Project title:	Asparagus purple spot: field evaluation of soil treatments to enhance crop debris degradation and reduce disease on the emerging new crop
Project number:	FV 341b
Project leader:	Angela Huckle, ADAS UK Ltd.
Report:	Final report, December 2013
Previous report:	None
Key staff:	Rob Drummond, Dennis Churchill (Senior Research Technicians) Chris Dyer (Statistician) Wilson Dyer (Independent asparagus consultant)
Location of project:	Grower sites in Norfolk and Hereford, with lab work carried out at ADAS Boxworth.
Industry Representative:	Ron Marshall, Cobrey Farms, Hereford.
Date project commenced:	23 November 2012
Date project completed (or expected completion date):	31 December 2013

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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

Urea applied as a spray to asparagus debris at 100 kg N/ha, either as a single application or as 2 applications of 50 kg N/ha post ridging, gave a significant reduction in spore release for up to 28 days post application.

Background

Stemphylium purple spot of asparagus caused by the fungus *Stemphylium vesicarium* often occurs on emerged spears during the harvest season and can lead to problems of quality and customer acceptability in the harvested product. Following harvest, the disease can also develop on asparagus fern affecting main stems, secondary branches and needles leading to premature defoliation and decreased yields in subsequent years where not adequately controlled. The sexual stage of the disease produces survival structures (pseudothecia) (Figure 1) which overwinter on fern debris and are the initial source of the disease in the following spring. Once purple spot is present in a crop, asexual spores (conidia) are produced on lesions in wet weather and are readily spread by wind and water splash leading to rapid disease increase. The disease can be very difficult to control once established, and most growers rely on preventative fungicide programmes to preserve green fern area through summer into autumn, the aim being to providing adequate carbohydrate return to the root system for harvest the following year. A typical fungicide programme consists of four or five applications and represents a substantial cost in time and effort to the grower.

Figure 1. Overwintering resting bodies (pseudothecia) of *Stemphylium vesicarium* which are seen as raised black dots on asparagus debris remaining on the soil surface in early spring, 2013.

Following spring cultivations, fern debris from the previous season is commonly found on the soil surface as spears emerge. Burial of this debris by ridging up after flailing to remove the fern reduces the potential amount of *Stemphylium* spore release in the following spring, but in older crops where crowns and roots develop closer to the soil surface this can be difficult due to the risk of damage to the extensive root system, so large amounts of debris may remain on the surface. Heavy rainfall can also cause the debris to become exposed again. Previous work in FV 341 indicated the potential of urea as a treatment for reducing *S. vesicarium* inoculum overwintering on asparagus fern debris. The aim of this project was to determine the efficacy of urea and Perlka (calcium cyanamide) debris treatments in field crops by examining their effect on (i) level and persistence of spore production from debris and (ii) occurrence of purple spot on spears.

Summary

Two field experiments were carried out in 2013 using commercial crops of asparagus cv. Gijnlim. The experiments were located in Norfolk and Herefordshire. Each field site had a known history of *Stemphylium* purple spot and resting bodies (pseudothecia) of the pathogen were present on the debris at each site and treatment timing. Nine treatments were applied to the crop over three timings as shown in Table 1 below.

Table 1. Detail of the urea and Perlka treatments applied to asparagus crops in 2013.

			Application rate and timing (kg N/ha)		
Treatment	Product	Water volume (l/ha)	T1 Jan/Feb (post flailing)	T2 Mar (post ridging but pre herbicide)	T3 + 4 weeks after T2
1		-	0	0	0
2 (standard)	urea*	1000	0	100	0
3	urea*	400	0	100	0
4	urea*	400	0	50	0
5	urea*	400	50	0	0
6	urea*	400	0	50	50
7	urea*	400	50	50	0
8	Perlka	top dressing	60	0	0
9	Perlka	top dressing	0	60	0

** urea applied with Silwett – L77 (wetter) at 0.025%*

There were three treatment timings as shown and these were as follows;

- **Timing 1 –** The first treatments were applied January/February after the grower had flailed the fern from the previous season
- **Timing 2 –** The second treatments were applied in March/April after ridging but before the pre-harvest residual herbicide application, and at least 4 weeks after T1
- **Timing 3 –** The third treatments were applied 4 weeks after Timing 2.

The treatment timings were chosen in relation to when the ascospores would be maturing or released based on the findings in of HDC project FV 341. This showed that from January to March mature pseudothecia contained abundant ascospores, which were starting to erupt through the epidermal stem tissues and that the proportion of pseudothecia erupting through skin tissue increased from January to March. Therefore Timing 1 and 2 were included to test whether treatment application to debris on the soil surface in late winter or early spring could delay or prevent release of ascospores. An application of urea at Timing 3 in addition to Timing 2 was included to compare if two later treatments post-ridging gave better efficacy than two treatments applied pre and post-ridging.

Effect of urea application on spore release

Urea applied as a spray to asparagus debris at the rate of 100 kg N/ha post ridging gave a statistically significant reduction in spore release for 28 days post application compared to the untreated (Table 2). Statistical significance was maintained when 100 kg N/ha was applied at either 1000 l/ha or 400 l/ha, or as 2 applications of 50 kg N/ha post ridging (Timing 2 and 3) at 400 l/ha, at both experimental sites. A rate of 50 kg N/ha applied at 400 l/ha as two early applications (Timing 1 and 2) or once only gave a reduction in spores but this was not consistently significant at all assessments. Urea applied as an early treatment at 50 kg N/ha at a volume of 400l/ha post-flailing, but pre-ridging gave a significant reduction in spore release at both sites for 28 days post application. Perlka did not consistently reduce spore release from the debris at either site.

Table 2. Summary of the effect of urea and Perlka treatments on production of *Stemphylium vesicarium* ascospores from asparagus crop debris – 2013

Treatment and application timing					% spore production (untreated = 100) at one and two months after treatment			
		T1	T2	T3	Hereford		Norfolk	
			kg N/ha		28 Days after T1	56 Days after T1	28 Days after T1	56 Days after T1
1	Untreated	0	0	0	100	100	100	100
2	urea	0	100*	0	**15**	**24**	**45**	108
3	urea	0	100	0	**63**	**67**	**34**	90
4	urea	0	50	0	94	95	76	82
5	urea	50	0	0	**69**	87	**51**	87
6	urea	0	50	50	**46**	**49**	**50**	**49**
7	urea	50	50	0	**52 (38)**	98 **(35)**	**32** (84)	47 (74)
8	Perlka	60**	0	0	108	133	97	113
9	Perlka	0	60**	0	73	89	**24**	**13**

Values in bold are significantly different from the untreated
Figures in brackets are days after T2 treatment was applied
*1,000 l/ha **top dressed

Effect of urea applications on purple spot in the emerging crop

The application of urea at 100 kg N/ha either at 400 l/ha or 1000 l/ha significantly reduced purple spot on the spears at one assessment out of three carried out at the Hereford site (Figure 2). These assessments were carried out during harvest and freshly emerged spears were assessed each time. When considering the data from the spear assessments, it is necessary to take into account movement of spores between plots in high winds as the crop is very sparse and open at this point. Although the plots are large, the openness of the crop may have led to the lack of differences between treatments in the earlier assessments as these were carried out just after periods where winds were recorded up to 20 mph.

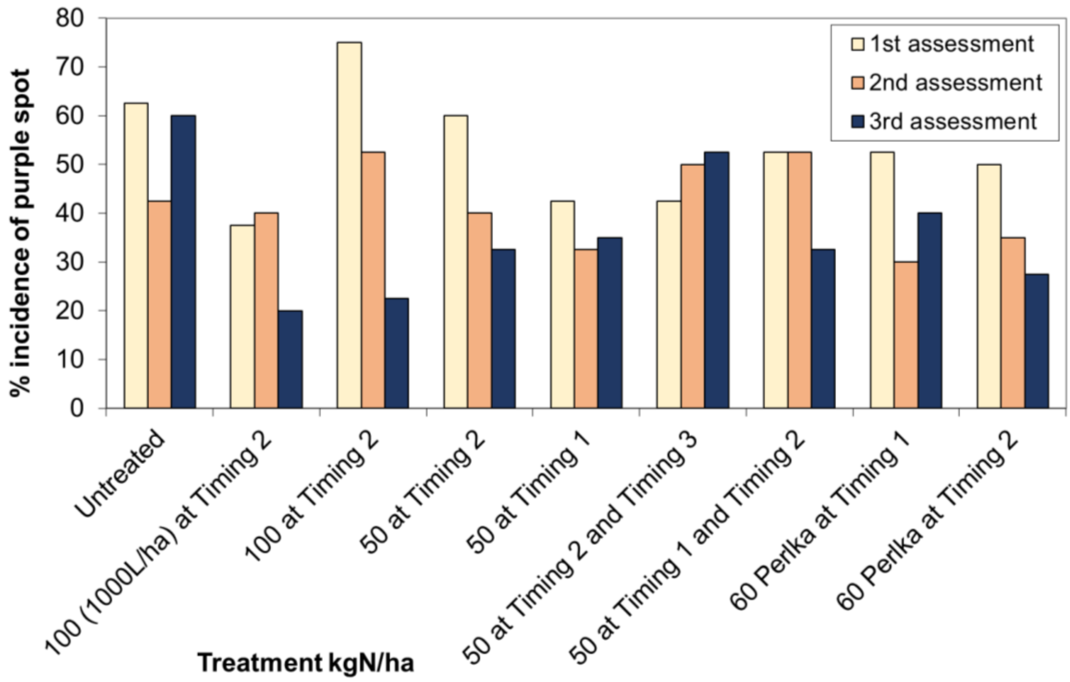

Figure 2. Incidence of purple spot on the emerging spears at assessments on 18 May, 31 May and 2 June, Hereford, 2013.

All other assessments of purple spot on the spears, the stem bases of ferns, or the fern canopy showed no significant differences. However urea did reduce purple spot, but this was not consistent across all assessments at either site. Some treatments (100 kg N/ha applied at either 1000 l/ha or 400 l/ha, or 50 kg N/ha applied at two timings) appeared to reduce incidence in the spears, on the stem bases, or in the fern canopy, but these reductions did not achieve statistical significance or consistent effects in assessments at both sites. This may be because disease incidence was generally low in 2013. However, these treatments warrant further investigation in another season where disease levels may be higher and differences easier to separate.

