# Methods for measuring Rhizoctonia stem canker severity in potato crops

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## Abstract

Soil-borne diseases, such as stem canker caused by *Rhizoctonia solani*, can be responsible for substantial yield losses in potato crops in New Zealand. Robust and repeatable methods to assess disease severity are essential in research or crop assessments for growers/industry. This approach ensures parity between bodies of independent research and that remediation decisions are relevant and efficient. Disease assessment methods to measure the severity of stem canker on potato plants are described. These methods were developed and refined *in situ* over several growing seasons in different case studies of field and glasshouse monitoring and experimentation. The experimental setups were varied in scale and complexity, and their aims were also contrasting: in some, stem canker severity was a main variable, while in others, it was measured to account for its impact on the main experimental variables (i.e. yield). The methods produced robust results under the different experimental setups, thus demonstrating their repeatability and flexibility when analysing the data collected. Furthermore, this research was carried out in New Zealand, and has provided valuable knowledge of disease severity impact on productivity for the research community and potato industries.

***Additional keywords:*** protocol, *Rhizoctonia solani*, *Solanum tuberosum*, stem canker

## Introduction

Soil-borne diseases, such as Rhizoctonia stem canker (RSC) caused by *Rhizoctonia solani*, are one of the major factors causing substantial yield losses in potato crops (*Solanum tuberosum*) in New Zealand (Sinton *et al.*, 2014; Michel *et al.*, 2015). Solutions to reduce the risk of soil-borne disease outbreaks can consist of in-furrow pesticide application at planting and/or the choice of land where potato crops are grown (e.g. avoiding paddocks with potentially high inoculum based on previous cropping history). To rely on those solutions and to test their success, it is necessary to assess disease incidence and severity. Research activities and scouting by growers and industry also rely on successful assessment of these disease parameters.

Previous studies have proposed protocols for assessing the severity of diseases caused by *R. solani* (Carling and Leiner, 1990; Simons and Gilligan, 1997; Woodhall *et al.*, 2008). Some of these studies focused mainly on the tuber disease: black scurf. However, crop yields can be seriously affected by RSC, with little black scurf occurring on harvested tubers (Woodhall *et al.*, 2008). Furthermore, there are different anastomosis groups (AGs) of *R. solani* and not all have been associated with both black scurf and RSC (Carling *et al.*, 2002; Campion *et al.*, 2003): AG 2.1 and AG3 are present in New Zealand and associated with stem canker; however, only AG3 is associated with black scurf.

Disease severity has often been measured using the scale of 0 to 4 described by Carling and Leiner (1990). That scale is based on sprout or stem area displaying RSC symptoms and their intensity. Simons and Gilligan (1997) scored RSC severity using the 1 to 4 scale described by Adams and Hide (1980), which is based on the area of each stem covered by symptoms. These scoring methods provide a simple basis for assessing severity of RSC on potato plants, but they can be limited for establishing thresholds that, if reached, indicate that the disease is affecting crop yields.

This paper describes detailed methods for scoring severity of RSC in plant samples. Those methods have been developed for use in crop surveys, and for field and glasshouse experiments. Examples of recent field and glasshouse research are presented that illustrate how the methods can help with experimentation, and can be used in crop scouting by growers or industry, for assessment of RSC. The quality of data produced and appropriate statistical analyses are also discussed.

## Materials and Methods

### Measuring Rhizoctonia stem canker severity

Severity of RSC was measured for defined experimental units (e.g. observation plot, plot, individual plant) in different case studies using the following methods to score individual underground stems from sampled potato plants.

