

A disease assay for *Rhizoctonia solani* on potato (*Solanum tuberosum*)

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Abstract *Rhizoctonia solani* is an important soilborne pathogen of potato, causing black scurf on tubers and shoot/stolon canker on young plants. These diseases reduce the quality of fresh and seed potatoes and may harm crop establishment. A pathogenicity test has been developed for the study of biological suppression of diseases caused by *R. solani*. Disease expression from two *R. solani* isolates was assessed in potato plants grown from tissue-cultured plantlets or from minitubers. The isolates were cultured in two media, bran+water or barley grain+V8 juice[®], which were compared as inoculants of potting mix at several standardised rates. The proportions of diseased stolons and necrotic shoots were determined after 7 weeks growth in a greenhouse. Significantly different levels of disease incidence were found between the isolates, between inoculum concentrations for one isolate, and (only with minitubers) between inoculum media. Rates of inoculum that produced disease levels suitable for use in biocontrol assays were determined.

Keywords pathogenicity, inoculum, canker, biological control.

INTRODUCTION

The soilborne fungal pathogen *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk), pathogenic to potato (*Solanum tuberosum* L.), is ubiquitous in potato production worldwide (Banville et al. 1996). The pathogen can cause lesions (canker) on below-ground plant tissues, and produces sclerotia on daughter tubers (black scurf). Both diseases reduce marketable tuber yields. Fungicide control is not always effective, especially when initial inoculum levels are high (Tsrer & Peretz-Alon 2005). Biological control of *R. solani* could play a valuable role in reducing economic losses from *Rhizoctonia* diseases on potato.

The aim of the present study was to establish an appropriate protocol for screening organisms

with potential to suppress *R. solani* diseases on potato. To assess tissue-cultured plantlets for suitability in screening experiments, their disease susceptibility was compared to that of plants from minitubers. Different pathogen isolates grown on different media were tested at various concentrations in potting mix, to determine optimum inoculation methods. Effects of these factors on plant and disease parameters were determined.

MATERIALS AND METHODS

Pathogen isolates and production of inoculum

Two *R. solani* isolates were used; one (Rs043-2) had been isolated from potato tuber sclerotia and the other (R73-13b) from soil (Sneh et al. 2004).

Isolates were maintained on sterilised barley seeds stored at 4°C.

Pathogen inoculum was produced on two media, barley grain+V8 juice® (1:1 w/v) or wheat bran+reverse osmosis water (1:2 w/v), which were mixed in 250 ml conical flasks then autoclaved. Flasks were inoculated with the respective *R. solani* isolates from potato dextrose agar cultures, then placed in an incubator for 13 days at 25°C. Uninoculated media were incubated in the same way, for use as experimental controls.

Experimental design

Two experiments investigated effects of different inoculum rates, inoculum media and *R. solani* isolates on disease incidence on potato. One experiment used tissue-cultured plantlets of cv. 'Gladiator' and the other used minitubers of cv. 'Desiree', both of which are susceptible to *R. solani*. Inoculum was added to potting mix (sphagnum peat:pumice (3:2 v/v) with added nutrients, lime and wetting agent) at the following five rates (treatments) for both isolates and inoculum media: 0.03, 0.1, 0.3, 1 and 3% w/w. Three controls were used in each experiment: no inoculum, uninoculated bran+water or uninoculated barley+V8, both at 3% w/w. The appropriate weights of potting mix and inoculum were thoroughly mixed and added to ten 0.9 litre plastic pots, each with a separate plastic saucer. This procedure was used for all treatments, except 0.03 and 0.1% barley+V8. For these, the required weight of inoculum was distributed evenly amongst the pots, with kernels placed at mid-depth in each pot. This was to ensure even distribution of the small number of infested barley kernels in these low rate treatments. Tissue-cultured plantlets were planted singly into pots. Minitubers were pre-sprouted (25:15°C, 16:8 h light:dark) for 3 days then planted one per pot. Each experiment was laid out in a randomised block design with 10 replicates, with two pots of each of the three experimental controls per replicate. The experiments were set up on consecutive weeks in one greenhouse unit, with temperature control,

and supplementary lighting (16:8 h light:dark). Temperature and relative humidity were recorded by data logger.

Disease assessment and plant parameters

After 7 weeks for both experiments, all plants were washed free of potting mix and total number of stolons and number of stolons with visible lesions were recorded on each plant. For the minituber experiment, the number of emerged shoots and number and proportion of non-emerged shoots with rotted or dead apices ('nipped') were also determined. The number and fresh weight of tubers, and root and shoot dry weights were recorded for each plant.

Statistical analyses

All data were subjected to analysis of variance for randomised block designs with treatment structures of 2×2×5 factorial + three controls. The factors were isolates, inoculum media and log₁₀-transformed initial inoculum concentrations, for which linear and quadratic polynomial components were included in the analyses. The statistical significance of these polynomial components was used to provide an appropriate order of approximating polynomial to fit to each data set for graphing trends.

