiospores and ascospores to the epidemic and timing and importance of leaf infection and secondary spread were investigated.

Materials and methods

There were three parts to this study – in-field crop monitoring and pot-based leaf and root inoculation experiments. Details of the methodology used to produce ascospores and pycnidiospores for the pot experiments are also included in this section.

Crop monitoring

The incidence and severity of symptoms on leaves and roots was monitored at monthly intervals in four swede crops. Two sites were located in Scotland and two sites in England. All sites were monitored from May 2014 to harvest. Records were taken on 100 plants (as 4 transects of 25 plants) for both leaf and root symptoms. Both the incidence (as the percentage of plants affected with foliar or root symptoms) and severity (as the percentage

leaf or root area affected per plant) were determined. Prior to harvest, dry rot was assessed on the visible root, at or above soil level. At harvest, roots were washed and dry rot was assessed on the whole root surface. Details of all sites are shown in Table 1. One hundred roots (25 from each transect) were stored for 1 month following harvest and assessed for the incidence and severity of dry rot assessed as described previously with the exception of the site in Spalding as cabbage root fly damage was too severe to allow long-term storage. All roots from commercial crops were sampled when the grower was harvesting the crop.

Table 1. Details for disease monitoring sites 2014/2015

Site	Location	Sowing date	Harvest date	Variety
1	Spalding, Peterborough	12 May 2014	8 December 2014	Magres
2	Selby, North Yorkshire	16 May 2014	3 December 2014	Magres
3	Perth, Scotland	15 May 2014	20 October 2014	Magres
4	Fife, Scotland	29 May 2014	28 November 2014	Magres

Inoculum production

The method used in this study for producing ascospores for inoculation is based on a method described by Huang *et al*., 2003. Stubbles with severe stem cankers from winter oilseed rape (cv. Catana) harvested on 18 July 2014 at ADAS Boxworth were collected. Stubbles were washed, cut into 30cm pieces and placed into a tray lined with a hessian sack with drainage holes in the base. The tray containing this stem debris was placed outside and watered daily (in the absence of rainfall) to encourage the production of psuedothecia. Once pseudothecia were produced, stem samples were removed and a random sample checked to ensure mature spores were present. Once confirmed, stem pieces were stored at -15˚C until required. When required for experiments, stem pieces were attached to the underside of a petri dish using petroleum jelly, sprayed with sterile distilled water until run off and incubated at 20˚C to encourage ascospore release. After 3 hours, the spore solution was checked for the presence of ascospores and adjusted to 1 x 10^4 ascospores/ml sterile distilled water. As ascospores were derived from stem cankers, it is likely that the species was *L.* maculans, however, the ratio of *L. maculans* and *L. biglobosa* derived from natural ascospores was not determined.

Pycnidiospores were produced in agar culture from both *L. maculans* (= Phoma A) and *L. biglobosa* (= Phoma B) using single spore isolate cultures supplied by Dr Yongju Huang (University of Hertfordshire). Both *L. maculans* and *L. biglobosa* cultures originated from

oilseed rape. Five mm discs were taken from the growing colony, plated onto V8 agar and grown at 20˚C (12h light/12h dark cycle) for 14 days. Prior to use for inoculation, plates were flooded with sterile distilled water and the surface of the plate rubbed gently with a glass rod to detach the pycnidiospores. The solution was filtered through 2 layers of muslin and the concentration adjusted to 1 x 10^7 pycnidiospores/ml sterile distilled water.

Plants for both experiments were grown in pots outside. Two hundred and thirty 5 litre pots (plus 20 spare pots) were filled with sterilized loam soil [Rothamsted weed mix (80% kettering loam + 20% grit + 2 kg osmacote slow release fertiliser] on 13 May 2014 and allowed to settle overnight. Soil was watered thoroughly the following day and 24 hours prior to sowing. Six swede seeds of cv. Magres were sown at 1 cm depth on 20 May. On 23 June, pots were thinned to three plants per pot then to two plants per plot on 9 July.

Leaf inoculation experiment

The purpose of this experiment was to determine whether naturally occurring ascospores and pycnidiospores of *L. maculans* and *L. biglobosa* infected swede, whether leaf infection caused dry rot on the root and whether timing of infection influenced dry rot development. The trial was laid out in a randomised block design with 5 replicates and 26 treatments (

Table **2**). Treatments consisted of two completely untreated controls two known isolates of *L. maculans* and *L. biglobosa*, and naturally occurring ascospores each with and without leaf wounding. Four inoculation dates, spaced 4 weeks apart, were included. Two plants were included in each replicate.

The last fully emerged leaf was selected at each inoculation timing and tagged by tying a piece of string around the base of the leaf. Where appropriate, the target leaf was wounded using sharp ended forceps towards the petiole end of the leaf. The tip of the forceps was opened and pressed in to the leaf surface to produce 2 wounds per leaf immediately prior to inoculation. One hour prior to inoculation, all plants were watered thoroughly. Ascospore and pycnidiospore suspensions were produced as described previously. Depending on the treatment being applied, a 10 µl drop of ascospore suspension (1 x 10^4 spores/ml) or pycnidiospore suspension (1 x 10^6 spores/ml) of *Leptosphaeria maculans* (Phoma A - *Phoma lingam* stage) or *Leptosphaeria biglobosa* (Phoma B – *Phoma lingam* stage), was placed at the inoculation site (following wounding as appropriate) taking care to avoid the droplet rolling off the leaf. After inoculation, the plants were covered in polythene bag which did not touch the plants but was sufficient to maintain high humidity and leaf wetness for 72h. After this, the polythene bags were removed.

It was not possible to store the roots following the experiment due to damage from cabbage root fly which had caused many of the roots to start to rot, therefore the harvest date was brought forward to 13 January 2015 and roots were assessed immediately before being disposed of.

Table 2. Phoma inoculation treatments applied to swede leaves – 2014.

Trt	Isolate/spore type	Wounding (Yes/No)	Inoculation date
1	No inoculation	Yes	-
2	No inoculation	No	-
3	*L. maculans* (pycnidiospores)	Yes	24 July 2014
4	*L. maculans* (pycnidiospores)	No	24 July 2014
5	*L. biglobosa* (pycnidiospores)	Yes	24 July 2014
6	*L. biglobosa* (pycnidiospores)	No	24 July 2014
7	Ascospores	Yes	24 July 2014
8	Ascospores	No	24 July 2014
9	*L. maculans* (pycnidiospores)	Yes	28 August 2014
10	*L. maculans* (pycnidiospores)	No	28 August 2014
11	*L. biglobosa* (pycnidiospores)	Yes	28 August 2014
12	*L. biglobosa* (pycnidiospores)	No	28 August 2014
13	Ascospores	Yes	28 August 2014
14	Ascospores	No	28 August 2014
15	*L. maculans* (pycnidiospores)	Yes	30 September 2014
16	*L. maculans* (pycnidiospores)	No	30 September 2014
17	*L. biglobosa* (pycnidiospores)	Yes	30 September 2014
18	*L. biglobosa* (pycnidiospores)	No	30 September 2014
19	Ascospores	Yes	30 September 2014
20	Ascospores	No	30 September 2014
21	*L. maculans* (pycnidiospores)	Yes	27 October 2014

22	*L. maculans* (pycnidiospores)	No	27 October 2014
23	*L. biglobosa* (pycnidiospores)	Yes	27 October 2014
24	*L. biglobosa* (pycnidiospores)	No	27 October 2014
25	Ascospores	Yes	27 October 2014
26	Ascospores	No	27 October 2014

The incidence (as the percentage of plants affected with foliar or root symptoms) and severity (as the percentage leaf/root area affected per plant) was assessed prior to the first inoculation. As leaf lesions were generally slow to develop, plants were assessed fully a month after each inoculation. The inoculated leaf and all leaves were assessed and scored separately where natural infection was found. Photographs of symptoms were taken as appropriate.

Root inoculation experiment

The purpose of this experiment was to determine whether dry rot can develop from spores applied directly to the roots and whether the timing of the infection affects the development of dry rot. The trial was laid out in a randomised block design with 5 replicates and 20 treatments (Table 3). Treatments consisted of two completely untreated controls, two known isolates of *L. maculans* and *L. biglobosa*, and naturally occurring ascospores each with and without leaf wounding. Three inoculation dates were included. Two plants were included in each replicate.

Table 3. Phoma inoculation treatments applied to swede roots – 2014.

Trt	Isolate/spore type	Wounding (Yes/No)	Timing
1	No inoculation	Yes	-
2	No inoculation	No	-
3	*L. maculans* (pycnidiospores)	Yes	28 August 2014
4	*L. maculans* (pycnidiospores)	No	28 August 2014
5	*L. biglobosa* (pycnidiospores)	Yes	28 August 2014
6	*L. biglobosa* (pycnidiospores)	No	28 August 2014
7	Ascospores	Yes	28 August 2014
8	Ascospores	No	28 August 2014
9	*L. maculans* (pycnidiospores)	Yes	30 September 2014
10	*L. maculans* (pycnidiospores)	No	30 September 2014
11	*L. biglobosa* (pycnidiospores)	Yes	30 September 2014
12	*L. biglobosa* (pycnidiospores)	No	30 September 2014
13	Ascospores	Yes	30 September 2014
14	Ascospores	No	30 September 2014
15	*L. maculans* (pycnidiospores)	Yes	27 October 2014
16	*L. maculans* (pycnidiospores)	No	27 October 2014
17	*L. biglobosa* (pycnidiospores)	Yes	27 October 2014
18	*L. biglobosa* (pycnidiospores)	No	27 October 2014
19	Ascospores	Yes	27 October 2014
20	Ascospores	No	27 October 2014

One hour prior to inoculation, all plants were watered thoroughly. An area on the upper part of the root, just below the leaf scars, was selected to be inoculated on each plant on each inoculation date. Where appropriate, the root was wounded using sharp ended forceps. The

tip of the forceps was opened and pressed in to the root surface which broke the surface to produce 2 wounds per root immediately prior to inoculation. Ascospore and pycnidiospore suspensions were produced as described previously for the leaf inoculation experiment. Depending on the treatment being applied, a 10 µl drop of ascospore suspension (1 x 10^4 ascospores/ml) or pycnidiospore suspension (1 x 10^6 pycnidiospores/ml) of *L. maculans* (Phoma A - *Phoma lingam* stage) or *L. biglobosa* (Phoma B – *Phoma lingam* stage), was placed at the inoculation site (wounded or unwounded as appropriate) taking care to avoid the droplet rolling off the root. After inoculation, the plants were covered in polythene bag which does not touch the plants but is sufficient to maintain high humidity for 72h. After this time, the polythene bags were removed.

It was not possible to store the roots successfully following this experiment due to severe damage from cabbage root fly and the resultant rotting of many of the roots, therefore the harvest date was brought forward to 13 January 2015 and roots were assessed immediately.

The incidence (as the percentage of plants affected with foliar or root symptoms) and severity (as the percentage leaf/root area affected per plant) was assessed on roots prior to inoculation. The experiment was inspected weekly for root infection (at or above soil level) after each inoculation, with a full disease assessment carried out once lesions were visible. After this, the experiment was assessed monthly. It was difficult to identify the success of root inoculation as naturally occurring lesions (those not associated with the inoculation site) became visible at a similar time to when inoculated lesions became visible. Attempts were made to separate these lesions to determine the success of the inoculation method but this was not always possible. Photographs of symptoms were taken as appropriate.

Nutrition, pest and weed control

On 9 July, cabbage root fly and cabbage stem flea beetle were observed in the trial. Products and rates applied to control both pests as well as other trial nutritional inputs are shown in Appendix 1.

Statistical analysis

Due to plant losses as a result of cabbage root fly, 38 plants from the leaf inoculation experiment and 46 plants from the root inoculation experiment were unable to be included in the final statistical analysis of data. As incidence data were proportional, a logit regression was applied to data, with significance determined using analysis of deviance and the standard error calculated for all treatments. All severity data were analysed as an unbalanced analysis of variance with the least significant difference used to compare treatments at the 5% level.

Results

Crop monitoring: England (North Yorkshire and Lincolnshire) and Scotland (Fife and Perth)

At Spalding in Lincolnshire, Phoma leaf spot was first observed on 17 July (Figure 1). Incidence of Phoma leaf spot on leaves remained low until 22 October when it increased to 18% plants affected. The epidemic continued to develop until 94% of plants were affected by Phoma leaf spot on 11 February 2015. An early attack of cabbage root fly meant that 35 out of the 100 plants originally tagged for assessment were lost prior to the final harvest assessment and roots were rotting extensively by 12 January making field assessments difficult to do. As a result, Phoma dry rot incidence was only assessed up until 8 December when roots were destructively sampled and no roots were stored. First dry rot symptoms were evident on 12 August, with incidence increasing to 15% by 22 October. In the following two months, there was only a small increase in incidence to 17% observed when assessed on 8 December.

At Selby, no Phoma leaf spot was observed until 3 December when 44% of plants were reported to have symptoms (Figure 2). No dry rot was observed after 6 weeks storage, with only a general report of minor cabbage root fly damage on the surface of several roots.

In Fife, no Phoma leaf symptoms were reported during the monitoring period. This crop received three fungicide applications, the first on 28 July 2014 [Azoxystrobin 1.0 L/ha (as Amistar: Syngenta Crop Protection)], the second on 12 September [Prothioconazole 0.4 L/ha (as Rudis: Bayer CropScience)] and the third on 30 September [Prothioconazole 0.4 L/ha (as Rudis: Bayer CropScience)]. First symptoms of dry rot on roots in the field was observed on 30 June, with incidence increasing to 15% by 4 August (Figure 3). At harvest on 28 November, the whole root was assessed and disease incidence increased to 33%. Assessing roots during storage proved challenging as softening of the root meant the skin shrivelled, hiding previously observed symptoms. Incidence during storage was variable and there was no clear increase in dry rot incidence, although severity of symptoms increased slightly.

In Perth, no Phoma leaf spot or dry rot symptoms were observed in the field prior to harvest on 20 October, when 75% of roots were reported to be affected (Figure 4). This crop received three fungicide applications, the first on 7 July 2014 [Azoxystrobin 1.0 L/ha (as Amistar: Syngenta Crop Protection)], the second on 4 August [Prothioconazole 0.4 L/ha (as Rudis: Bayer CropScience)] and the third on 27 August [Azoxystrobin 1.0 L/ha (as Amistar: Syngenta Crop Protection)]. No further increases in dry rot incidence or severity were noted during storage, although there were issues with roots softening and associated difficulties with assessing as described previously for the Fife site.

