

Final Report

Investigating soil pH and nutrition as possible factors influencing Pink Rot in potatoes – a pilot study

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Delivery partner: University of Tasmania

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PT19000

Project:

Investigating soil pH and nutrition as possible factors influencing Pink Rot in potatoes- a pilot study (PT19000)

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Public summary

Pink rot caused by *Phytophthora erythroseptica* is a key soil-borne disease characterized by pink colouration of the cut tubers and a distinctly unpleasant odour. The rapid rotting of the tubers, whether in ground or in storage, results in significant yield losses and product downgrades or rejection, impacting the financial viability of growers in key potato production regions of Tasmania, and to a lesser extent, small regions on mainland Australia.

With fungicides becoming less effective there is a need to assess other putative control options. A prior hydroponics study in the USA identified that Ca applications and/or the raising of soil pH to greater than 7.0 corresponded with reduced stolon and root infection by *P. erythroseptica*, although no work on pink rot tuber disease was undertaken. Combined with anecdotal evidence of the positive impacts of Ca application on soil and plant health in general, there was a motivation by industry to test whether Ca application and/or pH modification may provide a sustainable disease control option. Additionally, there was a need to test what other soil factors may contribute to increased likelihood of disease and catalogue through a review of the literature what is known of the disease and what knowledge and research gaps remain. Analysis of historical work on pink rot and industry perceptions were also utilized to confirm outcomes and identify future research priorities.

Field work from 19 different sites over the two growing seasons (2020/21 and 2021/22) provided ample opportunity to identify factors important in pink rot disease expression. While rainfall was a key driver, the intricacies of topography and importance of soil physical factors, including poor structural quality and reduced topsoil depth, were key determining factors of increased pink rot disease. Other physical factors, including row orientation, frequency of row intersections, and reduced mound height was also associated with a greater likelihood of pink rot. Physical crop damage, whether it be through tractor-traffic, irrigator runs, or stem wind damage also can predispose crops to a greater chance of pink rot as does the presence of other soilborne diseases including root galling and powdery scab, rhizoctonia canker, and Scerotinia, amongst others. All of the above factors are considered important avenues of future proposed work on pink rot.

Where specific chemical fertility treatments including calciprill, OzCal, nanocal, Platinum Plus, calcium nitrate, gypsum were tested at six different field sites and in two separate pot trials they provided no benefit in negating pink rot tuber symptoms and *Phytophthora* induced stem die-back. The trials also confirmed that non-practical large quantities of calcium product would be required in typical ferrosol potato growing soils to alter soil pH above 7.0.

Pink rot remains a complex disease with many factors contributing to potential epidemics. Future research should look at a number of these factors that will provide a better understanding of pathogen dynamics and disease outbreaks. Further research on pink rot should be supported within an agile longer-term research program that can quickly adapt to industry feedback. Key areas of focus should include interactions with other soilborne pathogens, the role of key physical soil factors, strategic soil amendments and managing inoculum through a better understanding of the roles of potato volunteers and alternative hosts.

Keywords

Soilborne disease; Pink rot; Potato; PREDICTA; Research gaps

Introduction

Phytophthora erythroseptica is a soil borne oomycete and the primary causal agent of pink rot in potatoes (*Solanum tuberosum*). The disease is characterized by pink colouration of the cut tubers and a distinctly unpleasant odour. The rapid rotting of the tubers, whether in ground or in storage, results in significant yield losses and product downgrades or rejection. Pink rot is a disease of increasing importance, particularly in key potato production regions of Tasmania, and to a lesser extent, small regions on mainland Australia.

Currently disease management relies on several integrated strategies with the usage of soil-applied fungicide treatments the key industry control strategy. However, consistent with work overseas where resistance to key chemistries targeting pink rot have been recorded, evidence has revealed fungicide resistance breaking strains of the pathogen in Tasmania (Chitrangi et al. 2019) which may help explain increasing industry concerns with pink rot disease over the last 5 years particularly. This highlights the need for a more integrated approach to management with pursuit of other putative control avenues. In addition, the usage and testing in field of molecular soil tests currently under development may enable better risk quantification (PT15008).