After thoroughly washing the root system of individual plants free of soil or potting medium, each stem was given a severity score using a 0 to 6 scale (Figure 1). Each score correlated with a range of proportions of stem surface covered by symptoms: 0 = no disease; 1 = 1 to 10% of stem surface affected; 2 = 11 to 30%; 3 = 31 to 50%; 4 = 51 to 80%; 5 = 81 to 100%; and 6 = dead stem. Each stem was also given a lesion type score: 0 = no symptoms; 1 = flecks on the stem surface; 2 = flecks and splits along the stem; and 3 = brown lesions and splits along the stem or a dead stem. Mean RSC severity per stem was calculated as:

$$Mean\ RSC\ severity = \frac{\sum_{S=0}^{6}\sum_{L=0}^{3}(n_{S,L} * S * L)}{n_T}$$

Where S is the severity score, L is the lesion type score, $n_{S,L}$ is the number of stems in the given severity, lesion type score combination, and $n_T$ is the total number of stems in the sample. As an indication, a mean RSC severity of 6 or greater, measured using the described methods, has been associated with severe yield losses in previous research (Michel *et al.*, 2015).

All statistical analyses of the case studies described below were carried out in Genstat version 17 (VSN International Ltd, UK).

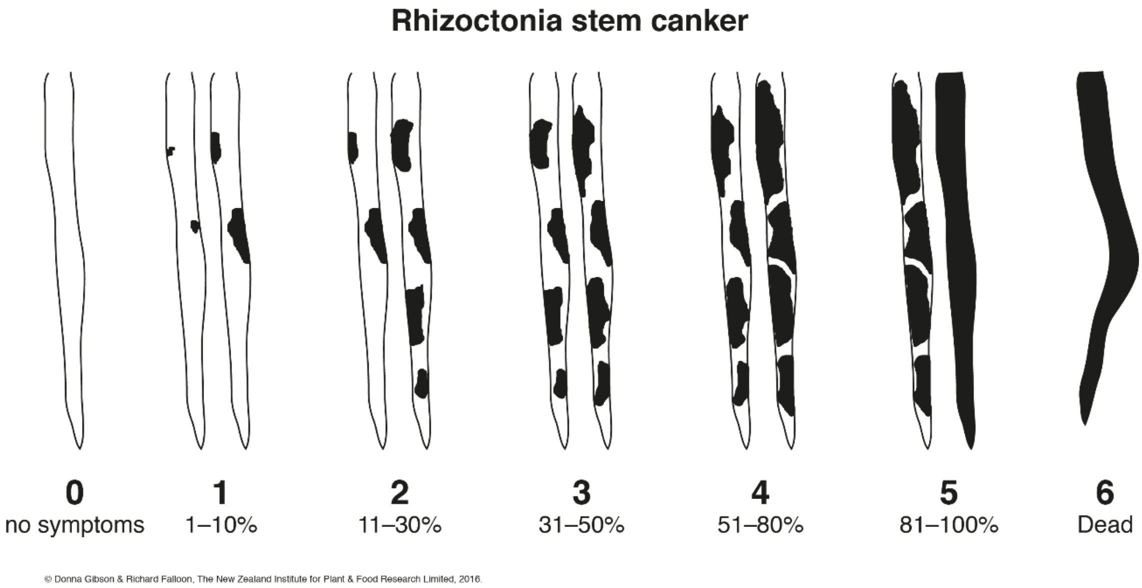


**Figure 1:** Key for assessment of severity of Rhizoctonia stem canker on individual underground stems of potato plants. Proportions of stem surface area affected for each score are indicated.

### Case study one

This was a two-year project (2016/17 and 2017/18 growing seasons) which aimed to identify relationships in soil-borne disease severity between seed crops in one year and subsequent main crops established with these seed tubers in the following year. The project took place in Canterbury, New Zealand in both years.

In 2016/17 (Year one), 11 potato seed crops were monitored from planting to harvest for soil-borne diseases, including RSC. Six crops were 'Agria' potatoes, and five were 'Innovator'. An area of six to nine rows by 15 m long (54 to 122 $m^2$) was marked out in each crop as an observation plot. This was visited every seven to 10 days, and RSC severity was measured using the methods described in Measuring Rhizoctonia stem canker severity, for a four-plant sample (one plant from each corner of the plot). At the end of the growing season, seed tubers were collected from the observation plot in each of the 11 crops.

