THE EFFECT OF INCREASED SOIL ORGANIC MATTER ON SEVERITY OF DISEASE CAUSED BY *RHIZOCTONIA SOLANI* AG3 ON POTATO

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Summary: The impact of incorporating a typical source of organic matter (OM) used in Scottish Agriculture on potato disease caused by *R. solani* AG3 was investigated in a field trial. Increasing soil OM in plots which had been infested with *R. solani* prior to planting, resulted in delayed emergence (11 weeks cf. 7 weeks), and significantly lower tuber yields (3.2 cf. 18.6 kg/plot, in high and low OM plots respectively). Additional soil OM increased the incidence (45% cf. 19%) but not severity of black scurf on progeny tubers in plots infested with *R. solani*, but had no effect on emergence and tuber yield in un-infested control plots. Therefore, in this study, the main effect of increased soil OM was to delay emergence when soil was infested with *R.solani*, and this delay had a subsequent impact on tuber yield. The results are discussed in relation to additional studies looking at the impact of soil OM on *R. solani* survival in soil through a typical rotation.

INTRODUCTION

The incorporation of organic matter into field soil has been reported to both increase and decrease severity of disease caused by soil-borne pathogenic fungi; see review by Noble (2011). This is reportedly due to differences in the composition of the OM which may possess antagonistic properties and thus repress disease, or alternatively provide a substrate for increased pathogen growth. In this study we investigated the impact of increasing soil organic matter, with a typical source used in Scottish Agriculture, on crop emergence and subsequent yield and disease on progeny tubers in field soil infested with *R. solani* AG3 (hereafter referred to as *R. solani*).

MATERIALS AND METHODS

Field Trial

A field was selected which was naturally very low in OM; 0.2 g OM per kg of soil. A split plot design was created, with high and low OM areas of the field as main plots. A high level of soil OM was created with the incorporation of cattle manure mixed with barley straw (approximately 35 t/ha) which increased the OM to 0.5 g per kg, which whilst being referred to as high, is in fact, still relatively low. Within each main-plot, plots were either infested with *R. solani* or un-infested (control), there were four replicate blocks.

To infest plots with *R. solani*, sclerotia were prepared by inoculating potato dextrose agar (PDA) plates with a single isolate, AG3PT Rs08 (Fera collection), which were then incubated in the dark at 20°C for 6 weeks. Sclerotia were removed from plates using a scalpel blade, and then left to air dry at room temperature for two days. Batches of 5 g sclerotia mixed with vermiculite were spread along each of the prepared drills in the infested treatment plots, seed tubers were then placed along the drill in direct contact with the inoculum. The inoculum was incorporated into the soil at planting depth when the seed potatoes were mechanically covered. No inoculum was added to the soil in the control (un-infested) plots. Mini-tubers of the cultivar Markies were used (no official resistance rating available, but considered relatively susceptible to black scurf). Twenty five tubers were peeled and assessed individually for *R. solani* contamination using real-time PCR, which confirmed absence of infection. Each plot consisted of a single row of 13 plants with a guard at either end, surrounded by a guard row. Irrigation and crop management were as for a commercial crop. Emergence was recorded at intervals of 2 to 3 days. Mid-season (14 weeks after planting) a single plant per plot was carefully dug up and the number of stems, stolons and pruned stolons recorded. At final harvest, the surface area of black scurf on progeny tubers was recorded using the black scurf severity key from Woodhall *et al*. (2008); 0; no sclerotia present, 1; less than 1% of the tuber surface area covered in sclerotia, 2; 1 to 10%, 3; 11 to 20%, 4; 21 to 50% and 5; 51% or more.

Soil samples

Soil samples were collected by taking the top 10cm of soil from at least 100 points in a W-shape across the selected portion of the field to give a total of approximately 1 kg. Two sets of soil samples were taken prior to the trial being planted, the first was taken to establish that the trial area had no detectable inoculum, the second to ensure that no inoculum was introduced with the addition of organic matter into the split plot design. A week after planting, a soil sample was taken from each of the plots (consisting of a bulk of 25 cores). Similarly, soil samples were taken from each plot during the growing season (14 weeks after planting) and immediately after the final harvest.