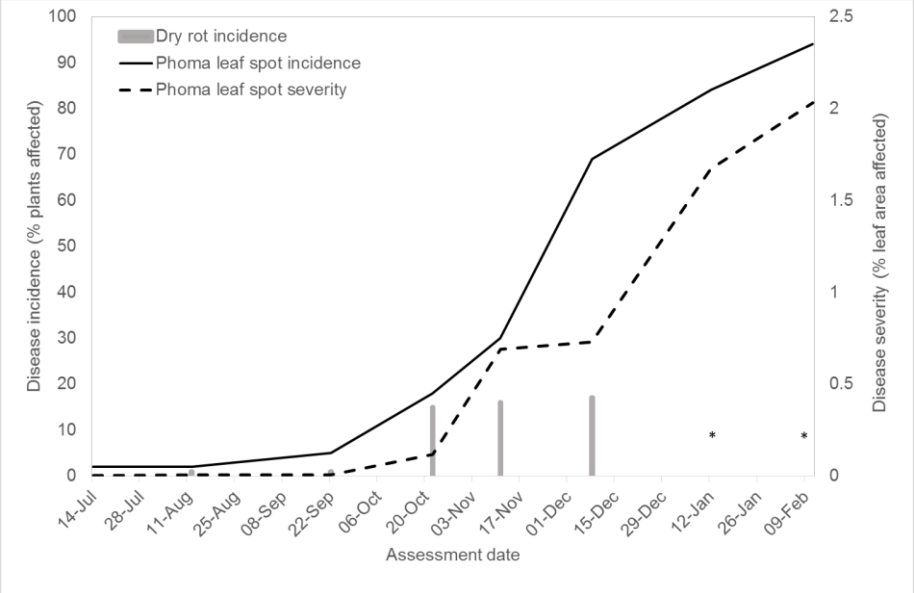


Figure 1. Progress of natural Phoma leaf spotting on swede in Spalding, Lincolnshire as the incidence (percentage of plants affected) and severity (percentage leaf area affected), and dry rot on roots, as the incidence (percentage of plants affected). *no dry rot assessment carried out. No fungicides were applied at this site.

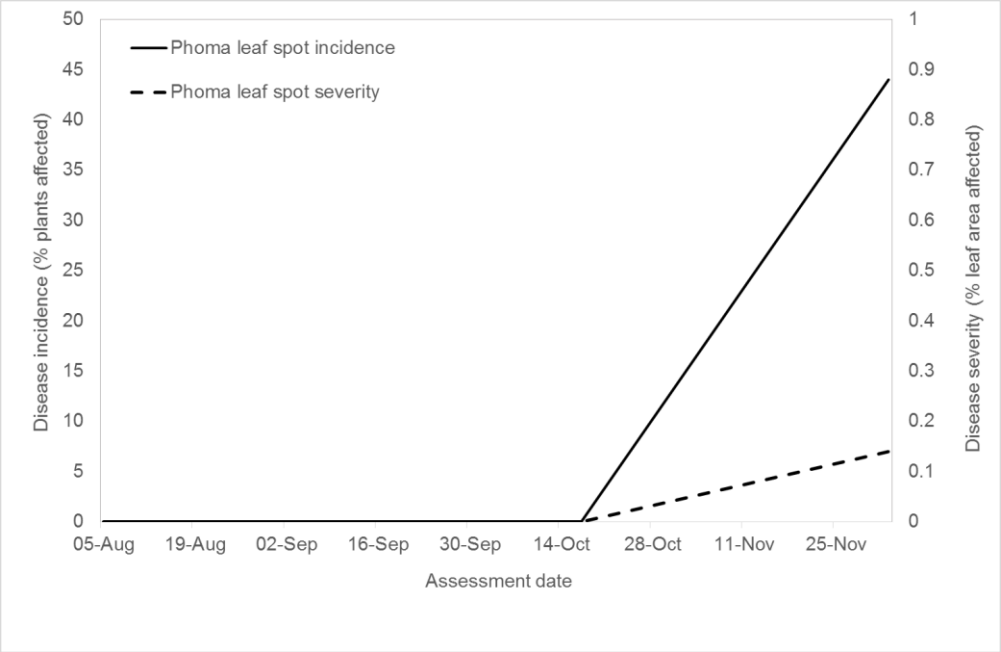


Figure 2. Progress of natural Phoma leaf spotting on swede at Selby, North Yorkshire, as the incidence (percentage of plants affected) and severity (percentage leaf area affected). No dry rot symptoms on roots were reported at this site. No fungicides were applied at this site.

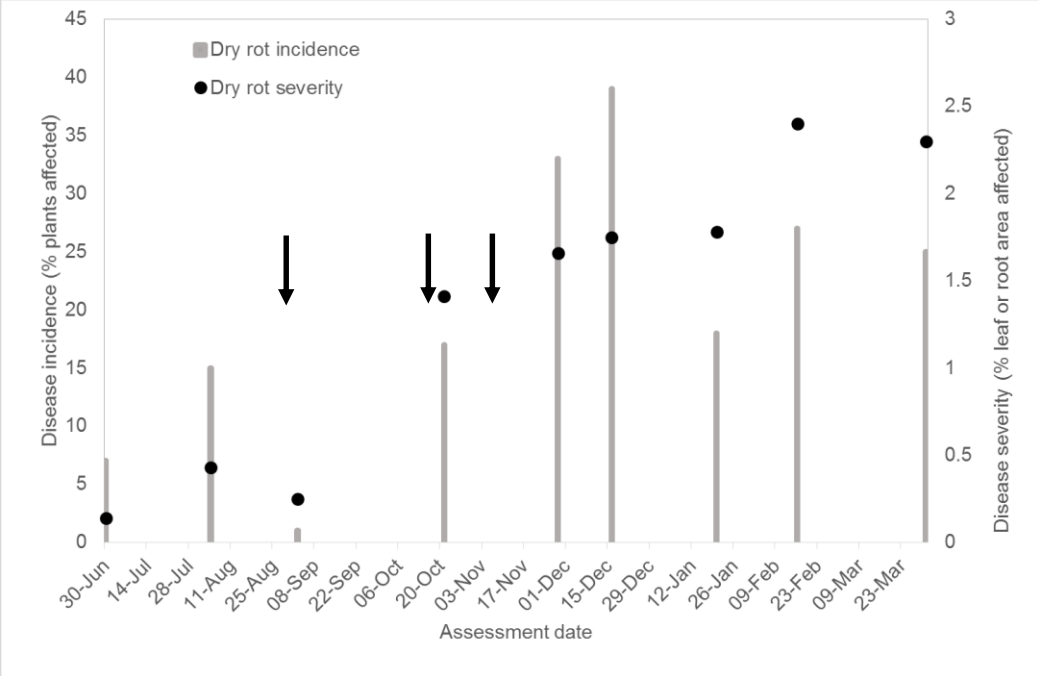


Figure 3. Progress of dry rot development in Fife, Scotland on roots [as the incidence (percentage of plants affected)] and severity (percentage root area affected)] on visible root area in the field until harvest on 28 November when the whole root was assessed. No leaf symptoms were observed. Arrows show when fungicides were applied.

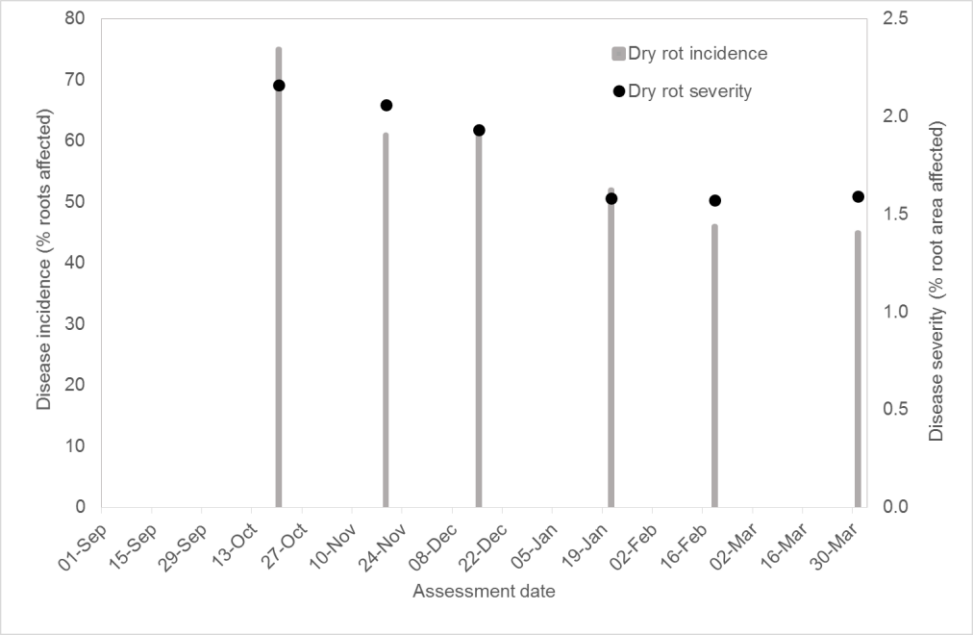


Figure 4. Progress of dry rot development in Perth, Scotland on roots, as the incidence (percentage of plants affected) and severity (percentage root area affected) on visible root area in the field until harvest on 20 October when the whole root was assessed. No leaf symptoms were observed. Fungicides were applied 4 July, 14 August and 27 August.

Leaf inoculation experiment

All leaves to be inoculated were assessed for visible naturally occurring Phoma leaf spot prior to inoculation. No naturally occurring Phoma leaf spot was reported on leaves due to be inoculated prior to any inoculation date. The turnover of leaves was rapid, particularly during early plant growth and in all instances inoculated leaves did not remain attached to the plants for more than 4 weeks after inoculation. Following the first inoculation on 24 July, leaves were no longer present 1 week after inoculation, therefore there are no data are available on lesion development from this date.

The second inoculation was carried out on 28 August and lesions were first noted 15 days after inoculation on 12 September, 15 days after inoculation (Figure 5). No lesions were found on control plants where leaves were not inoculated or where leaves had been inoculated with ascospores. Pycnidiospores of both *L. maculans* and *L. biglobosa* were both infective on swede, with a greater incidence of lesions occurring where leaves were wounded prior to inoculation for both fungal species. No further assessments were carried out as leaves had senesced a week later.

The third inoculation was carried out on 30 September and no lesions noted when plants were assessed on 10 October. First symptoms were observed on inoculated plants on 17 October (Figure 5)

Appendix 2.

Table 4). For wounded treatments, *L. maculans*, *L.biglobosa* and ascospores all caused lesions whereas no symptoms were observed where *L. biglobosa* and ascospores were used to inoculate in the absence of wounding. No lesions were observed on uninoculated control plants.

Natural infection was tracked on all plants after it was first observed on plants on 10 October (Figure 6). In the untreated uninoculated controls, leaf incidence increased rapidly with 11% plants affected on 10 October, increasing to 83% plants by 27 October. First root symptoms were observed 17 days after the appearance of leaf infection with 28% plants showing root symptoms and an average of 8.6% root surface area affected. The final inoculation was carried out on 27 October and lesions were again produced following inoculation with all spore types and species when assessed on 12 November (Figure 5).

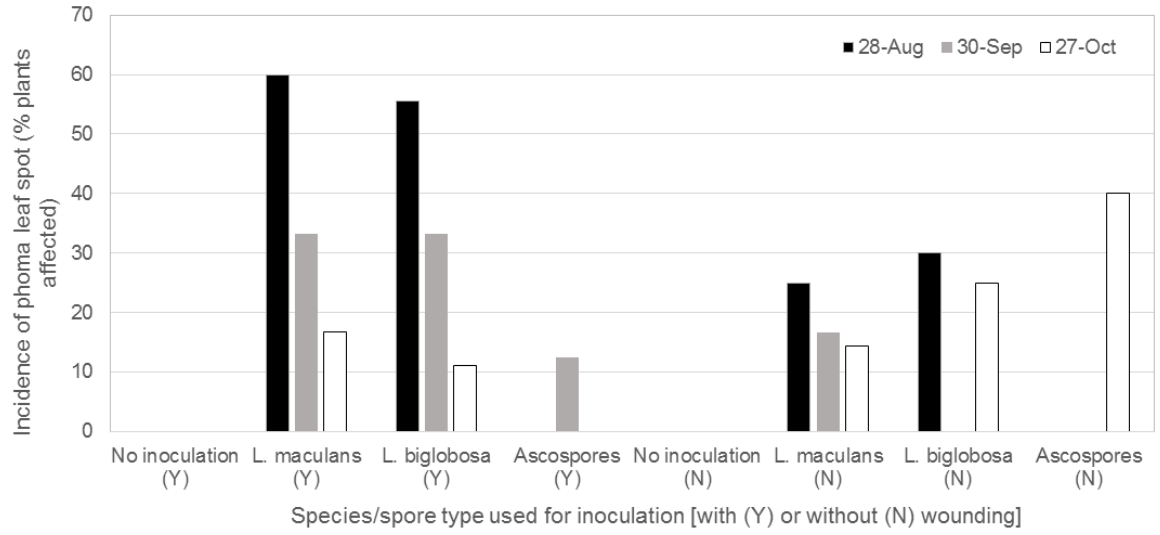


Figure 5. Incidence of Phoma leaf spot caused by *L. maculans, L. biglobosa* and ascospores following inoculation on swede. Plants were assessed 15 to 17 days after the inoculation date stated in the figure.

