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Factors affecting the development and control of black dot on potato tubers

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Field trials were carried out over a 4 year period (2004–2007) to determine the effect of agronomic factors, specifically cultivar resistance, irrigation, crop duration and chemical control (in-furrow application of azoxystrobin), on black dot development on potato tubers grown in fields where soilborne inoculum of *Colletotrichum coccodes* was present. In 2004, 2005 and 2006, two field trials were performed each year and in 2007, 19 mini-field trials were carried out across Scotland and England. Cultivar resistance was clearly demonstrated to be an effective method of reducing black dot disease severity on tubers (described here as the percentage of unmarketable tubers, i.e. those with symptoms covering a surface area of >10%). In the four field trials carried out in 2004 and 2005, in irrigated and fungicide-untreated plots, 43·8% of tubers of cv. Maris Piper were unmarketable, compared with 17·0% of tubers of cv. Sante. Assessments of disease development on underground plant parts (stems, stolons and roots) revealed that cultivar resistance acted only at the tuber level, as disease symptoms on other parts were often high irrespective of published disease resistance ratings. Irrigation increased the severity of disease on tubers in two trials (England 2004 and 2006), but its effect was less significant when rainfall was high. Delaying harvest by 2 weeks increased disease severity in all six trials, whilst application of azoxystrobin consistently reduced black dot severity. There were significant interactions between factors. The results clearly show how black dot disease severity can be reduced through an integrated approach to disease management.

Keywords: azoxystrobin, black dot, *Colletotrichum coccodes*, crop duration, disease management, irrigation

Introduction

Black dot, caused by *Colletotrichum coccodes*, is a tuber blemish disease of potato and an important constraint to the pre-pack potato industry in the UK due to development of unsightly brown necrotic lesions and microsclerotia on the tuber surface. In other countries, yield reductions as a result of the disease may be up to 30% in susceptible cultivars, but are inconsistent (Tsror (Lahkim) *et al.*, 1999a). Various aspects of black dot importance, epidemiology and control have been previously reviewed by Lees & Hilton (2003).

The potential for seed tubers infected by *C. coccodes* to cause disease on progeny tubers is long established (Dickson, 1926), and once the pathogen is introduced into previously uncontaminated soil the microsclerotia can survive for at least 8 years (Dillard & Cobb, 1998) and can act as an effective source of inoculum. Recent work in the UK (Lees *et al.*, 2010) showed that seed tuber inoculum of *C. coccodes* was relatively less important than soilborne inoculum in causing black dot and that the level of seed tuber inoculum did not significantly affect either the incidence or severity of disease. By contrast, soilborne inoculum had the potential to result in high levels of disease and the amount of *C. coccodes* inoculum was found to have a significant effect.

Cultivar resistance to black dot is the most sustainable control measure and one that can be easily used in combination with other approaches. However, the use of host resistance for the control of black dot has not been widely investigated. Black dot resistance has not been a major breeding target, and currently few resistant varieties are grown commercially in the UK, although some varieties do show some resistance (http://varieties.potato.org.uk/). In field experiments in the UK, significant differences between black dot disease symptom severity on daughter tubers of commercial potato cultivars was observed when disease-free seed was sown in naturally and artificially infected soils (Read, 1991). The cultivars Desirée, Maris Piper, Maris Peer and Record were among those most severely affected by disease, whereas cvs Cara, Pentland Crown and Romano were the least affected. Tsror (Lahkim) *et al.* (1999b) found that cultivars Cara and Nicola were less susceptible to tuber infection than cvs Alpha, Desirée and Agria. No work has

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previously been carried out to understand the interaction between soil inoculum level and cultivar resistance on resulting severity of progeny tuber disease.

The application of the strobilurin fungicide azoxystrobin (Amistar®; Syngenta Crop Protection UK Ltd) was previously shown to be effective in significantly reducing the incidence of black dot on progeny tubers and the disease severity on stems of cvs Russet Burbank and Norkotah Russet compared with non-treated controls (Nitzan *et al.*, 2005). Cummings & Johnson (2008) showed that a single foliar application of azoxystrobin applied at an early growth stage (34–62 days after planting) reduced the severity of black dot on stems whereas in-furrow application at planting had little effect. Since 2004, azoxystrobin has had full approval for soil incorporation or in-furrow treatment for control of soilborne black dot and *Rhizoctonia* in the UK. Therefore chemical control of soilborne inoculum is now an option, but guidance on how best to use chemical control as part of an integrated management scheme is required.

Potato crops are routinely irrigated to maintain crop growth and development and to control common scab caused by *Streptomyces* species. Whilst common scab is controlled through the application of water, increasing soil moisture can, conversely, increase the severity of powdery scab (Taylor & Flett, 1981; Adams *et al.*, 1987; de Boer, 2000; Tuncer, 2002). Similarly, Adams *et al.* (1987) found irrigation to increase black dot in a relatively dry season. Read & Hide (1988) compared the effect of irrigated and unirrigated treatments on black dot development in 2 years, which in retrospect were considered to be wet early in the season. They found that infection was reduced by irrigation early in the season, but by the time of the final harvest, when the unirrigated plots had dried out, irrigation significantly increased the incidence of black dot on progeny tubers.

Early harvesting of crops has been shown to reduce the severity of black dot on progeny tubers (Hide & Boorer, 1991; Hide *et al.*, 1994). Experimental results have shown that infection of daughter tubers by *C. coccodes* occurs early in the season (A. J. Hilton, SRUC, Aberdeen, UK, unpublished data). Therefore early harvesting of the crop reduces the length of time the tubers are in contact with the infested soil, and the length of time available for disease development. It has been shown recently that increasing the total crop duration (determined as the number of days from 50% emergence to harvest) significantly increased disease severity, whilst differences in the length of time between haulm destruction and harvest did not (J. C. Peters, Fera, York, UK unpublished data). Crop duration should therefore be considered a component of integrated disease management.

This study was carried out to assess the effects and interactions between a number of agronomic factors: cultivar resistance, azoxystrobin in-furrow treatment, irrigation and crop duration (defined here as the time from planting to harvest) on black dot development on tubers, where soil inoculum was present in the field. Combining this knowledge will enable the successful management of risk factors to facilitate the integrated control of black dot.