There has been little investment in pink rot research within Australia, in recent times. Substantive projects have occurred approximately 20 years ago (PT97004, PT01042), but since then research has been limited as noted in a recent review (PT13013) apart from some investment in soil detection and risk analysis (PT15008) and some anecdotal observations from industry suggesting a possible association between calcium addition and/or high usage of acidifying fertilisers and incidence of pink rot (PT19000, RFP). No major research projects within Australia have recently targeted control options for this disease. However, work in the USA in hydroponics has identified some possible beneficial effects of raising pH ≥7 (Benson et al. 2009a) and added Calcium (independent of pH, Benson et al. 2009b), in negating colonization of root and stolon tissue. Whilst this work was preliminary and didn't explore pink rot infection within the tuber, it is worthy of further investigation – particularly to see if the beneficial effect results in less tuber disease and to see whether the research is applicable to a soil system within Australia. All the key soil types in Tasmania that potatoes are grown on are acidic in nature (pH 5.5-6.5 – Cotching et al. 2009) with some highly buffered i.e. resistant to pH change to the levels suggested to be inhibitory to disease (ferrosols). Others, tennosols (sandy soils) are less buffered and more amenable to pH modification. The promising results from the US are worthy of independent detailed investigation. Additionally, the role of other physical soil factors (compaction, soil structure) is often noted as contributing to increased soilborne diseases were also considered worth measuring to see if they are associated with pink rot disease control.

Given the financial significance of pink rot, this small pilot project was initiated to determine if:

- pH and or Ca modification of soils can aid in disease control
- identify other additional factors (landform and/or soil physical factors) that may impact disease control
- catalogue by a literature review known information and clear research gaps of this pathogen/disease.
- Provide to industry clear direction for future project ideas

Methodology

This project linked heavily with industry and growers which provided a feedback mechanism to optimize and alter research initiatives that were industry relevant.

The work was broken up into several components:

Literature review. A key early component of the project was to comprehensively review the relevant literature (with a focus on peer reviewed journal articles and validated reports) on the role of pH and nutrients on pink rot and other soilborne potato diseases. The scope of this increased through the project to include broader topics of relevance to pink rot, given the limited literature on the role of pH and nutrients on the disease, and also included what are some key knowledge gaps and opportunities in managing this disease. The review utilised relevant literature from both Australian and International sources, and also incorporated relevant industry perceptions and ideas. In addition to collation for reporting for Hort Innovation and industry, the review is to be published in a peer-reviewed scientific journal to

authenticate the high level of independent scientific expertise used in its production. This review, along with industry consultation helped to optimise the specifics of the survey work, historical analysis and pot trials.

Field surveys/trials. In 2020/21 (Year 1) and 2021/22 (Year 2) eleven and eight field sites respectively, were chosen from areas with a known history of pink rot including Sisters Beach, Sassafras, Scottsdale and the Midlands. Survey assessments from every field included:

- Pathogen detection through the season using Predicta Pt
- Soil chemistry, with a focus on calcium and pH
- Soil structure and depth, and landform variation across paddocks
- Incidence of disease through the season and at harvest (pink rot and other tuber diseases).

In addition to the survey work in Year 1 and 2 five and one site respectively included specific field testing of calcium and or/alternate fertiliser products. This included the usage of either pre-spread Calcium products (e.g. OzCal or calciprill) and in-furrow treatments (Nanocal, Platinum Plus) with three to five replicates used to test treatment effects.

Of note methodology for pink rot disease assessment was altered slightly between Year 1 and 2 particularly for the survey work. While 3 to 5 larger replicates (10 plant plots) were assessed in Year 1 this was changed to 20 smaller replicates (3 plants per plot) in Year 2 to account for the highly variable expression of pink rot disease in the field.

Additionally, historical analysis of a prior trial (SA Grid Trial) was also conducted through further soil analysis and correlation with prior pink rot disease data to determine if a relationship between soil pH/Ca and pink rot existed.

Pot trials. The key objectives of the pot trials were to study the role of pH, Calcium level (independent of pH), other acidifying fertilisers on pathogen load & pink rot expression in controlled experiments.

The first series of pot trials (2021) examined pH and Calcium effects across two soil types (organic loam, ferrosol) on pathogen proliferation and disease development. For pH modification experiments soils were treated with OzCal to attain 4 different pH levels. For Calcium modification experiments (independent of pH) we applied calcium sulphate (gypsum) at four different levels as this will not modify soil pH, but provide differential calcium levels. We also tested three different pathogen levels and took PREDICTA single species pink rot samples 3 weeks post inoculum application. We quantified soil nutrients (from bulk samples) prior to planting Russet Burbank tubers and after completion of the pot trials (from each pot in 3 replicates).

