Project Report

Prediction and manipulation of black dot development in potato crops

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Preface

Losses due to black dot (*Colletotrichum coccodes*) are now estimated at £3 million each year in the UK, mainly caused by rejection of crops for the pre-pack market. One option for the management of the disease is early harvesting, which reduces symptom development. However, in order to select crops for early harvesting it is essential to know which crops are likely to be at risk from black dot. The British Potato Council commissioned a research project to examine the factors influencing black dot development.

The aims of this project were:

- To determine if assessing stems and stolons for the presence of the disease in the growing crop could be used to predict if black dot is likely to develop in harvested tubers
- To assess if haulm destruction methods could be used to reduce black dot
- To assess how conditions, particularly temperature and condensation events, during curing and storage, may effect development of black dot on tubers

The research has shown that the ability to predict the risk of black dot occurring varies with potato variety. The methods of haulm destruction and the different storage conditions studied during the project did not effectively reduce the development of black dot. The reasons for this, and its commercial implications are discussed.

Summary

As all underground parts of a potato plant can be infected with *C. coccodes* it was speculated that it may be possible to predict black dot development on harvested tubers by assessing stems and stolons for the presence of the disease in the growing crop. In a 3-year study stems and stolons were assessed for black dot in 124 potato crops and related to the disease on the tubers at harvest. In some years significant associations were observed in some cultivars. In 2000 and 2002 in the cv. Estima if stems or stolons showed black dot symptoms as the crops were starting to senesce then the disease was likely to be present on tubers at harvest. In cv. Maris Piper a significant association between black dot on stems in September 1999 with disease at harvest was observed. No relationship between black dot on stems and stolons in the growing crop and disease on tubers at harvest were observed in the cvs. Saxon and King Edward.

To explain these results a glasshouse and field experiment were performed to identify when symptoms of black dot develop on underground parts of a potato plant. These showed that infection by *C. coccodes* could occur soon after these plant parts had formed. Visual symptoms did not appear till just prior to senescence on stems and stolons and during senescence on tubers.

Methods of haulm destruction and different storage conditions did not effectively reduce development of black dot on tubers. Reasons for this and its commercial implications are discussed.

Introduction

In recent years, black dot (*Colletotrichum coccodes*) has become an economically important disease problem in potato. It is characterised by silvery lesions on the tuber surface resulting in a deterioration of skin quality (Lees and Hilton, 2003). Losses due to this disease are now estimated at £3 million each year in the UK mainly caused by rejection of crops for the pre-pack market. At present measures to control this disease in the field are limited to use of azoxystrobin (Amistar) to reduce infection resulting from soil-borne inoculum and early harvesting which reduces symptom development (Hide & Boorer, 1991; Hide *et al.,* 1994). In order to select crops for early harvesting it is essential to know which crops are likely to be at risk from black dot. As all underground parts of a potato plant can be infected with *C. coccodes* (Andrivion *et al*., 1998) it was speculated that it may be possible to predict black dot development on harvested tubers by assessing stems and stolons for presence of the disease in the growing crop. The first aim of this project was to assess if such a method could be used in commercial crops of different varieties to predict if black dot is likely to develop in harvested tubers. In-conjunction with this study other experiments were performed to identify at what stage in the crop development infection by *C. coccodes* and symptoms of black dot occurred on various plant parts.

Once harvest priorities have been decided it is important to consider methods of haulm destruction. Chemical haulm destruction in-conjunction with haulm pulling was found to reduce black scurf compared with other methods of haulm destruction (Dijst *et al*., 1987). The second aim of this project was to assess if haulm destruction methods could be used to reduce black dot.

After harvest it is known that drying tubers can reduce the development of black dot (Hide & Boorer, 1991). However, it is unclear how conditions in storage may effect development of the disease. The third aim is to assess how conditions, particularly temperature and condensation events, during curing and storage, may effect development of black dot on tubers. The commercial implications of the findings are discussed at the end of the report.

Milestones

Proposed Research	Comments
To quantify the relationship between infection of plant parts and disease levels on tubers	Completed
To determine the optimum timing for plant part examination	Completed
To determine soil characteristics that influence disease development	Completed
To determine the effects of variety, skin set, temperature and relative humidity on disease development prior to and in store	Completed

Prediction of Black dot disease at harvest 1999-2001

Aims

To predict black dot development on harvested tubers by assessing stems and stolons for presence of the disease in commercial crops of potato.

Materials and Methods

Site details

Over a 3-year period, 1999-2001, 124 potato fields from three growing regions (Somerset, Lincolnshire and Scotland) in the UK were sampled. In 1999, samples from 22 Estima, 20 Maris Piper and 5 King Edward crops were taken (47 in total). In 2000, samples from 16 Estima, 12 Maris Piper and 13 Saxon crops were taken (41 in total). In 2001, samples from 14 Estima and 16 Saxon crops were taken (30 in total). Information on whether these sites had a high or low risk of developing black dot and agronomic details was obtained from Branston and WCF potatoes.

Assessment of stems and stolons

Sampling was performed at various times during the growing season by taking five (1999) and seven plants (2000 and 2001) in a W shaped manner across the field using a clean fork. The below ground parts of the plant were washed and 1 stem and 2 stolons were assessed for the % area of these covered in silvering and sclerotia. Visual assessments were confirmed as black dot using a binocular microscope.

Assessment of tubers

From each site, 50 tubers were harvested and sent to SAC, Aberdeen. On arrival tubers were washed lightly to remove excess soil and the percentage area covered in silvering and sclerotia was assessed. Visual observations were confirmed as black dot using a binocular microscope.

Results and Discussion

This work was performed to develop an easy method by which growers could sample their own crops, assess the plants for disease and then make a prediction on whether individual stocks are at risk of developing black dot at harvest. Thus 5 (1999) and 7 (2000-2001) plants were sampled per crop to emulate the numbers of plants that may be sampled by a grower. Symptoms on stems and stolons were easily assessed but care must be taken with samples to prevent diseased tissue from being removed when washing infected plants.

Initially, in July, few black dot symptoms were observed on stems and stolons. As crops started to senescence in August so symptoms of black dot developed on all underground parts. As a result no association between symptoms on stems and stolons in July and that on tubers at harvest was detected. In both August and September significant associations were observed between black dot on stems (r =0.63 August 2000 and r = 0.41 September 1999) and stolons (r=0.55 August 2000) (Table 1) with incidence of black dot on tubers in the cv. Estima. It is suggested that assessing stems and stolons for black dot as the crop starts to senesce is a useful method to predict if harvested tubers will develop black dot symptoms in cv. Estima. Although this method can help predict incidence of black dot on tubers it can not be used to predict how severe these symptoms will be. As a result sites where a low incidence of black dot on stems was observed may have produced tubers with a high incidence of the disease (Figure 1).

Significant association (r = 0.9 and 0.79) were observed between incidence of black dot on stems and stolons in July and black dot on tubers in October in cultivar Saxon (Table 2). However, as there were only a few sites where the disease was found on underground parts it is suggested that this data may be skewed due to a reduced number of data points (Figure 2). A significant association (r = 0.52) was also observed between incidence of black dot on stems in September and black dot on tubers in October in cultivar Maris Piper (Table 3). However, at this late stage in the season it is too late to make decisions on haulm destruction or early harvesting of the crop. No significant associations were observed between the incidence of black dot on stems and stolons with incidence on tubers in other varieties. Reasons for this are unclear. However, symptoms of black dot do differ between cultivars. In Chapter 7 (Figures 8, 9) symptoms of black dot developed earlier on stems of cv. Estima, at tuber bulking, whilst, in Maris Piper symptoms were not observed till early senescence. In contrast, symptoms on stolons were not observed till early senescence in both varieties (Figures 10, 11). Symptom development on tubers (Hilton *et al*., 2000) and stems and stolons (Andrivion *et al.,* 1998) is also dependent on host resistance which may not be related. This may mean that in more resistant varieties symptoms may not develop as rapidly on stems and stolons.

TABLE 2: RELATIONSHIP BETWEEN INCIDENCE OF BLACK DOT ON STEM AND STOLONS WITH TUBERS ON CV. ESTIMA. STEMS AND STOLONS WERE ASSESSED FOR BLACK DOT IN JULY, AUGUST AND SEPTEMBER AND TUBERS IN OCTOBER BETWEEN 1999 AND 2001. ASSOCIATIONS ARE EXPRESSED AS CORRELATION CO-EFFICIENTS.

Month	Year	Stem	Stolon
July	2000	0.13	-0.02
	2001	-0.18	-0.18
August	2000	0.63*	0.55*
	2001	-0.18	0.10
September	1999	0.41**	0.36

*Significant to 5% level
** Significant to 1% level

FIGURE 1: RELATIONSHIP BETWEEN % INCIDENCE OF BLACK DOT ON STEMS AND TUBERS ON CV. ESTIMA IN 2000. STEMS WERE ASSESSED FOR BLACK DOT IN AUGUST AND TUBERS IN OCTOBER.

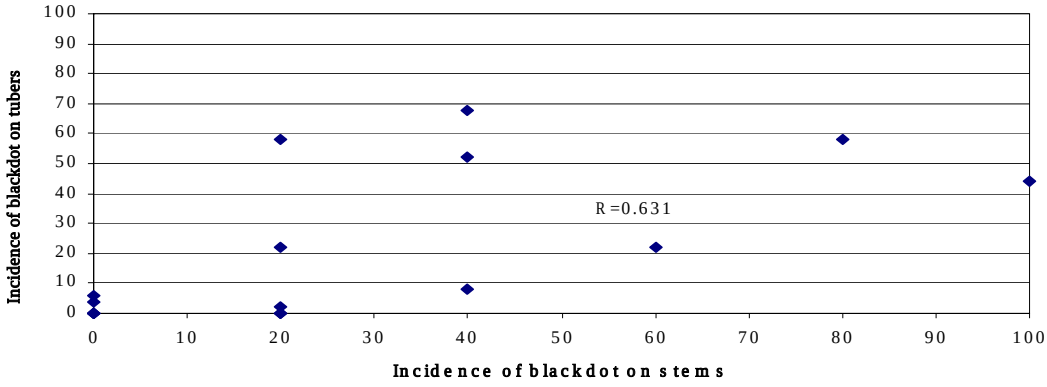


TABLE 2: RELATIONSHIP BETWEEN INCIDENCE OF BLACK DOT ON STEMS AND STOLONS WITH TUBERS ON CV. SAXON. STEMS AND STOLONS WERE ASSESSED FOR BLACK DOT IN JULY AND AUGUST AND TUBERS IN OCTOBER IN 2000 AND 2001. ASSOCIATIONS ARE EXPRESSED AS CORRELATION CO-EFFICIENTS.

Month	Year	Stems	Stolons
July	2000	0.59	0.79**
	2001	0.14	0.14
August	2000	0.44	0.35
	2001	-0.09	-0.12

FIGURE 2: RELATIONSHIP BETWEEN % INCIDENCE OF BLACK DOT ON STEMS AND TUBERS ON CV. SAXON IN 2000. STEMS WERE ASSESSED FOR BLACK DOT IN AUGUST AND TUBERS IN OCTOBER.