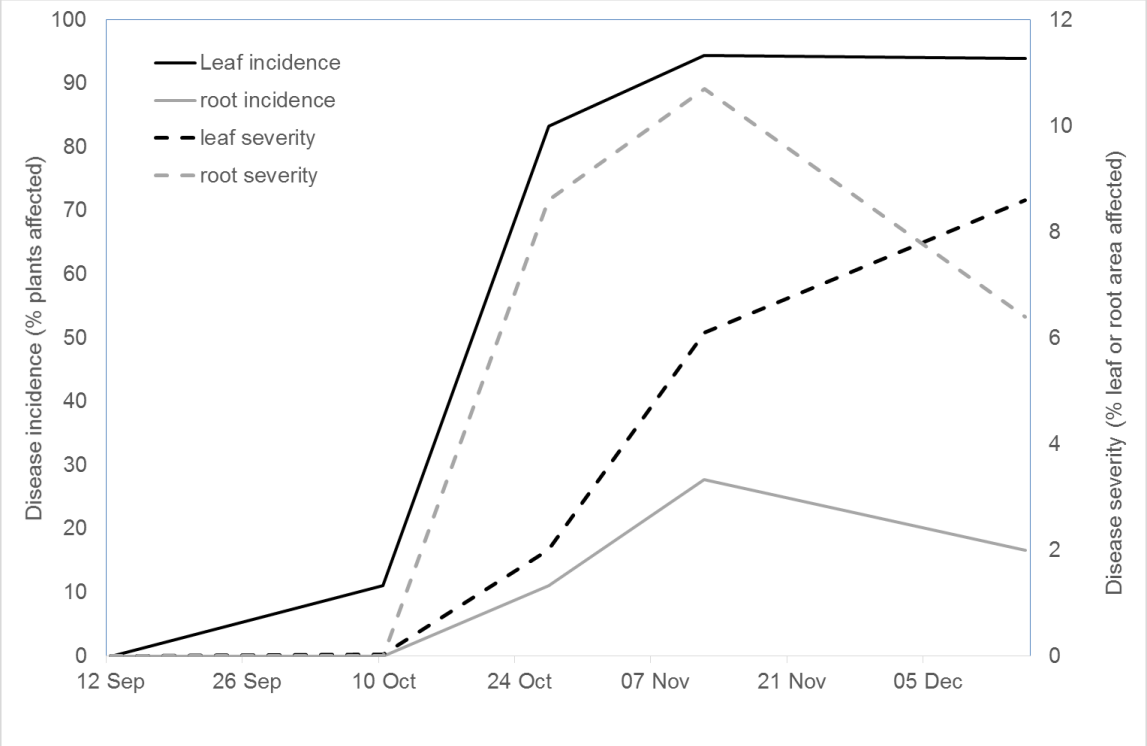


Figure 6. Progress of natural leaf infection and root infection in uninoculated treatments (Treatments 1 and 2) until the final non-destructive root assessment on 12 December 2014.

Root infection was assessed as the percentage above ground root area affected from 27 October onwards when root infection was visible (Figure 7). The incidence of plants affected increased from 19% to 31% in two weeks from 27 October to 12 November and disease severity increased slightly from 5.8% to 7.9% over the same two week period. There was no statistically significant isolate/spore type x wounding x inoculation interaction identified for the severity of root infection when assessed on 27 October or 12 November (Figure 8). It was noted that root infection was visible on plants that were not inoculated until 27 October. The entire root was assessed on 13 January 2015 and there were no statistically significant differences between treatments for the incidence of internal and external root symptoms (Figure 9). When the entire root was assessed for the severity of external and internal symptoms on 13 January, there was a significant interaction between isolate/spore type x wounding x inoculation date for external root symptoms but not internal root symptoms (Figure 10). Root symptoms were observed on all plants including the untreated controls. Generally, the severity of root symptoms were lower on plants that had not been inoculated (5.7 to 7.0% root surface area affected) compared to those that had been (7.6 to 26.9% root surface area affected).

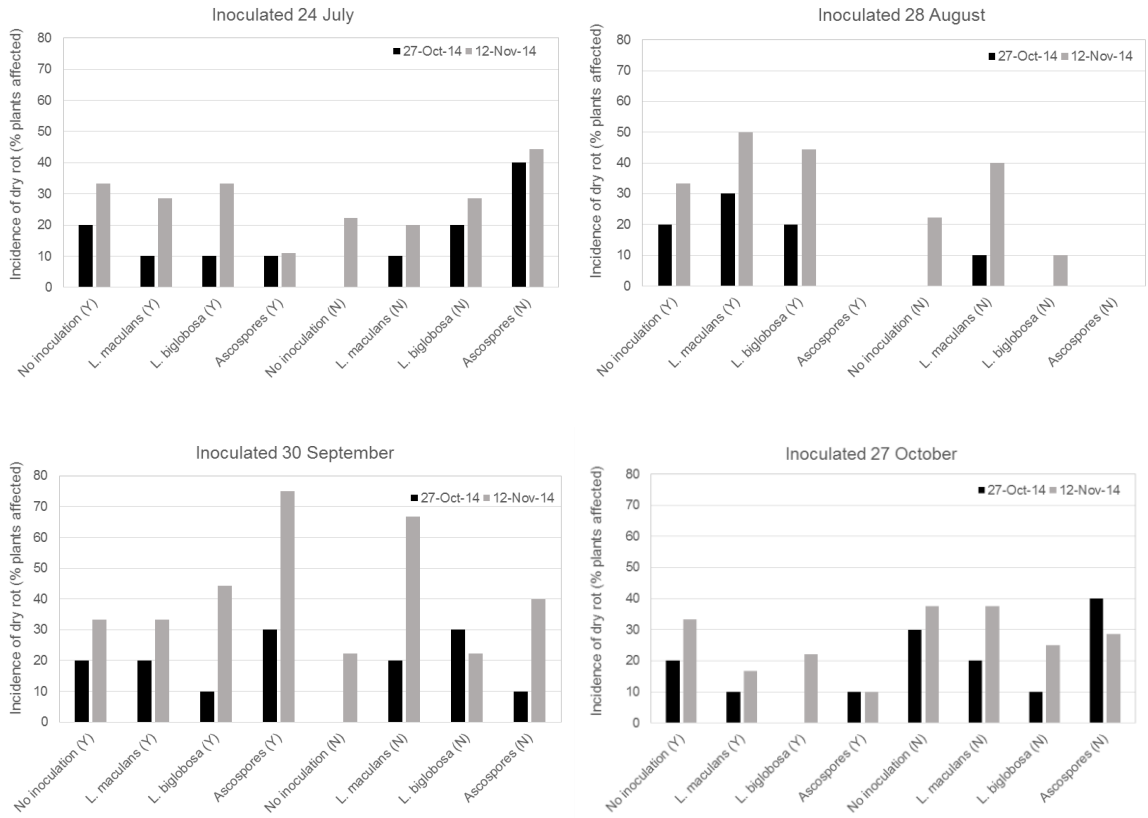


Figure 7. Incidence of root infection (visible from above without destructive sampling) on 27 October and 12 November 2014 for each inoculation date. X-axis specifies the species/spore type used for inoculation and whether inoculation was carried out with (Y) or without (N) wounding.

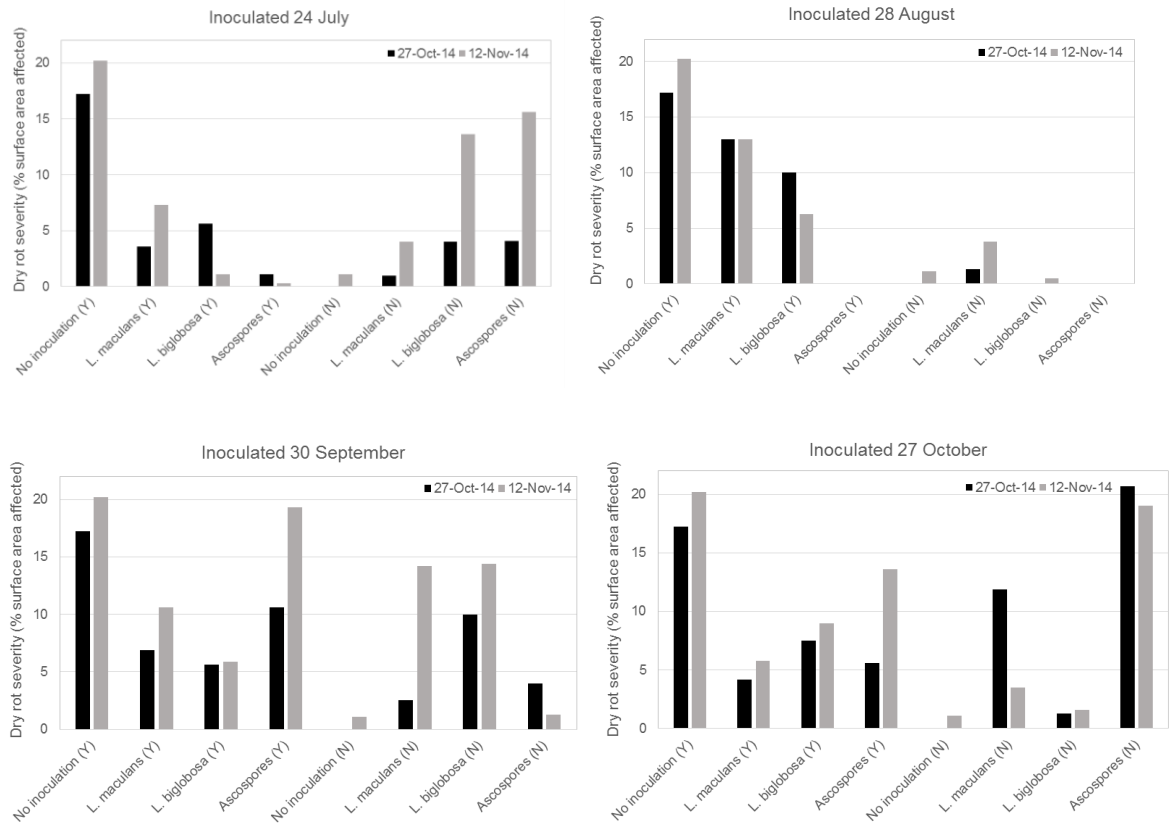


Figure 8. Severity of root infection (visible from above without destructive sampling) on 27 October and 12 November 2014 for each inoculation date. X-axis specifies the species/spore type used for inoculation and whether inoculation was carried out with (Y) or without (N) wounding.

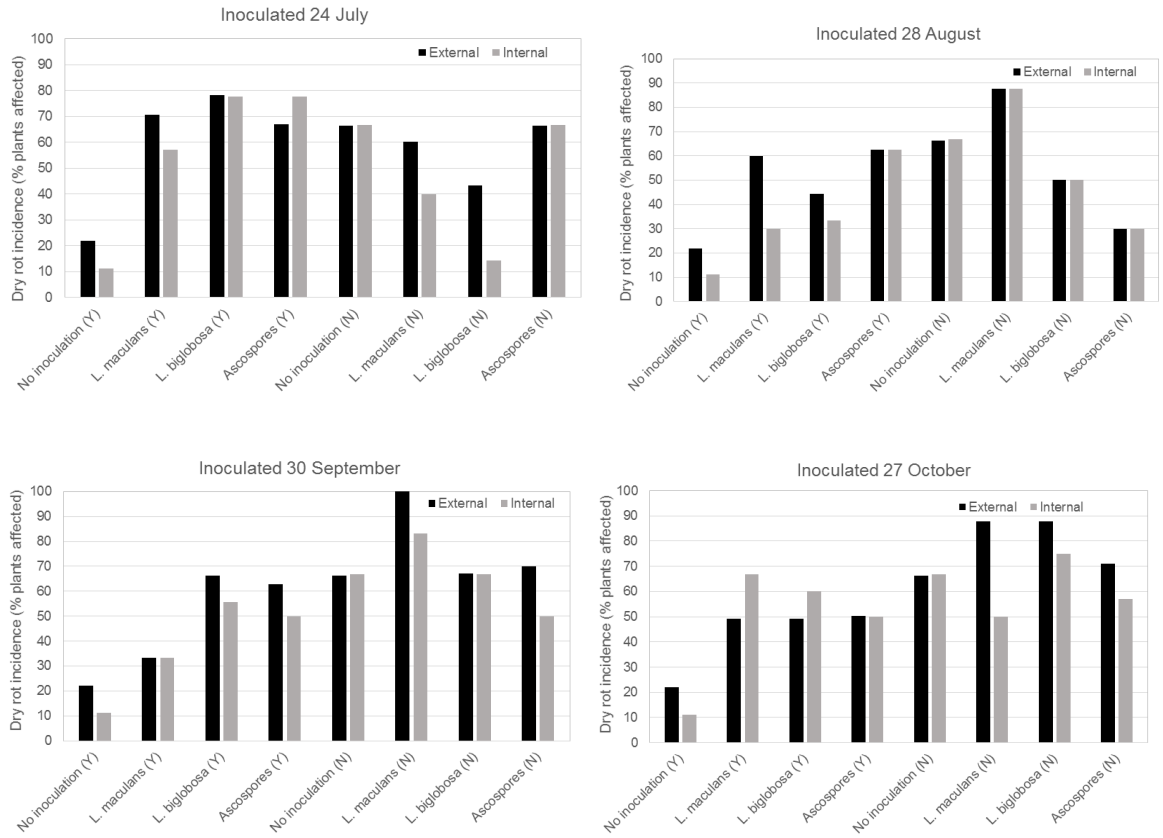


Figure 9. Incidence of external and internal dry rot symptoms on 13 January on whole swedes. X-axis specifies the species/spore type used for inoculation and whether inoculation was carried out with (Y) or without (N) wounding.