Materials and methods

Field trials

Seed

Certified seed stocks of the black dot susceptible potato cultivar Maris Piper and the more resistant cultivars Sante and Saxon were used in field trials in 2004, Maris Piper and Sante were used in 2005 and Maris Piper and Estima in 2006. In the 2007 field trials, six cultivars with a range of resistance ratings were compared. Cultivar resistance ratings and maturity classes (http://varieties.potato.org.uk/) and incidence and severity of black dot disease (visual inspection) on seed stocks used in each trial are shown in Table 1.

Soil samples

Soil was collected from all field trial areas prior to planting. Each bulk soil sample comprised at least 100 subsamples taken in a W-shape across the experimental areas of the field at a depth of 5–15 cm to give a total weight of *c.* 1 kg. Soil DNA extractions were carried out according to the method of Brierley *et al.* (2009) and *C. coccodes* soil inoculum was quantified using real-time PCR according to the method described by Cullen *et al.* (2002). The amount of *C. coccodes* DNA detected was expressed as pg DNA g^{-1} soil.

Progeny tuber sampling and assessments from field trials

In the 2004–2006 trials, early and late harvests took place *c.* 4 weeks after haulm destruction (once skin set had occurred), and 6 weeks after haulm destruction. In each plot, the subplot to be harvested first was determined at random. Yield and tuber

Table 1 Details of potato seed stocks used in field trials (2004–2007), cultivar resistance ratings to black dot, maturity class and results of visual assessments (incidence and severity of black dot) on seed stock used in each trial

Cultivar	Resistance rating[a]	Maturity class	Trial year	Visual black dot on seed stock	
				% incidence	% severity
Maris Piper	4	Maincrop	2004	2	0·1
			2005	0	0
			2006	0	0
			2007	0	0
Sante	5	Maincrop	2004	38	5·8
			2005	0	0
			2007	0	0
Saxon	7	Second Early	2004	4	1·5
			2007	0	0
Estima	5	Early Maincrop	2006	0	0
			2007	0	0
King Edward	6	Maincrop	2007	0	0
Lady Christl	2	First Early	2007	0	0
Pentland Squire	3	Early Maincrop	2007	58	10

[a]Ratings are on a 1–9 scale of increasing resistance.

number in each fraction (>45, 45–65, 65–85, >85 mm) were measured immediately after harvest. At grading, a total of 50 tubers, 25 from the 45–65 mm fraction and 25 from the 65–85 mm fraction were placed in paper sacks and stored at 4°C for a month before being assessed for black dot incidence and severity (% diseased surface area). In the 2007 trials, tubers were assessed shortly after harvest. Disease is expressed as the percentage of unmarketable tubers: rejections in the pre-packing market are not based on rigid criteria, but unmarketability is defined here as the percentage of tubers with more than 10% surface area covered in black dot.

Parallel field trials 2004, 2005 and 2006: effect of cultivar, fungicide, irrigation and harvest date on disease

Two parallel trials, one located in Scotland and one in England, were carried out in each of 2004, 2005 and 2006, in fields where the history of black dot was known, based on cropping history. Site details are given in Table 2. Each trial investigated the effect of irrigation, the in-furrow application of the strobilurin fungicide azoxystrobin, early and late harvest (different crop duration) and cultivar resistance on development of black dot on progeny tubers. Irrigation was applied to irrigated plots via trickle tape according to a schedule determined by ADAS using the package Irriguide (Bailey & Spackman, 1996). This model incorporates meteorological data with site-specific soil and agronomic data and determines when and how much water to apply. The total amount of irrigation applied to irrigated plots in each trial is given in Table 2. The fungicide treatment consisted of azoxystrobin applied in-furrow at planting, at a rate of 750 g ha^{-1} a.i. in a 100 L ha^{-1} solution using forward and backward facing nozzles. Trials were laid out in a split-plot design with irrigation as the main plot and cultivar/± azoxystrobin treatments as subplots and were harvested at two harvest dates (early and late, see Table 2 for details). All trials had four replicates for each treatment.

For all six field trials described here the plot size was four rows in ridges 11 m in length and tuber spacing was at 25 cm. Harvested tubers were taken from the two inner rows. Outer guard rows were planted with seed from a disease-free stock of the same cultivar. All trials were managed as for general ware crops with fertilizer, herbicide, blight and aphid control and haulm destruction methods as per local practice.

In the two trials carried out in 2004, plants were sampled destructively to determine the development of visual disease on below ground parts. Assessments were made 6, 4 and 2 weeks before, and just prior to, haulm destruction, and 2 weeks after haulm destruction. Samples comprised four adjacent plants within each treatment plot. Each group of plants sampled was separated from the next sampling area by a guard. The location of each subplot to be sampled was determined at random. All underground parts (stems, stolons and roots) from each plant sampled were placed in a separate paper bag. Five progeny tubers taken at random from each plant were also placed into each bag. Stems, stolons and tubers were assessed for the visual presence of black dot and disease was expressed as percentage incidence. Root infection was recorded as the percentage root area affected.

Field trials 2007: effect of cultivar resistance and soil inoculum level on progeny tuber disease

In 2007, a series of 19 field trial sites was established across England and Scotland to evaluate the interaction between cultivar resistance and soilborne inoculum density of *C. coccodes* in causing disease on progeny tubers. The sites were chosen both to provide a range of soil inoculum and to include soil types typically found in potato growing regions of the UK.

In order to identify ten suitable sites in England and nine in Scotland, a total of 58 soil samples were taken from fields destined to be planted with potatoes and the quantity of *C. coccodes* DNA in the samples was tested using real-time PCR. From these results, appropriate fields were selected for planting small replicated trials comprising six pre-packing cultivars with different resistance ratings to black dot (Table 1). Additional criteria used in selecting fields for trial sites besides soil type and *C. coccodes* inoculum density were that the fungicide azoxystrobin would not be used and that the field crop would be a long duration crop. Details of the sites selected for trials are shown in Table 3.