In 2017/18 (Year two), the seed tubers collected in Year one was planted in two field experiments, one for each cultivar, located within a commercial crop of the same cultivar. The experimental treatments were 11 seed lines (i.e. the crop from which the seed tubers originated), and three different combinations of storage and pre-planting treatments. The different combinations of storage and pre-planting treatments were likely to induce a range of potential loads of pathogen inoculum on the seed tubers. The details of the experimental treatments are not relevant for this paper. Therefore, each seed line was designated with a letter (A to F for 'Agria', A to E for 'Innovator') and the storage/pre-planting treatments were designated 1, 2 and 3. Plots were four rows by 10 m (36 $m^2$). The experiment was managed in the same way as the surrounding commercial crop (conventional practices). Severity of RSC was measured for each individual plot using the methods described in Measuring Rhizoctonia stem canker severity, for a four-plant sample (one plant from each corner of the plot) at three crop growth stages: canopy closure (CC), full canopy (FC) and late canopy (LC, 'Innovator' only, which was when senescence had commenced but the canopy was approx. 50% green).

Data of mean RSC severity score per stem from Year one was calculated for the observation plot for each seed line. Although the trial design was not replicated, the seed tubers used for Year two came from that area. Therefore, the data from Year one were not analysed, but were examined graphically to identify RSC severity trends between the respective seed crops and following main crops.

Mean RSC severity score per stem was analysed using a mixed model approach, fitted with restricted maximum likelihood (REML). Assumptions were checked via standard residual plots and log transformation was applied for 'Innovator' data, in order for assumptions to hold, but not 'Agria'. Fixed effects in the model were seed line, storage/pre-planting treatment, growth stage (CC, FC, LC ('Innovator' only)), and all interactions. Order was considered and the highest *p*-value are reported here. Random effects accounted for the position (Row, Column) within the field. For ease of interpretation, data of mean RSC severity score per stem for 'Innovator' were back-transformed from log for presentation here. As such, those tables do not have any LSD associated with the means displayed. Trends are indicated in the text (based on the log transformed data for 'Innovator') when $p<0.1$ and an estimate of the variation associated with predicted means provided by a 5% least significant difference ($LSD_{0.05}$).

### Case study two

To support field observations in the experiment described in case study one, two support experiments, one each for 'Agria' and 'Innovator', were established in a glasshouse. The aim was to assess if *R. solani* inoculum on seed tubers from each line was associated with RSC severity on the resulting plants.

For each seed line, eight randomly selected seed tubers were planted individually into separate planter bags filled with 24 L of sterile potting mix, giving eight replications for each seed line. Eight tubers from the same seed line, with no symptoms of black scurf, were also planted, after being dipped in 0.5% formaldehyde solution for four minutes, as an experimental control treatment in the 'Innovator' experiment. The resulting plants were grown in a controlled temperature glasshouse at 18°C (±2°C) to favour disease development. Plants were watered frequently to avoid water stress while also maintaining favourable conditions for disease development. Plants were grown for 84 days before the underground stems were collected to assess RSC severity on each plant using the methods described in Measuring Rhizoctonia stem canker severity.

Mean RSC severity score per stem was analysed using a mixed model approach, fitted with REML. The responses were transformed using log(x+1) for assumptions to hold. The fixed effect in the model was seed line. Random effects accounted for the position of pots (row, column) within the glasshouse.

For ease of interpretation, mean RSC severity score per stem data presented in tables were back-transformed from log(x+1). As such, those tables do not have any LSD associated with the means displayed. Trends indicated in the text are based on the log(x+1) transformed data when $p$<0.1 and an estimate of the variation associated with predicted means provided by a 5% least significant difference ($LSD_{0.05}$).

### Case study three

A field experiment was conducted in the 2017/18 growing season in Canterbury, New Zealand, to investigate effects of different potato bed architectures and seed tuber planting depths on yield and crop water use. Treatments consisted of two bed architectures (flat-top ridge/furrow bed or flatbed) and two seed tuber planting depths (shallow or deep), along with a conventional grower configuration for comparison.