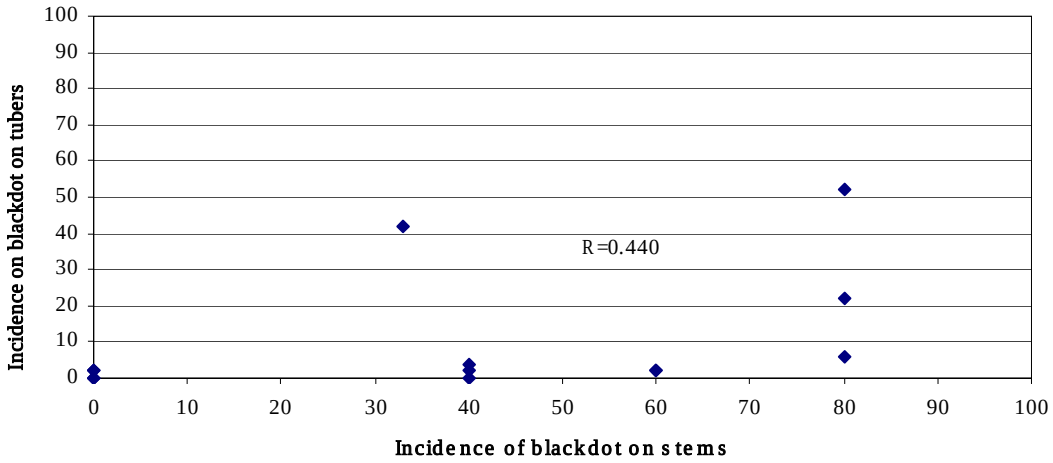


TABLE 3: RELATIONSHIP BETWEEN INCIDENCE OF BLACK DOT ON STEMS AND STOLONS WITH TUBERS ON CV. MARIS PIPER. STEMS AND STOLONS WERE ASSESSED FOR BLACK DOT IN JULY AND AUGUST AND TUBERS IN OCTOBER 1999 AND 2000. ASSOCIATIONS ARE EXPRESSED AS CORRELATION CO-EFFICIENTS.

Month	Year	Stems	Stolons
July	2000	0.30	0.05
August	2000	0.01	0.17
September	1999	0.52*	0.32

FIGURE 3: RELATIONSHIP BETWEEN % INCIDENCE OF BLACK DOT ON STEMS AND TUBERS ON CV. MARIS PIPER IN 2000. STEMS WERE ASSESSED FOR BLACK DOT IN AUGUST AND TUBERS IN OCTOBER.

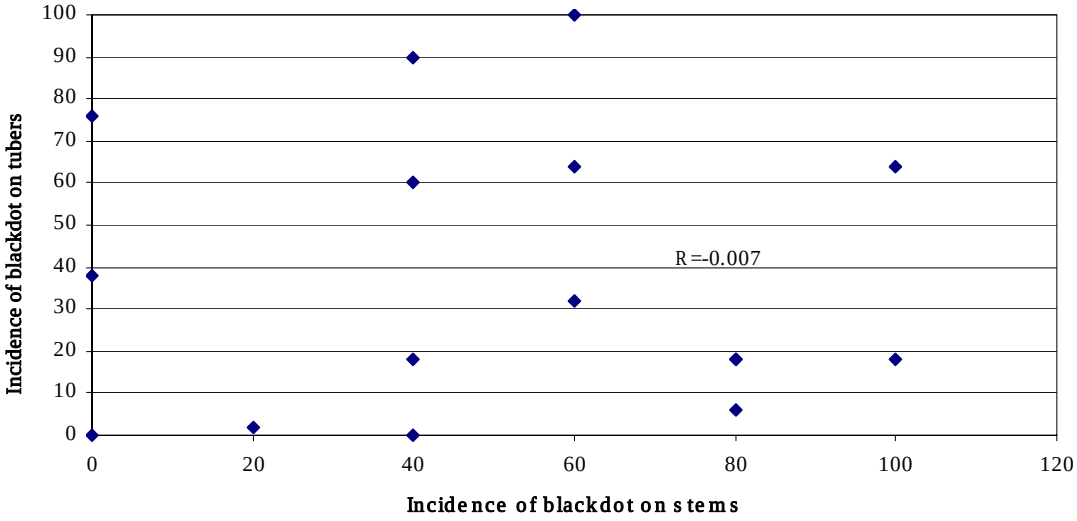


TABLE 4: RELATIONSHIP BETWEEN INCIDENCE OF BLACK DOT ON STEMS AND STOLONS WITH TUBERS ON CV. KING EDWARD. STEMS AND STOLONS WERE ASSESSED FOR BLACK DOT IN SEPTEMBER AND TUBERS IN OCTOBER 1999. ASSOCIATIONS ARE EXPRESSED AS CORRELATION CO-EFFICIENTS.

Month	Year	Stem	Stolons
September	1999	0.26	0.26

Effect of seed- and soil-borne inoculum on development of black dot on underground parts of potato under glasshouse conditions

Aims

To assess the effect of seed- and soil-borne inoculum on development of black dot and infection by *C. coccodes* on underground parts of potato under glasshouse conditions.

Materials and Methods

Experiment 1: Effect of seed-borne inoculum on black dot development on underground parts of potato

Seed

A seed stock of cv. Estima (SE3) (35-50mm) with black dot was obtained. Tubers were lightly washed and were sorted into those with 1-10%, 10-50% of surface area covered in black dot and no visual symptoms of black dot. Seed from a healthy stock of cv. Estima (SE2) (35-50mm) were also obtained and used as a clean control. To confirm that this stock was not infected with *C. coccodes* eye-plug tests were performed and results compared with the stock with black dot. This involved taking 2 eye cores from each of 25 tubers, using a 5 mm corer and placing them on square Petri dishes containing 25 separate compartments (2 plugs per compartment). Dishes were placed in a sealed plastic bag together with a damp tissue and incubated at 20^{o}C for 3 days. Eye plugs were assessed under a binocular microscope for the presence of microsclerotia.

Planting

Sandy loam soil from a field where potatoes had not been grown for 20 years was obtained and air-dried in a glasshouse. Once dried, soil was sieved through a 2 mm sieve into 6 inch pots containing a 1 inch layer of sterilised 5mm stone chippings and planted with one seed-tuber per pot at a depth of 5 cm. For each treatment 5 replicate pots were prepared for each of 5 sampling dates to give a total of 25 pots per treatment. Pots were placed on individual trays to avoid cross-contamination when watering and laid out in a randomised block design and blocked according to sampling date. The experiment was conducted in a glasshouse set at 20^{o}C (approx) with a lighting regime of 16 hours daylight and pots were watered to 60% water holding capacity twice weekly.

Glasshouse assessments

At planting the growth stage of the seed was assessed (Jefferies and Lawson, 1991). At each of 5 sample dates (2, 4, 6, 10 and 14 weeks after planting) plants were removed from pots, washed and underground plant parts assessed for disease as in Section 5.2.2

and growth stage according to Jeffries and Lawson (1991). Weight and number of tubers were assessed at each sampling date.

Laboratory assessments

At each sampling date, four stem, stolon and root sections were taken from each pot. Stem sections 5 mm in length were taken 5 cm below the soil surface. Stolon sections were selected that were growing approximately 5 cm below the soil surface and 5 cm from the stem to which it was attached. Root sections 5 mm in length were taken 5 cm from the seed tuber. Once daughter tubers were greater than 20 mm in diameter, tuber cores (5mm in diameter x 5mm in length) were also taken from 4 tubers per pot. All segments were surface sterilised in 96% ethanol for 30 seconds (Carnegie *et al*., 2003) and then plated onto Petri dishes containing semi-selective media (Farley, 1976) and incubated for 2 weeks at 25^{o}C. The incidence of *C. coccodes* was confirmed by identifying microsclerotia under a light microscope.

Experiment 2: Effect of soil-borne inoculum on development of black dot on underground parts of potato

Inoculation of soil

An isolate of *C. coccodes* pathogenic on potatoes was obtained from a tuber and maintained on Potato Dextrose Agar (PDA). For experimental purposes cultures were grown for one week at 20^{o}C. Cultures were macerated with a Braun hand held liquidiser together with 50ml of sterile water per agar plate. This was thoroughly mixed with air-dried sandy loam soil, from a site where potatoes had not been grown (Section 6.2.1), at a rate of 1/8, 1/16 and 1/32 plate of *C. coccodes* per kg soil. Soil was placed in 6 inch pots containing a 1 inch layer of sterilised 5mm stone chippings, to improve drainage and compared with soil which had not been contaminated. Seed cv. Estima (SE2) (35-50mm) free from black dot was obtained. The absence of black dot on this stock was confirmed with an eye-plug test (Section 6.2.1). Seed was planted at a depth of 10cm and placed in a glasshouse set at 20^{o}C.

For each treatment 5 replicate pots for each of 5 sampling dates were prepared, placed in individual trays to avoid cross-contamination when watering. Pots were laid out in a randomised block design and plant disease and laboratory assessments were taken as in Section 6.2.1.

Analysis of Variance was performed on both angular transformed and non-transformed data using Genstat, version 6.2 (Lawes Agricultural Trust).

Results

Experiment 1: Effect of seed-borne inoculum on black dot development on underground parts of potato

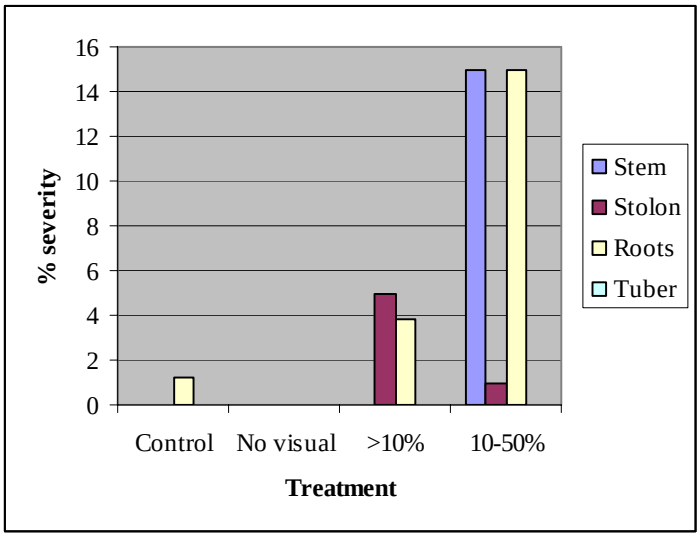
Symptoms of black dot were not observed till 14 weeks after planting when plant foliage started to senesce (growth stage = onset of flowering to completely dead). At this time severity of symptoms was 5% on roots with limited symptoms on stems (3.8%) and stolons (1.5%). No symptoms were observed on tubers.

C. coccodes was detected on plant tissue before any visual symptoms were observed. *C. coccodes* was first detected on a single stem segment 6 weeks after planting from a plant grown from seed with 1-10% black dot. Ten weeks after planting *C. coccodes* was detected on roots of plants grown from seed with 10-50% black dot.

Analysis of variance was performed on both angular transformed and non-transformed data. As results from the statistical analysis were similar for both sets of data results are presented as non-transformed data 14 weeks after planting. Planting seed with severe symptoms of black dot significantly ($P<0.05$) increased the severity of the disease on roots from 1.2% in control pots to 15% where seed with 10-50% black dot was planted (Figure 4). Where seed with visual symptoms of black dot were planted symptoms were also observed on stems and stolons. However, this was not significantly greater than the clean controls where no symptoms were detected.

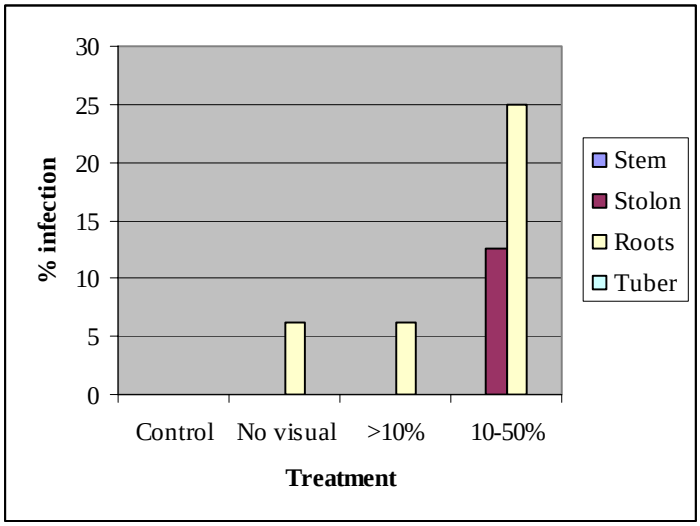
C. coccodes was most readily detected on roots. Where seed with 10-50% black dot was planted *C. coccodes* was detected on 25% of roots, whilst 6.25% of roots grown from seed with >10% were colonised by *C. coccodes* even though no visual symptoms were seen (Figure 5). *C. coccodes* was also detected on 12.5% of stolons where seed with 10-50% black dot was planted. No significant differences between treatments were observed.

