INTRODUCTION AND PERSISTENCE OF SEED AND SOIL-BORNE POTATO PATHOGENS WITHIN A ROTATION

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Summary: The Centre for Sustainable Cropping (CSC) is a long term experimental platform. It was established in 2009 to integrate all aspects of sustainability research on arable ecosystems at The James Hutton Institute and to study whole-system responses to sustainable crop management relative to current conventional crop husbandry practice. This study, utilizing the CSC platform, focuses on four seed and soil-borne potato pathogens, *Spongospora subterranea* (powdery scab), *Rhizoctonia solani* (black scurf), *Helminthosporium solani* (silver scurf) and *Colletotrichum coccodes* (black dot). Through quantifying pathogen levels in soil and on seed tubers we are investigating the impact of introducing inoculum into soil on subsequent disease levels on progeny tubers. The experimental platform also enables the effect of host resistance and treatments associated with the conventional and sustainable husbandry on both disease and soil contamination to be investigated. The persistence of soil-borne inoculum (once introduced through contaminated seed) is being monitored through the rotation.

INTRODUCTION

The Centre for Sustainable Cropping (CSC) is a long term experimental platform that was established in 2009 to integrate all aspects of sustainability research on arable ecosystems at The James Hutton Institute. The CSC provides a research facility to test and demonstrate the economic and environmental trade-offs, costs and benefits of sustainable arable land management to the whole arable ecosystem over many decades. This long-term, whole-systems approach is key if multiple benefits are to be identified and the potential conflicts between management for crop productivity and system resilience are to be resolved.

Sustainable management of arable systems for both agricultural production whilst minimising environmental impact must therefore achieve a balance between maximising crop production, conserving arable biodiversity and maintaining ecosystem functions. Thus, the general aims of the platform are:

- To design a sustainable cropping system that, over the course of a series of 6 year rotations, tests and demonstrates the optimisation of inputs (nutrients, water and agrochemicals), yield (quality and quantity), environmental services (water capture, greenhouse gas emissions, soil erosion, nutrient flows), biodiversity (soil microbes, plants and arthropods), and ecosystem processes (photosynthesis, carbon and nutrient transformations and fluxes, decomposition, community dynamics).

- To assess the effect of the sustainable system on long-term trends in yield and system health relative to standard conventional practice.
- To provide a broad framework for whole-systems research and a field-scale test-bed for new ‘sustainable’ crop varieties for enhanced nutrient and water use efficiency, weed suppression, and pest and disease resistance.
- To provide a demonstration site for knowledge transfer.