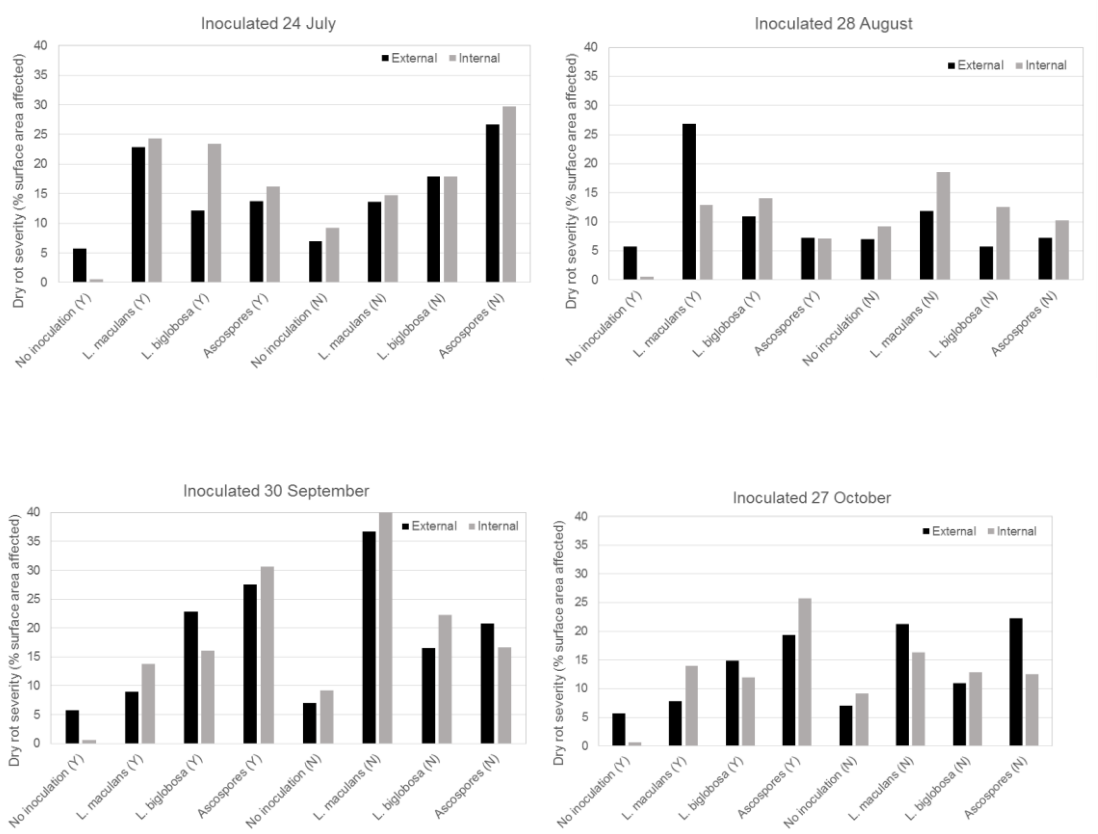


Figure 10. Severity of external (as % surface area affected) and internal (as % internal area affected) dry rot as assessed on 13 January on whole swedes. X-axis specifies the species/spore type used for inoculation and whether inoculation was carried out with (Y) or without (N) wounding.

Root inoculation experiment

Naturally occurring Phoma leaf spotting was observed in the experiment for the first time on 10 October (Figure 11). Disease development was tracked on the entire trial and on the untreated uninoculated plants (Treatments 1 and 2), where disease incidence increased over the next month to 100% plants affected. First sign of root infection was noted on 7 November, with both incidence and severity continuing to increase until 24 November. There appeared to be a slight decline in severity at the final non-destructive assessment on 10 December from 10% to 6%, however, incidence had increased to almost 50% suggesting new smaller lesions were now present.

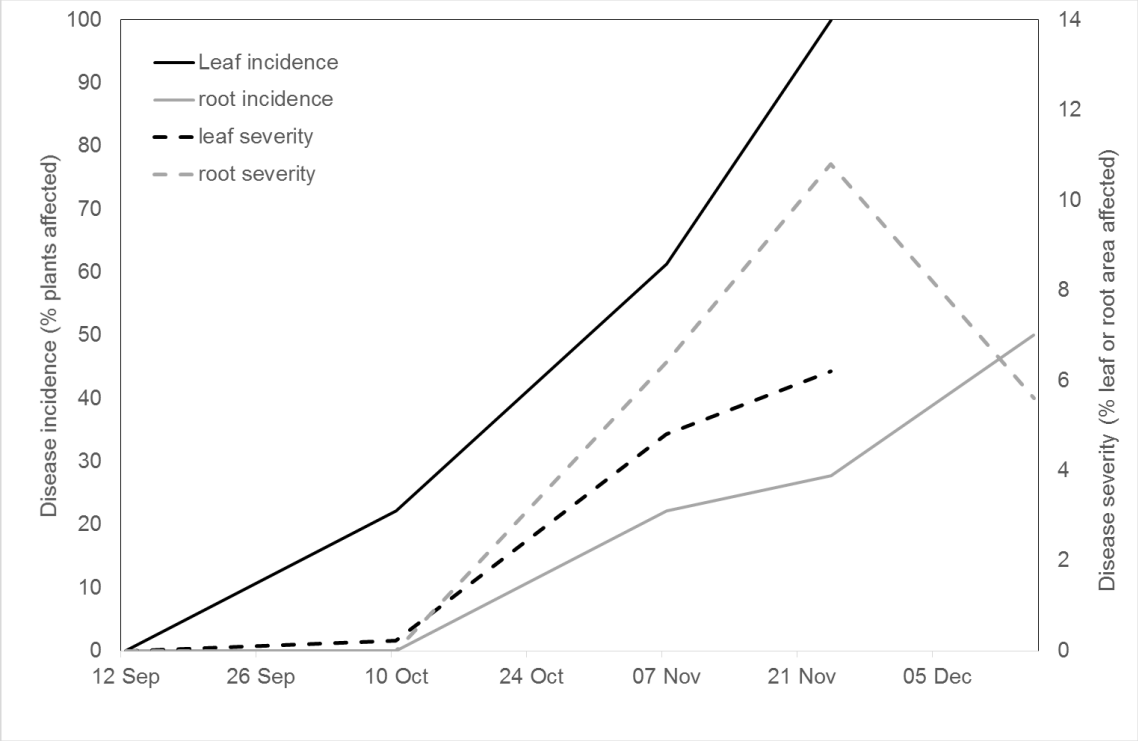


Figure 11. Progress of natural leaf infection and root infection in uninoculated treatments (Treatments 1 and 2) until the final non-destructive root assessment on 15 December 2014.

No dry rot symptoms were observed until 7 November regardless of inoculation date and lesions that could be directly associated with inoculation sites were identified. (dry rot (Figure 17).

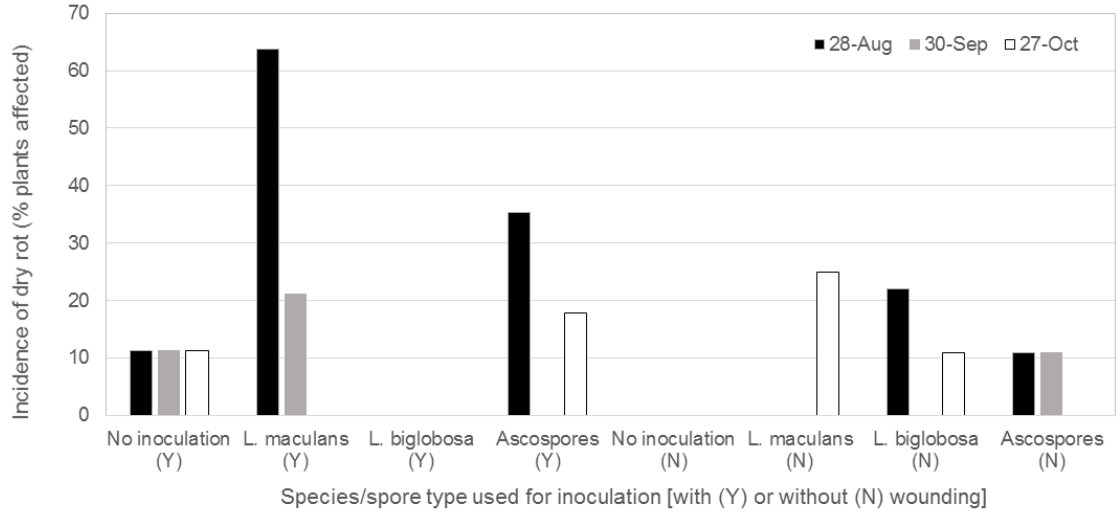
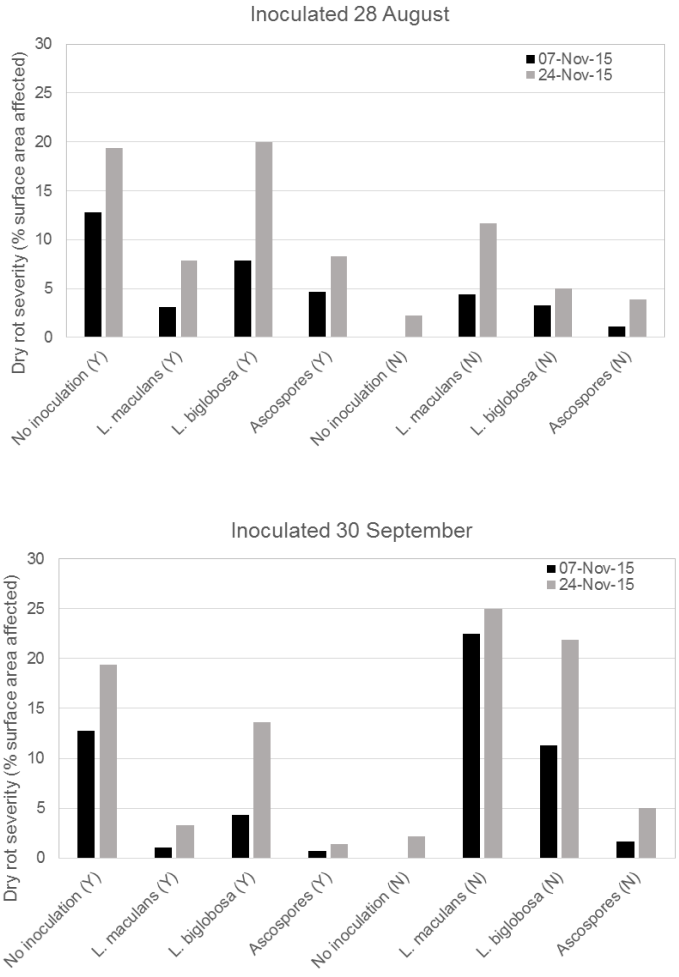


Figure 12). The earliest inoculation date (28 August) appeared to be most successful for causing dry rot, with majority of symptoms observed associated with this date. There was a significant interaction between isolate and wounding, with considerably greater incidence of

dry rot where *L. maculans* had been inoculated to wounded roots than *L biglobosa* or ascospores (Figure 13). Interestingly dry rot symptoms were found in the uninoculated wounded control on 7 November but not in the unwounded control until 24 November. There were no statistically significant differences in the incidence of dry rot for all treatments on 7 November or 24 November, however, there was a trend for increasing incidence over this 3 week period from 31.2% to 39.8% (Figure 14). Similarly, there was an increase in root disease severity over the same time period from 6.0% to 9.5% visible root area affected (



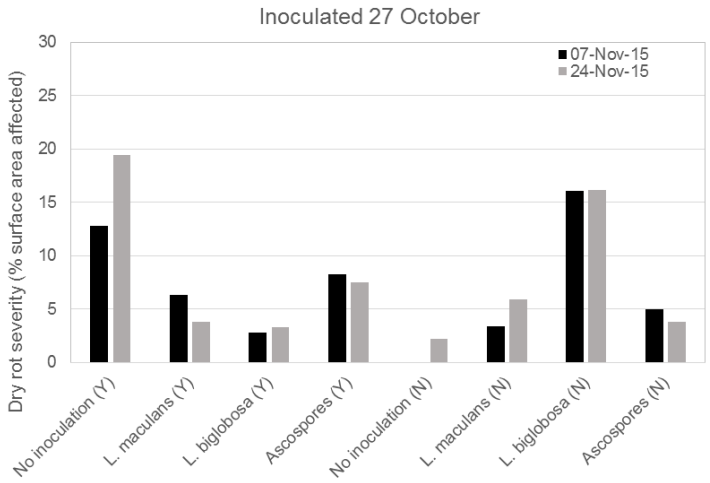


Figure 15).

At the final assessment on 13 January, dry rot incidence was high, even on roots that were not inoculated suggesting that some of the symptoms had been caused by the naturally occurring Phoma epidemic (Figure 16). There was no statistically significant interaction between treatments where incidence on the root surface was considered, however, there was for internal dry rot. There was a significant effect of wounding identified (*P*=0.007) for the incidence of internal dry rot with a mean of 41.4% plants affected in the absence of wounding and 65.3% of plants affected where the root was artificially wounded. There was no significant effect for external dry rot (Figure 17).

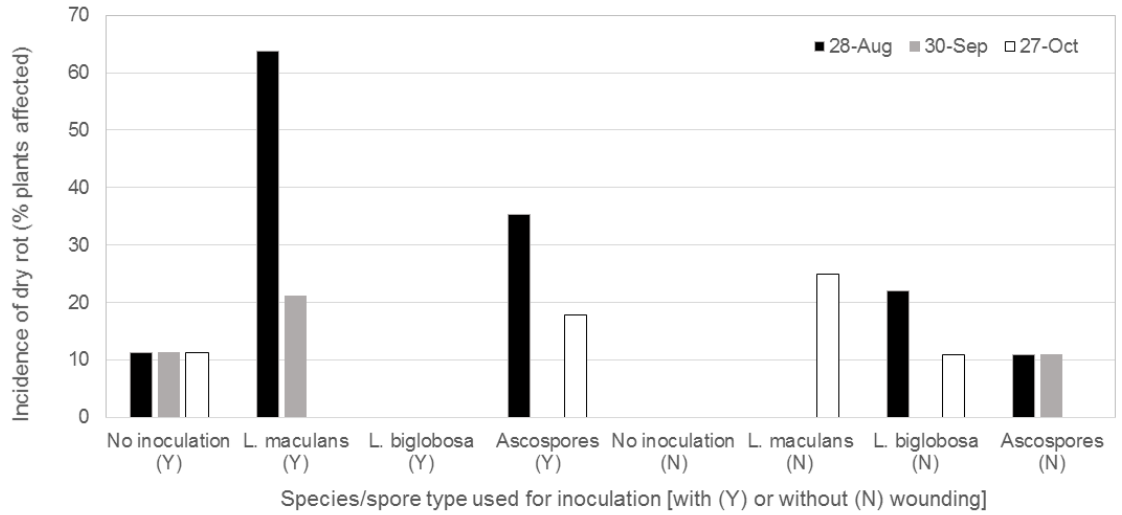


Figure 12. Incidence of dry rot associated with direct inoculation of roots on three inoculation dates (28 August, 30 September and 27 October) as assessed non-destructively on 7 November 2014.