Trials were placed within the farm crop and comprised three replicate plots of 20 tubers of each cultivar. Individual plots consisted of 10 tubers × two drills. A 1 m gap was left at the ends of the trial area and a blank drill between the farm crop and the trial area, where possible. Trials were planted as close to the date the field crop was planted as possible and seed was planted at the same depth and spacing as the farm crop. Trial

Table 2 Details of field trial sites in 2004, 2005 and 2006

	2004 Cambridge England	2004 Tayside Scotland	2005 Norfolk England	2005 Tayside Scotland	2006 Norfolk England	2006 Perthshire Scotland
Soil type	Sandy loam	Sandy loam	Silty clay loam	Sandy clay loam	Silty clay loam	Sandy loam
Soil inoculum[a]	124	25	2562	2593	3239	673
Planting date	7 May	18 May	5 May	12 May	4 May	26 Apr
Haulm destruction	2 Sep	7 Sep	6 Sep	30 Aug	5 Sep	11 Sep
Early harvest	30 Sep	5 Oct	3 Oct	25 Oct	4 Oct	26 Sep
Crop duration[b] (early harvest)	146	140	151	166	153	153
Late harvest	14 Oct	19 Oct	19 Oct	8 Nov	17 Oct	10 Oct
Crop duration[b] (late harvest)	160	154	167	180	166	167
Irrigation (mm)[c]	–	70	100	95	190	79

[a]Soil inoculum (pg DNA g^{-1} soil).
[b]Crop duration (days from planting to harvest).
[c]Total cumulative irrigation applied to irrigated plots (mm), data for England 2004 not available.

Table 3 Sites where field trials were established in 2007 to investigate the interaction between potato cultivar resistance to black dot and inoculum level across a range of soil types

Location	Soil type	Soil inoculum level (pg DNA g^{-1} soil)
Fife	Sandy clay loam	10
East Lothian	Sandy loam	20
Lincolnshire	Sandy clay loam	25
Cambridgeshire	Fen skirt	43
Aberdeenshire	Sandy clay loam	45
Lincolnshire	Silt loam	73
Norfolk	Silt clay loam	73
Tayside	Sandy loam	110
East Lothian	Sandy clay loam	126
Lincolnshire	Silt loam	142
Angus	Sandy loam	170
Norfolk	Sandy clay loam	230
Fife	Sandy clay loam	261
Angus	Sandy loam	267
Norfolk	Silty clay loam	516
Hereford	Clay loam	853
Norfolk	Sandy silt loam	2357
Lincolnshire	Silt loam	2357
East Lothian	Sandy loam	4707

plots received the same crop protection and husbandry treatments as the adjacent crop.

Statistical analysis

All statistical analysis was carried out using GenStat 16th edition (VSN International Ltd). In the six field trials (2004–2006), the effect of cultivar on percentage unmarketable tubers within each trial was analysed using general anova. Then for each cultivar, the effect of, and interaction between irrigation, azoxystrobin treatment, harvest date, trial site (England and Scotland) and year (where appropriate) on percentage unmarketable tubers was analysed using general anova.

Results

Cultivar resistance

When averaged across all treatments, significantly more disease developed on Maris Piper than on Sante ($P < 0{\cdot}01$) in the 2004 and 2005 trials in both England and Scotland (Table 4). In 2004 there was significantly more disease on Sante than on Saxon in both trials (Table 4).

In 2006, the severity of black dot on Maris Piper tubers was significantly greater than on Estima tubers at the English site but not at the Scottish site (Table 4). Although the difference in black dot resistance rating between Maris Piper (4) and Estima (5) is the same as that between Maris Piper and Sante, the relative disease levels from the 2004–2005 trials and the 2006 trials suggest that Sante exhibits greater resistance than Estima.

Across the 19 field trials harvested in 2007, the levels of disease found reflected the disease resistance ratings

Table 4 Effect of potato cultivar (mean of all treatments) on % unmarketable tubers in 2004, 2005 and 2006 field trials in England and Scotland

Year	Location			
	England		Scotland	
2004	Maris Piper	51a	Maris Piper	32a
	Sante	16b	Sante	15b
	Saxon	8c	Saxon	2c
	l.s.d.	4·9	l.s.d.	6·7
2005	Maris Piper	11a	Maris Piper	19a
	Sante	6b	Sante	3b
	l.s.d	3·4	l.s.d.	4·1
2006	Maris Piper	11a	Maris Piper	19a
	Estima	5b	Estima	18a
	l.s.d.	2·5	l.s.d.	3·8

Within a trial, values followed by different letters are significantly different ($P < 0{\cdot}01$).

for Pentland Squire (3), Maris Piper (4), Sante (5) and Saxon (7) (Fig. 1). King Edward was found to be more susceptible than its resistance rating (6) would suggest, and this finding was generally consistent across sites. Averaged across all sites, Lady Christl (a first-early maturing cultivar) was found to be less susceptible than predicted by its disease resistance rating (2). However at sites with higher levels of black dot soil inoculum (>100 pg DNA g^{-1} soil), the results for Lady Christl more accurately reflected the stated resistance rating (data not shown).

Black dot development on progeny tubers in 2007 was generally lower than in the previous 3 years, and this may be attributed to the timing of the disease assessments, which were made soon after harvest in 2007, whilst in 2004–2006 they were made at least 1 month after harvest.