RESULTS

Temperature and relative humidity

During both experiments, the mean temperature was 17.6°C (maximum 23.2°C, minimum 15.4°C) and the mean relative humidity was 70.5% (maximum 93.9%, minimum 41.7%).

Tissue-cultured plantlet experiment

The mean proportions of diseased stolons per plant (Figure 1) were significantly affected by isolate ($P < 0.001$) and inoculum rate ($P = 0.003$). Isolate R73-13b gave consistently high disease incidence (81 to 89% stolons affected) across all inoculum rates, with no significant trend. Isolate Rs043-2 gave greatest incidence (62 to 75%) from low inoculum rates (0.03, 0.1 and 0.3%), but reduced incidence (53 and 26%) from the two greatest inoculum rates, and this downward

trend was significant ($P < 0.001$). Both inoculum media gave similar disease incidence within each isolate for all inoculum rates.

Neither number of tubers nor tuber fresh weight correlated with increasing disease incidence or score for any of the experimental treatments. Mean root and shoot dry weights (per plant) were positively correlated ($P = 0.011$ and $P = 0.016$, respectively) with increased disease incidence for Rs043-2 cultured on barley+V8, but not for Rs043-2 on bran+water or R73-13b on both media.

Minituber experiment

Mean proportions of diseased stolons (Figure 2a) were significantly affected by isolate ($P < 0.001$), inoculum rate ($P < 0.001$) and inoculum media ($P = 0.026$). Isolate R73-13b gave consistently high disease incidence both from bran+water (76 to 89% stolons affected) and barley+V8 media (69 to 87%) across all inoculum rates, with no significant trends. Isolate Rs043-2 cultured on bran+water gave the greatest disease incidence (52 to 97%) from low inoculum rates (0.03, 0.1 and 0.3%), with reduced disease incidence

(32 and 33%) at the two greatest inoculum rates, and this downward trend was significant ($P < 0.001$). When cultured on barley+V8, Rs043-2 gave greatest disease incidence (80 and 61%) from inoculum rates of 0.03 and 0.3% respectively, and reduced incidence (2, 46 and 41%) from inoculum rates of 0.1, 1.0 and 3.0% respectively, but this trend was not statistically significant.

Mean proportions of 'nipped' shoots (Figure 2b) were significantly affected by isolate ($P < 0.001$), inoculum rate ($P = 0.002$) and inoculum media ($P = 0.023$). Compared with Rs043-2, isolate R73-13b caused less shoot 'nipping' at low inoculum rates of both inoculum media (Figure 2b), with no significant trends. Isolate Rs043-2 cultured on bran+water gave high proportions of shoot 'nipping' (68 to 73%) at low inoculum rates (0.03, 0.1 and 0.3%), and less 'nipping' (54 to 37%) at the two greatest inoculum rates, with this downward trend significant ($P = 0.005$). When cultured on barley+V8, Rs043-2 gave high proportions of shoot 'nipping' (66 to 87%) from the four lowest inoculum rates, but reduced 'nipping' (42 and 37%) from the greatest inoculum rate, with this downward trend significant ($P = 0.01$).

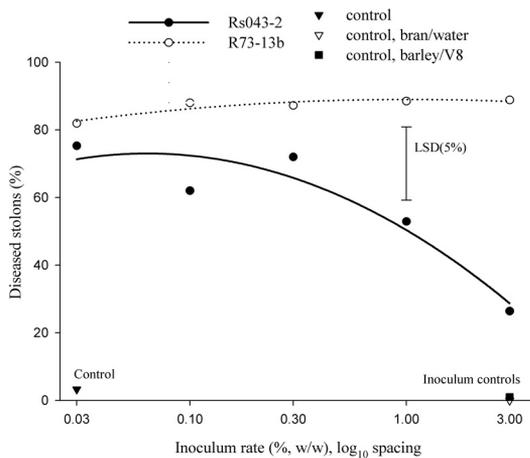


Figure 1 Mean percentage of diseased stolons on potato plants grown from tissue-cultured plantlets, inoculated with two different *R. solani* isolates, at different rates of inoculation (averaged over two inoculum media). Bar is LSD ($P = 0.05$) for comparisons between "non-control" points.

DISCUSSION

The effects of pathogen isolate, inoculum medium type and increasing rate of inoculum were reasonably consistent across the two experiments. This result indicates that plants grown from tissue-cultured plantlets are suitable for experiments to screen for suppressors of Rhizoctonia disease. Plants from plantlets gave similar results to plants grown from minitubers. Plants from minitubers produced multiple underground shoots, similar to the field situation where seed tubers are used for crop establishment. Differences in overall disease incidence between the two experiments are likely to be due either to differences in propagule type or potato cultivar.