The most promising treatments based on the reduction of spore release were 100 kg N/ha applied at either 1000 l/ha or 400 l/ha, or 50 kg N/ha applied at two timings. The former treatment is certainly too high a volume to be commercially practical, especially on a large scale. Results showed a water volume of 400 l/ha giving a result comparable to 1000 l/ha in terms of reduction of purple spot in the emerging crop. Although this represents an improvement, lower volumes would be preferable from a commercial point of view. Applying the majority of the nitrogen requirement as urea at 100 kg N/ha just prior to spear growth also represents poor environmental practice given that N uptake is very low in early spring and bearing in mind the N_{max} for asparagus in an NVZ is 180 kg N/ha. Such practice would increase the risk of N loss to leaching in a wet spring or to volatilization in a dry spring. To reduce spore release with fewer compromises in terms of crop nutrition or increased environmental risk, applying urea twice as 50 kg N/ha instead of rather than in addition to any other nitrogen fertiliser could be considered since this gave comparable results to 100 kg N/ha at 400l/ha (Table 2).

Further work

Although the significant reduction in spore release did not result in a consistent reduction of Stemphylium purple spot, the most promising treatments warrant further investigation in another season as it is difficult to make firm conclusions based on data from one year of study in a low disease year. Additionally, an application of urea between spear harvest and fern growth was not considered in this study, but could be a useful timing to look at in further work to see if additional control closer to fern growth could be more beneficial if spores are still being released from the resting bodies. There are also commercially available urea formulations available such as Nufol 20 which may be more practical to use than the technical grade urea used in the study, and work on their efficacy against spore release would be useful.

Financial Benefits

The project shows that urea reduces spore release from debris for 28 days post application. When applied instead of or as an additional fertiliser application after ridging and prior to spear emergence, urea has potential to reduce the inoculum pressure during harvest. The urea spray would most likely be best used as part of an integrated approach to disease control alongside existing fungicide and fertiliser programmes. It could be argued that with reduced inoculum pressure post-harvest, the first spray application could be delayed and numbers of sprays reduced. However since most available fungicides are primarily protectant in activity, then this would probably be inadvisable. The use of urea for

Stemphylium control therefore represents an additional input on top of any fungicide programs.

Applying an extra urea spray as well as ammonium nitrate and alongside a fungicide programme may well add to input costs for disease control by c. £60/ha using current urea costs of £280/tonne. However urea provides another potential method for *Stemphylium* control by reducing spore release and infection at the stem bases, in turn possibly reducing the risk of infection, in an area which is difficult to reach with fungicide sprays when the canopy is closed. Assuming an average yield of 1 tonne/ha and a farm gate price of £5,500/tonne, a yield loss of only 1.1 % represents a reduction in sales equivalent to the cost of the additional urea input. Therefore if urea provides a following year yield benefit greater than 1.1% by additional control of *Stemphylium*, it is worth considering as part of an integrated programme of *Stemphylium* control in asparagus at current urea prices.

Action Point

- Application of urea as a spray to asparagus debris post-ridging, reduces spore release from *Stemphylium* resting bodies seen on asparagus debris. The most effective rate and timing post-ridging is 100 kg N/ha in either 1000 l/ha or 400 l/ha water volume. However this is not the most practical rate and timing as N uptake by the crop at this time is low and the practice would lead to diffuse pollution. Growers should consider applying 50 kg N/ha as two applications pre-harvest as this still gives comparable reduction in spore release, and may be a useful addition to the fertiliser regime whilst reducing the risk to the environment.

SCIENCE SECTION

Introduction

Stemphylium purple spot of asparagus caused by the fungus *Stemphylium vesicarium* often occurs on emerged spears during the harvest season and can lead to problems of quality and customer acceptability in the harvested product. Following harvest, the disease can also develop on asparagus fern affecting main stems, secondary branches and needles and leading to premature defoliation and decreased yields in subsequent years where not adequately controlled. The sexual stage of the disease produces survival structures (pseudothecia) (Figure 1) which overwinter on fern debris and are the initial source of the disease in the following spring. Once purple spot is present in a crop, asexual spores (conidia) are produced on lesions in wet weather and are readily spread by wind and water splash leading to rapid disease increase. The disease can be very difficult to control once established, and most growers rely on preventative fungicide programmes to preserve green fern area through summer into autumn, with the objective of providing adequate carbohydrate return to the root system for harvest in the following year. A typical fungicide programme consists of four or five applications and represents a substantial cost in time and effort to the grower.

Following spring cultivations, fern debris from the previous season is commonly found on the soil surface as spears emerge. Burial of this debris by ridging up after flailing to remove the fern reduces the potential amount of *Stemphylium* spore release in the following spring, but in older crops where crowns and roots develop closer to the soil surface this can be difficult due to the risk of damage to the extensive root system, so large amounts of debris may remain on the surface. Heavy rainfall can also cause the debris to become exposed again. Work done in FV 341 highlighted the potential of urea as a treatment for reducing *S. vesicarium* inoculum overwintering on asparagus fern debris. The project aimed to determine the effectiveness of debris treatments in field crops by examining their effect on (i) level and persistence of spore production from debris and (ii) occurrence of purple spot on spears.

This was determined against the objectives:

1. To determine the efficacy of dormant season treatments for reduction of ascospores release from crop debris by *S. vesicarium*
2. To determine the effect of these treatments on occurrence of purple spot in the emerging crop.

Materials and methods

Site selection

Two trials were carried out on growers holdings in Norfolk and Herefordshire; consisting of nine treatments in two asparagus crops cv. Gijnlim with a history of *Stemphylium* and abundant pseudothecia present on the overwintering debris. The trial areas were located within commercial crops and arranged so that treatments could be applied by spraying equipment mounted on a Kawasaki Mule or by an Oxford Precision knapsack sprayer. The trial area at each site including surrounding discard was approximately 11, 000 m^2 and individual plots were marked out within these areas using a randomised block design, with each plot measuring 20 m long x two spray widths (3 crop rows) plus 1 row discard either side which equates to 8 rows (approximately 12 m width). Five metre buffers were used between the ends of each plot to limit cross-contamination through debris movement during farm operations, and only the central six rows within the 20 m treated area were assessed.

The *Stemphylium* was naturally occurring and present as resting bodies on the debris in the chosen crop at each site at the first application timing. The trial crops were managed as per commercial practice, e.g. routine treatments applied as well as crop husbandry and the crop was marketed as normal.

Treatments and trial design

The trial at both sites was laid out in a fully randomised block design with nine treatments including an untreated control replicated four times. Nine treatments were applied to the crop over three timings as in Table 3 below. All treatments fell within NVZ regulations current at the time of the trial.

Table 3. Detail of the urea and Perlka treatments applied in 2012/13.

Treatment	Product	Water volume (l/ha)	Application rate and timing (kg N/ha) T1 Jan/Feb (post flailing)	T2 Mar (post ridging but pre herbicide)	T3 + 4 weeks after T2
1		-	0	0	0
2 (standard)	urea*	1000	0	100	0
3	urea*	400	0	100	0
4	urea*	400	0	50	0
5	urea*	400	50	0	0
6	urea*	400	0	50	50
7	urea*	400	50	50	0
8	Perlka	top dressing	60	0	0
9	Perlka	top dressing	0	60	0

** urea applied with Silwett – L77 (wetter) at 0.025%*

There were three treatment timings as shown in Table 3 and detailed below. Each treatment required close liaison with the grower particularly at Norfolk in February to ensure that Timing 2 was applied within the very small window of opportunity. At this site a residual herbicide is applied within a few days after chain harrowing and ridging has taken place, therefore it was necessary to apply the timing 2 treatments within this short window to avoid disturbing the herbicide. At the Hereford site chain harrowing is not carried out, prior to the timing 2 application, rotavating and ridging was carried out in one pass at this site.

- **Timing 1 –** The first treatments were applied January/February after the grower had flailed the fern from the previous season
- **Timing 2 –** The second treatments were applied in March/April after ridging but before the pre-harvest residual herbicide application.
- **Timing 3 –** The third treatments were applied 4 weeks after Timing 2.

The urea treatments were applied to the plots as a spray over the debris and soil using a 6 m spray boom mounted on the back of a Kawasaki Mule (Figure 3). On the occasions before the mounted sprayer was available at the Norfolk site, the urea was applied to the plots as a spray using an OPS knapsack sprayer and the appropriate spray boom. All treatments were applied using an OPS knapsack and an appropriate spray boom at the Herefordshire site. The urea used was industrial specification technical grade urea supplied by Yara, and all treatments were dissolved in hot water before use. Urea treatments were applied at the water volumes detailed, using the appropriate nozzles and pressures to achieve the rates required. All the treatments were also applied with a wetter (Silwett – L77) to maintain continuity with previous work in FV 341, and encourage coverage of the debris. The Perlka treatments were applied by hand and at the same times as the urea for both Timing 1 and 2. All experimental treatments were applied by ADAS staff as detailed in the crop diary (Appendix 1).

Figure 3. Mounted spray equipment applying urea to a plot, Norfolk, 2013.

Assessments

Spore release was assessed 1, 7, 14, 28 and 56 days after each spray timing. This was done by collecting debris at random from the central six rows of each plot, and excluding 1m around the plot edges to avoid edge effects. The pieces were suspended above agar, with five pieces of debris on each plate, and four plates were prepared per plot to give 20 results per plot. Intensity of release of ascospores was estimated on the agar below. This was categorized into low, medium or high ascospore release. (Low = 0-50 spores per piece, Moderate = 50-150 spores per piece, High > 150 spores per piece). The results were categorized rather than counted, as counting the number of spores released was very time consuming, and there were clear differences between each category chosen.

Incidence was assessed as presence or absence of purple spots on the spears from 10 spears in each plot. At the same assessment timing, severity of purple spot was assessed as a visual % score on the spears at these points at appropriate timings throughout harvest when infection would be expected, e.g. after high rainfall events. Incidence and severity of purple spot on stem bases and in the canopy was also assessed during the fern growth stage. This was assessed on ten spears at five points in each plot

Statistical analysis

The debris data was obtained from four plates per plot, each having five assessments of the debris making a total of 20 assessments per plot. They were assessed as 0, L, M or H categories as described above. The numbers of assessments in each category were then analysed, using a generalized linear model (GLM) in Genstat, using the logistic regression model which is appropriate for proportions. The proportions in each category were analysed and tested for differences, and the proportions for each treatment were calculated along with the standard error of the proportion.