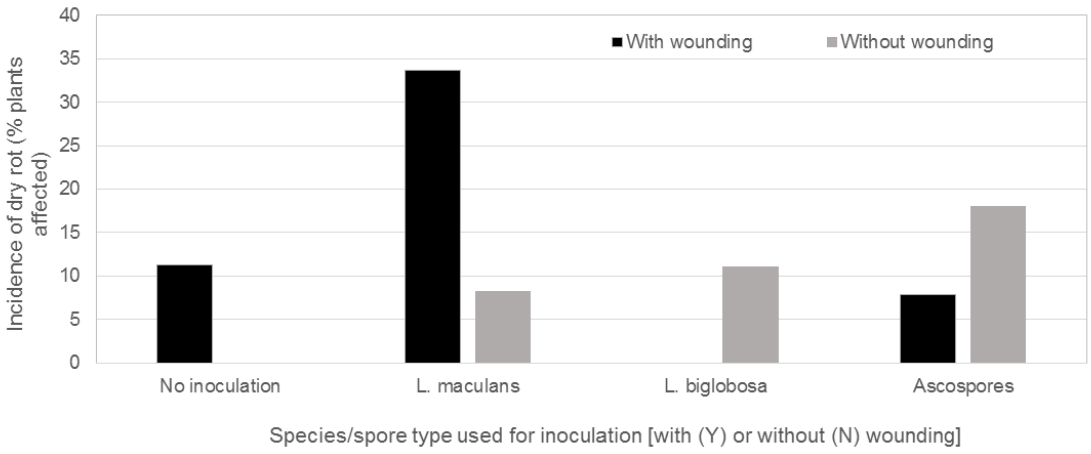


Figure 13. Incidence of dry rot on inoculated roots with and without wounding as assessed non-destructively on 7 November 2014.

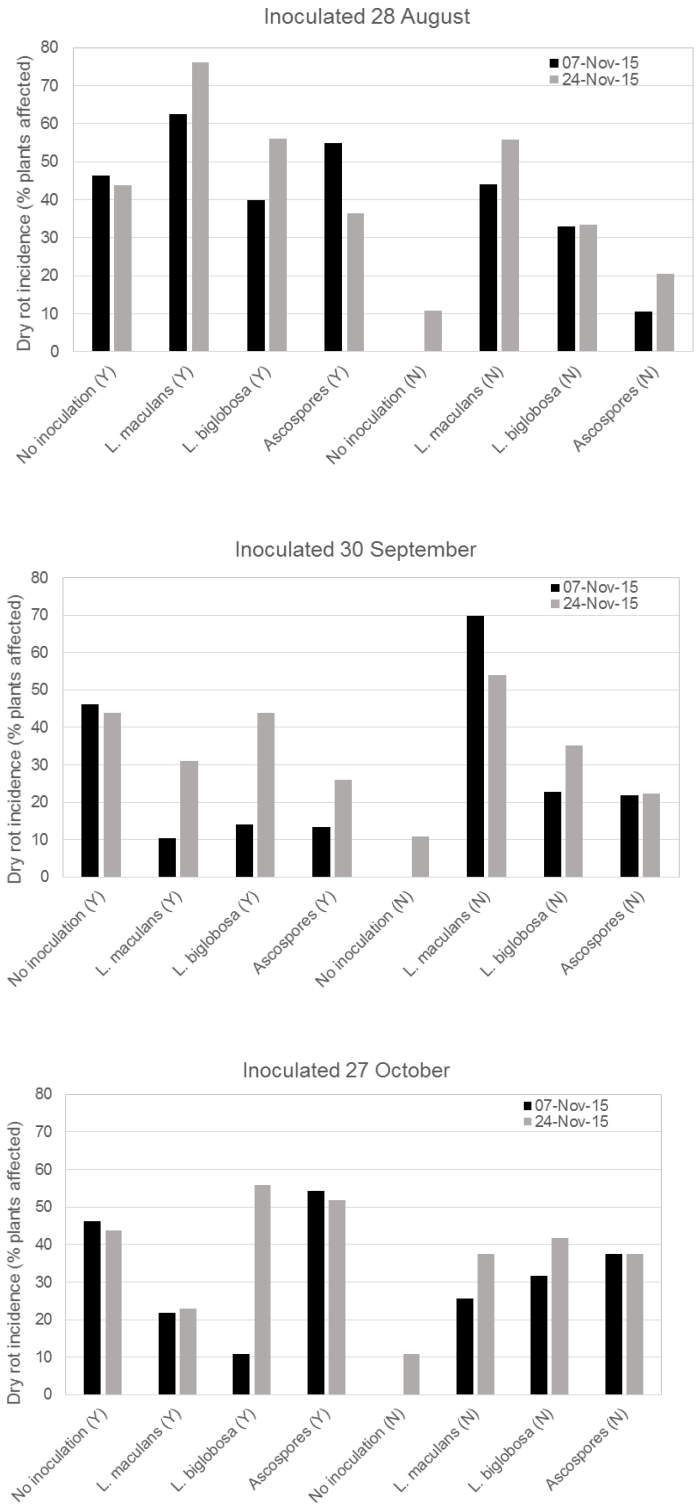


Figure 14. Incidence of dry rot (assessed without destructive sampling) associated with direct inoculation of roots on three inoculation dates (28 August, 30 September and 27 October) as assessed on 7 November 2014 and 24 November 2014. .X-axis specifies the species/spore type used for inoculation and whether inoculation was carried out with (Y) or without (N) wounding.

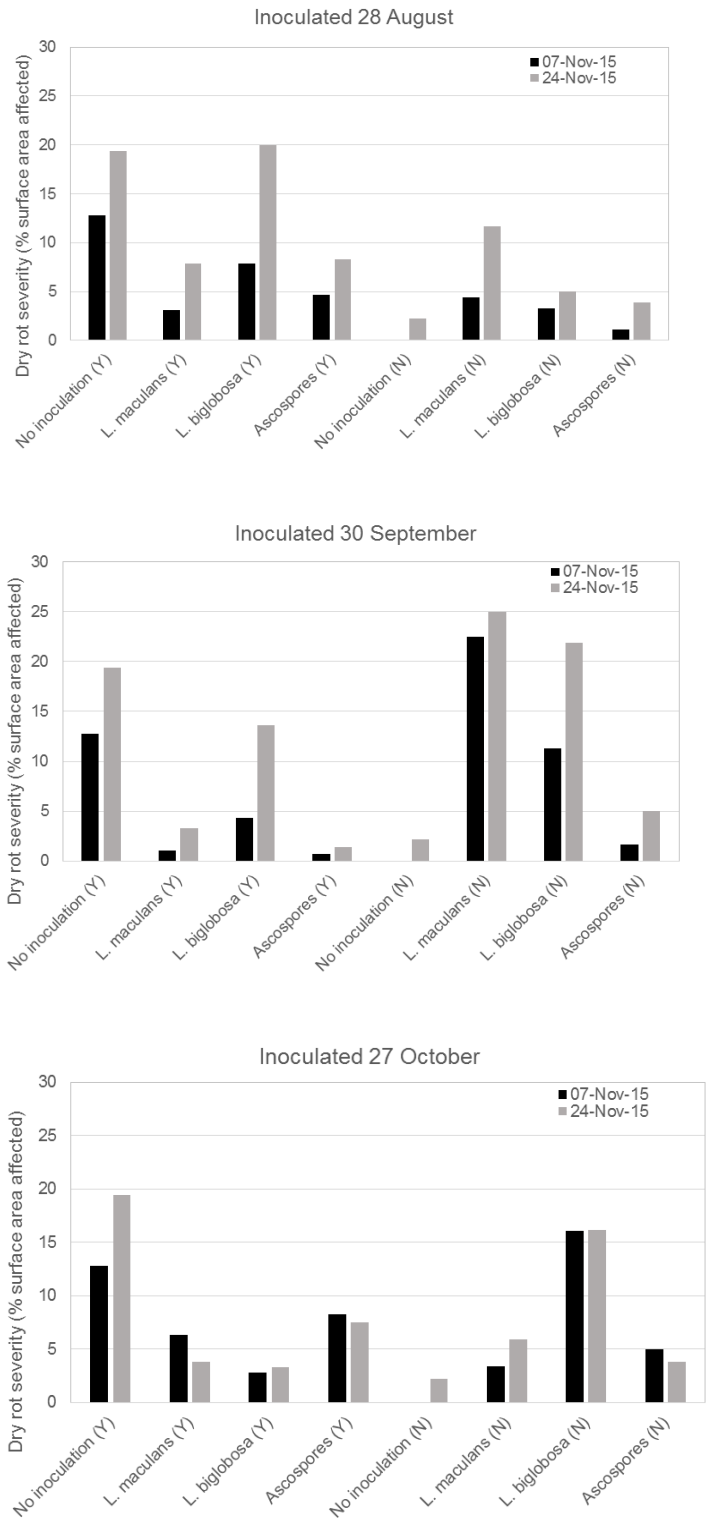


Figure 15. Severity of dry rot (assessed without destructive sampling) associated with direct inoculation of roots on three inoculation dates (28 August, 30 September and 27 October) as assessed on 7 November 2014 and 24 November 2014. X-axis specifies the species/spore type used for inoculation and whether inoculation was carried out with (Y) or without (N) wounding.

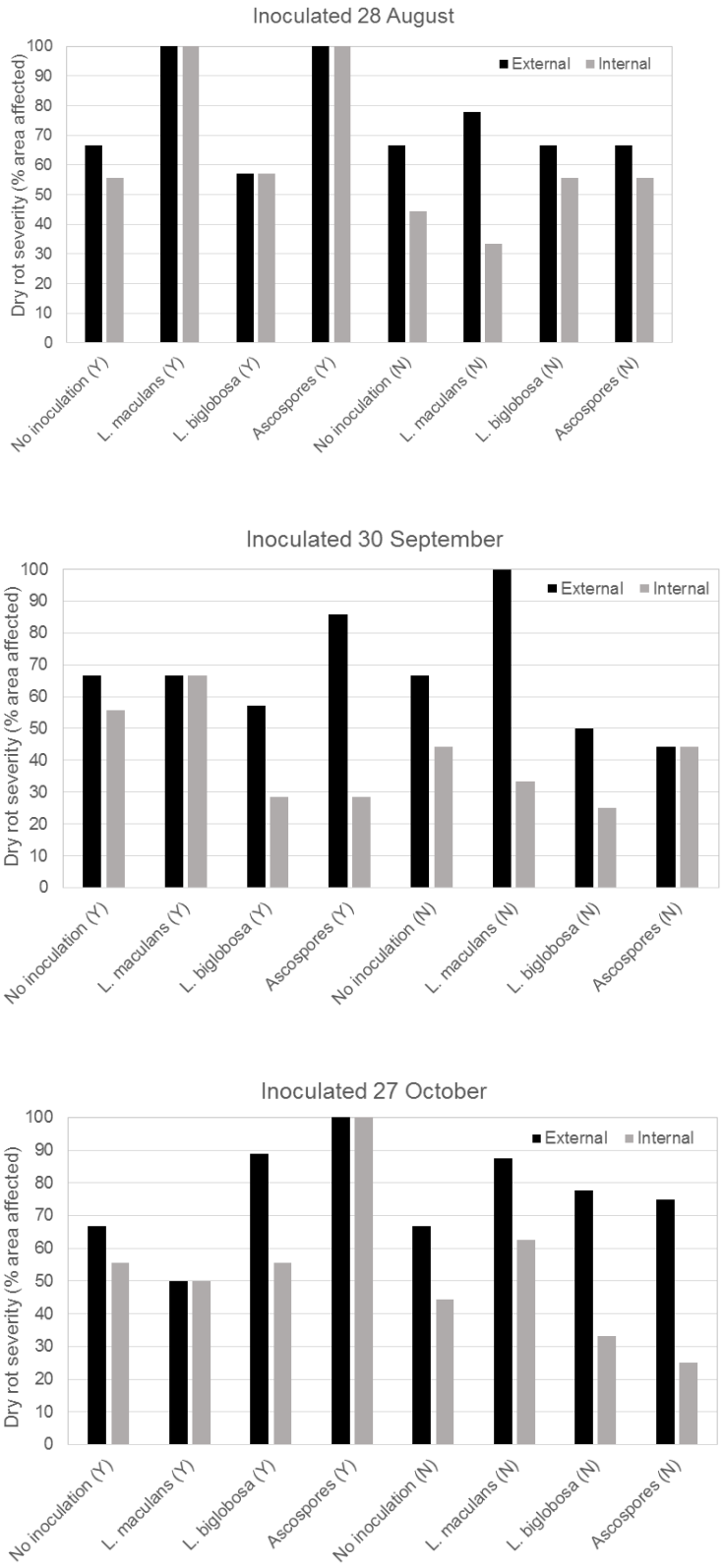


Figure 16. Incidence of dry rot (as % plants affected) as assessed on 13 January on whole swedes. X-axis specifies the species/spore type used for inoculation and whether inoculation was carried out with (Y) or without (N) wounding.