Plots were six rows by 20 m long (108 $m^2$). Severity of RSC was measured for each plot using the methods described in Measuring Rhizoctonia stem canker severity, for a four-plant sample (one plant from each corner of the plot) at the LC crop growth stage.

Mean RSC severity per stem was not a main variable in the experiment or directly related to the treatments. However, this was measured to quantify the variability of disease expression within the experiment, which could confound effects from the experimental treatments on main variables, such as tuber yield and crop water use.

Mean RSC score per stem was analysed using a mixed model approach, fitted with REML. Assumptions were checked via standard residual plots. Random effects accounted for the position (block) within the field. Bed architecture and seed tuber depth treatments were fixed effects in the models for the conventional grower configuration comparison analysis.

### Case study four

A suite of multi-site field experiments was set up in the 2016/17 growing season in Canterbury, New Zealand. The study was carried out across 15 field sites, and aimed to investigate relationships between soil physical conditions, soil-borne diseases in potato crops, and the respective previous 10-year cropping histories of the sites. Treatments consisted of cultivar, pre-planting seed tuber treatment, and site cropping history. Cultivars used were ‘Russet Burbank’ or ‘Innovator’. Pre-planting seed tuber treatment was a 0.37% formaldehyde solution dip for two minutes or untreated seed tubers. Field sites were organised in cropping history categories based on the previous 10 years: one site had previous potato crops and at least five years of pasture, six sites had previous potato crops and at least five years of arable crops, four sites had no previous potato crops and at least five years of pasture, and four sites had no previous potato crops and at least five years of arable crops. The distribution of cropping history categories was not uniform because of current conventional rotation practices and this was taken into account for the statistical analysis of the data.

Each site experiment was planted within a commercial crop. Cultivar and pre-planting seed tuber treatment combinations were randomised between sites. The experimental design resulted in four plots, each of six rows by 20 m long (108 $m^2$), at each site. The experiment in each field was managed conventionally by the grower, using methods applied to the surrounding crop.

Severity of RSC was measured for each plot at each site, using the methods described in Measuring Rhizoctonia stem canker severity, for a four-plant sample (one plant from each corner of the plot) at crop emergence, and at the FC and LC crop growth stages.

Mean RSC severity score per stem was analysed using a mixed model approach, fitted with REML. Fixed effects in the model were cultivar, pre-planting seed tuber treatment, presence/absence of potatoes in the cropping history, arable crop/pasture dominance in the cropping history, and all interactions. Order of the effects was considered, and the highest *p*-values are reported here. Random effects (site or plot) accounted for the location (site) and repeated measures.

## Results and Discussion

### Case study one

In the 'Agria' experiment, there was an interaction ($p$=0.026) between the growth stage at sampling and the storage/pre-planting treatment for RSC severity on sampled plants. At the CC growth stage, there were no significant differences in RSC severity between the storage/pre-planting treatments. However, significant differences were detected at the FC growth stage, when storage/pre-planting treatment 2 gave increased RSC severity on average (Table 1). These differences, however, were not consistent, thus demonstrating that the data collected using the described methods to measure RSC severity had sufficient precision for detection of small differences between treatments, even though said differences were not biologically relevant. Overall, the highest mean RSC severity score per stem was 1.7, which was unlikely to have affected crop yields.

There was also a strong seed line effect on RSC severity in resulting plants ($p$<0.001). On average, plants from seed line E had a greater mean RSC severity score per stem (0.9) than plants from all the other seed lines, for which the mean scores ranged from 0.1 to 0.4 ($LSD_{0.05}$ = 0.3). These results demonstrated again that using the described methods to measure RSC severity detected small differences between experimental treatments. Furthermore, data from Year one (Figure 2) showed similar trends: RSC severity measured in the seed tuber crop for seed line E was greater than for all other seed tuber crops.