Throughout the report only the 0 category was used to plot the line graphs reporting results as proportion of debris with ascospores released. The proportion of debris releasing ascospores was calculated by converting the predicted values reported for the treatment to percentages, by subtracting the predicted value from 1 and multiplying by 100 (see data analysis in Appendix 2 for predicted values and details of significant differences).

The standard errors vary for each treatment, so the 95% confidence intervals for each treatment were calculated to make it easier to identify differences between treatments. Confidence interval (CI) was estimated using standard errors (SE) for each predicted value ($CI = SE \times t_{95\%}$, where df = 9 after timing 1, 18 after timing 2, and 3 after timing 3). If when comparing two treatments, the confidence intervals do not overlap we can say that they are significantly different.

The incidence and severity of purple spot on spears, stem bases and fern canopy was analysed using ANOVA.

Results

The trials were carried out at two sites (Hereford and Norfolk) with differing climate conditions. Results from each site are presented with reference to the project objectives.

Efficacy of dormant season treatments for reduction of S. vesicarium ascospore release from crop debris.

Hereford site

The first urea and Perlka treatments were applied on 27th February after the fern was flailed and the beds ridged. Significant differences in spore release were seen at each assessment date between the untreated plots and those where urea was applied at 50 kg N/ha (P 0.046 to <0.001, full data analysis in appendix 2) (Figure 4). Perlka was not effective at reducing spore release from the February application, but this could be due to the low temperatures at application when warm temperatures and moisture are necessary for Perlka to work. At one day and 28 days after the first treatment application the urea at 50 kg N/ha reduced spore release from the resting bodies on the debris by 40%. Persistence of spore reduction continues significantly until 28 days (P < 0.001), but at 56 days post application all treatments have lost significant efficacy when compared to the untreated. Treatments

2,3,4,6 and 9 were untreated at the first timing, and therefore no samples were taken and they were the same as T1 and untreated at this stage.

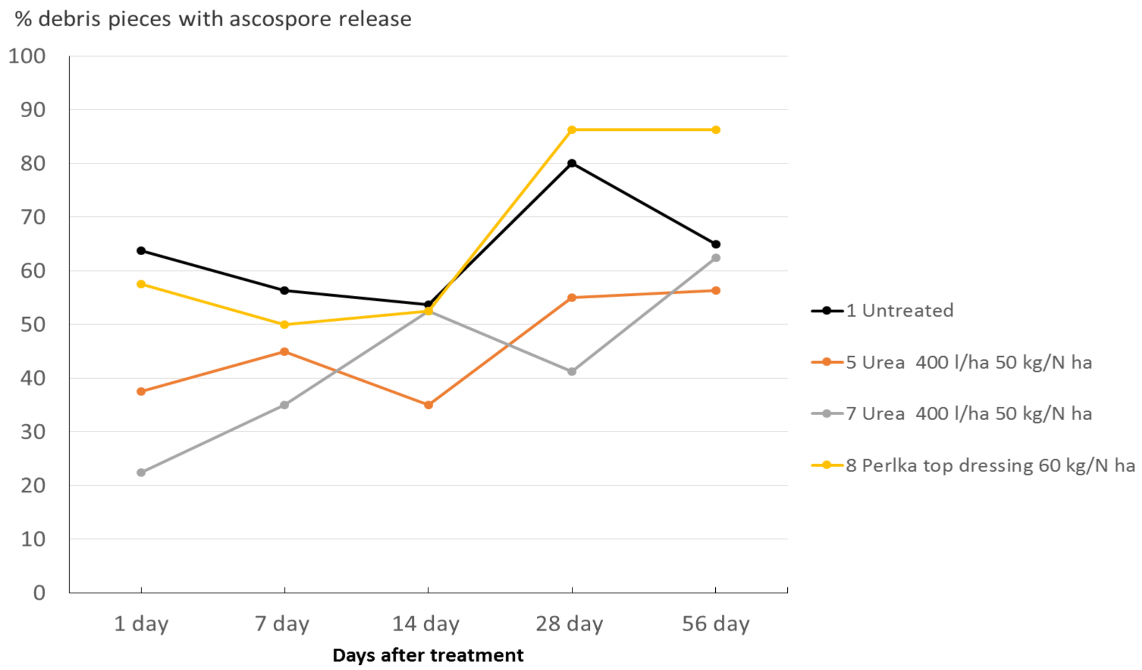

Figure 4. Trend in percentage of ascospores released from resting bodies on debris for a period of 8 weeks after urea and Perlka applied before ridging at the Hereford site, March to April 2013 (i.e. after the first treatment application and before the second).

All treatments showed an increase in active spore release with time, possibly due to maturation of pseudothecia spores and favourable weather periods for spore release. Therefore although there is a reduction in spore release with urea application, this is expressed relative to the intensity of spore release from the untreated. Perlka increased spore release compared with untreated, while urea decreased spore release during the first four assessments but was not associated with any significant difference at the final assessment.

The second urea and Perlka treatments were applied on 1st April, and again significant reductions of spore release at each timing were seen for some of the urea treatments (P <0.001, full data analysis in Appendix 2). At 14 days after the second application of treatments, spore release increased to give heavy release in the untreated and this trend was mirrored across all treatments, as weather conditions were favorable with 5.7 mm rain on 12 April, two days before the debris was collected. The urea treatments still showed less spore release compared with untreated debris despite the favourable conditions, with only 37 to 60% of spores released from the urea treated plots when compared with 81% spores released from debris in the untreated plots (Figure 5). This represents a reduction in spore release of 26 to 54% compared with the levels in the untreated plots. The urea treatments where the 100 kg N/ha rate was applied either at 1000 l/ha or 400 l/ha or where 50 kg N/ha was applied twice to give a total amount applied of 100 kg N/ha, were still significantly reducing spore release at 56 days post application (P <0.001). As seen previously in Figure 3, again the reduction of spore release over time by urea is relative to the intensity of spore release which is most likely influenced by the maturation of pseudothecia spores and favourable weather conditions for spore release.

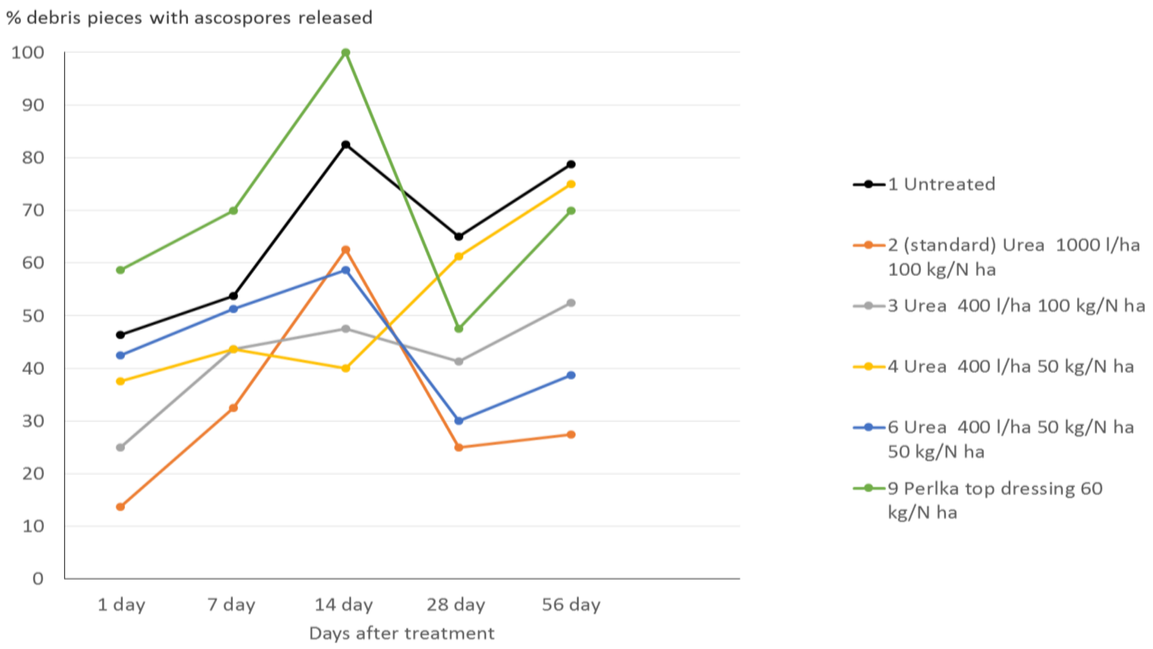

Figure 5. Trend in percentage of ascospores released from resting bodies on debris for a period of 8 weeks after the second timing application of urea and Perlka applied after ridging at the Hereford site, April to June 2013.

As the Timing 1 and Timing 2 treatments were applied approximately 28 days apart, it was possible to compare the data assessed 56 days post application of the Timing 1 treatments with the data assessed 28 days post application of the Timing 2 treatments (Figure 6). From this it can be seen that at this time of assessment the greatest suppression of spore release was from the 100 kg N/ha treatment applied at 1000 l/ha. Urea applied at a rate of 100 kg N/ha at 400 l/ha, and applied as two applications of 50 kg N/ha at 400 l/ha also gave a significant reduction in spore release compared with untreated debris (P <0.001).