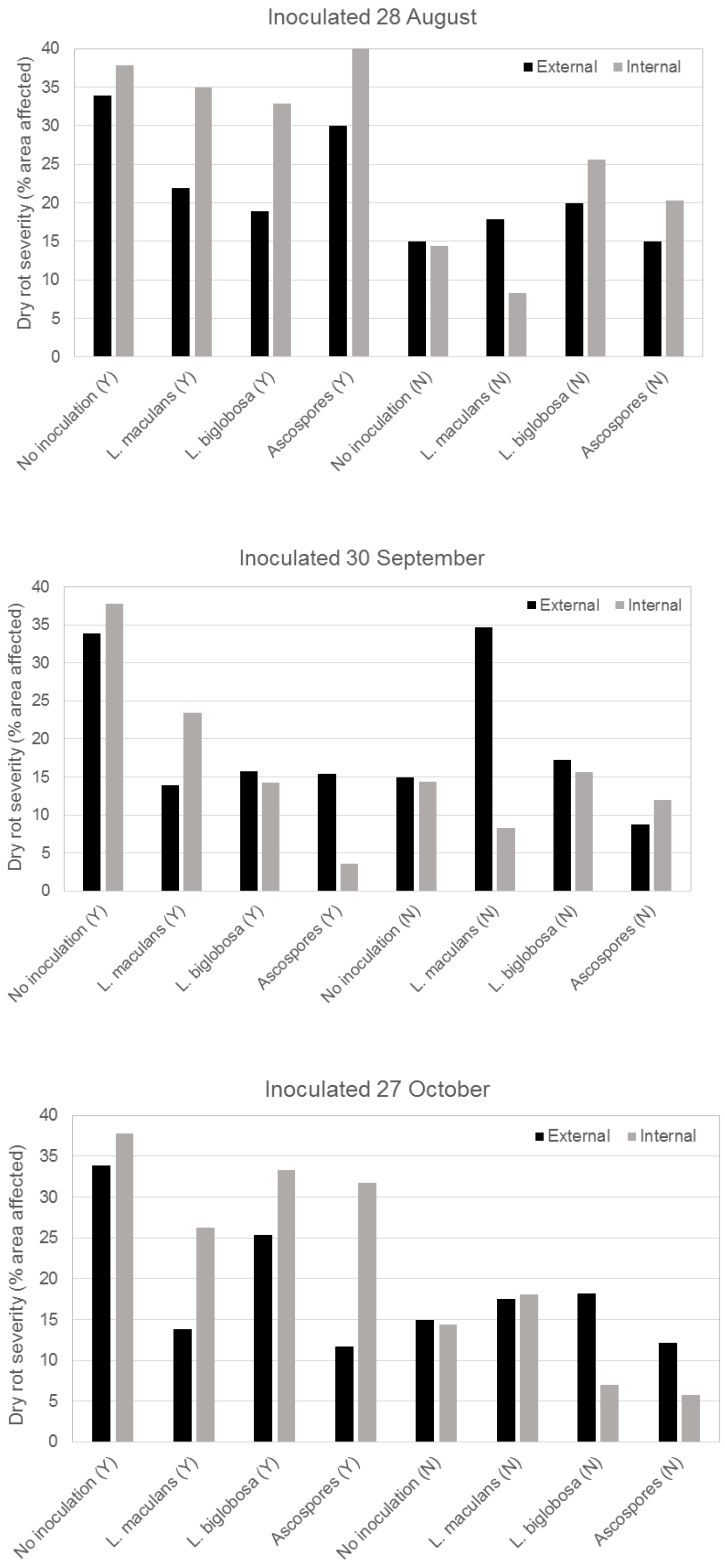


Figure 17. Severity of external (as % surface area affected) and internal (as % internal area affected) dry rot as assessed on 13 January on whole swedes. X-axis specifies the species/spore type used for inoculation and whether inoculation was carried out with (Y) or without (N) wounding.

Symptoms observed in the pot experiments and field monitoring sites

A range of Phoma symptoms were observed, from natural leaf infection to root infection with and without characteristic symptoms. This section contains a series of photographs depicting the range of symptoms observed on plants from the pot and field experiments to aid identification in future. Leaf infections appeared to be more severe than those usually observed on oilseed rape, with spread of the fungus through leaf veins clearly visible (Figure 18) and a large proportion of the leaf area was affected. On roots, direct infection of the root was observed on the upper portion of the root (Figure 19) as well as at or near the soil surface, where cracking of the root associated with the developing lesion was observed. The fungus was still active with a dark leading edge clearly visible on roots on 13 January 2015 (Figure 20). There were often differences in the development of Phoma inside the bulb compared to outside and in this example external roots symptoms were less severe than internal symptoms (Figure 21).

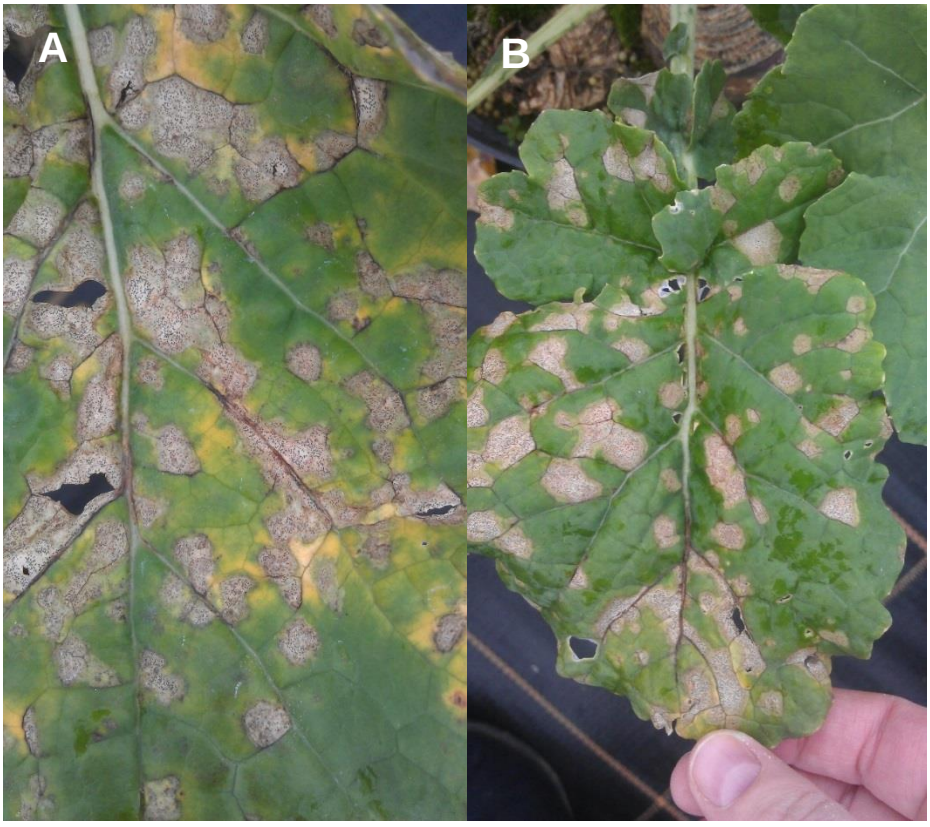


Figure 18. Phoma leaf spotting on leaves showing chlorosis of the leaves associated with lesion formation (A) and the extent of leaf damage with the movement of the fungus through the leaf veins (B) on 12 November 2014 in the pot experiment at Boxworth,

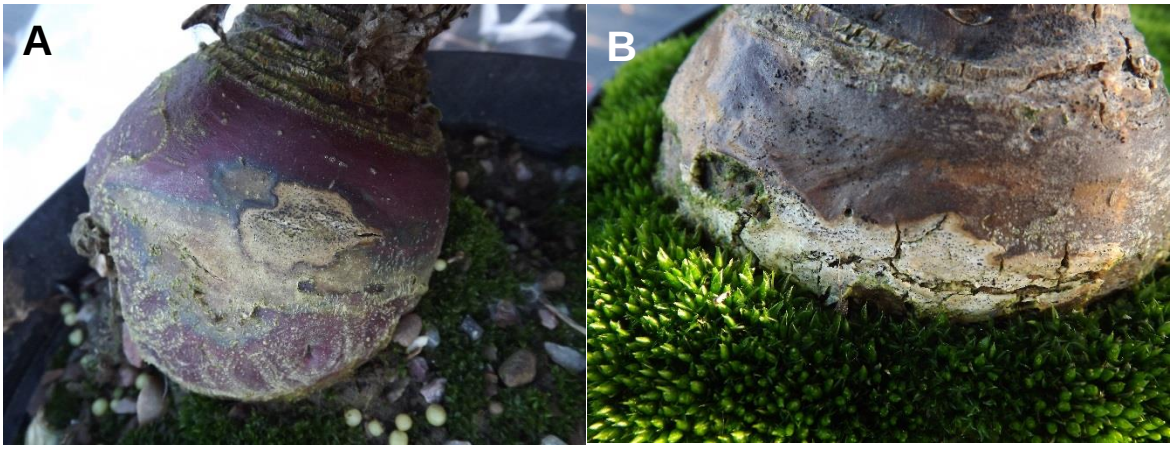


Figure 19. Different stages of dry rot development were observed on roots (not associated with leaf or root inoculations). Lesions were visible on the upper root and started in the absence of significant surface cracking (A). More advanced lesions were often associated with the soil surface, where extensive cracking and pycnidia were visible on 13 January 2015 in the pot experiment at Boxworth.

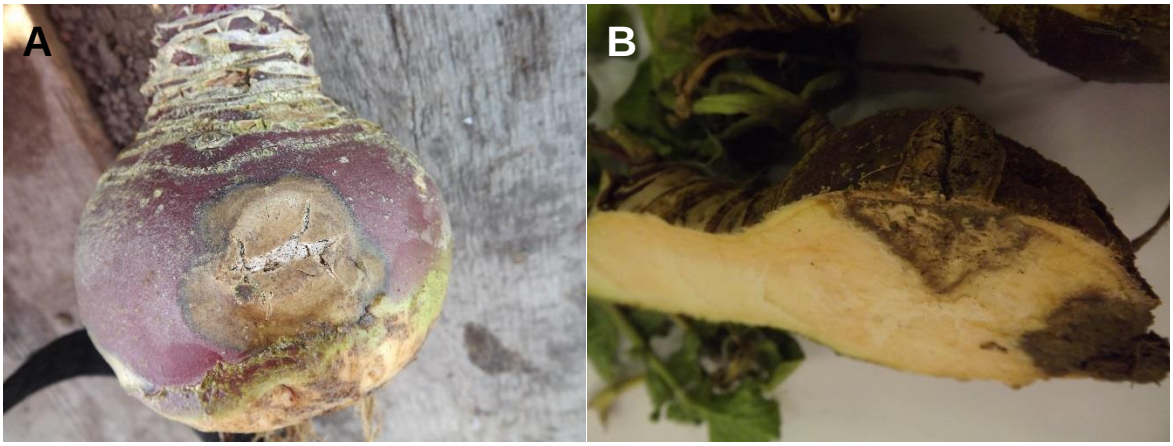


Figure 20. Extensive dry rot lesion with central cracking of the skin and pycnidia clearly visible (A) and cross section of a swede showing the difference in the extent of dry rot damage inside the root compared to outside the root (B), both taken on 13 January 2015 in the pot experiment at Boxworth.

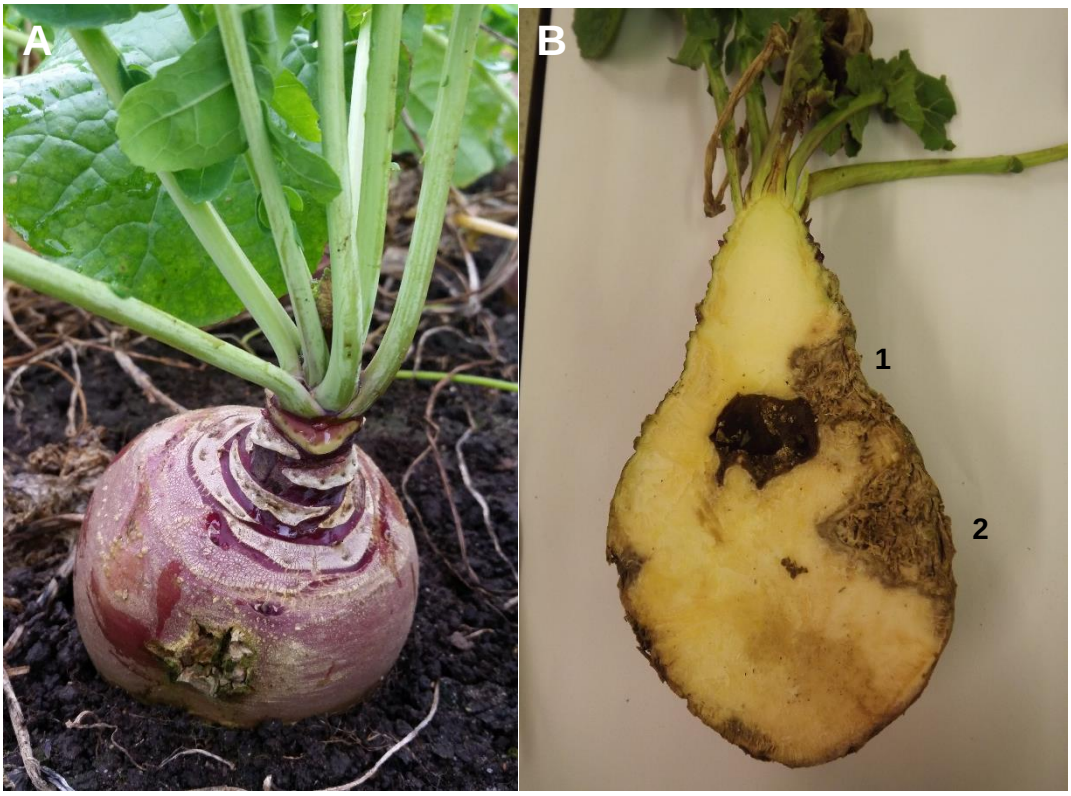


Figure 21. Dry rot observed on field grown swedes (A) on 27 October in Scotland (Photo: Tracy Yoxall, SRUC). Cross section of swede (B) from Boxworth pot experiment showing two lesions originating from different infections: near the upper root following inoculation (1) and the soil surface (2).

Discussion

From data presented here, there are parallels between the development of Phoma on swedes and oilseed rape, however, there are key differences that will influence the methods implemented to control the disease. These are discussed in relation to current knowledge of Phoma development on oilseed rape for each project objective below.

1. To improve understanding of the disease cycle in swedes

Anecdotal reports of dry rot on swede from growers previously suggested that this disease occurred on roots in the presence and absence of leaf infection. Observations from this study suggest that there is no requirement for leaf infection on swede to cause root infection and that direct infection of the root is possible. At the monitoring sites in Scotland, no leaf lesions were reported at either site however root infection was reported, with dry rot identified in the field prior to harvest and after storage. At one site, dry rot occurred in July, far earlier than would be expected to occur as a result of infection from ascospores. Whether these early symptoms were the result of seed infection or the early release of ascospores was not determined.