FIGURE 4: DEVELOPMENT OF BLACK DOT ON ROOTS, STEMS, STOLONS AND DAUGHTER TUBERS 14 WEEKS AFTER PLANTING SEED WITH NO VISUAL SYMPTOMS, >10% BLACK DOT, 10-50% BLACK DOT COMPARED WITH A HEALTHY CONTROL. DISEASE SYMPTOMS WERE ASSESSED AS % AREA OF UNDERGROUND PARTS COVERED IN SYMPTOMS OF NECROSIS AND MICROSCLEROTIA.



L.S.D – Stem 18.3 (ns), Stolon 7.9 (ns), Roots 10.8 ($P<0.05$), Tubers 0 (ns)

FIGURE 5: COLONISATION OF *C. COCCODES* ON ROOTS, STEMS STOLONS AND TUBERS 14 WEEKS AFTER PLANTING PLANTING SEED WITH NO VISUAL SYMPTOMS, >10% BLACK DOT, 10-50% BLACK DOT COMPARED WITH A HEALTHY CONTROL. % INFECTION WAS DETERMINED AS THE INCIDENCE OF *C. COCCODES* ON SECTIONS PLATED ONTO SELECTIVE MEDIA (FARLEY'S 1976).



L.S.D – Stem 0 (ns), Stolon 11.12 (ns), Roots 40.9 (ns), Tuber 0 (ns)

Experiment 2: Effect of seed-borne inoculum on black dot development on underground parts of potato

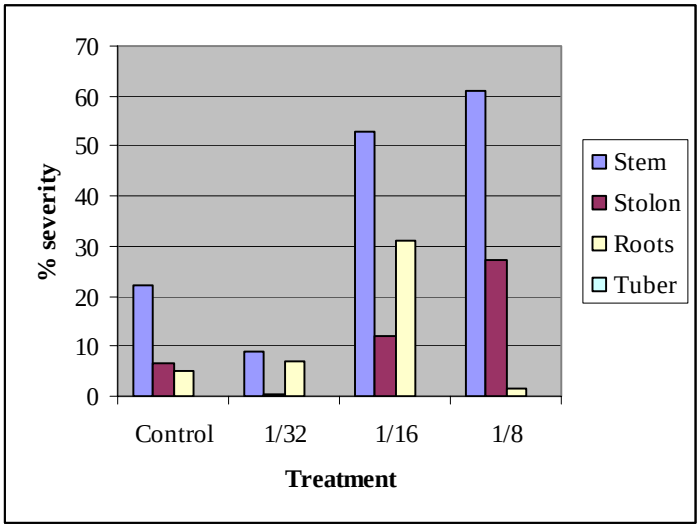
Symptoms of black dot on roots, stems and stolons were not observed till 14 weeks after planting (growth stage = onset of senescence to completely dead). Symptoms were most readily observed on stems (37% severity) but were also observed on roots (11.2%) and stolons (11.5%) (Figure 6). No symptoms were observed on tubers.

C. coccodes was detected on plant parts before visual symptoms were observed. On stems *C. coccodes* was obtained from one segment 2 weeks after planting. Six weeks after planting *C. coccodes* was observed on 44% of stems in soil contaminated with 1/16 of a plate per kg of soil and 6% where 1/32 and 1/8 of a plate were added per kg of soil. No *C. coccodes* was detected on stolon or tuber segments.

Fourteen weeks after planting, although symptoms of black dot appeared most severe in pots where soil was contaminated with 1/16 and 1/8 plate *C. coccodes* per kg soil no significant differences were observed between treatments. For example, in control pots stems showed a severity of 20% compared with 61% where seed was planted in soil contaminated with 1/8 plate *C. coccodes* per kg soil. On stolons, symptoms of black dot were most severe where seed was planted in soil contaminated with 1/8 plate *C. coccodes* per kg soil (27%) compared with control pots (6.7%).

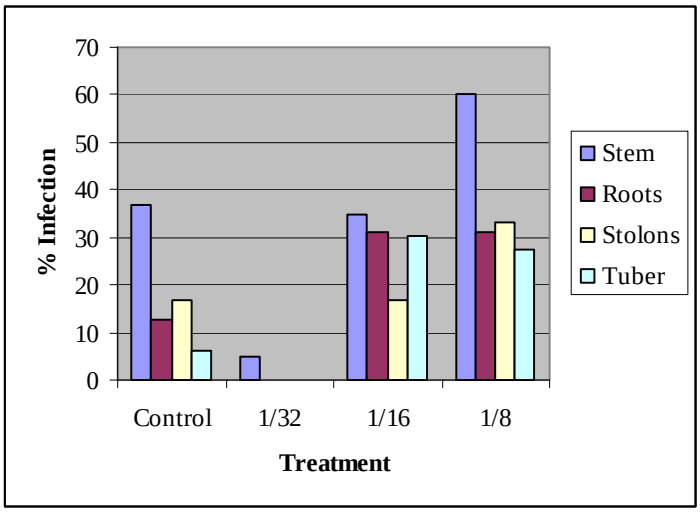
Similarly, *C. coccodes* was isolated more readily from plants where soil was contaminated with 1/16 and 1/8 of a plate per kg soil (Figure 7). However, these differences were not significant. For example, *C. coccodes* was isolated from 27% of tuber segments from pots when soil was contaminated with 1/8 plate per kg soil compared with 6% in control pots. In control pots *C. coccodes* was found on a number of stem segments (22%) compared with 61% of stem segments from pots where soil was contaminated with 1/8 culture per kg soil.

FIGURE 6: DEVELOPMENT OF BLACK DOT ON ROOTS, STEMS, STOLONS AND DAUGHTER TUBERS 14 WEEKS AFTER PLANTING HEALTHY SEED CV. ESTIMA IN SOIL AMENDED WITH 1/8, 1/16 AND 1/32 OF A CULTURE OF *C. COCCODES* PER KG OF SOIL AND COMPARED WITH UN-AMENDED SOIL. DISEASE SYMPTOMS WERE ASSESSED AS % AREA OF UNDERGROUND PARTS COVERED IN SYMPTOMS OF NECROSIS AND MICROSCLEROTIA.



L.S.D – Stem 78 (ns), Stolon 35 (ns), Roots 41.1 (ns), Tubers 0 (ns)

FIGURE 7: COLONISATION OF *C. COCCODES* ON ROOTS, STEMS STOLONS AND TUBERS 14 WEEKS AFTER PLANTING HEALTHY SEED CV. ESTIMA IN SOIL AMENDED WITH 1/8, 1/16 AND 1/32 OF A CULTURE OF *C. COCCODES* PER KG OF SOIL AND COMPARED WITH UN-AMENDED SOIL.. % INFECTION WAS DETERMINED AS THE INCIDENCE OF *C. COCCODES* ON STOLON SECTIONS PLATED ONTO SELECTIVE MEDIA (FARLEY'S 1976).



L.S.D – Stem 82.5 (ns), Stolon *, Roots 50 (ns), Tuber 31.2 (ns)

Discussion

Both infected seed and contaminated soil can act as a source of inoculum for black dot. In these experiments symptoms were observed on all underground part parts, except tubers, when healthy seed was planted in contaminated soil and where seed infected with *C. coccodes* was planted in non-contaminated soil. In these experiments symptoms were more severe where healthy seed was planted in soil contaminated with *C. coccodes*. In other studies more severe symptoms were observed on daughter tubers where seed was planted in soil amended with *C. coccodes* compared with seed with black dot planted in non-amended soil (Read and Hide, 1995). However, it is difficult to compare inoculum levels on seed with amounts of inoculum found in soil.

Initial symptoms were not observed until the haulm had started to senesce in both experiments. This confirms previous work which suggested that under British conditions symptom development was restricted to senescent plants or to plants weakened by some other disease (Jellis and Taylor, 1974). In contrast, symptoms of black dot were observed on roots 3 weeks after planting (Andrivion *et al.* 1998) and on stems 6 weeks after planting (Read and Hide, 1995), both prior to haulm senescence. When comparing results between these studies it is difficult to compare levels of seed or soil-borne inoculum to get a clearer picture on how these effect disease levels on underground plant tissues. Further work is required to relate levels of seed and soil-borne inoculum to symptom development under commercial field situations. To do this it is important to standardise methods of measuring levels of inoculum both on seed and in soil.

In this study the most susceptible plant organs were stems and roots where infected seed was planted and roots where healthy seed was planted in contaminated soil. In other studies initial symptoms were observed on roots in contaminated soil, whilst the first symptoms were observed on stems when infected seed was planted (Schmiedeknecht, 1956). It is suggested that this is due to the proximity of inoculum to the developing plant organs.

In contrast to symptom development, infection of underground plant tissue occurs before the haulm starts to senesce. Where healthy seed was planted in contaminated soil, *C. coccodes* was detected on stem tissue 2 weeks after planting. Where infected seed was planted in non-contaminated soil *C. coccodes* was detected on stem and root tissue 6 and 10 weeks after planting respectively. These results suggest that infection of underground plant parts can occur as these organs develop. There is then a latent phase when no symptoms are observed. Once haulm tissue starts to senesce symptom development occurs.

The effect of level of seed or soil-borne inoculum on infection was limited. No differences in severity of black dot or colonization of plant tissue by *C. coccodes* were observed between different levels of soil contamination. At present it is unclear what levels of soil-borne *C. coccodes* exist in agricultural soils. The levels of soil-borne inoculum used in this study were arbitrary and it is possible that in agricultural soils levels of *C. coccodes* inoculum may have a greater range than those used in this study.

Planting seed with severe black dot only increased severity of the disease on roots over lower levels. In earlier field studies (Read and Hide, 1995) planting seed with severe black dot resulted in an increase in the disease on roots, stems and tubers over a lower infection level. In these studies they used seed with >50% black dot compared with 10-50% upper limit in this present study.

Effect of seed- and soil-borne inoculum on development of black dot on underground tissue of potato under field conditions

Aims

To assess the effect of seed- and soil-borne inoculum on development of black dot and infection by *C. coccodes* and relate this to growth stage of the crop under field conditions.

Materials and Methods

Plant material

Seed stocks (35-50mm) with black dot of cvs Estima (SE1) and Maris Piper (SE2) were assessed for symptoms. This seed was used in plots planted with infected seed. Healthy stocks (35-50mm) of cvs Estima (SE2) and Maris Piper (SE2) were also visually assessed to confirm that no black dot was present. This seed was used in healthy control and soil inoculated plots.

Planting

The experiment was planted by hand on 17th May 2002 at a site near Oldmeldrum, Aberdeenshire. Plots consisted of 4 drills, 85 cm apart, each with 25 tubers planted at 25cm intervals. Tubers were planted at a depth of 15- 20cm and then covered with soil.

Soil inoculum was prepared by homogenising a 14 day old culture of *C. coccodes* and adding this to air dried and sieved soil from the field site at a rate of 1/8 plate per kg of soil. Forty grams of inoculated soil was then added around each seed tuber after planting but before covering with soil. Uninoculated plots received 40 g of unamended soil. For each of the three treatments (healthy seed planted with contaminated soil, infected seed planted in unamended soil and healthy seed planted in unamended soil) 4 replicate plots of each treatment were planted in a randomised block design.

Plant assessments

Using a key to determine growth stage (Jefferies and Lawson, 1991) plants were sampled at full emergence (300, 14th June), stolon initiation (510, 26th June), tuber initiation (530, 10th July), when daughter tubers were 10mm in diameter (540, 21st August), at onset of foliage senescence (600, 6th September), 4 and 8 weeks after haulm destruction (690 and 700, 26th October and 25th November). At each date five plants from each plot were removed, washed and weighed to determine fresh weight. Stolons, stems, roots and daughter tubers were assessed for black dot (Section 5.2.2). In the field % emergence and crop growth stage was measured at regular intervals.