Black dot development on underground plant parts was monitored in the two field trials carried out in 2004. In the English trial, black dot symptoms were first visible on stems 90 days after planting (DAP) (Fig. 2), following which the incidence rapidly increased, reaching 99% at 132 DAP. Black dot on stolons and roots was first observed 104 DAP, after which it increased less rapidly than on stems to a maximum (on average across cultivars) of 77% on stolons and 59% on roots at 132 DAP. In the Scottish trial, black dot incidence on stems, stolons and roots progressed slightly more slowly compared to the English site and peaked at slightly lower levels, 83, 68 and 39% on stems, stolons and roots, respectively (Fig. 2). The incidence of black dot on stems, stolons and roots did not reflect tuber resistance ratings, with the least resistant cultivar, Maris Piper (4), often having significantly lower levels of black dot on stems, stolons and roots than the more resistant cultivars, Sante (5) and Saxon (7), during the season. At the final sampling date, there was little difference in levels of stem, stolon and root disease between cultivars (Fig. 2). However, published resistance ratings to black

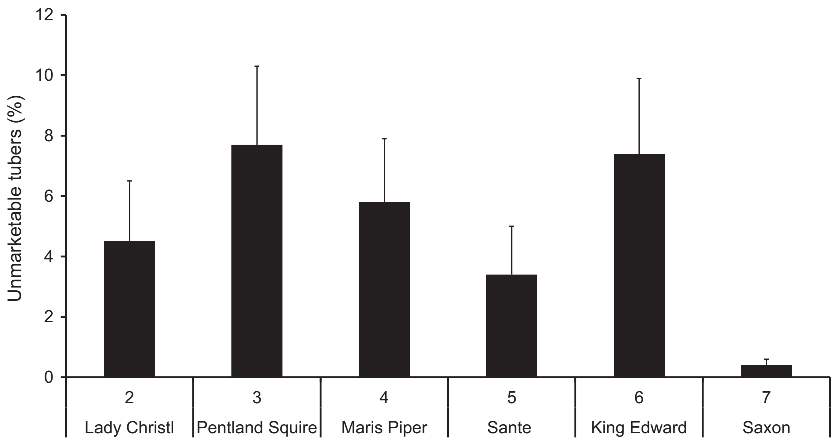

Figure 1 The percentage of unmarketable tubers of six potato cultivars with black dot resistance ratings ranging from 2 (least resistant) to 7 (most resistant) grown in 19 field trials. Bars represent mean across 19 field trials (+ standard error) carried out in 2007.

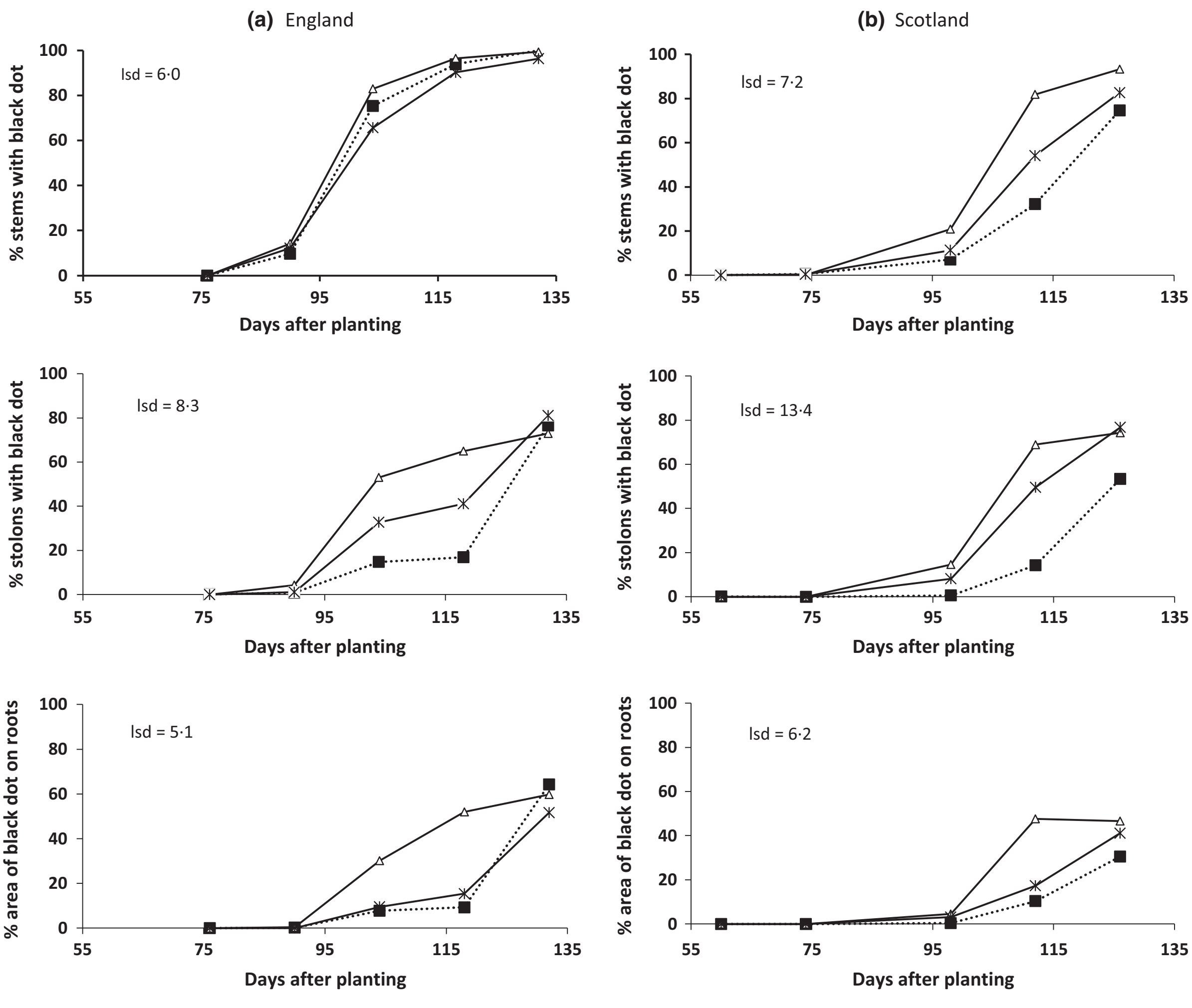

Figure 2 Black dot development on stems, stolons and roots, recorded as the percentage incidence, at 2-weekly intervals, in three cultivars with different disease resistance ratings, (■) Maris Piper (4), (Δ) Sante (5) and (×) Saxon (7); mean of all other treatments (n = 16), at the trial site in (a) England and (b) Scotland in 2004.