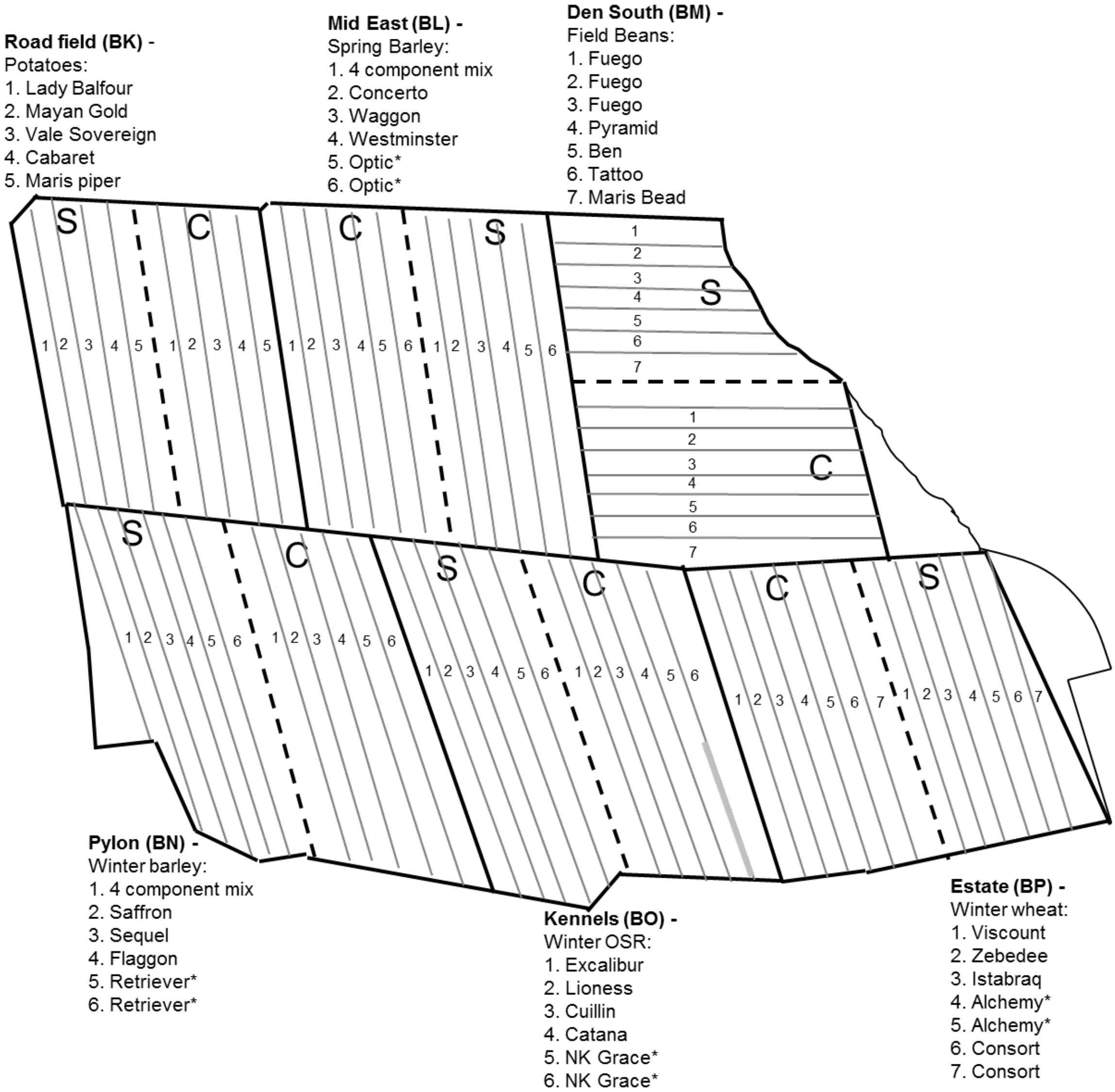

Figure 1. Field layout for 2011/2012 growing season showing the crop rotation, field splits (where C= conventional management and S = sustainable management) and cultivar strips for each of the six fields (BK to BP) of the CSC.

The platform is based on a six course rotation with six 5-6 Ha fields planted with potato, winter wheat, beans, spring barley, winter oilseed rape and winter barley each year (Fig. 1). One half of each field is managed according to current conventional crop husbandry practice and a sustainable management package is applied to the other half. Further details of the design, sampling and sustainable treatment can be found at www.hutton.ac.uk/csc.

By monitoring the levels of four potato pathogens in soil and on seed tubers, we are investigating the impact of inoculum sources and soil contamination on disease levels on progeny tubers. The CSC platform also enables the effect of host resistance and treatments associated with the conventional and sustainable husbandry to be investigated, although the latter will not be discussed here. The persistence of soil-borne inoculum (once introduced) is being monitored through the rotation. This study focuses on four soil-borne potato pathogens, *Spongospora subterranea* (powdery scab), *Rhizoctonia solani* (black scurf), *Helminthosporium solani* (silver scurf) and *Colletotrichum coccodes* (black dot).

This is a long-term project, and the results from the first three years (2011-2013) of monitoring are reported here. The data presented is from the conventional field plots only.