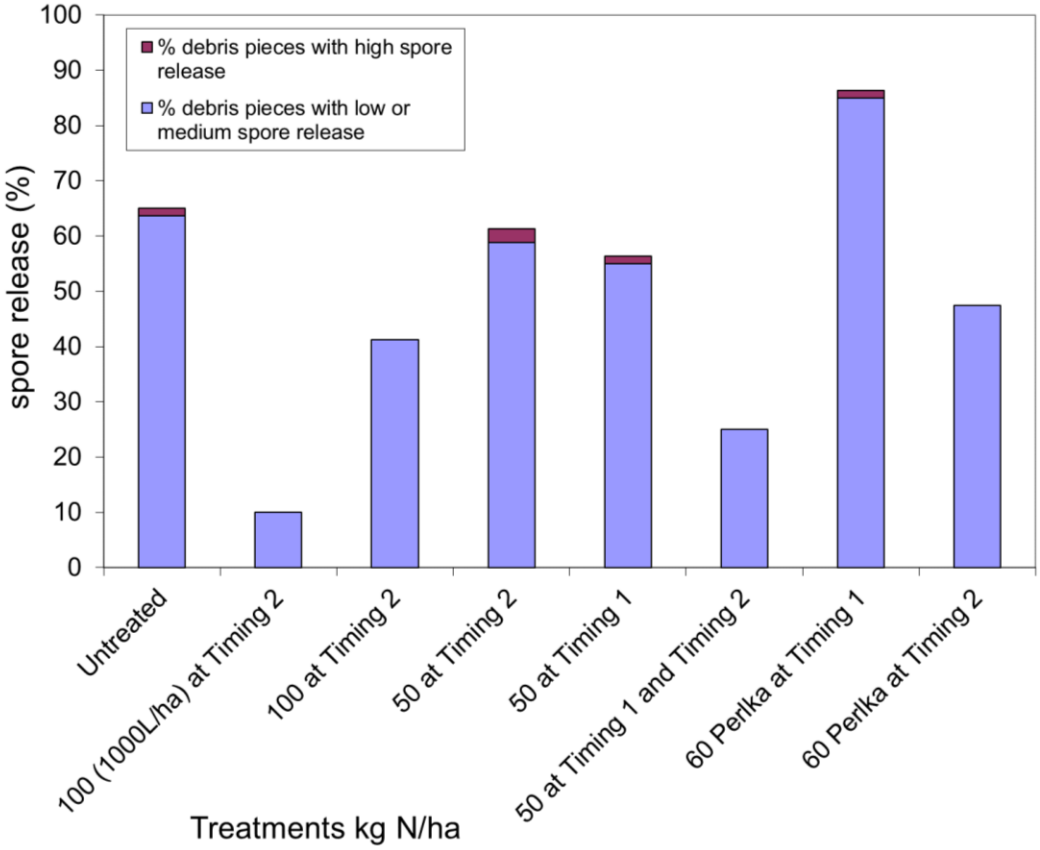

Figure 6. Percentage of debris from which ascospores were released 28 days after 2nd timing application, and 56 days after 1st timing application, assessed 31st April 2013, Hereford. The percentage of debris with high spore release are also shown (high spore release is > 150 spores per piece)

Norfolk site

At this site the first treatments were applied on 8th January after flailing and before ridging, and debris was fully assessed at 14, 28 and 56 days after application only. The debris was collected at 1 and 7 days after application and briefly assessed for spore release but very little spore release was seen at these assessments, due to possibly immature pseudothecia and unfavourable conditions for spore release. The 14 day collection of debris was delayed by snowfall and collected at 21 days post treatment, and at this timing 23% of the debris in the untreated plots was releasing spores in the lab tests (Figure 7). Again, the ascospore release from the resting bodies was significantly reduced at all assessments ($P = 0.002$ to <0.001). Following treatment, it appeared that Perlka had the most impact on ascospore release. This was unexpected since the weather was cool and calcium cyanamide requires warm, moist soil conditions for maximum efficacy. It is not clear in light of contrasting results at the Hereford site whether the reduction of ascospore release was due to the Perlka application.

At this site in the east, the untreated trend showed fewer ascospores released from the debris than in the west, possibly due to the drier climate. Also, this application was made seven weeks earlier than the treatments applied in the west and therefore, the pseudothecia may have been at a more immature stage of development.

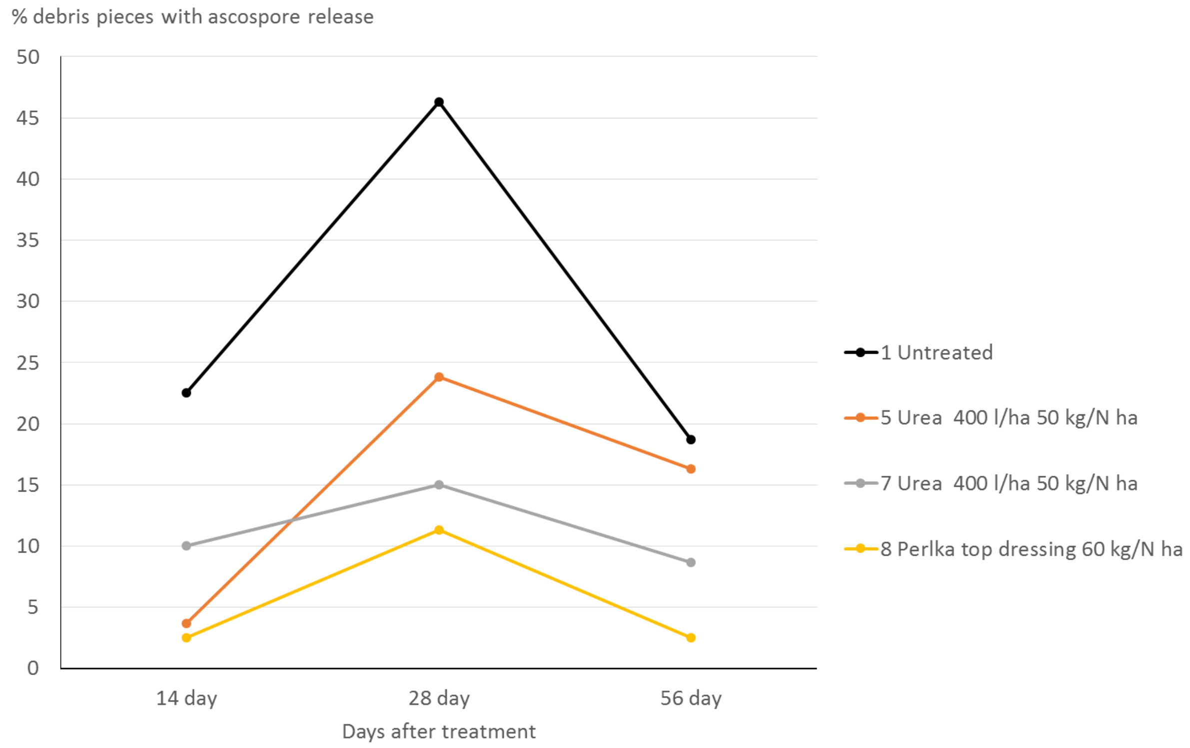

Figure 7. Trend in percentage of ascospores released from resting bodies on debris for a period of 8 weeks after urea and Perlka were applied before ridging at the Norfolk site, January to March 2013.

The second urea and Perlka treatments were applied on 8th April at the Norfolk site after ridging had taken place, and assessments for spore release were carried out. Again, the quantity of spores released from the untreated plots was less than seen at the Hereford site. The trend in spore release from the resting bodies on the debris increased steadily from April to June, from 26% of spores released in the untreated plots on 9th April to 49% spores released by the start of June. At assessments from seven to 28 days after the urea was applied, spore release was reduced significantly at the rate of 100 kg N/ha either applied at 1000 l/ha or 400 l/ha (Figure 8). Treatment 6 of the 50 kg N/ha treatments also significantly reduced spore release in comparison to the other treatments, as at this point in assessment a further application of urea had been applied as planned. Treatment 6 also continued to significantly reduce spore release up to 28 days after the second urea application was applied ($P < 0.001$).

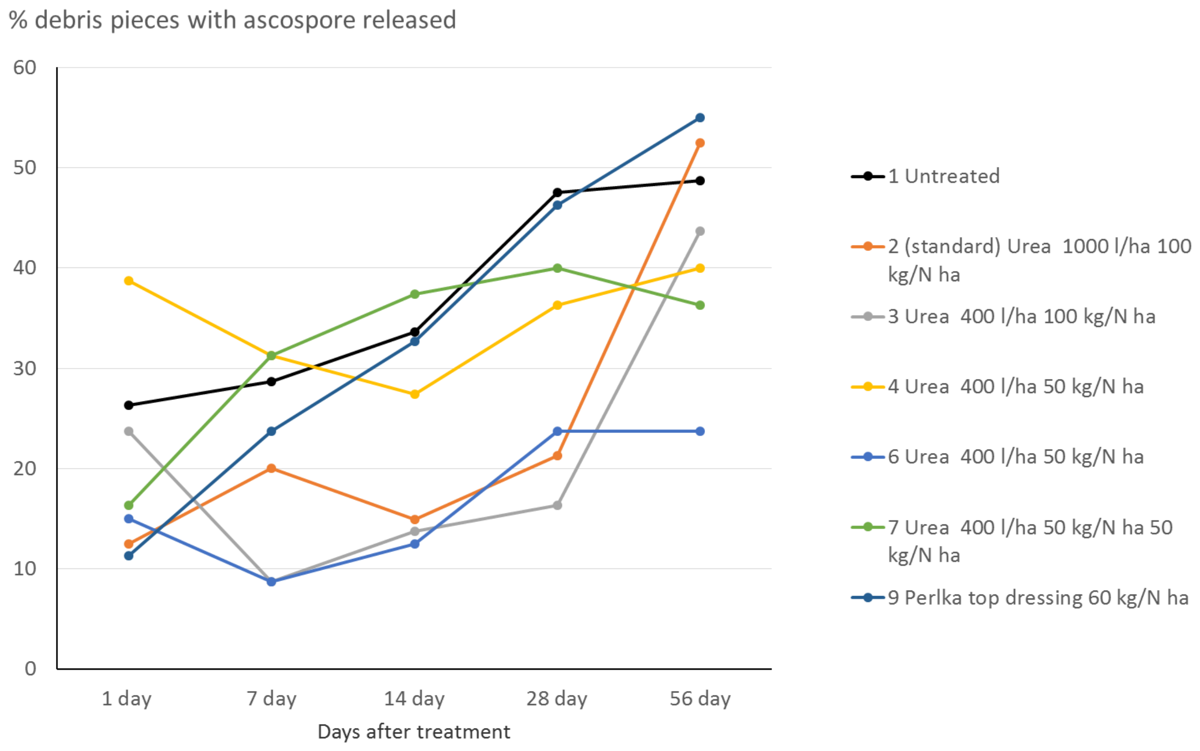

Figure 8. Percentage of ascospores released from resting bodies on debris for a period of 8 weeks after urea and Perlka were applied after ridging at the Norfolk site, April to June 2013.