In-vitro assessments

From each of the plants sampled for disease assessment, five (5mm segments) stem, stolon, root and tuber sections were taken. These were surface sterilised, by dipping, for 5 minutes in 1% sodium hypochlorite (0.1 % available chlorine) and then rinsed three times in sterile distilled water. The plant segments were then allowed to dry before being placed in Petri dishes containing selective media (Farley, 1972). Plates were sealed with Parafilm and then incubated for 2 weeks at 25^{o}C in darkness. Incidence of *C. coccodes* was recorded by assessing fungal colonies under a binocular microscope and looking for the characteristic microsclerotia.

Quantification of soil-borne inoculum

During the growing season, soil was collected from each plot at tuber initiation, when daughter tubers were 10mm in diameter, at onset of foliage senescence, and 2 weeks after haulm destruction. From each plant, 0.1 kg of soil that had been disturbed during the removal of plants (Section 7.2.3) was collected in a plastic bag and bulked to give a 0.5 kg soil sample from each plot.

The level of soil-borne *C. coccodes* was then assessed using a method adapted by Denner (1997). The soil was air-dried for 1-2 days at a temperature of 35 °C. Fifteen g of soil was then added to 500ml of distilled water in a 2 litre Labplex beaker and stirred for 2 minutes using a Braun hand blender. Once the soil had settled, the supernatant was decanted through a 90 µm mesh sieve. The debris retained on the sieve was re-suspended in 5ml sterile water and 0.5 ml of the suspension was then plated on to 90-mm diameter Petri dishes containing Farley's selective media. This was repeated 4 times for each plot. Plates were incubated at 25 °C in the dark for at least 14 days before counting *C. coccodes* colonies. The number of viable propagules of *C. coccodes* per g soil was calculated from the number of colonies that developed on all four plates.

Results and Discussion

As plants grown in control plots became infected with *C. coccodes* it can be assumed that soil-borne inoculum were present at this site prior to the start of this experiment, even though potatoes had not been grown here for 7 years. As a result few differences in infection or symptom development were observed between sources of inoculum.

Infection in stems and stolons had occurred by stolon development (Figure 14) and tubers became infected as they formed. The incidence of infection increased gradually over time reaching a peak at early senescence in stems and stolons and late senescence in tubers. Incidence of infection was lower in tubers than in stems and stolons. It is believed that as tuber development occurs later than other organs they have a shorter period in which to become infected. No differences in infection were observed between inoculum source and variety.

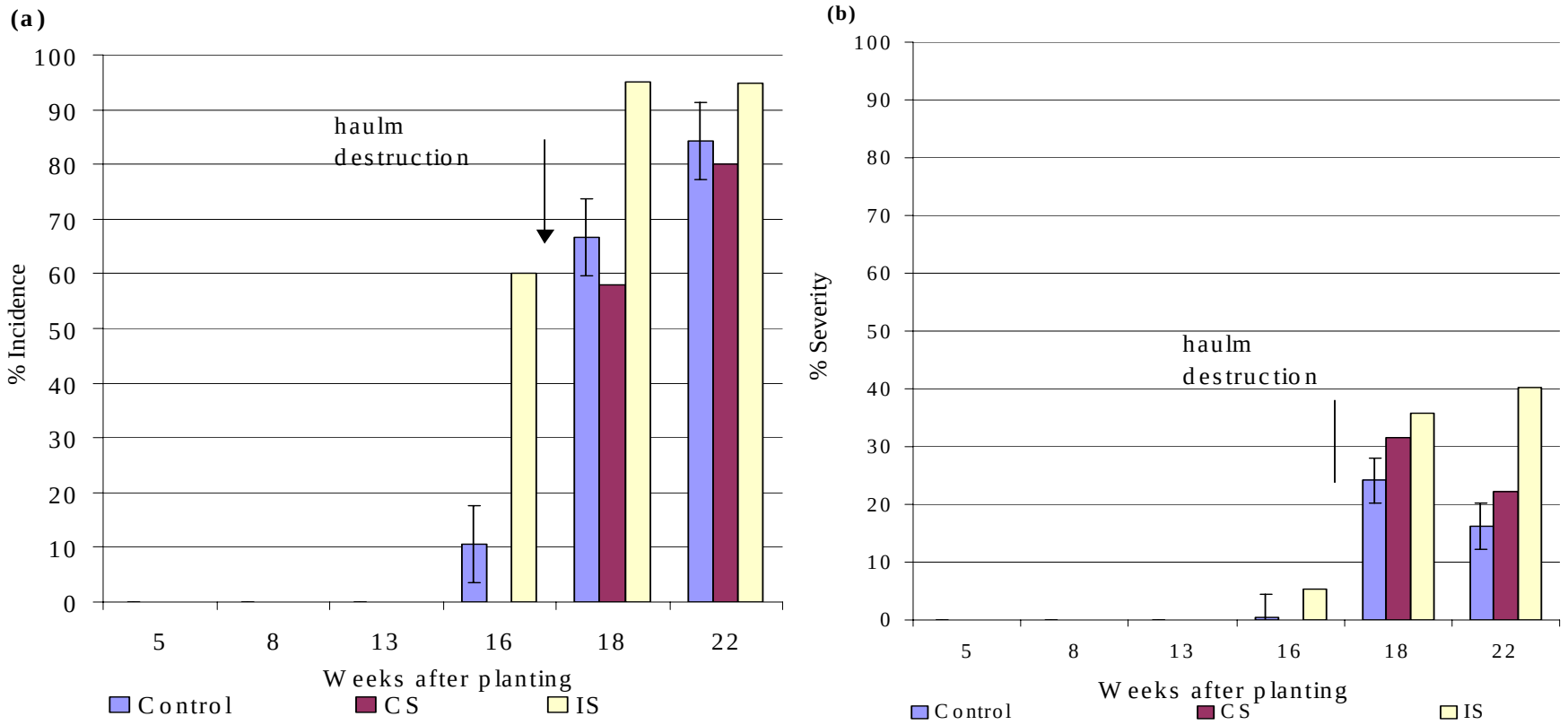
Symptoms developed on stems from tuber bulking in cv. Estima (Figure 8) and from early senescence in cv. Maris Piper (Figure 9) reaching a peak at late senescence. Symptoms on stems of cv. Estima were more severe from seed-borne inoculum whilst in contrast stems of cv. Maris Piper were more severe following inoculation of the

soil. Symptoms of black dot on stolons did not occur until early senescence in both cultivars reaching a peak at full senescence (Figures 10 & 11). No differences in symptom development on stolons were observed between inoculum source and variety.

Symptoms on tubers did not develop until late senescence (Figures 12 & 13), but even at this stage severity of symptoms were low. However, when tubers were sampled at full senescence (25th November) severity of black dot had increased to 8 % on cv. Estima and 15% on cv. Maris Piper. This highlights the need to harvest crops at risk as early as possible to reduce symptoms of black dot. No differences in severity of symptoms on tubers were observed between the sources of inoculum.

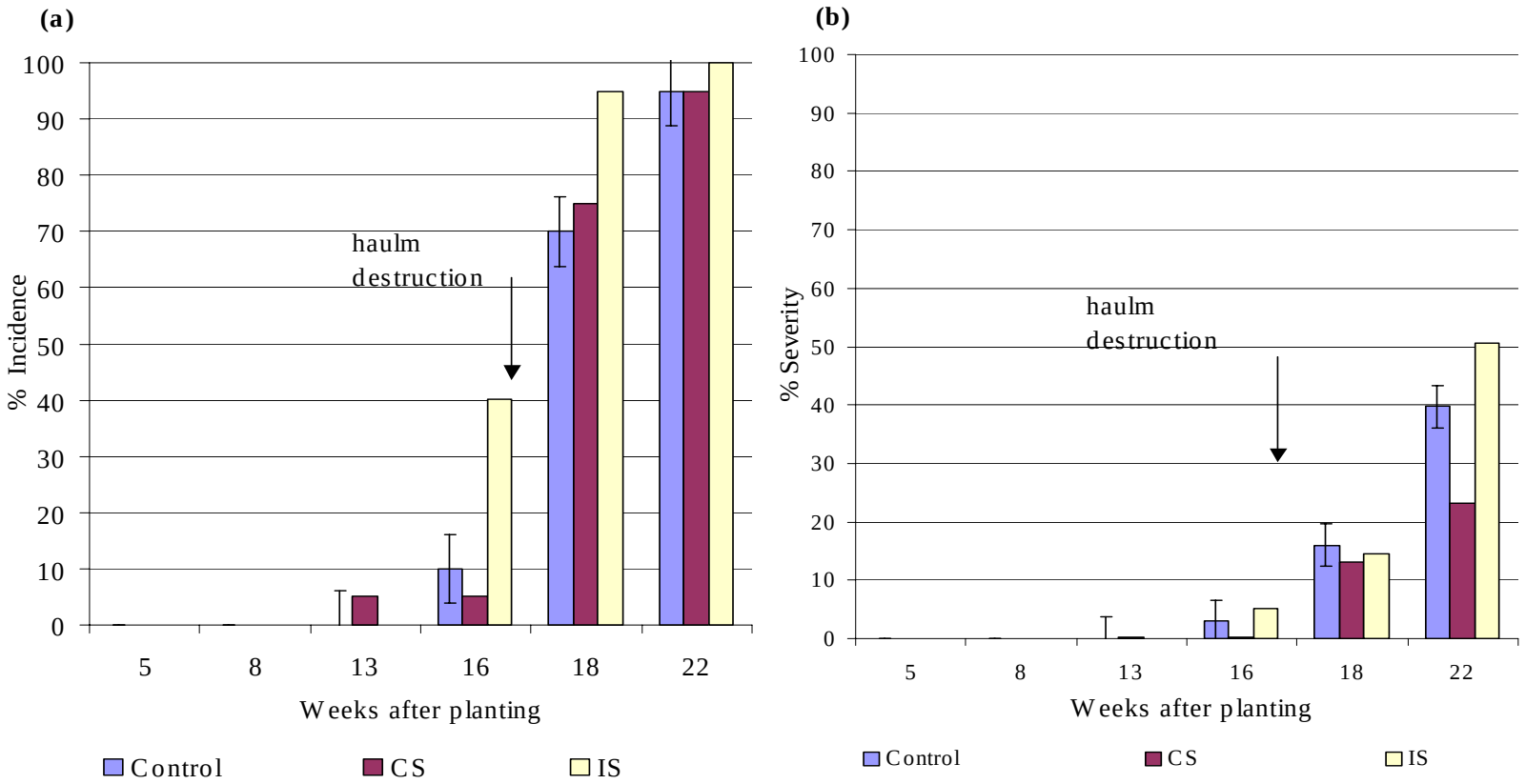
Using a method adapted by Denner (1997) the presence of *C. coccodes* in soil could be identified. (Figures 15 & 16). Using this method it was shown that as the crop and symptoms developed so levels of soil-borne inoculum increased reaching a peak of 1 colony forming units (per g soil) on November 25th in plots of cv. Estima. No differences between numbers of colony forming units were observed between inoculum types (Figures 15 & 16). It is suggested that high levels of soil-borne inoculum were already present prior to the start of this experiment making differences between treatments difficult to observe.

FIGURE 8 : INCIDENCE (A) AND SEVERITY (B) OF BLACK DOT ON STEMS OF CV. ESTIMA DURING THE GROWING SEASON†.