To establish if the incorporation of additional OM into the soil affected the persistence of inoculum, soil samples (a bulk of 100 cores) were taken from each of the two main-plot areas 10 and 18 weeks post-harvest.

Real-time PCR: quantification of soil and tuber inoculum

Tuber peel and soil DNA extractions were carried out according to the methods of Cullen *et al.* (2001) and Brierley *et al.* (2009) respectively and the amount of *R. solani* AG3 DNA detected using the assay of Lees *et al.* (2002) was expressed as ng DNA /ml tuber sap or pg DNA /g soil.

RESULTS

Emergence and mid-season sampling

Within 5 weeks of planting the control treatments in both the low- and high-OM plots had reached over 95 % emergence. The time taken to reach near complete emergence was progressively longer in infested plots with low OM and high OM respectively, with the later

taking over 11 weeks from planting to reach 90% emergence (Figure 1). The number of main and secondary stems and the total number of stolons per plant did not differ significantly different between treatments. No stolon pruning was observed in plants from the control treatments: however, the proportion of stolons which had been pruned was significantly higher ($P<0.05$) in plants from infested plots with high OM than in plants from infested plots with low OM (0.45 and 0.11 respectively); data not shown.

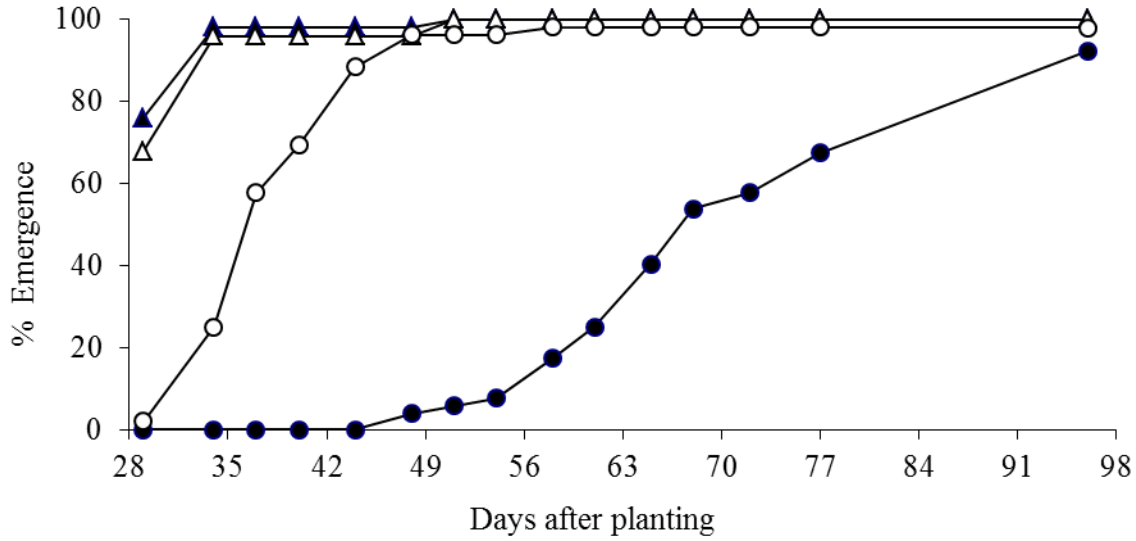

Figure 1. Percentage of plants emerged over time (days after planting) in un-infested control plots with low OM Δ and high OM ▲, and *R. solani* infested plots with low ○ OM and high ● OM. Mean of four replicate plots per treatment.

Tuber disease and yield

Black scurf (both incidence and severity) on progeny tubers was negligible in control (un-infested) plots (Figure 2A and B). Progeny tubers from high OM plots which had been infested with *R. solani* had a significantly ($P<0.05$) higher incidence and severity of black scurf than control plots (Figure 2). Additional soil OM significantly increased the incidence (45% cf. 19%) but not severity of black scurf on tubers grown in plots infested with *R.* solani (Figure 2).