In a second series of pot trials (2022) we also looked at the role of acidifying and less-acidifying nitrogen sources as well as tested a range of OzCal and gypsum levels from Year 1 trials. These treatments were across two soil types (sandy loam, ferrosol) but we only used one level of pathogen inoculum in Year 2 trials which was a sequential application at three separate dates. Soil nutrients were quantified as per the 2021 trial with Russet Burbank also the variety grown.

In both glasshouse experiments we examined plants at the early growth stage (2 weeks after emergence) for stolon/stem dieback with all tubers harvested at plant senescence for pink rot disease assessment.

Industry feedback and future R and D recommendations. Engagement with farmers, industry and the potato sector was through non-formal and formal discussions and collaborative field work activity, particularly with the growers most impacted by pink rot. Discussions and the identification of common themes was used to identify future R and D direction.

Results and discussion

There were four broad areas of work that were considered:

Literature review.

Presented as a condensed appendix this Review incorporated industry feedback and published literature. The initial literature review scope was increased through the project due to the very little information on pink rot management and very little evolving research. The identification of research and knowledge gaps in the review therefore became a major component of the review. The fact that there are only 27 recent peer reviewed articles worldwide (since 2010), on pink rot, with the majority of those on fungicide resistance, suggests that this soil-borne pathogen receives little critical study (Tegg and Wilson 2021). The review therefore provided a large section on knowledge and research gaps which will provide a scientific basis to future investment decisions.

Field surveys/trials.

From the nineteen field surveys conducted over two cropping seasons the detection of the pathogen *P. erythroseptica* just prior to planting was difficult with only 2 of the 19 sites recording a positive detection, this was despite 12 of the 19 different sites going on to record significant pink rot at harvest. Sequential pathogen assessment was able to detect presence of pink rot more accurately as the season progressed, most probably a function of increasing soil warmth and moisture. Soil sampling and risk detection pre-plant therefore needs to consider a greater density of sampling and/or enrichment techniques to better elucidate the presence of the pink rot pathogen. Additionally, the consideration of soil sampling timing may be warranted.

Significant disease occurred across 12 of the 19 sites and whilst site topography and aspect, water run on, was often associated with pink rot other soil factors were also important. Poor physical soil quality and reduced topsoil depth was often associated with the presence of pink rot (eroded slopes, poor structured and damaged headlands) and is worthy of further investigation, either through direct measurement or through associated tools (EM38 mapping). Physical attributes of some fields including frequent intersection of rows and headlands were also associated with a greater chance of pink rot disease and could be better managed by growers. Additionally damage by other soilborne pathogens was observed to also increase the likelihood of pink rot disease and is an area strongly supported for future study.

The impact of applied calcium products (Nanocal and/or Calciprill/OzCal) added either pre-spread, in-furrow and/or surface spray, at 6 research sites indicated that there was no discernable reduction in pink rot disease, compared to untreated control plots. While Calcium applications were able to slightly increase soil pH in a few circumstances the amount required to get soil pH's up to the reported efficacious range (7.0-7.4; Benson et al. 2009) was not considered viable or practical due to the large quantity of product required on a highly buffered typical ferrosol soil. The recommendation is that small, regular applications of suitable Calcium products is a proactive measure of maintaining overall soil health, in combination with regular soil nutritional testing.

Field trials and the inconsistent and sporadic nature of soilborne diseases can make the assessment of trial work difficult. Part of our field work identified that a greater number of replicates (up to 20) instead of traditional numbers (3 or 5) may be necessary to accurately determine the effectiveness of pink rot control options and experimental work in the future.

Separate analysis of a prior SA grid trial indicated that while pink rot was associated with wet areas where other rot pathogens were also present, analysis showed that the higher pH (>7 (H₂O) at harvest was more likely to see the presence of tubers expressing pink rot disease. Calcium levels weren't as obviously linked with pink rot disease. This work on a sandy loam provides some preliminary evidence that an increased pH does not negate pink rot disease expression, within the constraints of this specific trial site.

Pot trials.

Pot trial work indicated that extremely large quantities of given calcium products were required to alter pH to reported efficacious levels (pH 7.0 – 7.4; Benson et al. 2009). For organic and sandy loams the level required was less than that necessary for a typical ferrosol (t/ha) but well above the standard grower application. The impacts of the applied Ca and alternate products on both *Phytophthora* induced stem die-back and subsequent pink rot was non-significant with all treatments producing die back (particularly more obvious in the poorer structured loam soils) and pink rot on the tubers,

comparable with the non-treated controls. It should be noted that pink rot symptoms on the tubers were not recorded in Year 1 trials but were present in Year 2 trials, which was a functional of cooler environmental conditions in Year 1. As a result procedures in Year 2 were modified to include warmer glasshouse conditions and sequential application of the pathogen through the season. Future pot and controlled pot trials should endeavor to use this more frequent pathogen application to better guarantee disease outcomes for the testing of experimental treatments.