In the 'Innovator' experiment, growth stage at sampling had a strong effect on RSC severity ($p$<0.001). As the season progressed, mean RSC severity score per stem increased (Table 2). There was an interaction between seed line and storage/pre-planting treatments ($p$=0.025), and a weak interaction between seed line, storage/pre-planting treatments, and growth stage at sampling ($p$=0.099). For plants from seed line C, RSC severity was greater for storage/pre-planting treatment 3 than for the other two treatments (Table 2). For plants from seed line E, storage/pre-planting treatment 2 resulted in greater severity of RSC than treatment 3 from FC growth stage onwards. For storage/pre-planting treatments 1 and 2, plants from seed line C had less severe RSC than other seed lines. For storage/pre-planting treatment 2, plants from seed line B had less severe RSC than plants from seed line D. For storage/pre-planting treatment 3, plants from seed lines A and D had greater RSC severity than plants from seed lines B and E.

These results showed that using the outlined methods for assessing RSC severity allowed the statistical analyses to detect and identify differences between treatments in a moderate scale field experiment: there was a total of 60 plots and each plot was 36 $m^2$.

**Table 1:** Mean Rhizoctonia stem canker (RSC) severity scores for potato plants at two crop growth stages (canopy closure (CC) and full canopy (FC)), for six ‘Agria’ seed lines grown as main crops in Canterbury, New Zealand. The seed tubers were subjected to one of three different combinations of storage and pre-planting treatments. An estimate of the variation associated with predicted means is provided by an average of the 5% least significant difference ($LSD_{0.05}$).

| Seed line | Pre-planting treatment | CC | FC |
|---|---|---|---|
| A | 1 | 0.0 | 0.0 |
| | 2 | 0.1 | 0.6 |
| | 3 | 0.2 | 0.6 |
| B | 1 | 0.3 | 0.5 |
| | 2 | 0.0 | 0.1 |
| | 3 | 1.0 | 0.7 |
| C | 1 | 0.6 | 0.1 |
| | 2 | 0.1 | 0.8 |
| | 3 | 0.0 | 0.2 |
| D | 1 | 0.0 | 0.0 |
| | 2 | 0.0 | 0.2 |
| | 3 | 0.3 | 0.1 |
| E | 1 | 0.9 | 0.7 |
| | 2 | 0.3 | 1.7 |
| | 3 | 1.0 | 0.8 |
| F | 1 | 0.0 | 0.2 |
| | 2 | 0.1 | 0.3 |
| | 3 | 0.0 | 0.4 |
| Average $LSD_{0.05}$ | | 0.8 | |