The yield of tubers was significantly lower in treatments which had been infested with *R. solani* compared to control plots ($P<0.05$); however, the yield reduction was much less from infested plots with low OM than from plots with high-OM (Figure 3), reflecting the difference in time taken to reach near complete emergence between the treatments. The reduced tuber yields from infested plots were associated with variations in tuber size distribution (Figure 4); such that progeny from infested plots in the high-OM treatments were fewer in number and predominantly in the smaller (<45 mm) size class, whilst tubers from the infested plots in the low OM treatments were more numerous and predominantly in the 45-65 mm size class. The tuber size distribution from un-infested plots with low and high OM were very similar (Figure 4) with the majority of tubers being in the 65-85 mm size class.

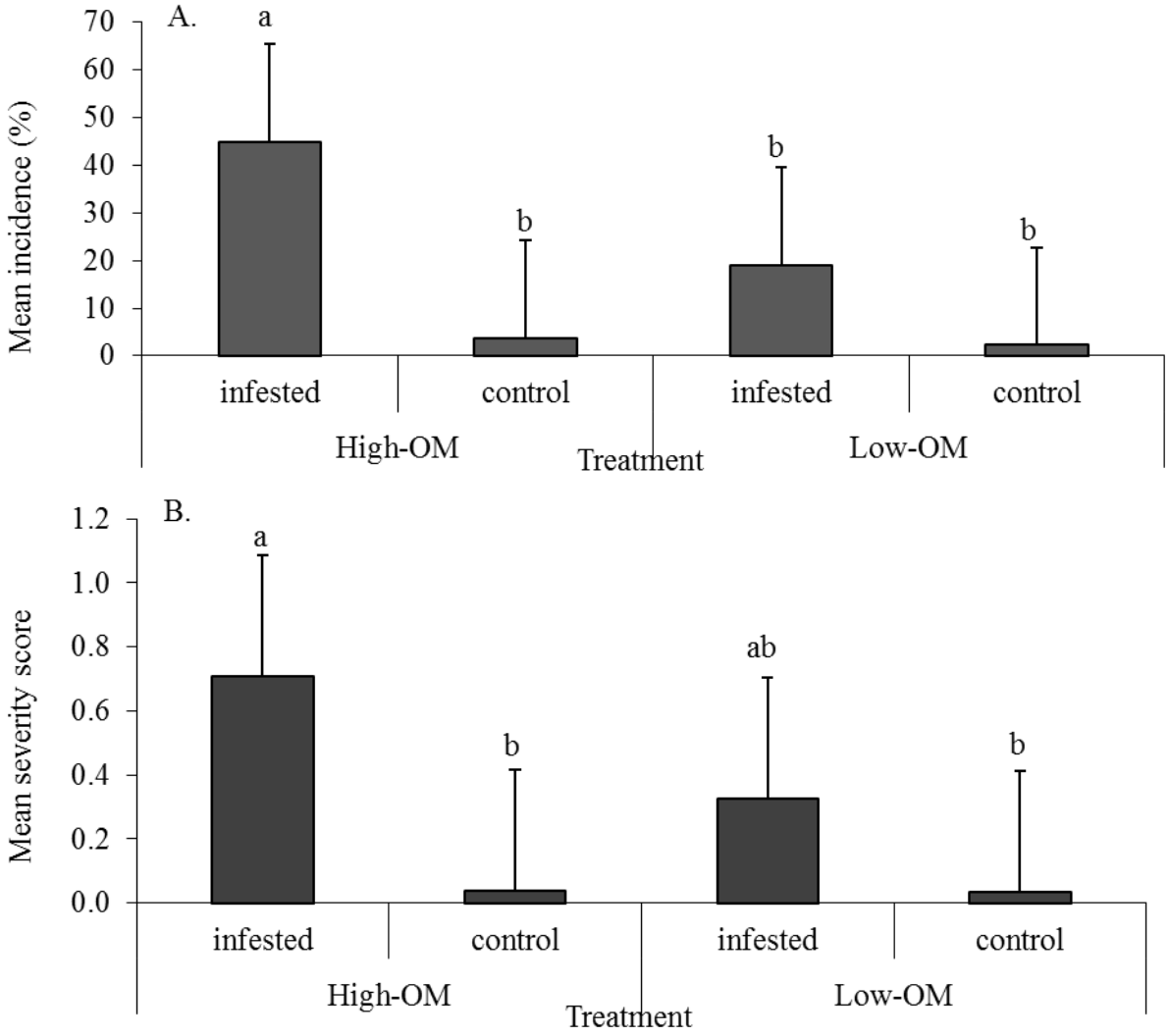

Figure 2. The effect of soil infestation with *R. solani* and the level of OM on A. the incidence and B. the severity of black scurf on progeny tubers at final harvest (mean of four replicates; bars represent lsd). Treatments not sharing the same letter are significantly different ($P<0.05$).