Effect of urea and Perlka on occurrence of purple spot in the emerging crop and ferns.

Hereford site

The spear assessments took place after spells of significant rainfall, after which infection by purple spot would be expected to take place. The incidence and severity of purple spot on spears was assessed as described in the materials and methods section, three times on 18 May, 31 May and 2 June. Severity was low (0.31% to 1.35%) at each assessment despite the rainfall in the week beforehand and no significant differences were seen. Full datasets can be found the Appendix 2.

There were weakly significant differences in incidence of purple spot at the assessment carried out on 2 June (P = 0.094). With the exception of the treatment where 50 kg N/ha was applied twice after ridging, the treatments that performed best in reducing incidence are those where spore release was reduced (Figure 9). As in the spore release results the treatments where 100 kg N/ha of urea was applied performed best and reduced incidence of purple spot on the emerging spears.

The assessments carried out in May 2013 do not follow a similar pattern but were carried out after a week of winds between 10 and 24 mph from 8 May to 13 May, and 5 and 23 mph from 23 May to 28 May which would have caused spores to be carried across plots and confound the results (Table 4). Winds had calmed to a maximum of 13 mph in the five days preceding the assessment on 2 June where the weakly significant differences were seen and the treatments of 100 kg N/ha reduced the incidence of purple spot on the spears compared to the untreated plots (Figure 9).

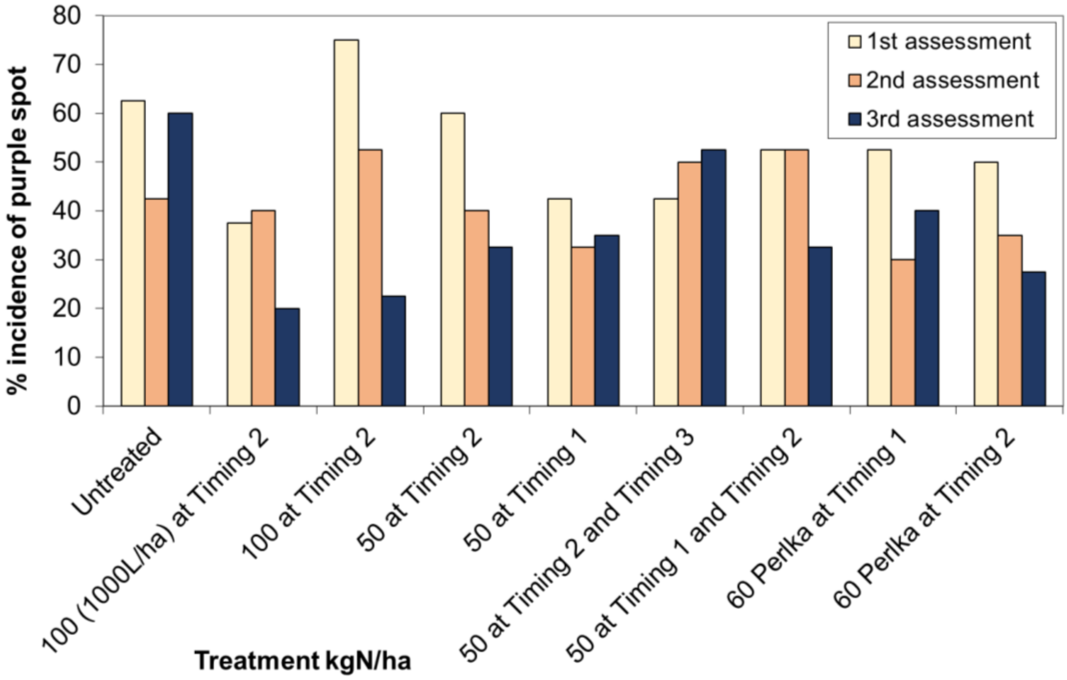

Figure 9. Incidence of purple spot on the emerging spears at assessments on 18 May, 31 May and 2 June, Hereford, 2013.

Table 4. Windspeed, temperature and rainfall around the time of the assessments of purple spot on the emerging spears on 18 May, 31 May and 2 June, Hereford, 2013.

DATE*	Windspeed at GMT hrs				Temperature (ºC)		Rain (mm)	
	0	600	1200	1800	max	min	0900-2100	0900-0900
02-May-13	7	2	4	3	16.2	0.8	0	0
03-May-13	4	5	11	15	14.9	1.4	0	0.6
04-May-13	11	13	16	14	14.8	8.4	0.8	0.8
05-May-13	6	11	12	10	16.5	5.3	0	0
06-May-13	5	2	6	7	19.5	1.5	0	0
07-May-13	3	0	10	11	21.5	3.5	0	2.9
08-May-13	7	13	18	15	15.9	10.7	0.7	1.1
09-May-13	9	14	24	20	11.9	6	3.7	4
10-May-13	18	10	19	16	14.6	8.1	0.6	0.7
11-May-13	11	11	16	16	12.6	4.8	2.4	2.9
12-May-13	7	7	12	13	12.2	3.7	1.2	1.2
13-May-13	12	11	17	15	11.4	6.1	3	3.1
14-May-13	7	10	11	7	8.8	2.6	17	39.7
15-May-13	15	14	16	10	12.4	3.6	1.2	1.3
16-May-13	4	2	5	7	13.9	1	0.1	3.1
17-May-13	5	6	6	9	12.4	5.1	0	0
18-May-13	**4**	**5**	**10**	**7**	**13.7**	**6.4**	**0**	**0**
19-May-13	2	2	6	9	17.5	3.2	0	0
20-May-13	6	8	12	9	15.9	6.4	0	0
21-May-13	9	9	9	11	13.5	9.5	0	0
22-May-13	4	9	15	17	13.7	4.6	0	1.2
23-May-13	10	11	16	17	11.2	3.8	4.2	5.4
24-May-13	7	11	23	14	10.8	3.7	0.6	0.6
25-May-13	7	5	4	5	15.5	1.8	0	0
26-May-13	5	2	4	12	17.6	1.7	0	0
27-May-13	5	7	18	17	14.2	2.8	0.4	4
28-May-13	9	4	6	10	10.8	7.2	21.7	23.2
29-May-13	6	4	9	11	14.8	7.3	4.3	7.1
30-May-13	8	10	13	11	15.2	9.2	3	3
31-May-13	**6**	**6**	**8**	**12**	**19.4**	**8.5**	**0**	**0**
01-Jun-13	7	8	13	12	15.8	4.8	0	0
02-Jun-13	**3**	**4**	**11**	**10**	**16.6**	**4.5**	**0**	**0**

*assessment dates in bold

Fern assessment was carried out once on 30 September and disease levels were low with 10% incidence in the untreated plots in stem bases and canopy and 0.01% severity. In the dry summer of 2013 *Stemphylium* was well controlled in the crop by the commercial fungicide programmes used at each site and no significant differences were seen from the addition of urea treatments. However, the 100 kg N/ha treatments did reduce the incidence of purple spot on the stem bases to 0% at the points where the crop was assessed (Figure 10).

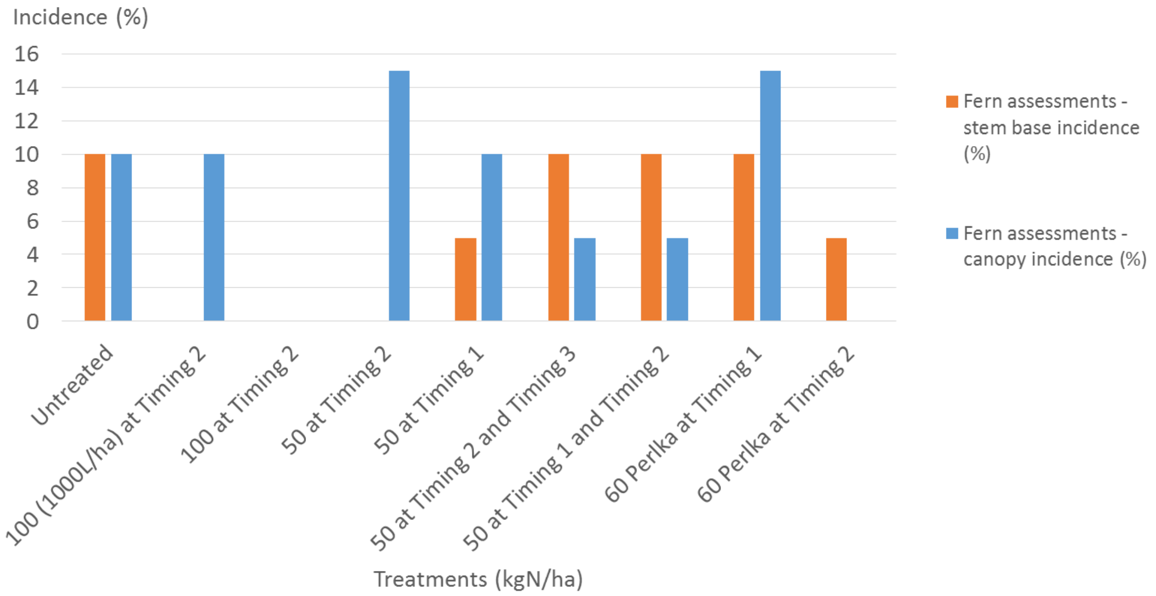

Figure 10. Incidence of purple spot on the stem bases and in the fern canopy at assessment on 30 September, Hereford, 2013.

Norfolk site

Only one spear assessment was carried out on 31 May as the disease levels were low in the crop at this growth stage with 15% of spears affected by purple spot in the untreated (Figure 11). The weather was considerably drier at the eastern site during harvest, and these less favourable conditions for *Stemphylium* infection led to less symptoms on the spears with severity of purple spot only 0.2% stem area affected in the untreated plots. Some treatments (100 kg N/ha applied at either 1000 l/ha or 400 l/ha, or 50 kg N/ha applied at two timings) appeared to reduce incidence on the spears, but these reductions did not achieve statistical significance or consistent effects in assessments at both sites.