The results of the pot trials supported the findings of the field trials in that Ca and/or pH adjustment provided no discernable control of pink rot symptoms.

Industry feedback and future R and D recommendations.

Through speaking with farmers, industry and the potato sector it is clear that a lot of knowledge relating to pink rot management sits with these people. Many speak of the significant financial impact of the disease and the need to accelerate and advance research through multiple avenues. Evidence within Australia suggests little peer reviewed and significant investment in pink rot since the 1990s where a SA project looked at putative management options. Since then, only smaller (pink rot detection diagnostics) and highly specific (this project) research has occurred; there is a distinct need to take a multi-faceted approach to combating this disease.

We have recommended a number of projects that would benefit the industry with 2 pages devoted to this in the final report. We recommend that such projects are longer-term, agile and adaptable, and provide a multi-pronged approach.

Outputs

The standard outputs from the RTP included the program logic, monitoring and evaluation plan, a project risk register and a stakeholder engagement/communication plan (at Milestone 102 – Sept 2020). Additionally, there was a 12 month milestone report (Milestone 103) and this final report presented here.

Key Outputs from this project also include:

Table X. Output summary

*Extension, communication and written articles included:

<https://ausveg.com.au/articles/pink-rot-in-potatoes-new-project-to-investigate-impact-of-soil-ph-and-nutrition/>

(AUsveg article – 3 Sept 2020)

<https://www.examiner.com.au/story/6861997/research-to-help-more-potatoes-end-up-on-plates/>

(Examiner article – 3 Aug 2020)

https://ausveg.com.au/app/uploads/2021/12/Potatoes-Australia-PotatoLink-Summer-2021_2022.pdf (Summer -2021/22) pp60-62.

<https://www.youtube.com/watch?v=PUrwAvYxAv8&t=14s> (PotatoLink Webinar – Nov 10 2022, R. Tegg)

Outcomes

The outcome of this project is that the potato industry have access to independent research results and analysis relating to pink rot. There are two parts to this. Firstly, Hort Innovation will have reasoned and informed recommendations for decisions on future investment by levy payers into whether management options such as pH and nutrient (fertiliser) manipulation for pink rot require further scientific study – in the case of this work it suggests no future specific investment in this area.

Secondly, Potato growers, and researchers, will have access to new information on pink rot (scientific information through the literature review, grower friendly articles through industry articles and the communication program – PT17002).

We expect that growers will use this information, where possible, to improve the productivity and long-term term sustainability of their business.

Table X. Outcome summary

Monitoring and evaluation

Table X. Key Evaluation Questions

Recommendations

This project has a clear mandate to identify research gaps and opportunities to address understanding of pink rot disease and better inform potential management options moving forward

From the key project aims; **1) investigate impact of soil pH and Ca formulations in field and pot**, there is little to recommend; such an approach is not a useful or practical means for pink rot control, but the application of smaller, regular and strategic applications of Ca formulations in improving general soil health may be worthwhile. **2) investigate impact of landform and soil structure**, identified that soil quality, topsoil depth and other features that impact water movement can impact pink rot, and further study in this area is recommended. **3) identify knowledge gaps and opportunities from literature and industry**, these are listed below and are a key outcome of this project:

Future areas of R and D include:

Improved detection and risk management of pink rot

- What sampling density and/or timing of sampling is optimal for pathogen detection in field soils given the sporadic and temperature sensitive nature of the pathogen
- Can the pink rot pathogen within soil samples be concentrated using targeted microbial concentration techniques and/or enriched for better detection using suitable bait plants to optimize pathogen detection and avoid false negative tests.
- What other tools (e.g. measurements of physical soil parameters) may complement pathogen detection to provide growers an improved pink rot risk indicator.

Understanding pink rot and P. erythroseptica across the landscape and in different production systems

- Is soil the only source of pathogen? What is the role of seed, irrigation water and water run-off in pink rot epidemics.
- What is the impact of cropping system on likelihood of pink rot disease (e.g. intensive cropping vs cropping integrated with livestock production).