The results indicate that plant parameters may not give good indications of disease, as most did not correlate with disease incidence, and the two that did were inconsistent across inoculum media types. In the minituber experiment,

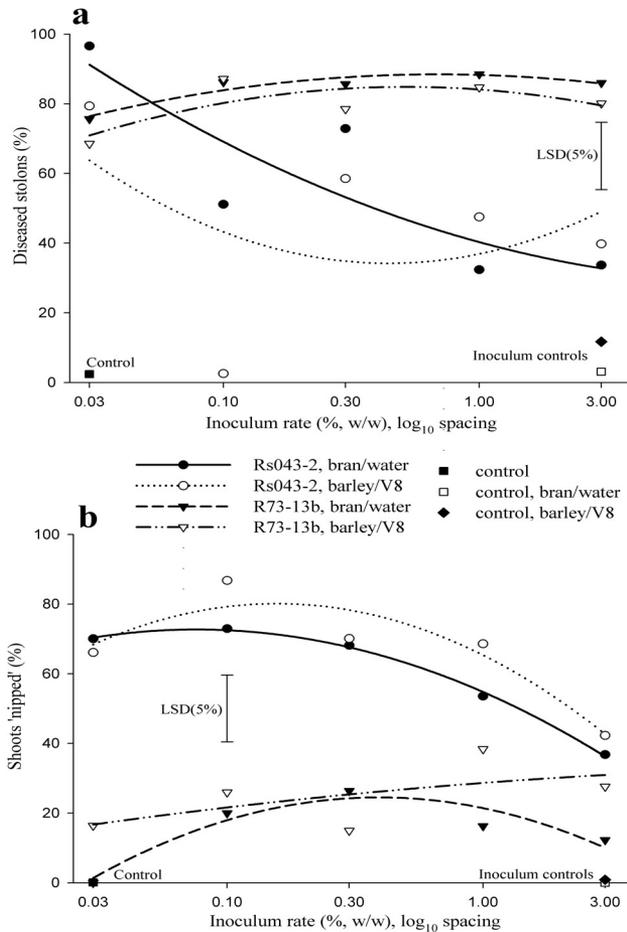


Figure 2 Mean percentage of (a) diseased stolons and (b) shoots ‘nipped’ on potato plants grown from minitubers, inoculated with two different *R. solani* isolates, at different rates of inoculation using two different media. Bars are LSDs (P=0.05) for comparisons between “non-control” points.

the proportion of diseased stolons did not clearly indicate disease effects because some inoculation rates prevented stolon formation. For instance, at the 0.1% inoculum of Rs043-2 cultured on barley+V8 (Figure 2a), only two of the ten replicates had stolons, indicating that this isolate severely affected stolon formation and/or survival. Trends for disease incidence (canker on stolons or ‘nipped’ shoots) with increasing inoculum rates were similar for the respective isolates in both the tissue-culture (Figure 1) and minituber (Figure 2b) experiments.

Although both isolates caused disease, they

behaved differently. R73-13b did not give reduced disease incidence with increasing inoculum rate, which occurred with Rs043-2, and caused far less shoot ‘nipping’ than Rs043-2. The decrease in stolon disease incidence and proportion of shoots ‘nipped’ with increasing initial inoculum concentration for isolate Rs043-2 was unexpected, and these results require further investigation of possible mechanisms. However, an inoculum rate giving intermediate disease incidence, which is more suitable for initial screening assays, can now be chosen to identify and distinguish disease suppression factors. The 1% (w/w) inoculum rate

for isolate Rs043-2 on both media was the most appropriate, but the mechanism causing reduced disease incidence with increasing inoculum rate is unclear. If this mechanism is found to interfere with biocontrol screening experiments, then lower inoculum rates could be used. If higher disease levels are required, the results indicate that this could be achieved either by adjusting inoculum rates of isolate Rs043-2, or by using isolate R73-13b, which consistently caused high stolon disease incidence. Rates below 0.03% may produce less disease, but in the experimental protocol used this was not practical.

The decline in disease incidence with increasing inoculum rate for isolate Rs043-2 could be explained by several mechanisms. For instance, the isolate could be using the inoculum medium as a nutrient source in preference to becoming pathogenic. Organic matter soil amendments have been shown to reduce the pathogenicity of certain *R. solani* isolates, but have no effect or increase pathogenicity of others (Bonanomi et al. 2007). In addition, it has been demonstrated that increased nutrient availability increases virulence of some *R. solani* isolates (Weinhold et al. 1972; Doornik 1980). Another possibility is that the nutrient source encouraged activity of suppressive micro-organisms present in the potting mix. Testing whether this explanation is correct could involve repeating the experiments using sterilised potting-mix. The decline could also be due to an isolate-specific population density effect regulating pathogenicity, perhaps quorum sensing or intra-population competition. Quorum sensing has so far only been reported for fungi with yeast-like phases in their lifecycles (Hogan 2006).

These experiments have shown that the consistency in disease susceptibility between plants grown from tissue-cultured plantlets and minitubers supports the use of plantlets as propagules for assays investigating disease suppression. Isolate Rs043-2, at a rate of 1% (w/w) of inoculum medium, gave intermediate levels of disease, and is therefore suitable for future disease suppression studies. The two pathogen inoculum media gave similar levels

of disease. These results provide a sound basis for future research on biological suppression of *Rhizoctonia* diseases of potato.

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