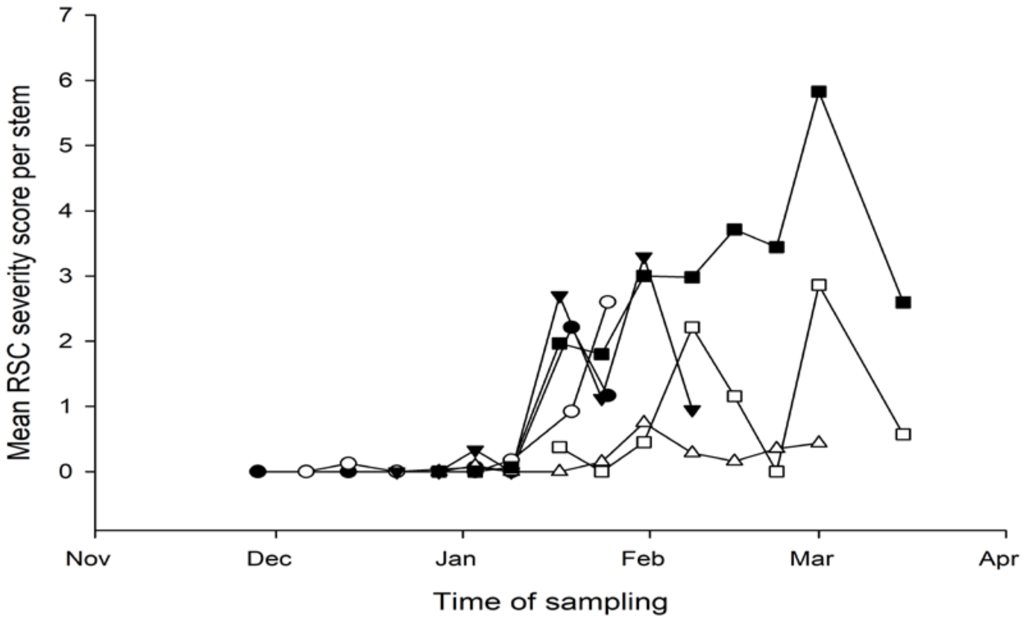


**Figure 2:** Mean Rhizoctonia stem canker (RSC) severity scores for potato plants, repetitively measured in six 'Agria' potato seed tuber crops (seed lines) during the 2016/17 growing season: ● seed line A; ○ seed line B; ▼ seed line C; Δ seed line D; ■ seed line E; □ seed line F.

**Case study two**

In the 'Agria' experiment, seed line had a strong effect on RSC severity ($p$<0.001). Plants from seed line A had a greater RSC severity than plants from all the other seed lines (Table 3). Overall, mean RSC severity score per stem was 1.7 or lower (back-transformed data), which is well below the threshold of 6 where moderate to severe tuber yield losses can incur (Michel *et al.*, 2015).

In the 'Innovator' experiment, seed line had a strong effect on RSC severity ($p$=0.031). The plant from the negative experimental control did not display any RSC symptoms. Plants from seed lines C, D, and E had significantly greater RSC severity than the negative control. Plants from seed line A had the lowest RSC severity.

These results differed from the field trial in case study one. For 'Agria', plants from seed line E had the highest RSC severity in the field but the lowest in the glasshouse; and plants from seed line A had low RSC severity in the field but the highest severity in the glasshouse. These different results could be attributed to the fact that the storage/pre-planting treatments from the field experiment were not imposed on the seed tubers used for the glasshouse experiment. Also, field conditions are more variable and can differ greatly from glasshouse conditions, where temperature was controlled. Finally, sterile potting mix was used in the glasshouse, whereas inoculum and other micro-organisms can be present in the soil where the field experiment was located. These differences in RSC severity were detected by the statistical analysis, however they do not necessarily relate to significant biological differences. Overall, the aim of the glasshouse work was to detect seed-borne inoculum; and general trends were similar between field and glasshouse experiments: RSC severity was low and well below the threshold of 6. Also, the methods did successfully measure a RSC severity of 0 on the negative control, which showed that, even though they allowed for detection of low RSC severity, they did not detect a false positive.

**Table 2:** Mean Rhizoctonia stem canker (RSC) severity scores for potato plants assessed at three crop growth stages (canopy closure (CC), full canopy (FC), and late canopy (LC)), for five 'Innovator' seed lines grown as main crops in Canterbury, New Zealand. The seed tubers were subjected to one of three different combinations of storage and pre-planting treatments. Data presented in the table was back-transformed (from log) and no least significant differences (LSDs) were associated with it.

| Seed line | Pre-planting treatment | CC | FC | LC |
|---|---|---|---|---|
| A | 1 | 2.9 | 4.0 | 4.6 |
| | 2 | 3.4 | 3.2 | 6.0 |
| | 3 | 3.3 | 3.8 | 5.3 |
| B | 1 | 3.9 | 3.5 | 4.8 |
| | 2 | 3.5 | 2.4 | 5.2 |
| | 3 | 2.9 | 3.1 | 3.3 |
| C | 1 | 2.2 | 2.2 | 3.2 |
| | 2 | 1.7 | 2.8 | 3.4 |
| | 3 | 2.6 | 3.3 | 4.8 |
| D | 1 | 4.6 | 3.1 | 3.7 |
| | 2 | 4.3 | 3.8 | 6.3 |
| | 3 | 3.4 | 3.8 | 5.9 |
| E | 1 | 2.7 | 3.2 | 4.4 |
| | 2 | 3.0 | 4.7 | 5.6 |
| | 3 | 3.6 | 1.7 | 4.7 |