Understanding interactions between soil-borne potato diseases, physical factors and disease management practices

Pathogen interactions –

- How does the presence of other soilborne pathogens (powdery scab, nematodes, black dot, Verticillium, Sclerotinia, bacteria and water rots) affect plant susceptibility and incidence of pink rot.
- What are the mechanisms of these interactions and how can this knowledge be used to develop more robust and integrated management tools for pink rot and other soil-borne diseases.

Physical factor interactions –

• What are the critical soil physical thresholds (of topsoil depth, soil structural quality, minimum mould height, other parameters) that lead to an increased likelihood of pink rot disease. What tools (EM38, others) may provide useful measures of soil physical capabilities and thus predict disease risk.

Disease management interactions

- What is the impact of the various registered chemicals commonly used in potato production (e.g. amistar, other fungicides, etc..) on non-intended targets (other pathogens, beneficials and soil biology) and on subsequent pink rot incidence.
- What novel soil amendments could assist in reducing risk of pink rot disease

Determining the role of alternative hosts and volunteer potatoes in maintenance of soil-borne pathogen populations

• To what extent does commonly occurring alternate hosts within the rotation (ryegrass, carrots, etc.) exacerbate

soil-borne pathogen levels and risk for the following potato crops.

• What density of potato volunteers supports pathogen populations that increase risk to a following potato crop; what life-cycle phase of the potato supports maximal pathogen population and when should volunteer control occur.

Healthy soils by management of inputs

- Sustainable management of cropping inputs (especially N fertilizer) and soil management practices to improve general soil health and the robustness of cropping systems.

Industry led activities

- Increased availability of cultivars with greater resistance to pink rot.
- Alternative chemical controls (fungicide options) and a better understanding of the effectiveness of current chemical controls.

Guiding principles for future R and D on soilborne diseases

Research programs should not be short-term (a minimum of 3 years duration, ideally 5 years and on-going), to ensure:

- Field based studies of soil borne disease (e.g. assessment of rotation/cropping systems) requires multi-year trials that cannot be accomplished in short-term projects
- Continuity in expertise and retention of expertise within the potato research sector
- Maintenance of key soil pathogens for future research (re-establishing such collections is time consuming)

Research should not have a single focus and needs to be agile and adaptive to industry feedback:

- This project showed that calcium applications and/or pH modification do not represent a silver bullet for pink rot control and that targeting only one aspect of disease control can be dangerous.
- This project partly evolved from a narrower to a broader perspective of adding more parameters of interest (pathogen quantification, soil quality and topography factors, cultural practices (row orientation) such that the outcomes would provide a greater insight into pink rot disease epidemics.
- In terms of pink rot there are multiple interacting factors that will need to be implemented to better reduce the financial impact of this disease and research in any one of these areas would be beneficial, although a broader program encompassing more than one factor would leverage better outcomes for industry.

Research needs to be locally relevant

- Although we are part of a global world and research insight from other countries provides much benefit there are circumstances where research needs to be locally focused and specific to our growers.
- In the case of pink rot management, volunteer control is regarded as critically important in reducing soilborne inoculum, yet in the US where cold frosts knock out the volunteers, this is not even considered or important.
- Additionally local paddock, climate and the vagaries of managing smaller and topographically challenging paddocks within Tasmania requires local, specific and a targeted approach.

Prioritization of R and D

It became clear that through discussion with industry and through scanning relevant literature there remain many unknowns related to understanding and managing pink rot. Targeting multiple avenues and projects would be beneficial, however we rate the urgent priorities for pink rot as:

- 1) Biological and physical interactions (especially between soil-borne pathogens, their current management tools and soil physical factors).
- 2) Determining the role of alternative hosts/volunteers.
- 3) Determining the impact of different production systems on disease risk.

We note that improved detection and risk management is important and would play a part within any of the projects above.

We also note the topic Sustainable use of N fertilizer may also provide indirect disease control benefits but provides more a sustainability benefit, through reduced inputs (costs) and less environmental impact.

Refereed scientific publications

Journal article

Tegg, R.S., Cotching, W.E., Rettke, M, Ravilojanan, C.K., Barsolte, E., Balotf, S., Leo, A., Wilson, C.R., 2023. Pink rot of potato – a review and identification of knowledge gaps. American Journal of Potato Research xxx, to be submitted.