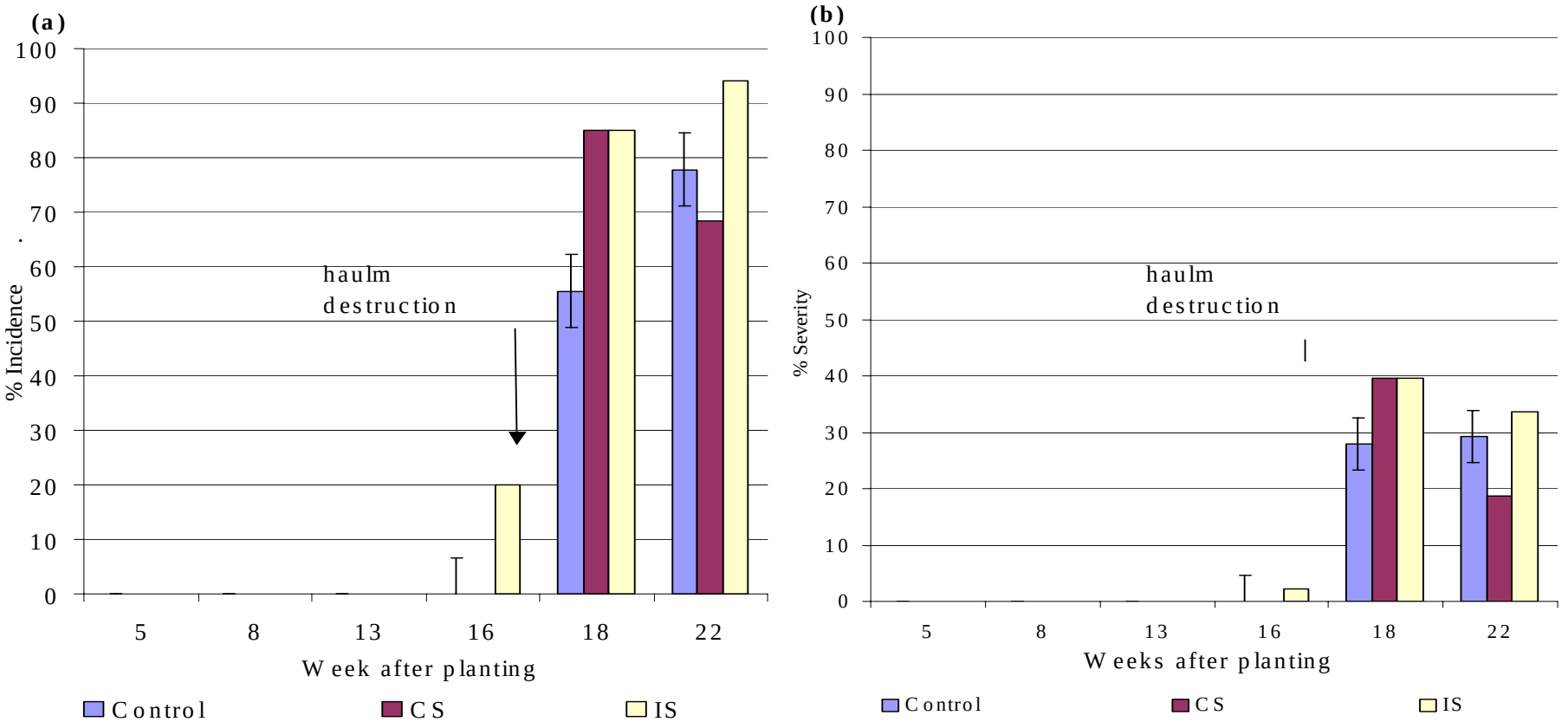
†Healthy seed was planted into plots where soil was amended with *C. coccodes* (CS) and compared with plots where seed was infected with *C. coccodes* (IS) (Table 3.2). Disease symptoms were assessed as % incidence and severity of necrosis and microsclerotia on 5 samples taken at random. Vertical bars (Error bars) are LSD values with 95 % confidence intervals.

FIGURE 9: INCIDENCE (A) AND SEVERITY (B) OF BLACK DOT ON STEMS OF CV. MARIS PIPER DURING THE GROWING SEASON†.



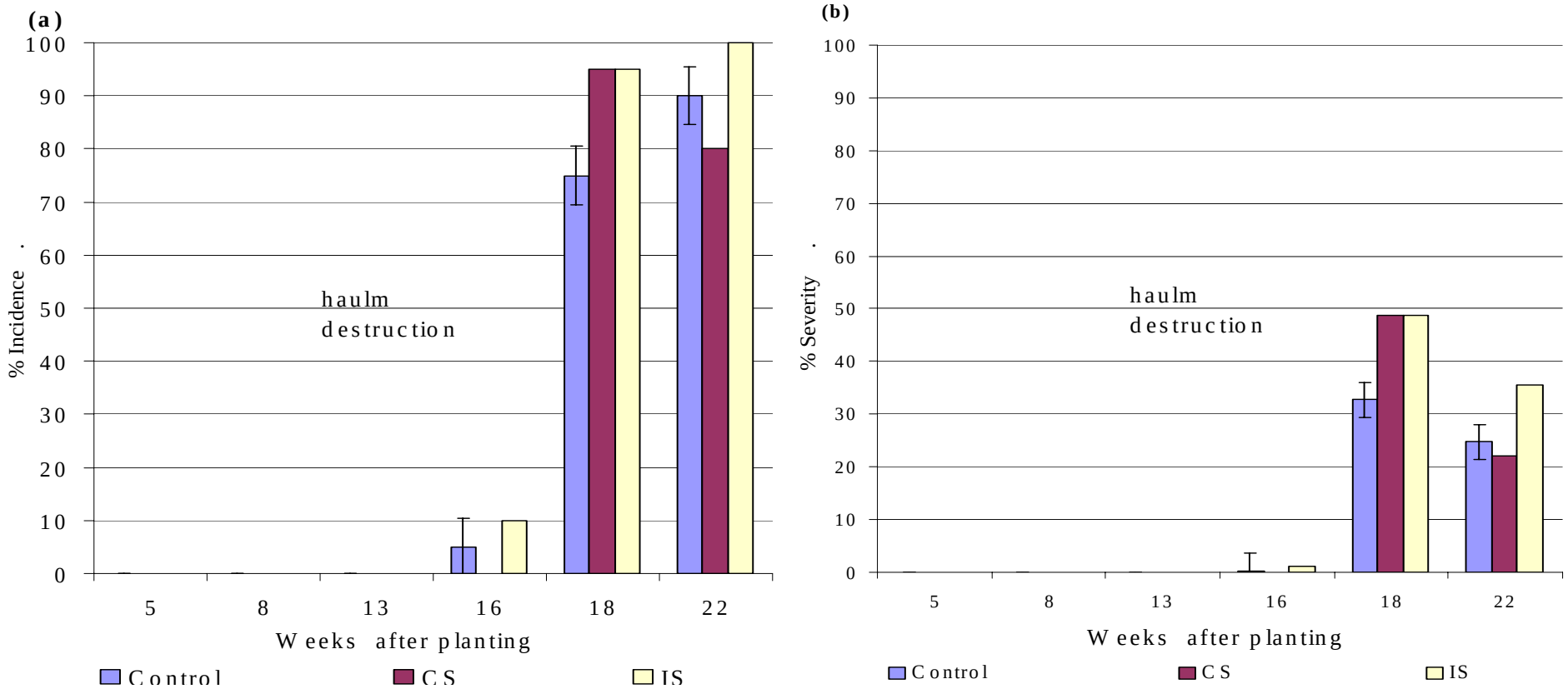
†Healthy seed was planted into plots where soil was amended with *C. coccodes* (CS) and compared with plots where seed was infected with *C. coccodes* (IS) (Table 3.2). Disease symptoms were assessed as % incidence and severity of necrosis and microsclerotia on 5 plants sampled at random. Vertical bars (Error bars) are LSD values with 95 % confidence intervals.

FIGURE 10: INCIDENCE (A) AND SEVERITY (B) OF BLACK DOT ON STOLONS OF CV. ESTIMA DURING THE GROWING SEASON†.



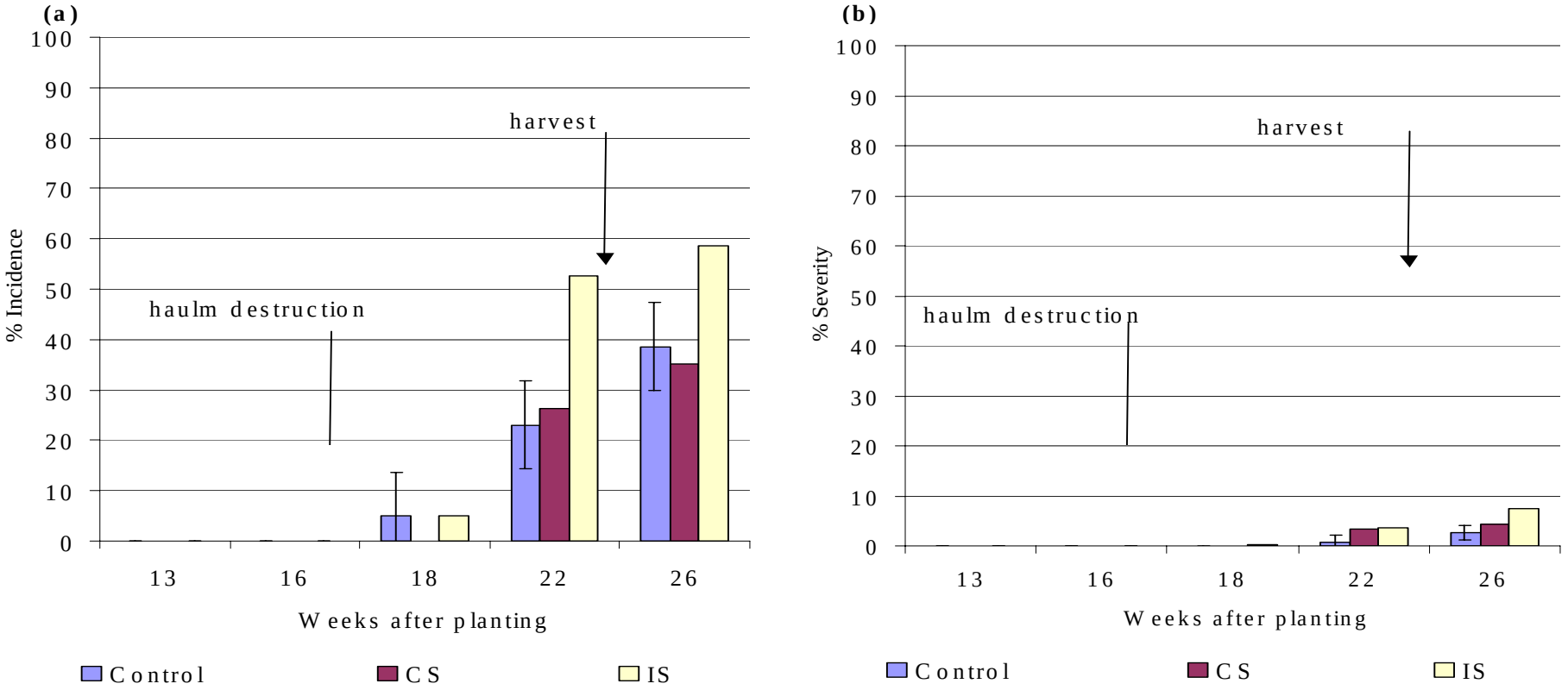
†Healthy seed was planted into plots where soil was amended with *C. coccodes* (CS) and compared with plots where seed was infected with *C. coccodes* (IS) (Table 3.2). Disease symptoms were assessed as % incidence and severity of necrosis and microsclerotia on 5 plants sampled at random. Vertical bars (Error bars) are LSD values with 95 % confidence intervals.

FIGURE 11: INCIDENCE (A) AND SEVERITY (B) OF BLACK DOT ON STOLONS OF CV. MARIS PIPER DURING THE GROWING SEASON†.