In contrast, at the monitoring site in North Yorkshire, leaf lesions were present but no root symptoms developed before or after storage. At the site in Lincolnshire, leaf lesions and root infection were visible and symptoms were present in the field in parallel. In oilseed rape which is sown in August/September, Phoma leaf infection in the autumn is responsible for initiating stem cankers by growing through leaf veins to the stem and then take 6 months to develop (Gladders *et al*., 1998). No such requirement for leaf infection to move from leaves to roots was observed for the development of dry rot on swede. It should be noted that the most severe Phoma epidemics occur in the south in the UK and this has been attributed to increasing winter/spring temperatures coupled with higher temperatures on average (Stonard *et al*., 2010). Dry rot on swede, however, did appear to be more severe at Scottish compared to English sites. This observation suggests that weather may be influencing dry rot but this was not investigated further in the current study.

In the inoculation experiments it was demonstrated that both ascospores and pycnidiospores can cause Phoma leaf spot and dry rot on swede. The leaf inoculation experiment has demonstrated for the first time that both species, *L. maculans* and *L. biglobosa*, can infect the leaves of swede and that infection can occur in the presence and absence of leaf wounding. Similarly, inoculating leaves of swede with ascospores taken from oilseed rape stubbles can also caused Phoma leaf spot and dry rot. Both *L. maculans* and *L. biglobosa* produce ascospores under UK conditions and are known to infect oilseed rape (Huang *et al*., 2005). This study has confirmed that both species are pathogenic on swede therefore measures to decrease the exposure of crops to ascospores in the autumn would be beneficial to decrease crop risk. This could be done through better targeting of fungicide applications in relation to ascospore release and an outline of future work is included in section 3.

2. To use artificial inoculation to identify key timings for infection and disease development

The purpose of the inoculation experiments was to determine whether Phoma leaf spot caused dry rot and whether timing of infection of leaves and roots affected disease development. In contrast to the field monitoring sites in Scotland where no leaf infection was observed, the first symptoms of naturally occurring leaf lesions in the swede pot experiments at ADAS Boxworth on 10 October coincided with first observations in oilseed rape on 22 October. In the UK, ascospores are the predominant source of infection for oilseed rape and likely to have been the source of these naturally occurring lesions.

Dry rot was not observed until 7 November regardless of the date roots were inoculated, however, some lesions could be linked to direct inoculation that had occurred over 5 weeks earlier. Where roots were inoculated in the pot experiment, the incidence of roots affected

and the severity of internal dry rot symptoms were significantly increased by wounding. Whether wounding is a requirement for dry rot development in field conditions has yet to be proven. The presence of ascospores, and therefore natural sources of infection at this time, made it difficult to determine whether lesions were absolutely derived from inoculation, however, this in conjunction with observations on roots from the monitoring studies does suggest that specific environmental conditions are required for root infection and these remain to be determined.

3. Comparison of findings in this project on swede with existing knowledge from oilseed rape

In oilseed rape, lesions produced on the leaves are responsible for causing stem cankers as the fungus grows through the leaf veins and down through the petiole to the stem (Gladders *et al*., 1998). Following the appearance of Phoma leaf spot in the autumn on leaves, the development of symptoms in the stem can take over 6 months (e.g. from leaf infection in October to first appearance of stem canker in March) with severity dependent on the timing of the onset of the epidemic in the autumn and subsequent temperatures (Evans *et al*., 2008). In contrast, dry rot was observed within one month of leaf infection or in the absence of leaf infection at monitoring sites. This suggests weather may be a factor in dry rot development and demonstrates that leaf infection is not required for dry rot to develop on swede.

It has been suggested that fungicides are applied during August to October in Ireland for Phoma control (Teagasc Technical Note, July 2012, Dry rot of swedes). Azole fungicides have mainly been used for the control of Phoma in oilseed rape and swede but there is strong activity in other fungicide groups (Fungicides for Phoma control in winter oilseed rape: AHDB cereals and oilseeds update, October 2014). To aid growers with fungicide application decisions for Phoma control in oilseed rape, a forecast is produced every year to guide growers as to when a critical threshold is reached (10 to 20% plants affected in a field) in order to apply fungicides and minimise crop losses. This is used alongside regular crop monitoring to decide on the appropriate fungicide application timing to control the disease. The model used to predict the date this threshold will be reached uses rainfall and temperature criteria to predict when spore release occurs http://www.rothamsted.ac.uk/Phoma-leaf-spot-forecast/Phoma-forecast. Rainfall in August and September is known to be factor in determining ascospore release, with more rain resulting in an earlier release date (Huang *et al*., 2005). Future work should focus on measures to help predict when high risk periods for ascospore release or root infection occur e.g. through tracking of temperatures and/or ascospore release occurs to aid better targeting of fungicide applications.

Notes on experimental methodology: although the following were unlikely to have had an impact on the outcomes of this project, it should be noted that swede grown in containers were very susceptible to insect pests including cabbage root fly and aphids. Steps should be taken in future experiments to prevent cabbage root fly infestation prior to emergence. Regular monitoring was required to treat aphid infestations. Two swedes per pot were used in this project, however, for future studies one plant per 5 litre pot may be better for growth and nutrition. The inclusion of a substrate mixed through the soil to minimise compaction and improve drainage may also aid better growth.

Conclusions

- The two Phoma species pathogenic to oilseed rape present in the UK (*L. maculans* and *L. biglobosa*) can cause Phoma leaf spotting and dry rot on swede;
- Ascospores can initiate epidemics on swede in the UK;
- Inoculation experiments showed that both ascospores and pycnidiospores can produce both leaf lesions and dry rot;
- Leaf infection is not required for dry rot to be observed in the crop;
- Wounding of the root appeared to increase the incidence of roots affected as well as the severity of internal dry rot symptoms.

Recommendations for future work

This project has identified three areas to investigate to improve the control of dry rot in swede:

4. Identify weather variables affecting dry rot development, whether those criteria are related to timing of spore release and can be used to predict crop risk;
5. Improve the targeting of fungicide programmes for dry rot control using weather based risk criteria or a leaf disease threshold;
6. Evaluate the efficacy of fungicides currently used to control Phoma in oilseed rape against dry rot on swede.

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Appendix 1. Details of pesticide and nutritional inputs applied to inoculated pot experiments at ADAS Boxworth.

Product	Active(s)	Rate of use*	Date
Bandu	deltamethrin	0.3 L/ha	11 July 2014
Osmacoat Exact Standard	15-8-12 + 2MgO	17.5 g per pot	18 July 2014
Bandu	deltamethrin	0.3 L/ha	5 August 2014
Nemasys	Steinernema feltiae	125 ml per pot	6 August 2014
Bandu	deltamethrin	0.3 L/ha	19 September 2014
Plenum + Codicide	pymetrozine + adjuvant	0.4 + 2.5 L/ha	30 September 2014

*insecticides applied in 400 L/ha water unless otherwise indicated.

Appendix 2.

Table 4. Infectivity of different spore types of *L. maculans, L. biglobosa* and ascospores derived from oilseed rape debris on swede on 12 September, 15-17 days after inoculation.

Trt	Species (spore type)	Inoculation timing	Wounding (Yes/No)	Incidence (% plants with lesion)	Lesion severity (% leaf area affected)
1	No inoculation	12 Sep 14	Yes	0.0	0.00
2	No inoculation		No	0.0	0.00
9	*L. maculans* (pycnidiospores)		Yes	60.0	0.09
10	*L. maculans* (pycnidiospores)		No	25.0	0.04
11	*L. biglobosa* (pycnidiospores)		Yes	55.6	0.07
12	*L. biglobosa* (pycnidiospores)		No	30.0	0.04
13	Ascospores		Yes	0.0	0.00
14	Ascospores		No	0.0	0.00
1	No inoculation	17 Oct 14	Yes	0.0	-
2	No inoculation		No	0.0	-
15	*L. maculans* (pycnidiospores)		Yes	33.3	-
16	*L. maculans* (pycnidiospores)		No	16.7	-
17	*L. biglobosa* (pycnidiospores)		Yes	33.3	-
18	*L. biglobosa* (pycnidiospores)		No	0.0	-
19	Ascospores		Yes	12.5	-
20	Ascospores		No	0.0	-
1	No inoculation	12 Nov 14	Yes	0.0	0.00
2	No inoculation		No	0.0	0.00
21	*L. maculans* (pycnidiospores)		Yes	16.7	0.17
22	*L. maculans* (pycnidiospores)		No	14.3	0.43
23	*L. biglobosa* (pycnidiospores)		Yes	11.1	0.67
24	*L. biglobosa* (pycnidiospores)		No	25.0	0.19
25	Ascospores		Yes	0.0	0.00
26	Ascospores		No	40.0	0.60

Table 5. Incidence of root infection (visible from above without destructive sampling) on 27 October and 12 November 2014.

Trt	Isolate/spore type	Wounding (Yes/No)	Inoculation date	27 October 2014	12 November 2014
1	None	Yes	-	20.0 ± 12.6	33.3 ± 14.3
2	None	No	-	0.0 ± 0.0	22.2 ± 12.6
3	*L. maculans*	Yes	24 July 2014	10.0 ± 9.5	28.6 ± 12.6
4	*L. maculans*	No	24 July 2014	10.0 ± 9.5	20.0 ± 12.6
5	*L. biglobosa*	Yes	24 July 2014	10.0 ± 9.5	33.3 ± 14.4
6	*L. biglobosa*	No	24 July 2014	20.0 ± 12.6	28.6 ± 12.6
7	Ascospores	Yes	24 July 2014	10.0 ± 9.5	11.1 ± 9.5
8	Ascospores	No	24 July 2014	40.0 ± 15.3	44.4 ± 15.4
9	*L. maculans*	Yes	28 August 2014	30.0 ± 14.4	50.0 ± 15.7
10	*L. maculans*	No	28 August 2014	10.0 ± 9.5	40.0 ± 15.4
11	*L. biglobosa*	Yes	28 August 2014	20.0 ± 12.6	44.4 ± 15.4
12	*L. biglobosa*	No	28 August 2014	0.0 ± 0.0	10.0 ± 9.4
13	Ascospores	Yes	28 August 2014	0.0 ± 0.0	0.0 ± 0.0
14	Ascospores	No	28 August 2014	0.0 ± 0.0	0.0 ± 0.0
15	*L. maculans*	Yes	30 September 2014	20.0 ± 12.6	33.3 ± 14.4
16	*L. maculans*	No	30 September 2014	20.0 ± 12.6	66.7 ± 15.4
17	*L. biglobosa*	Yes	30 September 2014	10.0 ± 9.5	44.4 ± 15.4
18	*L. biglobosa*	No	30 September 2014	30.0 ± 14.4	22.2 ± 12.6
19	Ascospores	Yes	30 September 2014	30.0 ± 14.4	75.0 ± 15.4
20	Ascospores	No	30 September 2014	10.0 ± 9.5	40.0 ± 15.4
21	*L. maculans*	Yes	27 October 2014	10.0 ± 9.5	16.7 ± 14.4
22	*L. maculans*	No	27 October 2014	20.0 ± 12.6	37.5 ± 12.6
23	*L. biglobosa*	Yes	27 October 2014	10.0 ± 9.5	10.0 ± 9.5
24	*L. biglobosa*	No	27 October 2014	10.0 ± 9.5	25.0 ± 12.6
25	Ascospores	Yes	27 October 2014	30.0 ± 14.4	37.5 ± 14.4
26	Ascospores	No	27 October 2014	40.0 ± 15.3	28.6 ± 12.6
			FPr	0.622 (ns)	0.653 (ns)

Table 6. Severity of root infection (visible from above without destructive sampling) on 27 October and 12 November 2014.

Trt	Isolate/spore type	Wounding (Yes/No)	Inoculation date	29 October 2014	12 November 2014
1	None	Yes	-	17.2	20.2
2	None	No	-	0.0	1.1
3	*L. maculans*	Yes	24 July 2014	3.6	7.3
4	*L. maculans*	No	24 July 2014	1.0	4.0
5	*L. biglobosa*	Yes	24 July 2014	5.6	1.1
6	*L. biglobosa*	No	24 July 2014	4.0	13.6
7	Ascospores	Yes	24 July 2014	1.1	0.3
8	Ascospores	No	24 July 2014	4.1	15.6
9	*L. maculans*	Yes	28 August 2014	13.0	13.0
10	*L. maculans*	No	28 August 2014	1.3	3.8
11	*L. biglobosa*	Yes	28 August 2014	10.0	6.3
12	*L. biglobosa*	No	28 August 2014	0.0	0.5
13	Ascospores	Yes	28 August 2014	0.0	0.0
14	Ascospores	No	28 August 2014	0.0	0.0
15	*L. maculans*	Yes	30 September 2014	6.9	10.6
16	*L. maculans*	No	30 September 2014	2.5	14.2
17	*L. biglobosa*	Yes	30 September 2014	5.6	5.9
18	*L. biglobosa*	No	30 September 2014	10.0	14.4
19	Ascospores	Yes	30 September 2014	10.6	19.3
20	Ascospores	No	30 September 2014	4.0	1.3
21	*L. maculans*	Yes	27 October 2014	4.2	5.8
22	*L. maculans*	No	27 October 2014	11.9	3.5
23	*L. biglobosa*	Yes	27 October 2014	7.5	9.0
24	*L. biglobosa*	No	27 October 2014	1.3	1.6
25	Ascospores	Yes	27 October 2014	5.6	13.6
26	Ascospores	No	27 October 2014	20.7	19.0
			Mean	5.8	7.9
			Fpr	0.109 (ns)	0.052 (ns)
			SED	7.84	9.77
			LSD	15.47	19.28

Table 7. Incidence of external and internal symptoms on destructively harvested roots on 13 January 2015.