MATERIALS AND METHODS

Five potato cultivars were planted in each year, both in the conventional and the sustainable field sections: cv. Maris Piper, the top ranked cultivar based on planted area in GB, cv. Mayan Gold which is resistant to a number of pathogens including powdery scab, cv. Vales Sovereign which is known to be drought resistant and yields well in lower nitrogen conditions, cv. Lady Balfour, which is popular in the organic market, and cv. Cabaret which is suitable for both ware and processing.

Soil samples

Soil samples were taken in March (prior to planting of the potato crop) and in October/November (post-harvest of the potato crop) from all the fields within the rotation, except in 2011 when the post-harvest soil samples were not taken until the following March. In the non-potato fields within the rotation, soil samples are taken from each half field. In the fields which have been planted with potato, soil samples are taken from each cultivar strip in the post-harvest sampling. Soil samples taken from the half field consisted of a bulk of 100 x 10 g samples taken from across the field area in a W-shape. When the areas under different cultivars were sampled, 20 x 10 g cores, taken across the area in a W-shape, were bulked.

Soil DNA extractions were carried out according to the method of Brierley *et al.* (2009). Target pathogen DNA was quantified using real-time PCR; *S. subterranea* (van der Graaf *et al.* 2003), *C. coccodes* (Cullen et al, 2002), *H. solani* (Cullen et al., 2001) and *R. solani* (Lees et al., 2002). Soil inoculum is expressed as pg DNA / g soil except for *S. subterranea* where inoculum is expressed as sporeballs / g soil. Amounts of *C. coccodes* DNA: <100, 100-1000, >1000 pg DNA/g soil and *S. subterranea* DNA: 0, <10 and >10 spore balls/ g soil are referred to as low, medium and high as determined in Lees et al (2010) and Brierley et al (2013) for *C. coccodes* and *S. subterrranea* respectively.

Potato tuber samples

A visual disease assessment was made on 100 tubers of each seed stock prior to planting in each year. Per cultivar 24 tubers were assessed individually using real-time PCR. The peel from each tuber was weighed and placed into a grinding bag with 15 mL CTAB-PO_4 Buffer and ground using a Homex 6 grinder (BIOREBA AG.). A 1.5 ml aliquot of the supernatant was removed and processed according to the method of Cullen *et al*. (2001). Target pathogen DNA was quantified using real-time PCR assays described above. The amount of pathogen DNA detected was expressed as ng DNA / g tuber peel. Within a week of the potato harvest, a visual disease assessment was made on 100 tubers of each cultivar from each field treatment and is reported as disease incidence (% of tubers with symptoms) and mean severity (percentage area of tuber covered in symptoms).

RESULTS

Road Field

At the onset of monitoring soil-borne potato pathogens in March 2011, the field in which potatoes were to be planted (Road Field), had medium levels of *S. subterranea* and *C. coccodes* but no detectable *R. solani* or *H. solani* inoculum (Table 1).

Visual assessment of the potato seed stocks planted in 2011 revealed that all five had some silver scurf (44-99 % incidence). Maris Piper and Cabaret had high incidences of black dot (>81%). All cultivars except Mayan Gold (which was free from powdery scab symptoms) had a relatively low incidence of powdery scab (<17%). No powdery scab, black dot or silver scurf were recorded on the progeny tubers of any cultivar, and the amount of detectable soil inoculum of *S. subterranea* and *C. coccodes* did not increase and remained undetectable for *H. solani* post-harvest (Table 1). A more detailed account of *R. solani* inoculum and black scurf on potato grown in Road Field is presented in Figure 2.