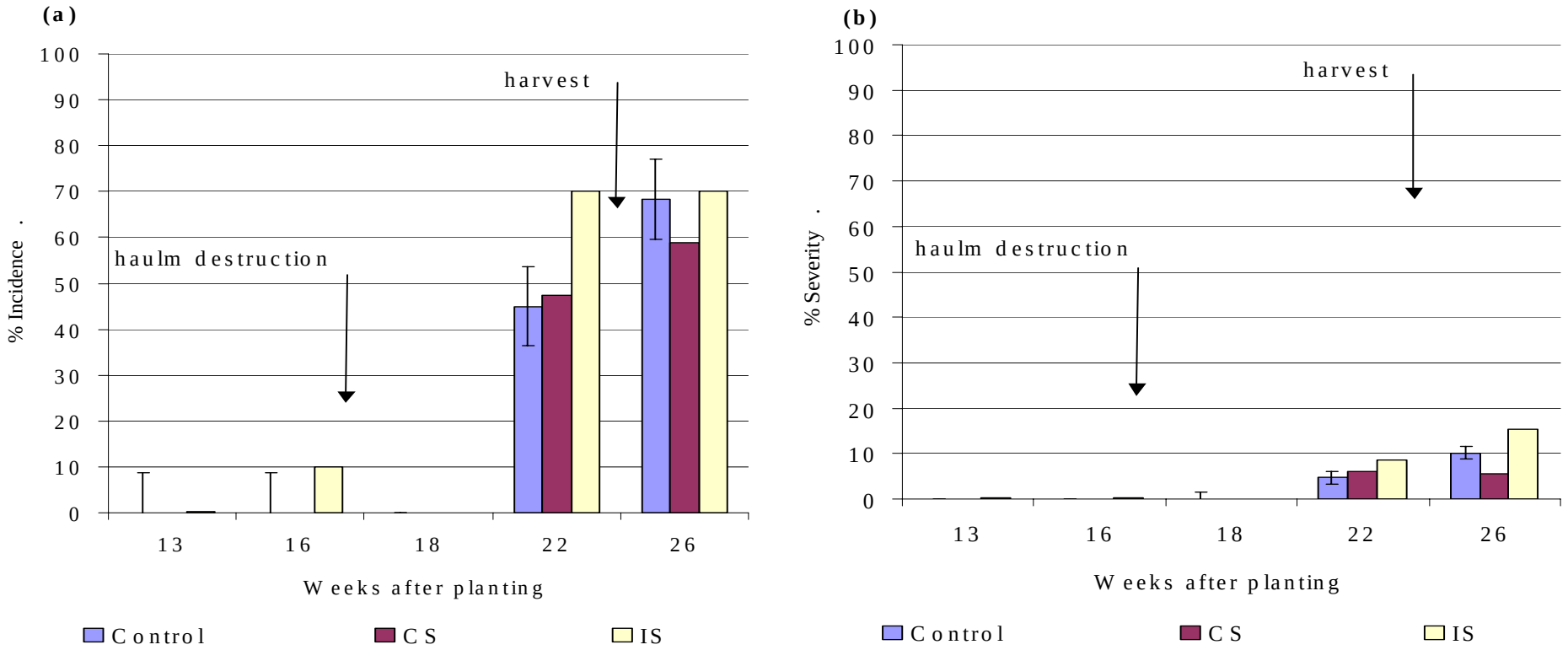
†Healthy seed was planted into plots where soil was amended with *C. coccodes* (CS) and compared with plots where seed was infected with *C. coccodes* (IS) (Table 3.2) was planted. Disease symptoms were assessed as % incidence and severity of necrosis and microsclerotia on 5 plants sampled at random. Vertical bars (Error bars) are LSD values with 95 % confidence intervals

FIGURE 12: INCIDENCE (A) AND SEVERITY (B) OF BLACK DOT ON TUBERS OF CV. ESTIMA DURING THE GROWING SEASON†.



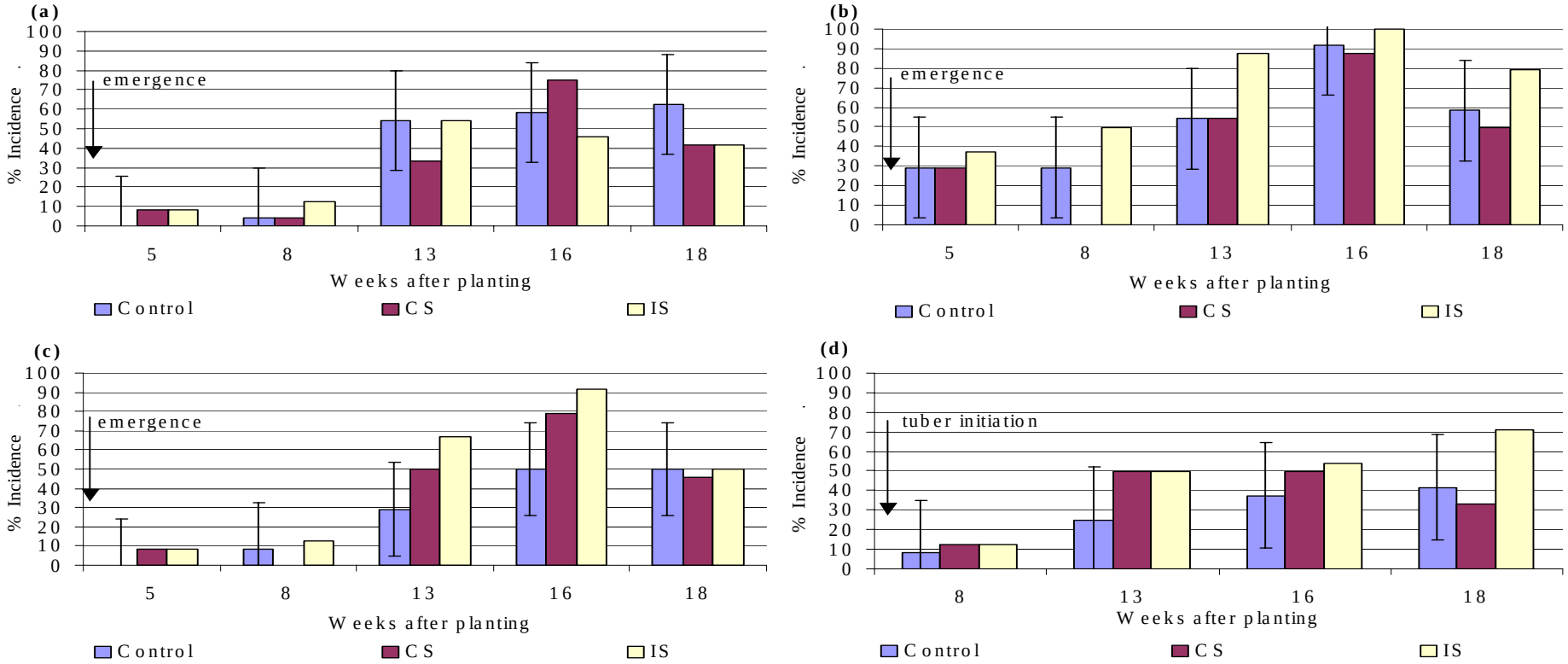
† Healthy seed was planted into plots where soil was amended with *C. coccodes* (CS) and compared with plots where seed was infected with *C. coccodes* (IS) (Table 3.2). Disease symptoms were assessed as % incidence and severity of necrosis and microsclerotia on 5 plants sampled at random. Vertical bars (Error bars) are LSD values with 95 % confidence interval

FIGURE 13: INCIDENCE (A) AND SEVERITY (B) OF BLACK DOT ON TUBERS OF CV. MARIS PIPER DURING THE GROWING SEASON†.



†Healthy seed was planted into plots where soil was amended with *C. coccodes* (CS) compared with plots where seed was infected with *C. coccodes* (IS) (Table 3.2). Disease symptoms were assessed as % incidence and severity of necrosis and microsclerotia on 5 plants sampled at random. Vertical bars (Error bars) are LSD values with 95 % confidence intervals.

FIGURE 14: INCIDENCE OF *C. COCCODES* ON ROOTS (**A**), STEMS (**B**), STOLONS (**C**), AND TUBERS (**D**) OF CV. MARIS PIPER, PLANTED BY HAND ON 17TH MAY 2002 AND MEASURED OVER A 21 WEEK PERIOD AT OLDMELDRUM FARM, ABERDEENSHIRE†.



†Healthy seed was planted into plots amended with *C. coccodes* (CS) and seed infected with *C. coccodes* (IS), compared with an uninfected control (Table 3.2). Infection was determined as the incidence of *C. coccodes* on stolon sections plated onto selective media (Farley's 1976). Vertical bars (Error bars) are LSD values with 95 % confidence intervals.

FIGURE 15: CONTAMINATION OF FIELD PLOTS OF CV. ESTIMA WITH *C. COCCODES* AFTER PLANTING. COLONY FORMING UNITS WERE MEASURED USING A METHOD ADAPTED BY DENNER (1997) AND ARE REPRESENTED AS NUMBER PER G OF SOIL.

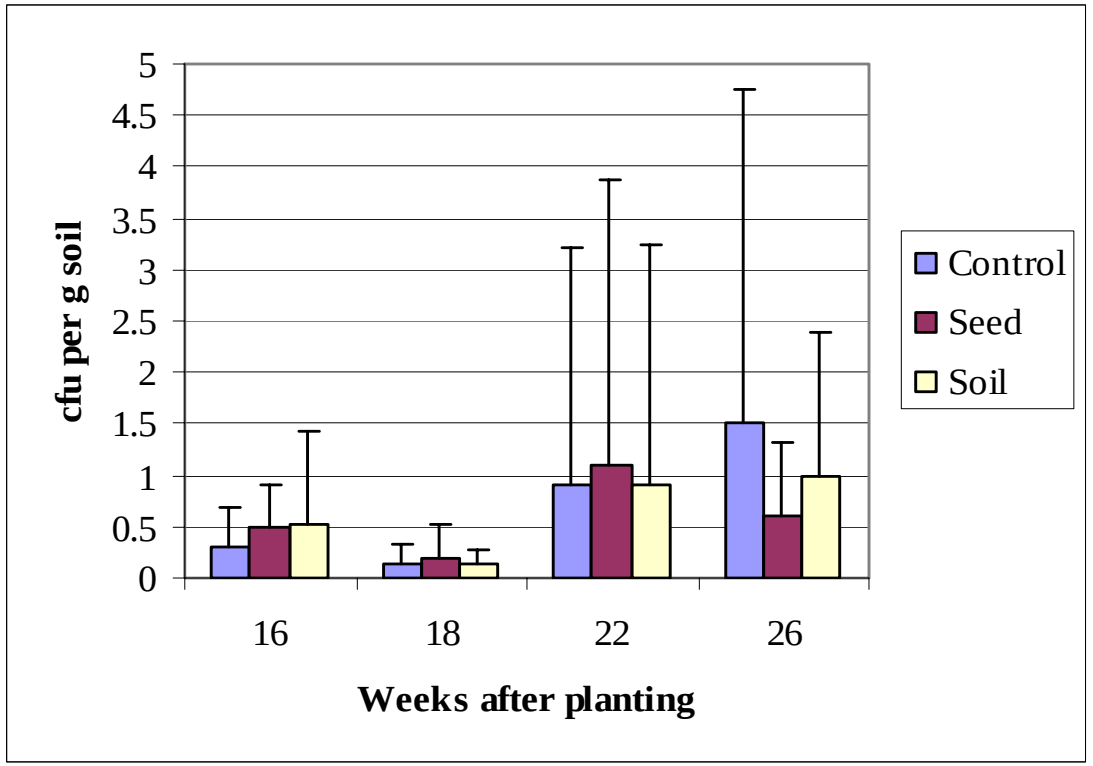
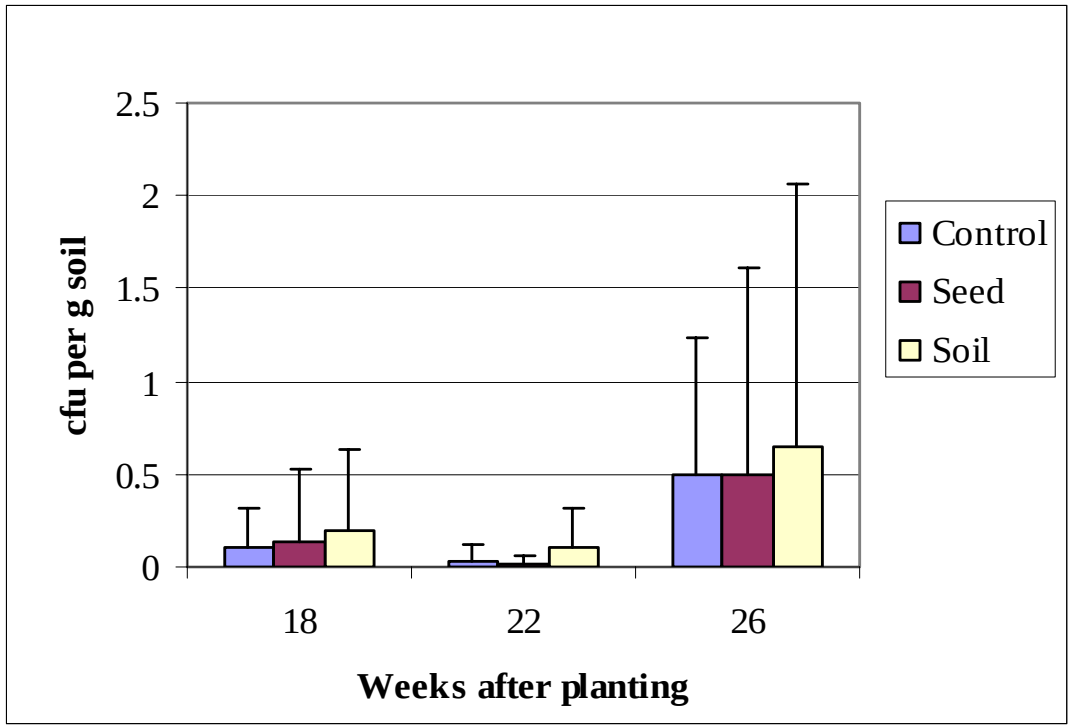