dot on tubers were well reflected by differences in disease incidence on tubers, particularly at the Scottish site, where incidence of black dot was significantly lower on tubers of cv. Saxon compared with cvs Sante and Maris Piper (Fig. 3). At the English site, where disease pressure was higher (based on soil inoculum levels), there was no

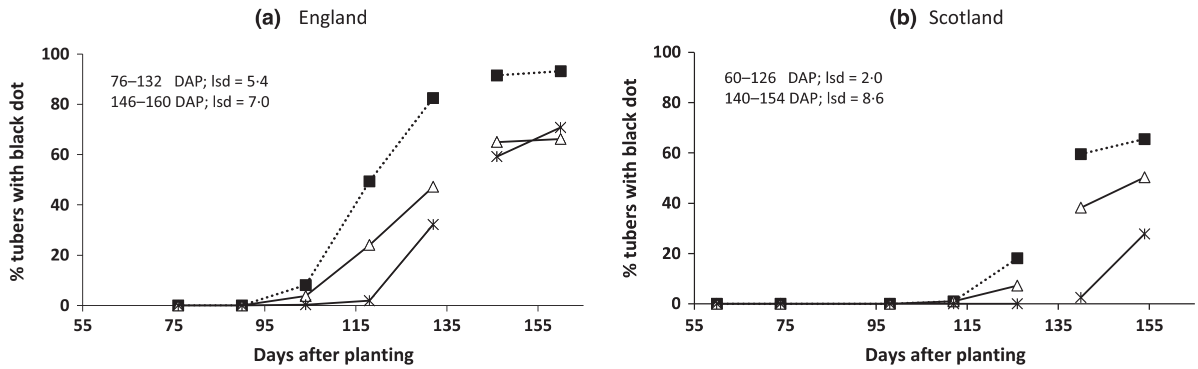

Figure 3 The incidence of black dot on tubers recorded at 2-weekly intervals and at the early and late harvests at the trial sites in (a) England and (b) Scotland in 2004, in three cultivars with different disease resistance ratings, (■) Maris Piper (4) and (Δ) Sante (5), (×) Saxon (7); mean of all other treatments (n = 16).

significant difference in the incidence of disease on tubers of Sante and Saxon (Fig. 3). However, the severity of disease, as reflected by the % unmarketable tubers, was significantly lower in Saxon than Sante (Table 4).

Effect of azoxystrobin, irrigation and harvest date on unmarketable tubers

Averaged across all trials, there were significant differences in the mean percentage unmarketable tubers between different harvest dates for all four cultivars (19 cf. 29, 8 cf. 12, 2 cf. 8, and 5 cf. 9 for Maris Piper, Sante, Saxon and Estima at early and late harvests, respectively; Table 5). Azoxystrobin treatment significantly reduced severity of disease in all cultivars except Estima (15 cf. 33, 7 cf. 13 and 2 cf. 7 for Maris Piper, Sante and Saxon with and without azoxystrobin treatment, respectively). Irrigation significantly increased severity of disease in all cultivars except Saxon (29 cf. 20, 14 cf. 7 and 10 cf. 5 for Maris Piper, Sante and Estima with and without irrigation, respectively).

Where the risk of disease was high, azoxystrobin consistently reduced the percentage of tubers deemed unmarketable due to levels of black dot. High levels of disease potential were associated with seasonal/trial site variations (Table 4), and there were significant interactions between azoxystrobin treatment and year for both Maris Piper and Sante (Table 5), with the impact of azoxystrobin on disease being greatest in 2004, the year in which disease was most severe (Table 6a,b). Similarly, for Saxon, there was a significant interaction between azoxystrobin and trial site, with azoxystrobin treatment having more of an effect in the English trial where disease severity was generally higher than at the Scottish trial site (Table 6c). Where factors such as irrigation and delayed harvest increased disease risk, then the use of azoxystrobin reduced the severity of black dot disease. In Saxon, for example, there was a significant interaction between azoxystrobin treatment and harvest date, as the impact of azoxystrobin on disease severity was greater at the second harvest date than the first (Tables 5 & 6c).

Delaying harvest increased disease severity, except when other control options were effective at keeping disease low. In Estima for example, there was a significant interaction between harvest date and trial site (Tables 5 & 6d), as there was no impact of delaying harvest in the English trial where disease severity was generally low, whilst in Scotland delaying harvest increased severity of disease. Similarly, for Sante, there was a significant interaction between azoxystrobin treatment, irrigation and harvest date (Tables 5 & 6b); harvest date had little impact on disease severity when disease was being controlled through a combination of no irrigation and azoxystrobin treatment, or when disease levels were relatively high due to the application of irrigation without the application of azoxystrobin. When only one disease

Table 5 Analysis of variance of the main effects azoxystrobin, harvest date and irrigation on the percentage unmarketable potato tubers, and interactions between main effects, trial site and year. Only interactions significant at $P < 0.05$ in at least one analysis (which were performed separately for each cultivar) are shown

		Cultivar			
Source of variation	d.f.	Maris Piper	Sante	Saxon	Estima
Main effects					
Azoxystrobin	1	<0·01	<0·01	<0·01	ns
Harvest date	1	<0·01	<0·01	<0·01	<0·05
Irrigation	1	<0·01	<0·01	ns	<0·01
Interactions					
Azoxystrobin/Harvest date	1	ns	ns	<0·05	ns
Azoxystrobin/Trial site	1	ns	ns	<0·01	ns
Harvest date/Trial site	1	ns	ns	ns	<0·05
Azoxystrobin/Harvest date/Irrigation	1	ns	<0·01	ns	ns
Azoxystrobin/Year	2	<0·01	<0·01	–	–
Irrigation/Trial site/Year	2	<0·05	<0·01	–	–

d.f.: degrees of freedom; ns: not significant; –: year not a factor in the analysis.