Cultivars Cabaret, Vales Sovereign and Lady Balfour seed stocks had some black scurf (28, 7 and 1 % incidence respectively), but stocks of all cultivars including Maris Piper and Mayan Gold had some measurable amount of *R. solani* contamination as determined by real-time PCR (Figure 2). Black scurf was found on cvs Cabaret, Lady Balfour, Vales Sovereign and Maris Piper progeny tubers. *R. solani*, which was undetectable in the field soil pre-planting, was now found in the cultivar strips where disease had developed when sampled the following year, March 2012 (Figure 2). Only cv. Mayan Gold had no black scurf on progeny tubers and no detectable soil inoculum post- harvest. Cv. Mayan Gold is highly resistant to black scurf, although no official resistance rating is available. We are not yet able to determine whether the black scurf on progeny tubers developed from *R. solani* in the soil that was present but undetected, or from seed infections, which in the case of cv. Maris Piper were symptomless. When the soil from this field was sampled again 8 months year later (November 2012) no *R. solani* inoculum was detected (Figure 2).

Table 1. Inoculum levels of four soil-borne pathogens of potato quantified after sampling soil on a number of occasions between March 2011 and November 2013 in three of the fields within the rotation.

Field	Crop	Sampling time	Inoculum level ([a] spore balls/ g soil and [b] pg DNA / g soil/)			
			S. subterranea [a]	*C. coccodes* [b]	*H. solani* [b]	*R.solani* AG3 [b]
Road Field	2011 Potatoes	March 2011	4.3	138.6	0.0	0.0
	2012 Winter wheat	March 2012	0.1	123.3	0.0	226.3
		November 2012	0.1	204.8	0.0	0.0
	2013 Winter OSR	March 2013	0.0	0.0	0.0	0.0
		November 2013	0.7	8.8	0.0	0.0
Mid East	2011 Spring barley	March 2011	0.8	0.0	0.0	0.0
	2012 Potaotes	March 2012	0.1	40.5	0.0	0.0
		November 2012	1.8	0.0	0.0	0.0
	2013 Winter wheat	March 2013	1.2	0.0	0.0	0.0
		November 2013	1.0	0.0	0.0	0.0
Den South	2011 Field beans	March 2011	0.0	151.3	0.0	0.0
	2012 Spring Barley	March 2012	0.0	0.0	0.0	0.0
		November 2012	0.0	0.0	0.0	0.0
	2013 Potatoes	March 2013	1.9	10.4	0.0	131.4
		November 2013	0.3	1.1	0.0	0.0

2011 Soil Pre-planting	Cultivar	Seed (% inc: PCR)	Disease (% inc./ sev.)	2012 Soil March	2012 Soil November	2013 Soil March	2013 Soil November
0	L. Balfour	1% : 0.2	7 / 3	226	0	0	0
	M. Gold	0% : 2.3	0 / 0	0			
	V.Sovereign	7% : 1.2	2 / 1	897			
	Cabaret	28% : 0.2	46 / 2	685			
	M. Piper	0% : 4.2	12 / 5	242			

Figure 2. Monitoring of *R. solani* inoculum in soil (pg DNA/ g soil) pre-planting and seed inoculum levels (black scurf incidence (%) and *R. solani* contamination (ng DNA / g peel)) for seed stocks of five cultivars planted in Road Field in 2011. Disease (% incidence and severity of black scurf) on progeny stocks, and soil inoculum in the cultivar strips following harvest of the potato crop are shown (March 2012), following which detectable *R. solani* soil inoculum from the whole field at subsequent sampling occasions is shown.

Mid East

In 2012 Mid East field was planted with potato. Prior to planting, low levels of *S. subterranea* and *C.coccodes* but no *R. solani* or *H. solani* inoculum were detected (Table 1). Seed stocks of cvs Maris Piper and Mayan gold had a high incidence of black dot (92 and 69 % respectively), and cvs Lady Balfour, Vales Sovereign, Cabaret and Maris Piper had relatively low incidences of powdery scab (< 12%). Cultivar Lady Balfour was the only one with any black scurf (4% incidence). The progeny tubers of all five cultivars were free of black dot, black scurf and powdery scab. More details of *H. solani* inoculum and silver scurf on potato grown in Mid East field are presented in Figure 3.