FIGURE 16: CONTAMINATION OF FIELD PLOTS OF CV. MARIS PIPER WITH *C. COCCODES* AFTER PLANTING. COLONY FORMING UNITS WERE MEASURED USING A METHOD ADAPTED BY DENNER (1997) AND ARE REPRESENTED AS NUMBER PER G OF SOIL.



Effect of haulm destruction on development of black dot

Aims

To assess if the method and date of haulm destruction would influence the extent of black dot on tubers

Materials and Methods

Site details

A commercial ware crop of Maris Piper with high-perceived risk of black dot was identified in East Lothian, Scotland. In this crop 28 plots consisting of 2 drills, 1.8m in length with seed planted 35cm apart were marked out. Seven treatments were laid out in a randomised block design with 4 replicate plots. Treatments included;
1) 4 l/ ha of Reglone in 500 l/ ha water applied on 6th September, 2001
2) 4 l/ ha of Reglone in 500 l/ ha water applied on 20th September, 2001
3) Sulphuric Acid at 330 l/ ha applied on 10th September, 2001
4) Whole haulm removed by hand leaving tubers intact on 7th September, 2001
5) Stolons severed using a spade pushed down the side of plant on 7th September,2001
6) Haulm cut 5 cm above ground level with shears on 7th September, 2001
7) Control - plants left to senesce naturally.

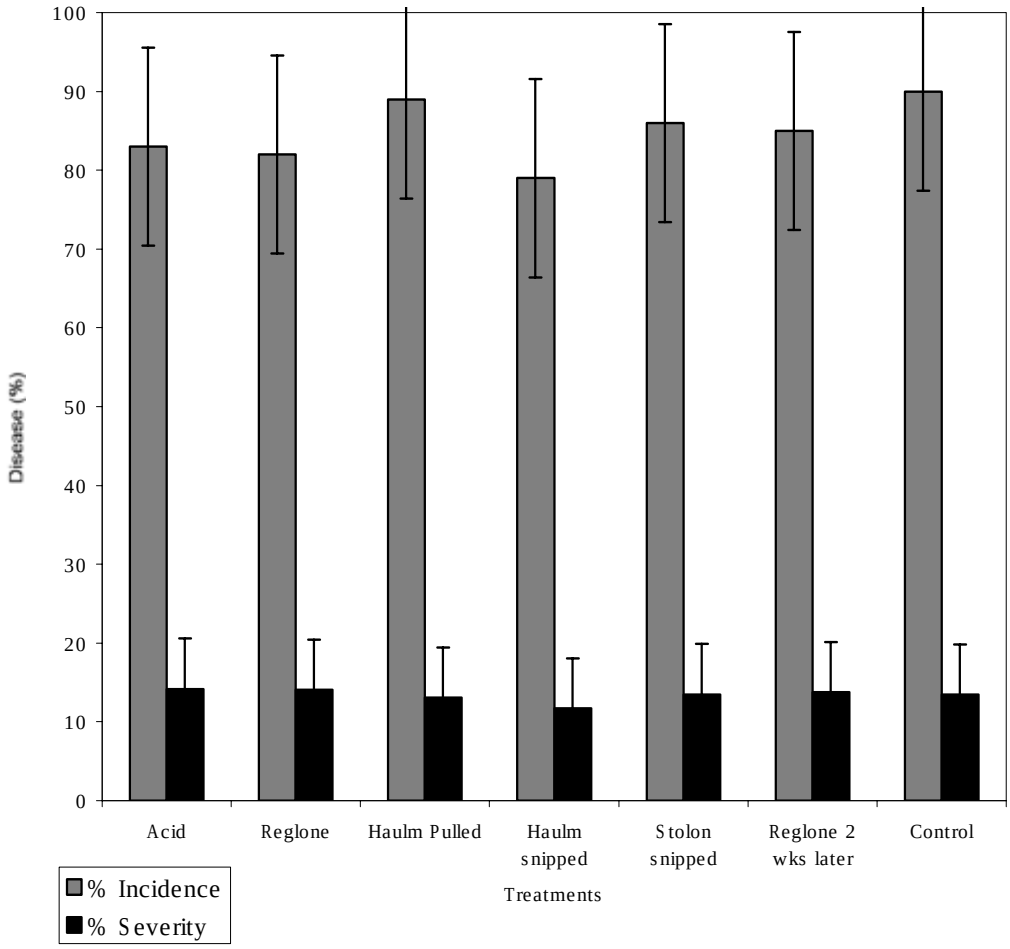
Plant Sampling

On the 7th October 2001, 25 ware sized tubers were harvested at random from each plot by hand. Tubers were placed inside a 25kg paper sack and transferred to SAC Aberdeen where they were washed and the incidence and percentage surface area of black dot was assessed (Section 5.2.3).

Results and discussion

A high incidence of black dot was observed in all treatments (+85%)(Figure 17). The control showed the highest incidence of black dot (90%) and the haulm cutting treatment the least (79%) with no method causing a significant reduction in incidence or severity compared to the control. In this experiment it can be concluded that haulm destruction methods cannot be used to significantly reduce severity or incidence of black dot on tubers.

FIGURE 17: EFFECT OF HAULM DESTRUCTION METHOD ON INCIDENCE AND SEVERITY OF BLACK DOT ON TUBERS.



Effect of storage conditions on black dot development

Aims

To identify conditions which favour black dot development in storage.

Material and Methods

Effects of condensation and temperature

In December 2001 a stock of cv. Maris Piper (SE2 35-45mm) infected with *C. coccodes* was divided into those tubers which showed no symptoms of black dot (symptomless) and those with 1-10% (average 3%) surface area covered in silvering and sclerotia of black dot (infected). Within the symptomless and infected groups tubers were split into three sub-groups of 64 and placed into plastic bags containing damp tissue paper to maintain humidity. To imitate a condensation event, tubers were removed from the bag and sprayed with de-ionised water at a rate of $3cm^3$ per sub-group every 3 hours using an atomiser. Tubers were then removed from the bags after 0, 9, 18 and 27 hours and were immediately air dried under a cool fan divided into replicates of 4 tubers and placed in a paper bag. Replicates were incubated at temperatures of 5, 10 and 15^OC. For each treatment there were 4 replicates.

Tubers were assessed for disease every 2 weeks by assessing the % incidence and surface with black dot symptoms (Section 5.2.3).

Effects of condensation during curing

In October 2003 freshly harvested tubers of cv. Estima (SE2 35-50mm) were obtained and visually assessed for black dot (Section 5.2.3) Twenty-five tubers were placed in each of 35 wooden boxes and held in an incubator at 15°C for 1 week. On day 1 the boxes were split into 3 treatment groups where tubers were exposed to 4 or 8 hour condensation events and control tubers which were not exposed to condensation. To simulate condensation of potato periderm, boxes were periodically sprayed with sterile de-ionised water at a rate of $3cm^3$ per box every 2 hours. All boxes were cured in a controlled environment chamber (temperatures reduced from 15 to 3°C over 21 days). One month after the condensation event tubers were assessed for disease (Section 5.2.3).

Results and discussion

At harvest symptoms of black dot may not be seen. However, once in store it is possible for symptoms to develop over time. In this study apparently uninfected (symptomless) tubers developed black dot in storage. In Chapter 7 it was observed that although tubers may be infected with *C. coccodes* they do not necessarily show symptoms of black dot. It is speculated that it is possible for stocks infected with *C. coccodes* to be placed in storage and that these symptoms can develop thereafter. It is suggested, that the majority of tubers are infected with *C. coccodes* prior to harvest and depending on conditions symptom development may develop in storage. Attempts

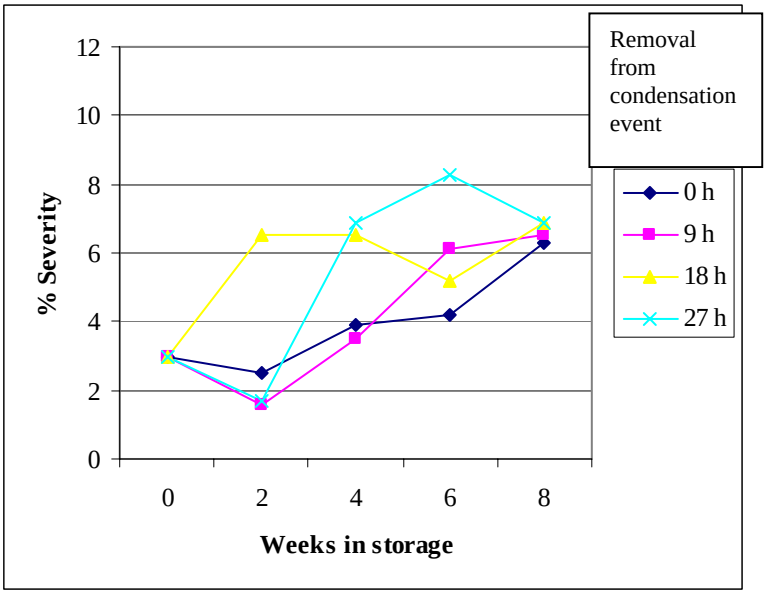
to infect healthy tubers during storage by placing them next to tubers with black dot failed to cause symptom development (data not presented).

On diseased tubers symptoms of black dot also became more severe (Figure 18) with time. In the absence of a condensation event and at 10^{o}C the severity of black dot increased from 3% at the beginning of the experiment to 10% after 6 weeks (Figure 18b).

Storage conditions only had limited effect on black dot development. Duration of condensation had no significant effect on black dot development at 5 and 15^{o}C (Figure 18). At 10C, after 6 weeks, significantly more symptoms of black dot were observed where tubers had not been exposed to condensation (9.8 %) with those tubers that had been subjected to condensation events lasting 18 (3.9 %) and 27 hours (2.4 %) (Figure 18b). This may suggest that where tubers are not maintained at a high humidity or where condensation events are not occurring then the periderm dries more rapidly out encouraging symptoms of black dot to develop. Temperature had no significant on disease development in storage. Condensation events during curing had no significant effect on black dot development (Figure 19).