Trt	Isolate/ spore type	Wounding (Yes/No)	Inoculation date	Incidence external symptoms (% plants affected)	Incidence internal symptoms (% plants affected)
1	None	Yes	-	22.0 ± 13.7	11.2 ± 10.6
2	None	No	-	66.3 ± 15.8	66.8 ± 15.6
3	*L. maculans*	Yes	24 July 2014	70.8 ± 17.2	57.1 ± 18.6
4	*L. maculans*	No	24 July 2014	60.1 ± 15.4	40.0 ± 15.5
5	*L. biglobosa*	Yes	24 July 2014	78.3 ± 13.6	77.8 ± 13.7
6	*L. biglobosa*	No	24 July 2014	43.3 ± 18.7	14.3 ± 13.3
7	Ascospores	Yes	24 July 2014	67.1 ± 15.6	77.8 ± 13.7
8	Ascospores	No	24 July 2014	66.4 ± 15.8	66.7 ±1 5.6
9	*L. maculans*	Yes	28 August 2014	60.0 ± 15.4	30.0 ± 14.5
10	*L. maculans*	No	28 August 2014	87.5 ± 11.7	87.5 ± 11.5
11	*L. biglobosa*	Yes	28 August 2014	44.3 ± 16.5	33.3 ± 15.5
12	*L. biglobosa*	No	28 August 2014	50.0 ± 15.7	50.0 ± 15.7
13	Ascospores	Yes	28 August 2014	62.5 ± 17.0	62.5 ± 17.1
14	Ascospores	No	28 August 2014	30.0 ± 14.5	30.0 ± 14.4
15	*L. maculans*	Yes	30 September 2014	33.2 ± 15.7	33.3 ± 15.5
16	*L. maculans*	No	30 September 2014	100.0 ± 6.5	83.3 ± 15.2
17	*L. biglobosa*	Yes	30 September 2014	66.4 ± 15.8	55.6 ± 16.5
18	*L. biglobosa*	No	30 September 2014	67.1 ± 15.6	66.7 ± 15.6
19	Ascospores	Yes	30 September 2014	62.8 ± 17.0	50.0 ± 17.4
20	Ascospores	No	30 September 2014	70.0 ± 14.4	50.0 ± 15.8
21	*L. maculans*	Yes	27 October 2014	49.1 ± 20.4	66.7 ± 19.2
22	*L. maculans*	No	27 October 2014	87.8 ± 11.5	50.0 ± 17.7
23	*L. biglobosa*	Yes	27 October 2014	49.1 ± 20.4	60.0 ± 15.4
24	*L. biglobosa*	No	27 October 2014	87.7 ± 11.5	75.0 ± 15.4
25	Ascospores	Yes	27 October 2014	50.2 ± 17.6	50.0 ± 17.6
26	Ascospores	No	27 October 2014	71.0 ± 17.2	57.1 ± 18.6
			FPr	0.411 (ns)	0.121 (ns)

Table 8. Severity of internal and external symptoms on destructively harvested roots on 13 January 2015.

Trt	Isolate/spore type	Wounding (Yes/No)	Inoculation date	External symptoms (% root surface area affected)	Internal symptoms (% root internal area affected)
1	None	Yes	-	5.7	0.6
2	None	No	-	7.0	9.2
3	*L. maculans*	Yes	24 July 2014	22.9	24.3
4	*L. maculans*	No	24 July 2014	13.6	14.8
5	*L. biglobosa*	Yes	24 July 2014	12.1	23.4
6	*L. biglobosa*	No	24 July 2014	17.9	7.9
7	Ascospores	Yes	24 July 2014	13.7	16.2
8	Ascospores	No	24 July 2014	26.7	29.7
9	*L. maculans*	Yes	28 August 2014	26.9	12.9
10	*L. maculans*	No	28 August 2014	11.9	18.5
11	*L. biglobosa*	Yes	28 August 2014	10.9	14.0
12	*L. biglobosa*	No	28 August 2014	5.8	12.5
13	Ascospores	Yes	28 August 2014	7.3	7.1
14	Ascospores	No	28 August 2014	7.2	10.3
15	*L. maculans*	Yes	30 September 2014	9.0	13.8
16	*L. maculans*	No	30 September 2014	36.7	40.0
17	*L. biglobosa*	Yes	30 September 2014	22.8	16.1
18	*L. biglobosa*	No	30 September 2014	16.5	22.3
19	Ascospores	Yes	30 September 2014	27.5	30.6
20	Ascospores	No	30 September 2014	20.8	16.6
21	*L. maculans*	Yes	27 October 2014	7.8	14.0
22	*L. maculans*	No	27 October 2014	21.3	16.3
23	*L. biglobosa*	Yes	27 October 2014	14.9	12.0
24	*L. biglobosa*	No	27 October 2014	10.9	12.9
25	Ascospores	Yes	27 October 2014	19.3	25.7
26	Ascospores	No	27 October 2014	22.3	12.5
			Mean	16.1	16.7
			Fpr	0.029	0.150 (ns)
			SED	10.81	13.21
			LSD	21.32	26.06

Table 9. Incidence of root infection directly associated with previous inoculations as assessed on 7 November 2014.

Trt	Isolate/spore type	Wounding (Yes/No)	Inoculation date	7 November 2014
1	None	Yes	-	11.3 ± 10.6
2	None	No	-	0.0 ± 0.0
3	*L. maculans*	Yes	28 August 2014	63.8 ± 16.8
4	*L. maculans*	No	28 August 2014	0.0 ± 0.0
5	*L. biglobosa*	Yes	28 August 2014	0.0 ± 0.0
6	*L. biglobosa*	No	28 August 2014	22.0 ± 13.7
7	Ascospores	Yes	28 August 2014	35.4 ± 19.7
8	Ascospores	No	28 August 2014	10.9 ± 10.2
9	*L. maculans*	Yes	30 September 2014	21.2 ± 13.6
10	*L. maculans*	No	30 September 2014	0.0 ± 0.0
11	*L. biglobosa*	Yes	30 September 2014	0.0 ± 0.0
12	*L. biglobosa*	No	30 September 2014	0.0 ± 0.0
13	Ascospores	Yes	30 September 2014	0.0 ± 0.0
14	Ascospores	No	30 September 2014	11.0 ± 10.3
15	*L. maculans*	Yes	27 October 2014	0.0 ± 0.0
16	*L. maculans*	No	27 October 2014	25.0 ± 15.1
17	*L. biglobosa*	Yes	27 October 2014	0.0 ± 0.0
18	*L. biglobosa*	No	27 October 2014	10.9 ± 10.1
19	Ascospores	Yes	27 October 2014	17.9 ± 16.1
20	Ascospores	No	27 October 2014	0.0 ± 0.0
			FPr	0.064 (ns)

Table 10. Incidence of dry rot on inoculated roots with and without wounding prior to inoculation.

Isolate/spore type	With wounding	Without wounding
No inoculation	11.3 ± 10.6	0.0 ± 0.0
L. maculans	33.7 ± 8.5	8.3 ± 5.1
L. biglobosa	0.0 ± 0.0	11.1 ± 5.8
Ascospores	7.9 ± 5.2	18.1 ± 8.6
FPr	0.017	

Table 11. Incidence of root infection (visible from above without destructive sampling) on 7 November and 24 November 2014.

Trt	Isolate/spore type	Wounding (Yes/No)	Inoculation date	7 November 2014	24 November 2014
1	None	Yes	-	46.3 ± 15.8	43.8 ± 16.0
2	None	No	-	0.0 ± 0.0	10.8 ± 10.1
3	*L. maculans*	Yes	28 August 2014	62.5 ± 15.7	76.1 ± 14.4
4	*L. maculans*	No	28 August 2014	44.0 ± 15.8	55.9 ± 15.9
5	*L. biglobosa*	Yes	28 August 2014	39.8 ± 17.4	56.1 ± 17.8
6	*L. biglobosa*	No	28 August 2014	32.9 ± 15.0	33.5 ± 15.3
7	Ascospores	Yes	28 August 2014	55.0 ± 19.4	36.5 ± 19.6
8	Ascospores	No	28 August 2014	10.5 ± 9.8	20.6 ± 12.8
9	*L. maculans*	Yes	30 September 2014	10.5 ± 9.8	31.1 ± 14.6
10	*L. maculans*	No	30 September 2014	69.7± 17.3	54.0 ± 19.4
11	*L. biglobosa*	Yes	30 September 2014	14.1 ± 12.8	43.8 ± 18.2
12	*L. biglobosa*	No	30 September 2014	22.7 ± 13.8	35.2 ± 16.0
13	Ascospores	Yes	30 September 2014	13.3 ± 12.2	26.1 ± 15.7
14	Ascospores	No	30 September 2014	21.9 ± 13.3	22.3 ± 13.6
15	*L. maculans*	Yes	27 October 2014	21.8 ± 18.7	23.0 ± 19.8
16	*L. maculans*	No	27 October 2014	25.6 ± 15.1	37.4 ± 16.7
17	*L. biglobosa*	Yes	27 October 2014	10.9 ± 10.2	55.9 ± 15.9
18	*L. biglobosa*	No	27 October 2014	31.6 ± 14.6	41.7 ± 15.6
19	Ascospores	Yes	27 October 2014	54.3 ± 19.2	51.9 ± 19.5
20	Ascospores	No	27 October 2014	37.6 ± 16.4	37.4 ± 16.4
			FPr	0.674 (ns)	0.946 (ns)

Table 12. Severity of root infection (visible from above without destructive sampling) on 7 November and 24 November 2014.

Trt	Isolate/spore type	Wounding (Yes/No)	Inoculation date	7 November 2014	24 November 2014
1	None	Yes	-	12.8	19.4
2	None	No	-	0.0	2.2
3	*L. maculans*	Yes	28 August 2014	3.1	7.9
4	*L. maculans*	No	28 August 2014	4.4	11.7
5	*L. biglobosa*	Yes	28 August 2014	7.9	20.0
6	*L. biglobosa*	No	28 August 2014	3.3	5.0
7	Ascospores	Yes	28 August 2014	4.7	8.3
8	Ascospores	No	28 August 2014	1.1	3.9
9	*L. maculans*	Yes	30 September 2014	1.1	3.3
10	*L. maculans*	No	30 September 2014	22.5	25.0
11	*L. biglobosa*	Yes	30 September 2014	4.3	13.6
12	*L. biglobosa*	No	30 September 2014	11.3	21.9
13	Ascospores	Yes	30 September 2014	0.7	1.4
14	Ascospores	No	30 September 2014	1.7	5.0
15	*L. maculans*	Yes	27 October 2014	6.3	3.8
16	*L. maculans*	No	27 October 2014	3.4	5.9
17	*L. biglobosa*	Yes	27 October 2014	2.8	3.3
18	*L. biglobosa*	No	27 October 2014	16.1	16.2
19	Ascospores	Yes	27 October 2014	8.3	7.5
20	Ascospores	No	27 October 2014	5.0	3.8
			FPr	0.172 (ns)	0.505 (ns)
			SED	8.07	11.57
			LSD	15.96	22.89

Table 13. Incidence of external and internal symptoms on destructively harvested roots on 13 January 2015.

Trt	Isolate/spore type	Wounding (Yes/No)	Inoculation date	Incidence external symptoms (% plants affected)	Incidence internal symptoms (% plants affected)
1	None	Yes	-	66.7 ± 15.8	55.6 ± 16.6
2	None	No	-	66.7 ± 15.8	44.4 ± 16.6
3	*L. maculans*	Yes	28 August 2014	100.0 ± 0.0	100.0 ± 0.0
4	*L. maculans*	No	28 August 2014	77.8 ± 13.4	33.3 ± 19.4
5	*L. biglobosa*	Yes	28 August 2014	57.1 ± 18.3	57.1 ± 18.8
6	*L. biglobosa*	No	28 August 2014	66.7 ± 15.2	55.6 ± 16.5
7	Ascospores	Yes	28 August 2014	100.0 ± 0.0	100.0 ± 0.0
8	Ascospores	No	28 August 2014	66.7 ± 16.0	55.6 ± 16.7
9	*L. maculans*	Yes	30 September 2014	66.7 ± 15.9	66.7 ± 15.8
10	*L. maculans*	No	30 September 2014	100.0 ± 0.0	33.3 ± 19.4
11	*L. biglobosa*	Yes	30 September 2014	57.1 ± 18.1	28.6 ± 17.1
12	*L. biglobosa*	No	30 September 2014	50.0 ± 17.2	25.0 ± 15.3
13	Ascospores	Yes	30 September 2014	85.7 ± 13.7	28.6 ± 17.0
14	Ascospores	No	30 September 2014	44.4 ±16.3	44.4 ± 16.6
15	*L. maculans*	Yes	27 October 2014	50.0 ± 24.1	50.0 ± 25.0
16	*L. maculans*	No	27 October 2014	87.5 ± 11.4	62.5 ± 17.1
17	*L. biglobosa*	Yes	27 October 2014	88.9 ± 10.1	55.6 ± 16.5
18	*L. biglobosa*	No	27 October 2014	77.8 ± 14.5	33.3 ± 15.6
19	Ascospores	Yes	27 October 2014	100.0 ± 0.0	100.0 ± 0.0
20	Ascospores	No	27 October 2014	75.0 ± 15.2	25.0 ± 15.3
			Mean	74.2	52.7
			FPr	0.082 (ns)	0.009

Table 14. Severity of internal and external symptoms on destructively harvested roots on 13 January 2015.

Trt	Isolate/spore type	Wounding (Yes/No)	Inoculation date	External symptoms (% root surface area affected)	Internal symptoms (% root internal area affected)
1	None	Yes	-	33.9	37.8
2	None	No	-	15.0	14.4
3	*L. maculans*	Yes	28 August 2014	21.9	35.0
4	*L. maculans*	No	28 August 2014	17.9	8.3
5	*L. biglobosa*	Yes	28 August 2014	18.9	32.9
6	*L. biglobosa*	No	28 August 2014	19.9	25.6
7	Ascospores	Yes	28 August 2014	30.0	41.2
8	Ascospores	No	28 August 2014	15.0	20.3
9	*L. maculans*	Yes	30 September 2014	13.9	23.4
10	*L. maculans*	No	30 September 2014	34.7	8.3
11	*L. biglobosa*	Yes	30 September 2014	15.7	14.3
12	*L. biglobosa*	No	30 September 2014	17.3	15.6
13	Ascospores	Yes	30 September 2014	15.4	3.6
14	Ascospores	No	30 September 2014	8.8	12.0
15	*L. maculans*	Yes	27 October 2014	13.8	26.3
16	*L. maculans*	No	27 October 2014	17.5	18.1
17	*L. biglobosa*	Yes	27 October 2014	25.4	33.3
18	*L. biglobosa*	No	27 October 2014	18.2	7.0
19	Ascospores	Yes	27 October 2014	11.7	31.8
20	Ascospores	No	27 October 2014	12.1	5.8
			FPr	0.416 (ns)	0.242 (ns)
			SED	12.66	20.15
			LSD	25.04	39.86