All seed stocks planted in 2012 had silver scurf symptoms and the DNA on the seed stocks ranged from 3 to 498 ng DNA / g peel, all cultivars had silver scurf on the progeny (Figure 3). Whilst cv. Cabaret had the lowest seed contamination level and the least disease on progeny tubers and cv. Lady Balfour the highest amount of seed contamination and the most disease on progeny tubers, there was little difference between the other three cultivars (Figure 3). Despite the relatively high amounts of contamination on seed stocks and resulting progeny, there was no inoculum detected in the soil post-harvest.

Den South

In 2013, Den South (the third field in the rotation) was planted with potatoes. In soil sampled immediately prior to the planting, *S. subterranea*, *C. coccodes* and *R. solani* were detected but not *H. solani* (Table 1). Silver scurf was common (>55% incidence) on all seed stocks planted in 2013, and cvs Lady Balfour and Vales Sovereign had relatively low incidences of black scurf (<13%). Cultivar Cabaret was the only seed stock to have any black dot, and the incidence was low (3%). Details of detectable *S. subterranea* and powdery scab on seed and progeny are given in Figure 4. All five seed stocks planted in 2013 had detectable *S. subterranea* inoculum (real-time PCR), and all but cv. Maris Piper had powdery scab symptoms, with cv. Cabaret having the greatest incidence (95%). Despite the levels of disease on the seed, none of the progeny stocks had powdery scab, most however had severe common scab (data not shown).

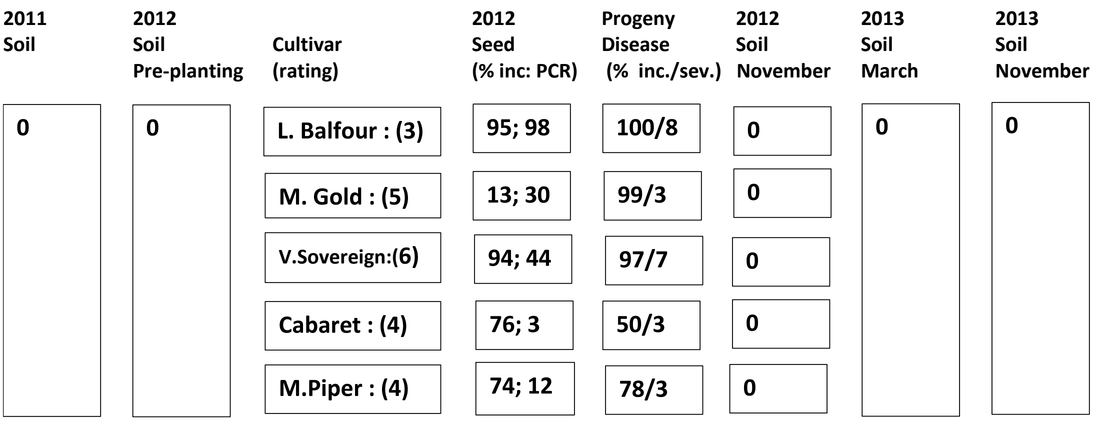

Figure 3. Monitoring of *H. solani* inoculum in soil (pg DNA/ g soil) pre-planting and seed inoculum levels (silver scurf incidence (%) and *H. solani* contamination (ng DNA / g peel)) for seed stocks of five cultivars planted in Mid-East Field in 2012. Disease (% incidence and severity of silver scurf) on progeny tubers, and soil inoculum in the cultivar strips following harvest of the potato crop are shown (November 2012), following which detectable *H. solani* soil inoculum from the whole field is shown for subsequent sampling occasions.