Table 6 The effect of azoxystrobin, irrigation and harvest date, trial site (England and Scotland) and year (where appropriate) on the percentage unmarketable potato tubers

				England		Scotland	
				Azoxystrobin		Azoxystrobin	
Cultivar	Year	Harvest date	Irrigation	No	Yes	No	Yes
(a) Maris Piper	2004	Early	No	51	16	37	17
			Yes	76	50	41	9
		Late	No	61	16	72	15
			Yes	78	56	45	17
	2005	Early	No	2	1	16	4
			Yes	12	5	39	4
		Late	No	15	16	33	9
			Yes	26	13	33	10
	2006	Early	No	6	1	10	9
			Yes	16	11	18	14
		Late	No	10	5	32	22
			Yes	35	24	30	22
(b) Sante	2004	Early	No	6	1	6	11
			Yes	32	21	25	7
		Late	No	7	0	25	5
			Yes	28	33	29	11
	2005	Early	No	3	1	3	4
			Yes	3	2	8	0
		Late	No	17	9	5	1
			Yes	7	8	3	0
(c) Saxon	2004	Early	No	4	0	0	0
			Yes	8	5	0	0
		Late	No	8	3	6	2
			Yes	23	7	3	2
(d) Estima	2006	Early	No	0	0	8	5
			Yes	4	3	12	9
		Late	No	1	0	12	11
			Yes	5	3	29	14

control treatment (either application of azoxystrobin or no irrigation) was applied, disease severity increased at the later harvest date compared to the earlier date (Tables 5 & 6b).

Irrigation did not increase disease severity in the 2005 season in England or in any season in Scotland, as illustrated by the significant interaction between irrigation, trial site and year for both Maris Piper (Tables 5 & 6a) and Sante (Tables 5 & 6b). The effects of irrigation were less significant in trials receiving high levels of rainfall, and consequently little irrigation was applied to distinguish between irrigated and unirrigated treatments.

Effect of azoxystrobin, irrigation and harvest date on underground disease development in Maris Piper

The effects of azoxystrobin treatment and irrigation on disease development on underground parts of cv. Maris Piper, recorded at the trials in England and Scotland in 2004, are shown in Figures 4 and 5. In the Scottish trial (Fig. 4b), incidence of black dot on stems, stolons and roots increased until the final sample harvest, with significant differences between treatments being apparent from the fourth sample harvest taken 112 DAP onwards. The irrigation plus azoxystrobin treatment significantly reduced black dot on stems and stolons compared with all other treatments. Azoxystrobin treatment with or without irrigation was effective in reducing the incidence of black dot on roots. At the English trial site, where the soil inoculum level pre-planting was greater than at the Scottish site, black dot symptoms on stems occurred relatively early (90 DAP) and increased rapidly to near 100% by 118 DAP in all treatments. The incidence of black dot on stolons and roots progressed at a similar rate to that found at the Scottish site, with irrigation (with or without azoxystrobin) reducing stolon and root disease.

It therefore appears that the development of black dot on stems, stolons and roots is reduced by both azoxystrobin and irrigation. The effects of irrigation were particularly evident at the English site where azoxystrobin treatment had little effect on the incidence of black dot on underground plant parts. At the Scottish site, irrigation, in combination with azoxystrobin, reduced black dot on stems and stolons whilst azoxystrobin was effective against black dot on roots. In the absence of azoxystrobin and irrigation, the incidence of black dot on stems, stolons and roots reached 100, 84 and 79%,