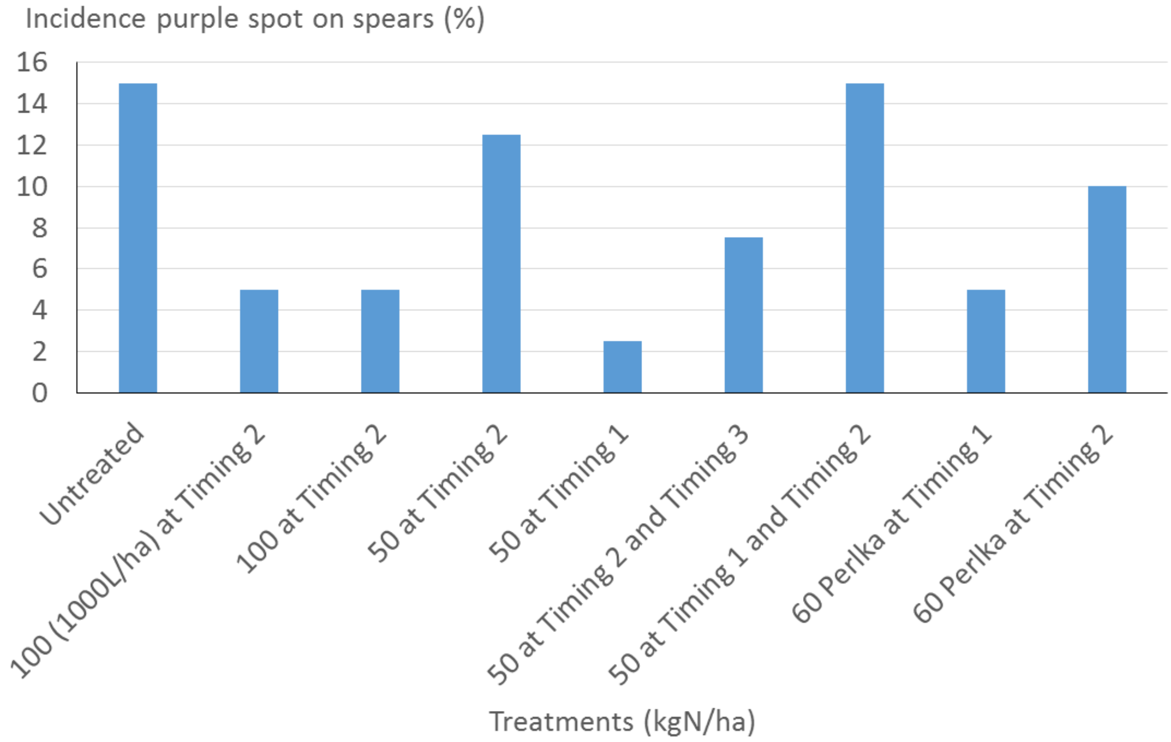

Figure 11. Incidence of purple spot on the emerging spears at assessments on 31 May, Norfolk, 2013.

Once the crop was left to develop fern following harvest, there was a greater incidence of purple spot in the ferns on the stem bases and canopy at the Norfolk site with 90% incidence on the stem bases in the untreated plots at the 1st assessment on 29 August, and 85% in the untreated plots at the 2nd assessment on 24 September. However, there were no significant differences between treatments in the incidence of purple spot. Incidence of stem base infection varied between 80 - 95% in the first assessment and 55 – 85% at the 2nd assessment. Severity of the purple spot on 24 September recorded as % area of the stem base or canopy was low at 4.05% in the untreated on the stem bases and 1.72% in the canopy in the untreated plots. There were no significant differences between treatments for incidence or severity at either assessment. Nevertheless, a trend for reduction in incidence was seen in some treatments at the 2nd assessment of purple spot in the canopy, as well as a reduction in severity on the stem bases at both assessment timings. (Figure 12 and 13).

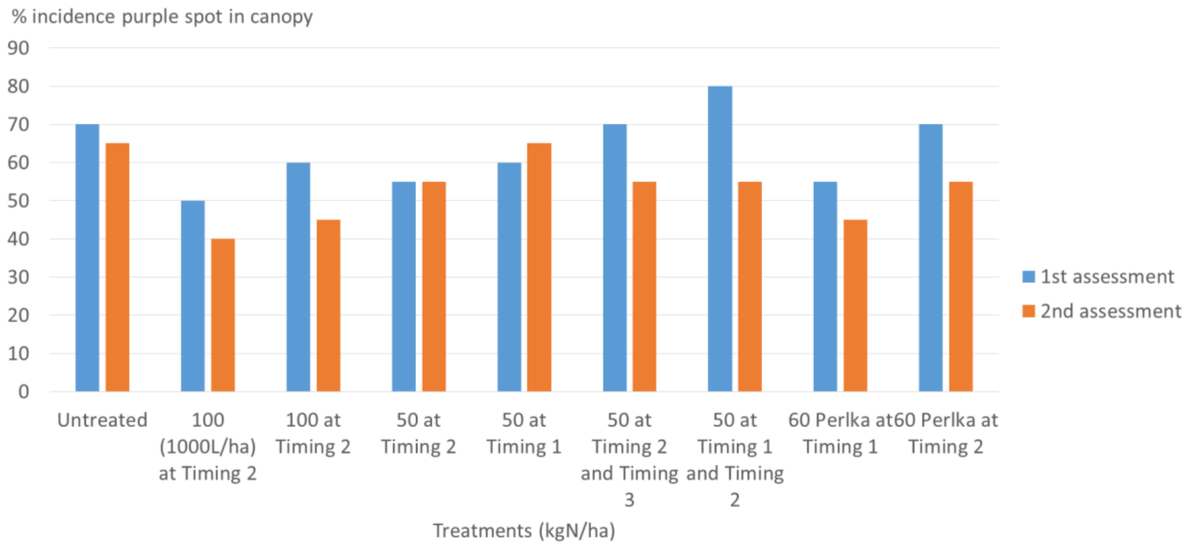

Figure 12. Incidence of purple spot in the canopy at assessment on 29 August and 24 September, Norfolk, 2013.

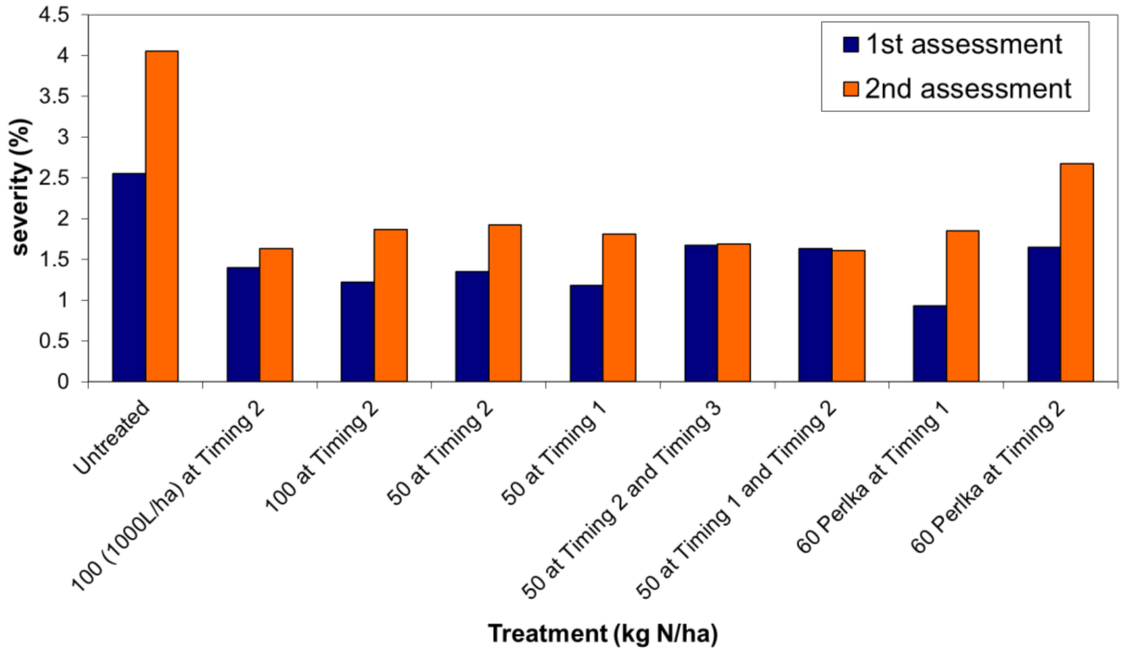

Figure 13. Severity of purple spot on the stem bases at assessment on 29 August and 24 September, Norfolk, 2013.

Discussion

The aim of this project was to improve control of S*temphylium* purple spot on asparagus by reduction of overwintering inoculum on crop debris. The specific objectives were:

- To determine the efficacy of dormant season soil treatments for reduction of *S. vesicarium* ascospore release from crop debris.
- To determine the effect of these treatments on occurrence of purple spot in the emerging crop.

Efficacy of dormant season treatments for reduction of ascospore release from crop debris by S. vesicarium

Urea applied as a spray to asparagus debris at the rate of 100 kg N/ha post ridging gave a significant reduction in spore release for up to 28 days post application. There was a significant reduction in spore numbers when 100 kg N/ha was applied at either 1000 l/ha or 400 l/ha, or as 2 applications of 50 kg N/ha post ridging (Timing 2 and 3) at 400 l/ha at both experimental sites. Lower rates of 50 kg N/ha applied at 400 l/ha as two early applications (Timing 1 and 2) or once only gave a reduction in spores but this was not consistently significant at all assessments. Urea applied as an early treatment at 50 kg N/ha at a volume of 400l/ha post flailing but pre-ridging gave a significant reduction in spore release at both sites up to 28 days post application.