Soil samples

Inoculum was detected in very few soil samples on any of the sampling occasions. No inoculum was detected in the samples taken 1 week after planting or during the growing season. Immediately post-harvest, inoculum was detected in only a single plot, a high-OM soil amended with sclerotia.

In the soil samples taken from each of the main-plots in December and February after the trial was harvested, only a trace amount of inoculum was detected in two samples; in December this was in the low-OM main-plot, and in February in the high-OM main-plot. Therefore the results indicate that very low levels of inoculum (generally below the threshold of detection) were present in the soil both during the trial and in the months following harvest, but increasing the level of soil OM did not have an effect on the amount of inoculum detected.

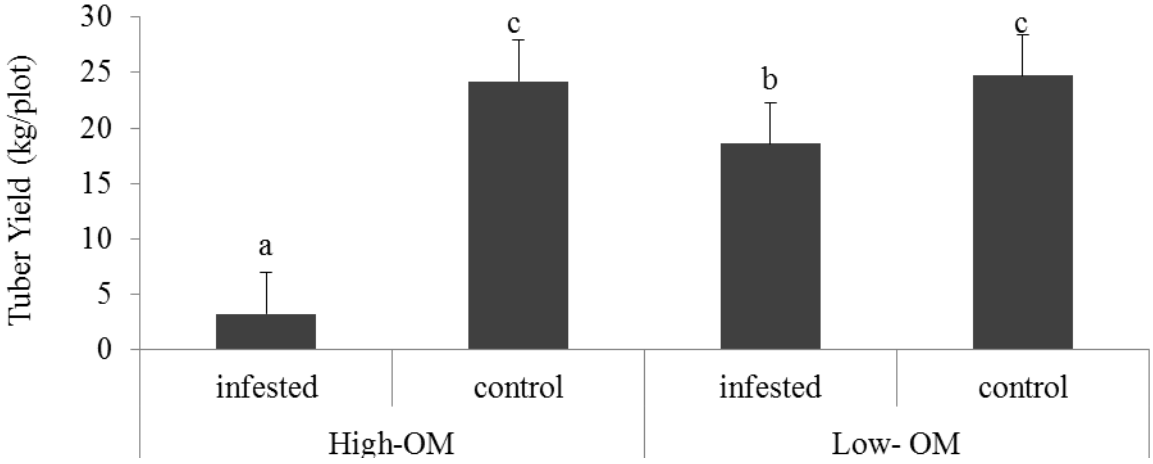

Figure 3. The effect of soil infestation with *R. solani* AG3 and the level of OM on the yield of tubers (kg per plot) at final harvest (mean of four replicate plots; bars represent lsd). Treatments with different letters are significantly different (P<0.05).