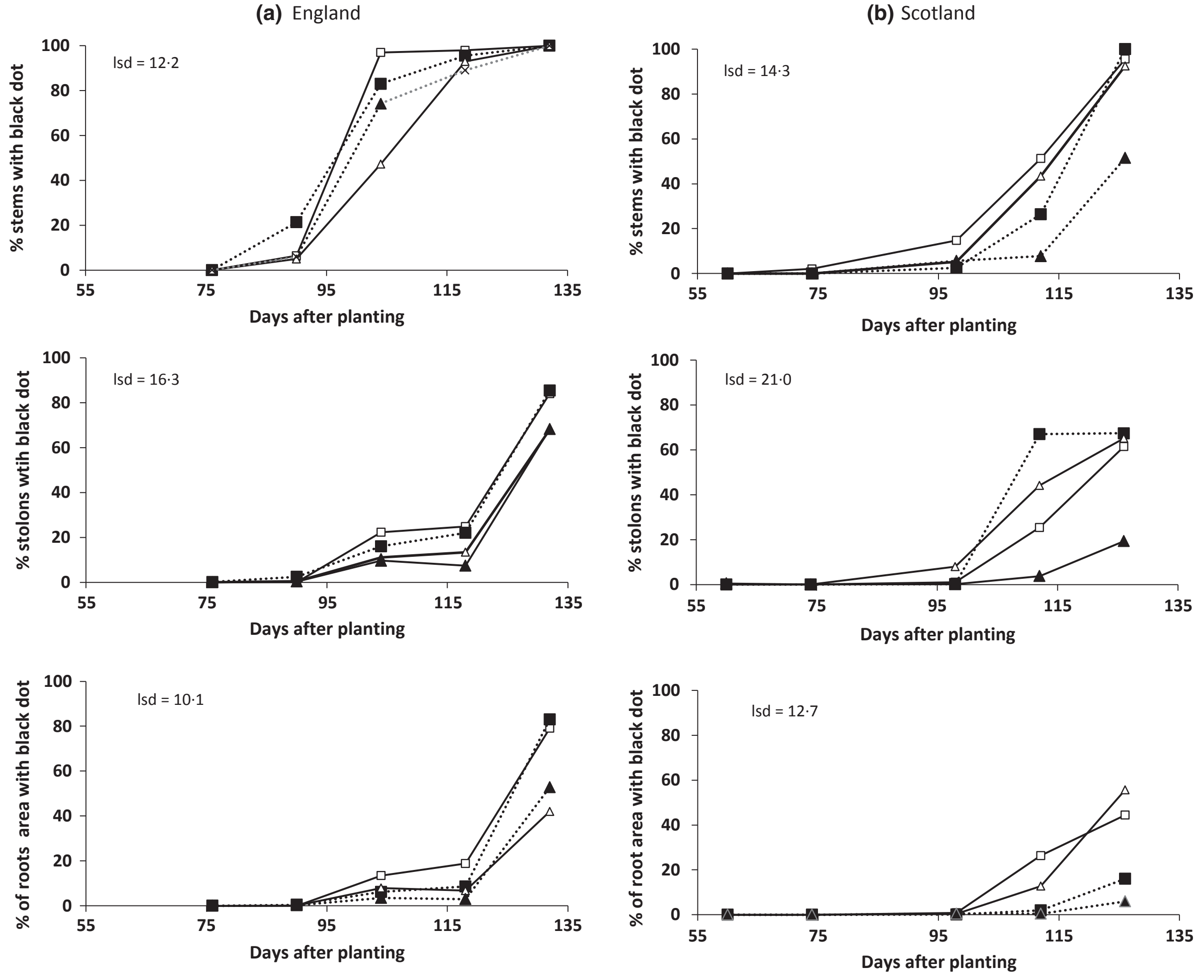

Figure 4 Percentage incidence of black dot on stems, stolons and roots of Maris Piper, at 2-weekly intervals at the trial sites in (a) England and (b) Scotland in 2004. No irrigation/no azoxystrobin (□), no irrigation/with azoxystrobin (■), irrigation/with azoxystrobin (▲), irrigation/no azoxystrobin (Δ).

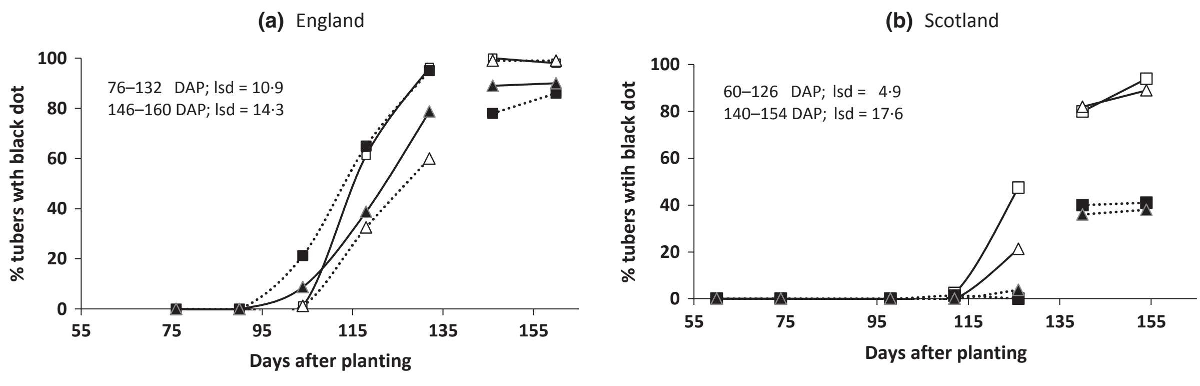

Figure 5 The incidence of black dot on Maris Piper tubers recorded at 2-weekly intervals and at the early and late harvests at the trial site in (a) England and (b) Scotland in 2004. No irrigation/no azoxystrobin (□), no irrigation/with azoxystrobin (■), irrigation/with azoxystrobin (▲), irrigation/no azoxystrobin (Δ).

respectively by the fifth sampling occasion (132 DAP) in England, but was lower for stems (96%), stolons (62%) and roots (44%) at the Scottish site 126 DAP.

The incidence of black dot on tubers increased steadily at both sites from 104 and 126 DAP at the English and Scottish sites, respectively (Fig. 5). By 132 DAP, black dot incidence was reduced by irrigation at the English site (Fig. 5a), whilst azoxystrobin had little effect. Whilst irrigation contributed to reduced black dot incidence at 126 DAP at the Scottish site, azoxystrobin was the main contributor. However, at both sites, by the time of the harvests (early and late) the effect of irrigation was no longer apparent, and azoxystrobin was the main contributor to reducing the incidence of black dot on tubers at both sites, but was particularly effective at the Scottish site (Fig. 5b).

The effect of irrigation and azoxystrobin on the incidence of black dot on underground parts (Fig. 4) and tubers (Fig. 5) of Maris Piper differed somewhat from their effect on the percentage unmarketable tubers at harvest (Fig. 3a). At the English site, where soil inoculum was relatively high in comparison to the Scottish site, azoxystrobin appeared to have little effect on the incidence of black dot on stems, stolons, roots and even tubers during the growing season. The effect appeared to be in limiting the severity of black dot on tubers, as reflected in the percentage of unmarketable tubers (Fig. 3a). Irrigation at the Scottish site in 2004 generally decreased the incidence of black dot on stems, stolons and roots and tubers during plant development, although the effect on tubers was no longer apparent at the early and late harvest. Azoxystrobin was more effective in reducing the incidence of black dot on roots and tubers and, when combined with irrigation, also on stems and stolons.

Discussion

Soil inoculum levels of *C. coccodes* can now be reliably quantified using a real-time PCR assay (Cullen *et al.*, 2002; Brierley *et al.*, 2009), and results have been shown to relate to risk of disease (Lees *et al.*, 2010). This development enables field selection to be a principal component of integrated disease control. Where a number of fields are tested for black dot, those with the most soil inoculum can be avoided. This is particularly useful on rented land where cropping history may not be known. On land which does not contain inoculum, or where there is a low risk associated with soil inoculum level, planting of seed infected with *C. coccodes* should be avoided to prevent further soil contamination.

Where soilborne inoculum is present and cannot be avoided, cultivar resistance is an effective way to reduce the risk of black dot. In the field trials reported here, swapping from a more susceptible cultivar to a more resistant one (even by one unit of resistance rating) resulted in a significant reduction in black dot on progeny tubers.