**Table 3:** Mean Rhizoctonia stem canker (RSC) severity scores for potato plants from six 'Agria' and five 'Innovator' seed lines, including a negative experimental control (seed tubers dipped for four minutes in 0.5% formaldehyde solution), grown in a glasshouse at 18°C (±2°C). Data presented in the table was back-transformed (from log(x+1)) and no least significant differences (LSDs) were associated with it.

| Cultivar | Seed line | Mean RSC severity per stem |
|---|---|---|
| 'Agria' | A | 1.7 |
| | B | 0.4 |
| | C | 0.5 |
| | D | 0.3 |
| | E | 0.2 |
| | F | 0.2 |
| 'Innovator' | A | 0.4 |
| | B | 0.5 |
| | C | 1.0 |
| | D | 1.7 |
| | E | 1.0 |
| 'Agria' | Negative control (dipped B) | 0.0 |

### Case study three

There was no evidence of interactions between bed architecture and seed tuber planting depth treatments on RSC severity (*p*=0.165). There was also no evidence of a treatment effect for the conventional grower comparison (*p*=0.587). Mean RSC severity score per stem ranged from 6.9 to 8.1 ($LSD_{0.05}$=1.8), but these were not affected by the treatments. Furthermore, incidence of RSC was uniform across the experiment, with 100% of the plants affected in all plots (data not shown), except three plots for which incidence ranged from 92 to 95%. The overall RSC severity in this trial was great enough to affect yield, thus possibly confounding the experimental treatment effects on tuber yields. However, this was unlikely because RSC incidence and severity were uniform across the experiment plots.

In this case study, the methods used to measure RSC severity showed that disease severity, coupled with high disease incidence, probably affected subsequent tuber yields (one of the main variables measured in the experiment). However, as RSC severity and incidence were uniform across the experiment, the disease was unlikely to have confounded the experimental treatment effects.

### Case study four

There was an interaction between presence/absence of previous potato crops and growth stage at sampling on RSC severity (*p*=0.029). At crop emergence, RSC severity was low and unaffected by the presence or absence of potatoes in the previous cropping history (Table 4). At the FC growth stage, RSC severity was greater than at crop emergence and remained similar at the LC growth stage. At the FC and LC growth stages, RSC severity was greater in fields that had previously grown potatoes. These results, associated with data of incidence of RSC and tuber yields (not presented), were key for determining if potatoes in the cropping history resulted in a greater probability of RSC occurring in a potato crop, and with greater RSC severity. However, crop yields were unaffected, and were highest in fields with at least five previous years of pasture, which was attributable to better soil physical conditions than for heavily cropped soils.

The methods used to score RSC severity provided key data for identifying relevant interactions between soil-borne disease pressure in potato crops and soil physical conditions in particular fields.

**Table 4:** Mean Rhizoctonia stem canker (RSC) severity scores for potato plants assessed at three crop growth stages (crop emergence, full canopy (FC), and late canopy (LC)), for ‘Russet Burbank’ and ‘Innovator’ crops (means are the average of both cultivars), grown in fields with or without potatoes in their last 10 years’ cropping history, in Canterbury, New Zealand. An estimate of the variation associated with predicted means is provided by an average of the 5% least significant difference ($LSD_{0.05}$).

| Growth stage | No potatoes | Potatoes |
|---|---|---|
| Crop emergence | 0.1 | 0.3 |
| FC | 4.3 | 6.3 |
| LC | 3.8 | 6.0 |
| $LSD_{0.05}$ | 1.4 | |

## Conclusions

The methods described here to measure severity of RSC have provided robust and relevant results in several case studies. In each case study, RSC severity was either a key variable or was measured to support results obtained for other key variables such as yield. The methods were used in situations where different amounts of RSC occurred, for individual plants in a glasshouse to plots from field experiments with areas greater than 100 $m^2$. In all the case studies described, the methods were able to measure low RSC severity, and did not detect false-positives.

All the data collected in these case studies resulted in key findings for New Zealand’s potato industries. These methods provide standardised measurements of RSC severity in potato crops, which are likely to make data collected by different users comparable, and can be used for meticulous and large scale analyses (e.g. meta-analysis).

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