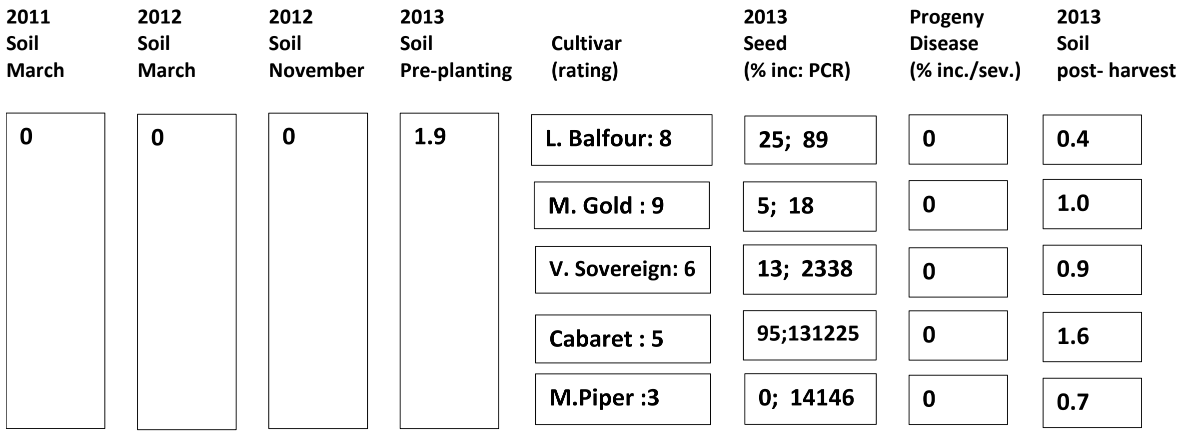

Figure 4. Monitoring of *S. subterranea* inoculum in soil (sporeballs / g soil) pre-planting and seed inoculum levels (powdery scab incidence (%) and *S. subterranea* contamination (sporeballs / g peel)) for seed stocks of five cultivars planted in Den-South Field in 2013. Disease (% incidence powdery scab) on progeny stocks and soil inoculum in the cultivar strips following harvest of the potato crop (November 2013) are shown.

DISCUSSION

With time we plan to establish a data set that will give us a greater understanding of what happens in the soil when diseased seed is planted, which factors prolong the persistence of a pathogen in the soil and thus the long-term impact of contaminating soil on future potato crops. We are already seeing interesting trends in the results, for example, all seed stocks planted in each year (2011-2013) had silver scurf. DNA on the seed stocks ranged from 1 to 240 ng DNA/g peel in 2011, 3 to 498 ng DNA / g peel in 2012 and just 1 to 20 ng DNA / g peel in 2013. The absence of detectable *H. solani* in the soil indicates that seed inoculum was the source of disease where it occurred. However there were marked seasonal variations in disease severity, no disease symptoms were found on progeny tubers in 2011. In 2012, all cultivars had silver scurf on the progeny and finally in 2013, three of the five cultivars had silver scurf on the progeny.

The seed sourced for planting in 2013 had powdery scab symptoms (particularly on cv. Cabaret), in addition, the soil into which potato was planted had detectable inoculum. However, despite both seed and soil-borne inoculum being present, the warm dry weather of 2013 reduced the risk of powdery scab, but conversely, all progeny stocks in 2013 had common scab, it being particularly severe in the susceptible cultivar Maris Piper (100% incidence and mean severity of 51%).

As the project develops, the effects of differences between the sustainable and conventional husbandry practices on seed and soil-borne pathogens of potato will be examined. For example, the use of municipal compost in the sustainable treatment may be a source of inoculum or the increased organic matter may have an impact on pathogen epidemiology in comparison to the conventional treatment. Additionally, the use of any seed treatments and in-furrow applications of azoxystrobin (Amistar) in the conventional treatment can be compared to the sustainable treatments where these applications may be reduced or not used at all. In the future, pathogen and disease results will be related to other datasets that are being collected alongside to see if there are any relations with other components of the system. For example, there will be opportunities to see if there are any effects of soil chemistry and biophysics, weed abundance etc. We will also in the longer term be able to relate trends in pathogens to meteorological data that is being collected at the site.

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