Table 2. Summary of the effect of urea and Perlka treatments on production of *Stemphylium vesicarium* ascospores from asparagus crop debris – 2013

Treatment and application timing					% spore production (untreated = 100) at one and two months after treatment			
		T1	T2	T3	Hereford		Norfolk	
			kg N/ha		28 Days after T1	56 Days after T1	28 Days after T1	56 Days after T1
1	untreated	0	0	0	100	100	100	100
2	urea	0	100*	0	**15**	**24**	**45**	108
3	urea	0	100	0	**63**	**67**	**34**	90
4	urea	0	50	0	94	95	76	82
5	urea	50	0	0	**69**	87	**51**	87
6	urea	0	50	50	**46**	**49**	**50**	**49**
7	urea	50	50	0	**52 (38)**	98 **(35)**	**32** (84)	47 (74)
8	Perlka	60**	0	0	108	133	97	113
9	Perlka	0	60**	0	73	89	**24**	**13**

Values in bold are significantly different from the untreated
Figures in brackets are days after T2 treatment applied
*1,000 l/ha **Top dressed

Perlka applied as a top dressing gave a significant reduction in spores after the post flailing application (Timing 1) at the Norfolk site, but this was not repeated at the Herefordshire site or after the post ridging applications. This could be due to the way the product was applied, as the manufacturers advise that it needs moisture and warmth to be activated, and when applied as a top dressing especially in the cooler winter months this may have contributed to the lack of efficacy. Incorporating the product may lead to better efficacy with Perlka but this was not done in order to avoid the potential confounding effect of burying debris at the same time as incorporating Perlka. Burial of debris has the potential to provide a control method against spore release in itself which is exploited by growers using cultural disease control methods such as ridging.

The results from the 2013 trials, where spore release from mature pseudothecia of *S. vesicarium* on fern debris was reduced by the application of urea at 100 kg N/ha in a volume of 1000 l/ha repeats the result from the experiment carried out as part of FV 341 in 2012. Research on suppression of ascospore release using urea has also been carried out by Humpherson-Jones and Burchill (1982) and Gladders (1980) to control blackleg (*Leptosphaeria maculans*) on oilseed rape. This also showed that treatment of stubble with products such as urea and certain adjuvants could reduce inoculum survival between seasons by suppressing ascospore release. Urea has also been used for a number of years in apple orchards for control of apple scab (*Venturia inaequalis*). Although the main mode of action is to hasten the breakdown of leaves, and so destroy the host on which the pathogen needs to survive, it is also acknowledged that the urea changes the microbial and chemical populations on the leaf which could be affecting ascospore release (Ross and Burchill, 1968). In work by Sutton and MacHardy (1993) bacterial populations on leaves of apple trees were increased by the urea, and this could play a part in spore suppression of some pathogenic organisms. Other work has shown urea to have a direct effect on the pathogen *V. inaequalis*, reducing the maturation of pseudothecia (Mezska and Bielenin, 2006). However, the exact mode of action of urea remains unknown.

In all treatments effective at reducing ascospore release in this project the reduction of spore release over time by urea was relative to the intensity of spore release from untreated debris, which is in turn influenced by the maturation of pseudothecia spores and favourable weather conditions for spore release. However, the greatest potential reduction in risk to the emerging crop would be obtained by applying urea at or just prior to the main period of ascospore release, as in the most effective treatments spore release was reduced by up to 85% compared to the untreated. Urea was also persistent at significantly reducing spore release for up to 28 days post application.

Effect of urea and Perlka on occurrence of purple spot in the emerging crop and ferns.

The application of urea at 100 kg N/ha either at 400 l/ha or 1000 l/ha significantly reduced purple spot on the spears at one assessment out of three carried out at the Hereford site. All other assessments at either site, of purple spot on either the spears, stem bases of the ferns, or of the fern canopy, showed no significant differences. Some treatments (100 kg N/ha applied at either 1000 l/ha or 400 l/ha, or 50 kg N/ha applied at two timings) appeared to reduce incidence in the spears, on the stem bases, or in the fern canopy, but these reductions did not achieve statistical significance or consistent effects in assessments at both sites. It was difficult to achieve significant differences between treatments when disease levels were low in 2013, as conditions were dry, and not conducive to infection with *Stemphylium* purple spot. However, the above treatments warrant further investigation in another season where disease levels may be higher and differences easier to separate.

These trends of reduction are useful in the spears to improve product quality, but the key time to reduce purple spot infection in the crop is during the fern stage. This is because severe infections can have an impact on the yield in the following year by causing early senescence and defoliation, reducing the time that the plant has to build carbohydrate stores in the crown.

Conclusions

The trends appear to indicate that the reduction in spore release from the debris may lead to a reduction in purple spot in the spears and ferns, but in the 2013 field trials no significant differences were seen. However, in a low disease year it was difficult to demonstrate significant effects. The treatments that reduced the spore release by the greatest amount, also tended to appear to show a reduction in purple spot in the spears and ferns, though not consistently. The most promising treatments were 100 kg N/ha applied at either 1000 l/ha or 400 l/ha, or 50 kg N/ha applied at two timings. The former treatment is certainly too high a volume to be practical, especially on a large scale, but 400 l/ha appears to give as good a result in reduction of purple spot in the following crop as applying the urea at 1000 l/ha. Applying the majority of the asparagus crop's nitrogen requirement, (bearing in mind the NVZ N_{max} is 180 kg N/ha) as urea at 100 kg N/ha just prior to spear growth is also not environmentally advisable given that N offtake by the spears at this point in growth is very low, and would increase the risk of loss of N to leaching in a wet spring. Therefore, to reduce spore release and possibly purple spot in the emerging crop without compromising crop nutrition or environmental risk, applying urea twice at 50 kg N/ha instead of nitrogen as a fertiliser could be considered.

An application of urea between spear harvest and fern growth was not considered in this study, but could be a useful timing to look at in further work to see if additional control closer to fern growth could be more beneficial if spores are still being released from the resting bodies. The use of urea for reduction of spore release shows promise, and further tests in a year with higher disease pressure could show useful results. There are also many commercially available urea products available such as Nufol 20 which may be more practical to use than the technical grade urea used in the study.

Knowledge and Technology Transfer

Presentation at the Asparagus Growers Agronomy Day - 26th June 2013 Barfoots, Romsey.
Presentation/project update at the Asparagus Growers panel meeting – 11th October, Stilton

Acknowledgements

The support of the UK asparagus growers who hosted trials and supported this project is gratefully acknowledged.

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Appendices

Appendix 1: Crop Diary

Norfolk site

Date	Task/application
23rd November 2012	Trial marked out
8th January 2013	**Timing 1 treatments applied**
9th January	T1 Day 1 debris collected
14th January	Spore count – little evidence of spore release. No counts
15th January	T1 Day 7 debris collected
21st January	Spore count – little evidence of spore release. No counts
28th January	T1 Day 14 debris collected on Day 21 due to snowfall in previous week
31st January	Spore count
6th February	T1 Day 28 debris collected
11th February	Spore count
6th March	T1 Day 56 debris collected
11th March	Spore count
Late March	Ridging carried out by grower
8th April	**Timing 2 treatments applied**
9th April	T2 Day 1 debris collected
14th April	Spore count
15th April	T2 Day 7 debris collected
22nd April	Spore count
22nd April	T2 Day 14 debris collected
29th April	Spore count
29th April	T2 Day 28 debris collected
29th April	**Timing 3 treatments applied**
30th April	T3 Day 1 debris collected
6th May	Spore count from T3 day 1
6th May	Spore count from T2 day 28
6th May	T3 Day 7 debris collected
11th May	Spore count
13th May	T3 Day 14 debris collected
18th May	Spore count
28th May	T2 Day 56 debris collected
28th May	T3 Day 28 debris collected
31st May	Spear disease assessment
3rd June	Spore count from T2 Day 56
3rd June	Spore count from T3 Day 28
24th June	T3 Day 56 debris collected
28th June	Spore count from T3 Day 56
29th August	Fern disease assessment mid fern stand
24th September	Fern disease assessment just before senescence
5th December 2013	Trial completed and markers collected

Hereford site

Date	**Task/application**
4th December 2012	Trial marked out
25th February 2013	Ridging carried out by grower – agreed fine to take place earlier as more debris will have become exposed due to ridges weathering by Timing 2 and still allows for a difference in early and late timings to be seen
27th February	**Timing 1 treatments applied**
28th February	T1 Day 1 debris collected
5th March	Spore count
6th March	T1 Day 7 debris collected
11th March	Spore count
13th March	T1 Day 14 debris collected
18th March	Spore count
27th March	T1 Day 28 debris collected
1st April	**Timing 2 treatments applied**
2nd April	T2 Day 1 debris collected
2nd April	Spore count T1 Day 28 debris
8th April	T2 Day 7 debris collected
9th April	Spore count T2 Day 1 debris
15th April	Spore count T2 Day 7 debris
15th April	T2 Day 14 debris collected
22nd April	Spore count T2 Day 14 debris
24th April	T1 Day 56 debris collected
28th April	Spore count T1 Day 56 debris
29th April	T2 Day 28 debris collected
2nd May	**Timing 3 treatments applied**
3rd May	T3 Day 1 debris collected
4th May	Spore count from T2 day 28 debris
9th May	Spore count from T3 day 1 debris
9th May	T3 Day 7 debris collected
15th May	Spore count T3 day 7 debris
16th May	T3 Day 14 debris collected
18th May	Spear disease assessment
22nd May	Spore count T3 day 14 debris
27th May	T2 Day 56 debris collected
29th May	T3 Day 28 debris collected
31st May	Spear disease assessment
2nd June	Spear disease assessment
3rd June	Spore count from T2 Day 56
4th June	Spore count from T3 Day 28
26th June	T3 Day 56 debris collected
2nd July	Spore count from T3 Day 56
30th September	Fern disease assessment just before senescence
5th December	Trial completed and markers collected

Appendix 2: Statistical tables – spore counts

The standard errors vary for each treatment, so the 95% confidence intervals for each treatment were calculated to make it easier to identify differences between treatments. If when comparing two treatments, the confidence intervals do not overlap we can say that they are significantly different. This is denoted where the letter is different from the untreated.

Confidence interval (CI) was estimated using standard errors (SE) for each predicted value (CI = SE x $t_{95\%}$, where df = 9 after timing 1, 18 after timing 2, and 3 after timing 3).

The predictions were converted to % ascospores released by converting the fractions to percentages, by subtracting the predicted value from 1 and multiplying by 100 to plot the graphs in the main body of the report.