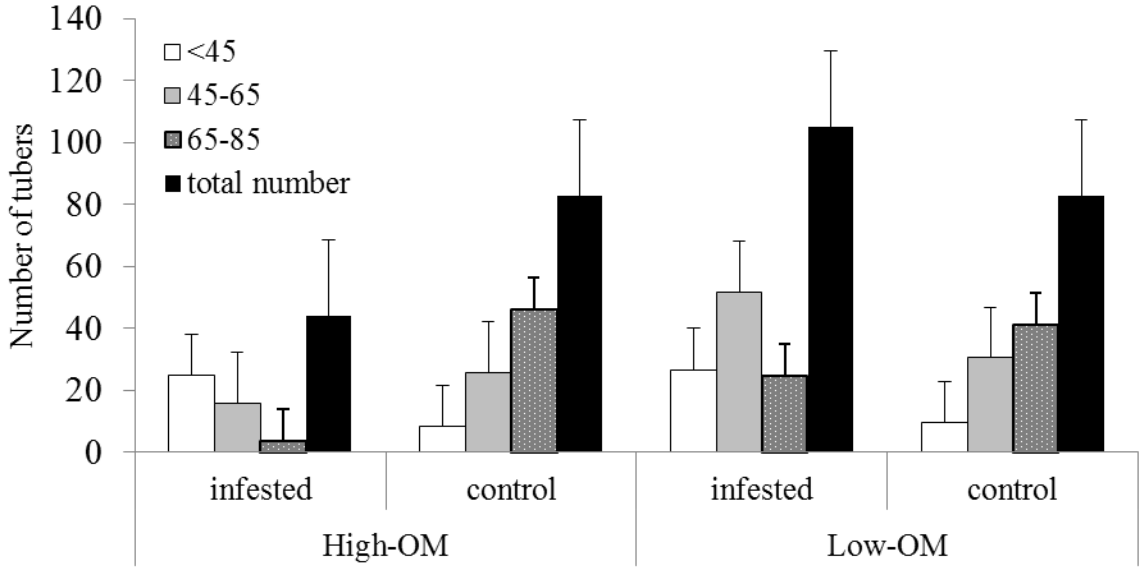

Figure 4. The effect of soil infestation with *R. solani* and the level of OM on the number of tubers in three different size grades (<45, 45-65 and 65-85 mm) at final harvest (mean of four replicate plots; bars represent lsd).

DISCUSSION

Early infection of newly emerging stolons and stems with *R. solani* can result in lesions, referred to as canker, and in more severe infections this results in girdling and pruning. Infection results in a delay in emergence and generally results in a decrease in tuber yield (Carling *et al.*, 1989) and can cause a greater number of non-target tuber sizes (Simons and Gilligan, 1997). The delayed emergence, evidence of stolon pruning and reduced yield in

infested treatments in this trial are in agreement with symptoms of early season infection. The effects were exacerbated in plots into which OM had been incorporated. This indicates the additional OM acted as a food source for the inoculum source used in this trial, and subsequently increased the severity of infection symptoms. The yield reduction in the high OM infested plots was quite severe, but had the tubers been left in ground longer they may have continued to bulk, thereby decreasing the apparent impact on yield.

The addition of soil-borne *R. solani* inoculum caused black scurf symptoms on progeny tubers, although the severity was generally low even in the infested high OM plots (mean severity score 0.7 on a scale of 1 to 5 of increasing severity). However, detection of inoculum in plots was rare, even shortly after the incorporation of sclerotia into the soil and post-harvest. Brierley *et al*. (2014) found that when black scurf developed on progeny tubers (mean severity equivalent to a score of 2) in a field crop, the soil remained infested with *R. solani* at detectable levels 5 months after harvest, but no inoculum was detected when soil was re- sampled 7 months later, indicating that inoculum is either short lived in the soil, or survives at levels below the threshold of detection. The findings reported by Brierley *et al*. (2014) are part of a long term study looking at the introduction of soil-borne pathogens of potato, including *R. solani*, into uncontaminated soils via contaminated seed and how this impacts on progeny disease and amounts of detectable inoculum in future years. This research will also investigate the impact of incorporating municipal compost into field soils on the introduction and persistence of soil-borne potato pathogens through a six year rotation.

ACKOWLEDGEMENTS

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