The Pentland Squire seed stock in 2007 and the Sante stock in 2004 were contaminated with black dot and may have increased the disease level slightly on the progeny tubers, but Lees *et al.* (2010) demonstrated that seed inoculum was of relatively low importance compared to soil inoculum in causing black dot disease. When six varieties with a spectrum of resistance rating were grown across sites in England and Scotland in 2007, four varieties appeared to develop disease according to their ratings. However, two varieties, King Edward and Lady Christl, did not conform to expectation. Averaged across 19 field sites with a range of soil inoculum levels, Lady Christl appeared more resistant than its rating suggests, despite it being an early maturing cultivar. However, it appears that the susceptibility of cv. Lady Christl differs according to inoculum pressure, and at high inoculum levels it is very susceptible. Given that in black dot disease resistance tests, high levels of inoculum are used when evaluating varietal resistance, it is possible to explain the low resistance rating based on the results from the three fields with the highest level of soil inoculum. In these trials the maincrop cv. King Edward was more susceptible than its rating suggests. Additionally, there were instances where there was little difference between Estima and Maris Piper (Scotland 2006). Estima is an earlier maturing cultivar than Maris Piper and this may have contributed to higher disease levels in this cultivar than predicted from its resistance rating.

In this study, an irrigation schedule (designed to restrict the development of common scab and to maximize yield) had little impact on disease in the 2005 season in England and in all seasons in Scotland. In contrast, irrigation significantly increased severity of black dot at the English site in 2006: this site received almost twice the amount of irrigation required in other trials. Lees *et al.* (2010) reported that in controlled environment experiments black dot severity was higher in the damp treatment compared to the treatment with restricted water (half that given to damp treatments). Olanya *et al.* (2010) looked at the effect of various irrigation regimes on disease incidence in field trials over a number of years. Although the results were often non-significant, in one year supplemental irrigation did increase the incidence of black dot on tubers. In practice, there is a major commercial benefit to applying irrigation and thus where a risk of black dot is perceived, and irrigation is to be applied, a grower should consider other control measures.

Azoxystrobin has full UK approval for control of black dot when applied in-furrow at planting or incorporated prior to planting. However, application of azoxystrobin can only be justified where inoculum is present. The results of a soil test can be used to make informed decisions on the use of this product at planting. The in-furrow treatment using azoxystrobin was evaluated in six field trials (2004–2006). In these trials, azoxystrobin resulted in significant reductions in the incidence and severity of disease and increased marketability. There was some evidence to suggest that where disease pressure was high (e.g. high soil inoculum level, susceptible cultivar, irrigation applied) the level of control achieved was attenuated, and conversely where disease was controlled through other factors such as resistant cultivar, no irrigation, then the use of azoxystrobin had little benefit. However, it was apparent in most trials that the effectiveness of the treatment persisted where harvest was delayed by 2 weeks. Over all the trials, azoxystrobin reduced the percentage of tubers unmarketable due to black dot from 26·7 to 14·6% in cv. Maris Piper, and from 12·9 to 7·1% in cv. Sante. Since its introduction, in-furrow or soil incorporation of azoxystrobin has been widely adopted in the potato industry, particularly the pre-packing sector. The uptake of the fungicide treatment reflects the levels of soilborne inoculum of *C. coccodes* in the main potato-growing regions of Great Britain. With black dot persisting in soil longer than the length of typical rotations, the continued use of azoxystrobin can be anticipated. As cultivar resistance appears to be expressed only in tubers, and infection of underground stems, stolons and roots can occur on all cultivars, soil inoculum is likely to increase irrespective of cultivar grown.

The longer a crop remains in the ground, the greater the risk of black dot developing, particularly where a high level of soil inoculum is present (J. C. Peters, unpublished data). Therefore, avoiding sites with high levels of inoculum for long duration crops will reduce risk of black dot. Where a crop is planted in soil containing a high level of inoculum, prioritizing this crop for early lifting will reduce the risk of black dot. In these trials, delaying harvest by 2 weeks after haulm destruction often resulted in significant increases in the proportion of unmarketable tubers.

Andrivon *et al.* (1998) studied colonization of underground parts by *C. coccodes* following inoculation of the seed tuber. They found that disease originating from seed inoculum was found first on roots (2 weeks after inoculation), then stolons and only on stems some 7–10 weeks after inoculation. Read & Hide (1995) compared seed stocks with varying levels of inoculum to clean stocks planted into inoculated soil. They found increased incidence of black dot on roots as early as 5 weeks and on stems 8 weeks after planting into inoculated soil, with lower incidence of disease with decreasing levels of seed inoculum. In the present study, where inoculum was soilborne (but naturally occurring at low levels), there was little difference in the timing of disease occurring on underground plant parts, which was first evident 12 weeks after planting. Johnson & Miliczky (1993) recorded below ground stem infection (colony-forming units in stem sap) at around 6–8 weeks after planting in plants sampled from commercial potato fields. In the Scottish trial in the present study, it was found that application of azoxystrobin in-furrow followed by irrigation significantly reduced the incidence of black dot on stems and stolons, and azoxystrobin alone significantly reduced black dot incidence on roots. In the English trial, only irrigation significantly reduced disease on stolons and roots. Read & Hide (1988) also recorded reduced incidence of black dot on stems and roots in plots that were irrigated compared to those which were not.

When comparing two cultivars, Andrivon *et al.* (1998) found similarly high levels of root and stem disease symptoms on inoculated plants of both cvs Bintje and Roseval, but cv. Roseval developed less disease on progeny tubers. Although the resistance ratings of these two cultivars to black dot are not known, cv. Roseval was considered to be more resistant to tuber disease than cv. Bintje, but this resistance did not extend to disease on other underground parts, a pattern also found in the present study. Assessments of the development of black dot on below ground plant parts demonstrated that cultivar resistance ratings appear to relate only to tubers. Differences in disease development on stems, stolons or roots between varieties do not relate to resistance ratings. Nitzan *et al.* (2010) investigating stem and stolon resistance to *C. coccodes* found no correlation between stem and stolon resistance and speculated that they were controlled by different genes. The monitoring of disease progression on underground parts during the growing season has shown that various disease control methods (cultivar resistance, treatment with azoxystrobin and reduced irrigation), whilst being effective in reducing the development of disease on tubers, do not impact on disease development on stems, stolons and roots in the same way and disease on these plant parts cannot be used as a predictor of likely disease development on tubers.

The results presented here highlight the options available to growers to reduce the risk of black dot on potato crops. By quantifying soil inoculum, growers can target cultivars to fields and reduce risk using a combination of reduced irrigation, in-furrow application of azoxystrobin and early harvest.

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