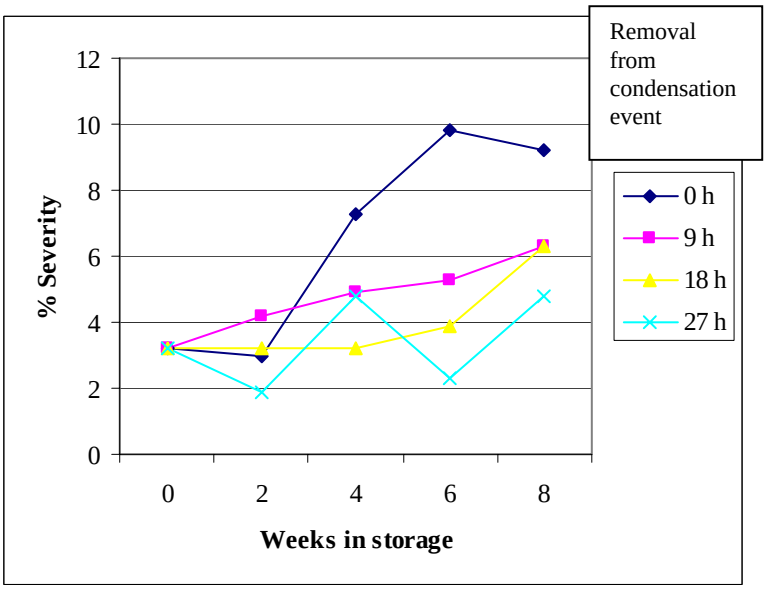
FIGURE 25: EFFECT OF STORAGE TEMPERATURE AND DURATION OF A CONDENSATION EVENT ON DEVELOPMENT OF BLACK DOT IN STORAGE. TUBERS FROM A SINGLE STOCK OF CV. MARIS PIPER WITH BLACK DOT SYMPTOMS (3%) WERE SELECTED AND EXPOSED TO A CONDENSATION EVENTS LASTING 9, 18 AND 27 HOURS. TUBERS WERE STORED AT 5 (A), 10 (B) AND 15C (C) FOR 8 WEEKS. DISEASE SEVERITY IS MEASURED AS THE SURFACE AREA COVERED IN BLACK DOT SYMPTOMS.

A) 5°C



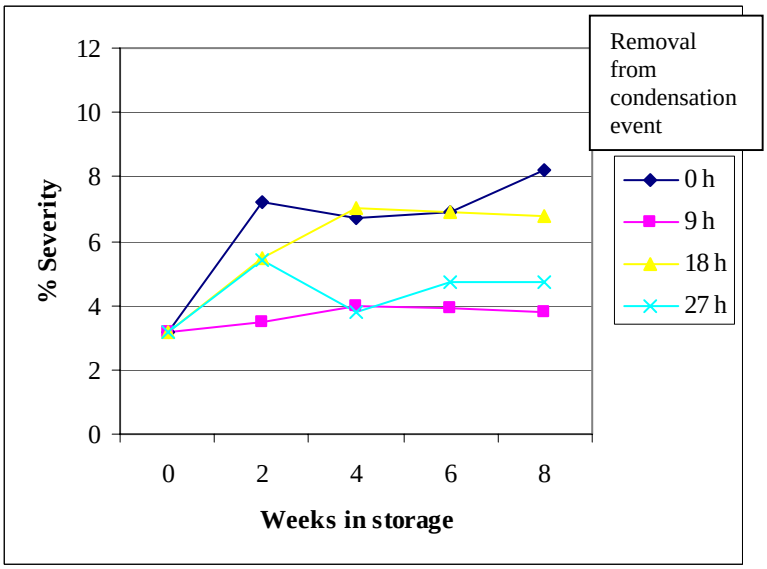
L.S.D – 5.6

b) 10°C



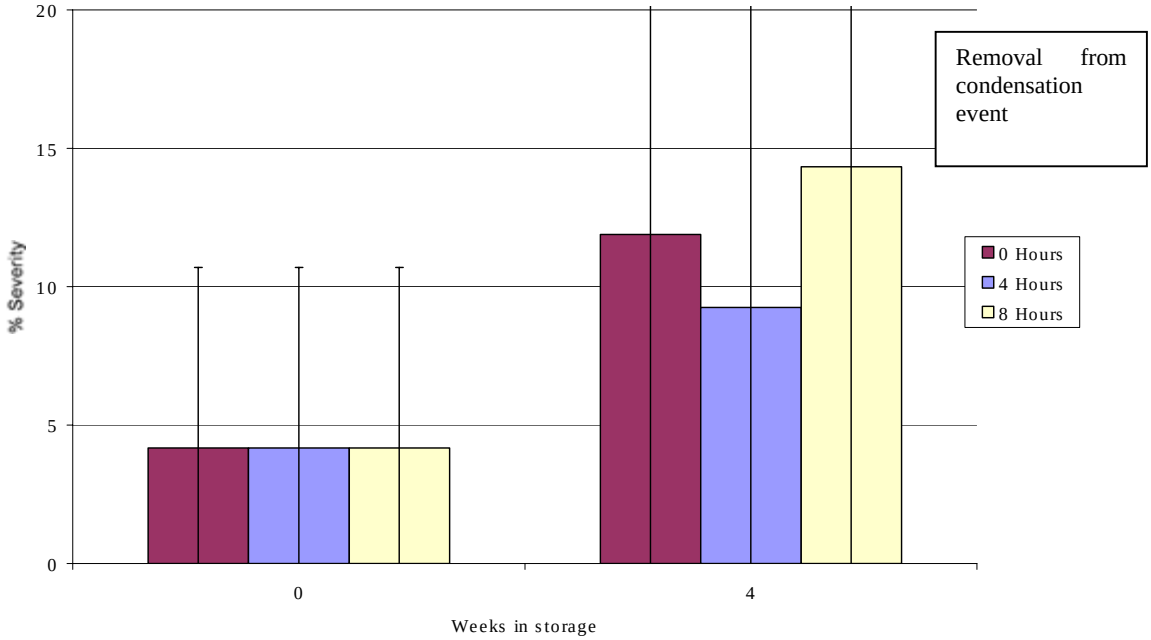
L.S.D – 5.7

c) 15°C



L.S.D – 5.2

FIGURE 19: EFFECT OF DURATION OF A CONDENSATION EVENT ON DEVELOPMENT OF BLACK DOT DURING CURING. TUBERS FROM A SINGLE STOCK OF CV. ESTIMA WITH BLACK DOT SYMPTOMS (4 %) WERE SELECTED AND EXPOSED TO CONDENSATION EVENTS LASTING 0, 4 AND 8 HOURS. DISEASE SEVERITY IS MEASURED AS THE SURFACE AREA COVERED IN BLACK DOT SYMPTOMS.



Key Findings

1. Infection of stems, stolons, roots and tubers by *C. coccodes* occurs as these organs develop.
2. Symptoms of black dot do not develop on stems, stolons or tubers until the crop canopy has started to senesce
3. Symptoms of black dot become progressively more severe on tubers the later they are harvested
4. Level of seed or soil-borne inoculum had only limited impact on disease on stems, roots and daughter tubers
5. It is possible to predict the risk of black dot occurring on tubers of cv. Estima by assessing stems and stolons of this cultivar as the crop starts to senesce
6. Assessing stems and stolons for black dot in the cvs Maris Piper, Saxon and King Edward can not be used to predict the development of black dot on tubers. It is suggested that symptoms of black dot on stems and stolons may develop at a later stage in these cultivars
7. Haulm destruction methods did not influence black dot development on tubers.
8. Symptoms of black dot can increase in storage. At harvest tubers maybe infected with *C. coccodes* without showing symptoms of black dot. Once in store symptoms may develop on these tubers. Where black dot symptoms already exist they may become increasingly severe during storage.
9. Storage conditions had little effect on symptom development

Implications for grower

Control measures to this disease can be separated into two distinctive phases, a) preventing initial infection and b) reducing symptom development

Preventing infection by *C. coccodes*

1. Identify if a field site is contaminated with *C. coccodes* prior to planting by knowing the history of the site. In the future diagnostic tools may be available for detecting what is present in soil.
2. Once established in soil, *C. coccodes* is relatively persistent and soil-borne inoculum is more important than seed infection.
3. If planting at a 'uncontaminated' site consider using 'healthy' seed

Reducing development of black dot symptoms

1. Harvest tubers as early as practically possible if black dot symptoms appear on stems or stolons at early senescence
2. Haulm destruction method will not reduce the development of black dot on tubers
3. Regularly inspect stocks for development of black dot in storage to ensure that they are sold before the quality deteriorates and their value declines. Incubate a sample at 10C to determine risk
4. Storage conditions have little effect on development of black dot

Relevant output

Refereed Papers

Lees AK, Hilton AJ, 2003. Black dot (*Colletotrichum coccodes*): an increasingly important disease of potato. *Plant Pathology* **52,** 3-12

Conference Proceedings

Danaher JE, McDonald K, Clayton R, Blackwood J and Bingham I, 2000. Prediction and manipulation of black dot (*Colletotrichum coccodes*) in potato crops. Proceeding of Brighton Crop Protection Conference 1-3 519-522

Danaher JE, Hilton AJ, Clayton RC, 2002. The prediction on black dot on harvested potato tubers. Proceedings 15th Triennial Conference of the European Association of Potato Research, Hamburg, 2002, p. 147

Other relevant output

Hilton AJ, 2002 Prediction of black dot in harvested tubers. Potatoes in Practice, SCRI, Dundee, August 2002

Hilton AJ. 2003. Update on Black dot. *Potato newsletter* March 2003 p.29-30

Hilton AJ and Peters J, 2003. Predicting and manipulating black dot in growing and stored crops. BPC fact sheet

Hilton AJ, 2004. Control of soil-borne diseases. Talk at 7th Potato Colloquium, 20th April 2004, The De Berre Belfry, Tamworth, UK

References

Andrivon D, Lucas JM, Guérin C, Jouan B, 1998. Colonization of roots, stolons, tubers and stems of various potato (*Solanum tuberosum*) cultivars by the black-dot fungus *Colletotrichum coccodes*. *Plant Pathology* **47**, 440-45.

Carnegie SF, Choiseul JW, Roberts AMI, 2003. Detection of *Colletotrichum coccodes* and *Helminthosporium solani* in soils by bioassay. *Plant Pathology* **92,** 287-303

Denner FDN, 1997. Black dot and silver scurf of potatoes in South Africa. *PhD Thesis*. 115p. University of Pretoria, South Africa.

Dijst G, Bouman A, Mulder A, Roosjen J, 1986. Effect of haulm destruction supplemented by cutting off roots on the incidence of black scurf and skin damage, flexibility of harvest period and yield of seed potatoes in field experiments. *Netherlands Journal of Plant Pathology* **92**, 287-303.

Farley JD, 1972. A selective medium for assay of *Colletotrichum coccodes* in soil. *Phytopathology* **62**, 1288-93.

Hide GA, Boorer KJ, Hall SM, 1994. Effects of watering potato plants before harvest and of curing conditions on development of tuber diseases during storage. *Potato Research* **37**, 169-72.

Hide GA, Boorer KJ, Hall SM, 1994. Effects of watering potato plants before harvest and of curing conditions on development of tuber diseases during storage. *Potato Research* **37**, 169-72.

Hilton AJ, Linton S, Lees AK, 2000. Resistance to potato blemish diseases. Report for the Research and Development Committee of the British Potato Council.

Jefferies RA, Lawson HM, 1991, A key for the stages of development of potato (*Solanum tuberosum*). *Annals of Applied Biology* **119,** 387-399.

Jellis GJ, Taylor GS, 1974. The relative importance of silver scurf and black dot: two disfiguring diseases of potato tubers. ADAS *Quarterly Review* **14,** 1075-8

Lees AK, Hilton AJ, 2003. Black dot (*Colletotrichum coccodes*): an increasingly important disease of potato. *Plant Pathology* **52,** 3-12

Read PJ, Hide GA, 1995. Development of black dot disease [*Colletotrichum coccodes*] (Wallr.) Hughes] and its effects on the growth and yield of potato plants. *Annals of Applied Biology* **127,** 57-72

Schmiedeknecht M, 1956. Unterersuchungen des Parasitismus von *Colletotrichum atramentarium* (B. et Br.) Taub. An Kartoffelstauden (*Solanum tuberosum* L.). *Phytopathologische Zeitschrift* **26**, 1-30