Norfolk site

Assessments after Timing 1 application

Day 14 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.775	0.04397	0.09946014	0.68	0.87	a
400L/50	5	0.9625	0.02099	0.04747938	0.92	1.01	b
400L/50	7	0.9	0.03259	0.07371858	0.83	0.97	a
Perlka	8	0.975	0.01721	0.03892902	0.94	1.01	b
					F prob	<0.001	

Day 28 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.5375	0.0552	0.1248624	0.41	0.66	a
400L/50	5	0.7625	0.04726	0.10690212	0.66	0.87	b
400L/50	7	0.85	0.03973	0.08986926	0.76	0.94	b
Perlka	8	0.8875	0.03499	0.07914738	0.81	0.97	b
					F prob	<0.001	

Day 56 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.8125	0.043	0.097266	0.72	0.91	a
400L/50	5	0.8375	0.04071	0.09208602	0.75	0.93	a
400L/50	7	0.9125	0.03135	0.0709137	0.84	0.98	a
Perlka	8	0.975	0.01728	0.03908736	0.94	1.01	b
					F prob	0.002	

Assessments after Timing 2 application

Day 1 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.7375	0.04813	0.10112113	0.64	0.84	a
1000L/100	2	0.875	0.03651	0.07670751	0.80	0.95	a
400L/100	3	0.7625	0.04662	0.09794862	0.66	0.86	a
400L/50	4	0.6125	0.05279	0.11091179	0.50	0.72	a
2 late apps	6	0.85	0.03934	0.08265334	0.77	0.93	a
2 early apps	7	0.8375	0.04066	0.08542666	0.75	0.92	a
Perlka	9	0.8875	0.03493	0.07338793	0.81	0.96	a
					F prob	<0.001	

Day 7 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.7125	0.04883	0.10259183	0.61	0.82	a
1000L/100	2	0.8	0.04341	0.09120441	0.71	0.89	a
400L/100	3	0.9125	0.03109	0.06532009	0.85	0.98	b
400L/50	4	0.6875	0.04996	0.10496596	0.58	0.79	a
2 late apps	6	0.9125	0.03109	0.06532009	0.85	0.98	b
2 early apps	7	0.6875	0.04996	0.10496596	0.58	0.79	a
Perlka	9	0.7625	0.04605	0.09675105	0.67	0.86	a
					F prob	<0.001	

Day 14 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.6625	0.0516	0.1084116	0.55	0.77	a
1000L/100	2	0.85	0.03934	0.08265334	0.77	0.93	b
400L/100	3	0.8625	0.03769	0.07918669	0.78	0.94	b
400L/50	4	0.725	0.04885	0.10263385	0.62	0.83	a
2 late apps	6	0.875	0.0364	0.0764764	0.80	0.95	b
2 early apps	7	0.625	0.05278	0.11089078	0.51	0.74	a
Perlka	9	0.675	0.05114	0.10744514	0.57	0.78	a
					F prob	<0.001	

Day 28 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.525	0.05427	0.11402127	0.41	0.64	a
1000L/100	2	0.7875	0.0449	0.0943349	0.69	0.88	b
400L/100	3	0.8375	0.04058	0.08525858	0.75	0.92	b
400L/50	4	0.6375	0.05237	0.11002937	0.53	0.75	a
2 late apps	6	0.7625	0.04663	0.09796963	0.66	0.86	b
2 early apps	7	0.6	0.05331	0.11200431	0.49	0.71	a
Perlka	9	0.5375	0.0542	0.1138742	0.42	0.65	a
					F prob	<0.001	

Day 56 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.5125	0.05349	0.11238249	0.40	0.62	a
1000L/100	2	0.475	0.05357	0.11255057	0.36	0.59	a
400L/100	3	0.5625	0.05295	0.11124795	0.45	0.67	a
400L/50	4	0.6	0.05222	0.10971422	0.49	0.71	a
2 late apps	6	0.7625	0.04553	0.09565853	0.67	0.86	b
2 early apps	7	0.6375	0.05121	0.10759221	0.53	0.75	a
Perlka	9	0.45	0.05346	0.11231946	0.34	0.56	a
					F prob	<0.001	

Assessments after Timing 3 application

Day 1 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.3625	0.04765	0.15166995	0.21	0.51	a
6	0.5375	0.04819	0.15338877	0.38	0.69	a
				F prob	0.011	

Day 7 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.4625	0.04866	0.15488478	0.31	0.62	a
6	0.625	0.04767	0.15173361	0.47	0.78	a
				F prob	0.018	

Day 14 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.3125	0.03569	0.11360127	0.20	0.43	a
6	0	0.00001	0.00003183	0.00	0.00	b
				F prob	<0.001	

Day 28 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.2375	0.04515	0.14371245	0.09	0.38	a
6	0.2875	0.04765	0.15166995	0.14	0.44	a
				F prob	NS	

Day 56 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.2125	0.0456	0.1451448	0.0673552	0.3576448	a
6	0.35	0.05311	0.16904913	0.18095087	0.51904913	a
				F prob	NS	

Hereford site

Assessments after Timing 1 application

Day 1 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.3625	0.05268	0.11916216	0.24	0.48	a
400L/50	5	0.625	0.05303	0.11995386	0.51	0.74	b
400L/50	7	0.775	0.04598	0.10400676	0.67	0.88	b
Perlka	8	0.425	0.05411	0.12239682	0.30	0.55	a
					F prob	<0.001	

Day 7 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.4375	0.05501	0.12443262	0.31	0.56	a
400L/50	5	0.55	0.05516	0.12477192	0.43	0.67	a
400L/50	7	0.65	0.05289	0.11963718	0.53	0.77	a
Perlka	8	0.5	0.05544	0.12540528	0.37	0.63	a
					F prob	0.046	

Day 14 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.4625	0.05337	0.12072294	0.34	0.58	a
400L/50	5	0.65	0.05123	0.11588226	0.53	0.77	a
400L/50	7	0.475	0.05344	0.12088128	0.35	0.60	a
Perlka	8	0.475	0.05344	0.12088128	0.35	0.60	a
					F prob	0.036	

Day 28 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.2	0.03145	0.0711399	0.13	0.27	a
400L/50	5	0.45	0.03692	0.08351304	0.37	0.53	b
400L/50	7	0.5875	0.03464	0.07835568	0.51	0.67	b
Perlka	8	0.1375	0.03062	0.06926244	0.07	0.21	a
					F prob	<0.001	

Day 56 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.35	0.03325	0.0752115	0.27	0.43	b
400L/50	5	0.4375	0.02521	0.05702502	0.38	0.49	b
400L/50	7	0.375	0.03183	0.07199946	0.30	0.45	b
Perlka	8	0.1375	0.0326	0.0737412	0.06	0.21	a
					F prob	<0.001	

Assessments after Timing 2 application

Day 1 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.5375	0.05512	0.11580712	0.42	0.65	a
1000L/100	2	0.8	0.04444	0.09336844	0.71	0.89	b
400L/100	3	0.75	0.04804	0.10093204	0.65	0.85	b
400L/50	4	0.625	0.05358	0.11257158	0.51	0.74	a
2 late apps	6	0.575	0.05467	0.11486167	0.46	0.69	a
2 early apps	7	0.8625	0.03831	0.08048931	0.78	0.94	b
Perlka	9	0.4125	0.05439	0.11427339	0.30	0.53	a
					F prob	<0.001	

Day 7 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.4625	0.05537	0.11633237	0.35	0.58	a
1000L/100	2	0.7	0.05094	0.10702494	0.59	0.81	b
400L/100	3	0.5625	0.05509	0.11574409	0.45	0.68	a
400L/50	4	0.5625	0.05509	0.11574409	0.45	0.68	a
2 late apps	6	0.4875	0.05551	0.11662651	0.37	0.60	a
2 early apps	7	0.675	0.05205	0.10935705	0.57	0.78	b
Perlka	9	0.3	0.05095	0.10704595	0.19	0.41	a
					F prob	<0.001	

Day 14 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.175	0.04163	0.08746463	0.09	0.26	a
1000L/100	2	0.8125	0.04228	0.08883028	0.72	0.90	b
400L/100	3	0.525	0.0534	0.1121934	0.41	0.64	b
400L/50	4	0.6	0.05237	0.11002937	0.49	0.71	b
2 late apps	6	0.4125	0.05287	0.11107987	0.30	0.52	b
2 early apps	7	0.375	0.05212	0.10950412	0.27	0.48	b
Perlka	9	0	0.00033	0.00069333	0.00	0.00	a
					F prob	<0.001	

Day 28 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.35	0.05294	0.11122694	0.24	0.46	a
1000L/100	2	0.9	0.03317	0.06969017	0.83	0.97	b
400L/100	3	0.5875	0.0546	0.1147146	0.47	0.70	b
400L/50	4	0.3875	0.05405	0.11355905	0.27	0.50	a
2 late apps	6	0.7	0.05088	0.10689888	0.59	0.81	b
2 early apps	7	0.75	0.04811	0.10107911	0.65	0.85	b
Perlka	9	0.525	0.05538	0.11635338	0.41	0.64	a
					F prob	<0.001	

Day 56 Proportion with score 0

	Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
UT	1	0.2125	0.04447	0.09343147	0.12	0.31	a
1000L/100	2	0.8125	0.04285	0.09002785	0.72	0.90	b
400L/100	3	0.475	0.0539	0.1132439	0.36	0.59	b
400L/50	4	0.25	0.04695	0.09864195	0.15	0.35	a
2 late apps	6	0.6125	0.05283	0.11099583	0.50	0.72	b
2 early apps	7	0.725	0.04874	0.10240274	0.62	0.83	b
Perlka	9	0.3	0.04956	0.10412556	0.20	0.40	a
					F prob	<0.001	

Assessments after Timing 3 application

Day 1 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.225	0.04587	0.14600421	0.08	0.37	a
6	0.5125	0.0544	0.1731552	0.34	0.69	a
				F prob	NS	

Day 7 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.3625	0.05168	0.16449744	0.20	0.53	a
6	0.6	0.05284	0.16818972	0.43	0.77	a
				F prob	NS	

Day 14 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.3125	0.05072	0.16144176	0.15	0.47	a
6	0.5375	0.05435	0.17299605	0.36	0.71	a
				F prob	NS	

Day 28 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.2875	0.05018	0.15972294	0.13	0.45	a
6	0.45	0.04765	0.05505	0.39	0.51	a
				F prob	NS	

Day 56 Proportion with score 0

Treatment	Prediction	S.E.	Confidence Interval	Lower Confidence Limit	Upper Confidence Limit	
1	0.2125	0.04565	0.14530395	0.07	0.36	a
6	0.3625	0.05362	0.17067246	0.19	0.53	a
				F prob	NS	