Chapter in a book or paper in conference proceedings

Tegg, R.S., Wilson, C.R., 2022. Management of major fungal and fungal-like soilborne diseases of potato. In: Chakrabarti, S.W., Sharma, S., Shah, M.A. (Eds.), Sustainable Management of potato pests and diseases. SpringerLink, Singapore, pp. 525–540.

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- Benson, J 2008, 'Effect of Ca and pH on disease severity of pink rot *Phytopthora erythroseptica* in Russet Norkotah potato *Solanum tuberosum*', MS Thesis, Brigham Young University, USA.
- Chitrangi, R., et al. 2019. Pink rot of potato, a re-emerging problem in Tasmania: isolate diversity, fungicide resistance, pathogenicity & population dynamics. EAPR Pathology & Pests Symposium, Switzerland, 2 Sep 2019.

Intellectual property

'No project IP or commercialisation to report'.

Acknowledgements

We acknowledge the grower and industry support in assistance with particularly the field trials/surveys over both seasons – individuals are noted in that Appendix. We also appreciate the funding from the potato growers levy and Hort Innovation.

Appendices

Appendix 1A. Succinct Literature Review Summary

Appendix 1B. Submitted peer review paper

Appendix 2. Field surveys/trials

Appendix 3. Historical Grid Trial

Appendix 4. Pot trials<

Appendix 2 -Field trial summary – Season 2020/21 & 2021/22

Final Hort Innovation - 10th January 2023

Parent project - PT17000- Investigating soil pH and nutrition as possible factors influencing pink rot in potatoes – a pilot study

Project Funder – Hort Innovation

Project Team - Robert Tegg, Bill Cotching, Michael Rettke & Calum Wilson

Project Institutes - Tasmanian Institute of Agriculture, University of Tasmania, South Australian Research & Development Institute

Project in kind - Serve Ag (Doug Green) and Rob Stanic (Oro Agri) – knowledge and chemicals.

- Simplot Australia Pty Ltd (knowledge, field access, treatment application)

- J. Tubb, E. Blanchard, M. Bowen, D. Wise, D. Briggs, W. Meagher, B. Prins

- McCains Pty Ltd. (knowledge, field access) – Rodney Smith

Potato growers (trial sites, treatment application)

- L. Elphinstone, D. Lohrey, P. Van Es, F. & K. Wagner, N. Whelan, M. McDougall, O. Smith, Coates, Vaucluse Estate, A. Perry, L. Sattler, G. Howard, J. Millwood.

Summary

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Materials and Methods.

Sites and treatments.

Season 1. Sites chosen included specific zones (sites) known to have a recent history of pink rot (Sisters Beach, Scottsdale & surrounds, Midlands) with the following treatment applications applied at each site (Table 1a). Seven of the sites had specific Calcium treatments applied including nanocal (in furrow, Fig. 1) at seven sites and calciprill (pre-spread) at five of those sites as well. All eleven sites were utilised for the field survey work (which included soil chemistry/pathogen/disease assessment).

Table 1a. Overview of the eleven field sites used in the 2020-21 season for field survey work. Also shown are the calcium treatments (calciprill/nanocal) applied at selected sites.

Season 2. Sites chosen based on industry feedback focused on the key Scottsdale and surrounds area with one site (Whelan -upper) used to test a range of Calcium and fertiliser alternatives (Table 1b). These included nanocal (as applied in Season 1), calcium nitrate (applied in furrow as an alternative to standard fertiliser practice) and Platinum Plus (applied in furrow as an alternative to standard fertiliser practice). All eight sites were utilised for the field survey work (which included soil chemistry/pathogen/disease assessment).

Table 1b. Overview of the eight field sites used in the 2021-22 season for field survey work.

* this site included the testing of nanocal, calcium nitrate and Platinum Plus.

Soil sampling for chemistry and pathogen detection – for field surveys

Prior to planting soil samples were taken (in a W pattern – 30 cores per sample) in duplicate (recommendation from PT 15008) from the 1 ha sites for PREDICTA Pt testing and soil chemistry. The fertilizer and the irrigation regime (plan) from the grower was recorded. Site specifics including soil type, structure, topsoil depth, slope, aspect were also recorded.

An in crop assessment (soil, stems) was made at approximately 90 days after emergence, this coincided with early senescence of the crop, which is likely to occur in areas infected with pink rot. As the pathogen inoculum build up in the soil can be seasonal and temperature/moisture dependant we will take soil samples for PREDICTA testing. We will also collect representative stem samples for PREDICTA tissue assessment and record paddock observations (wet, patchy areas, measurement of compaction, die back and eroded areas, etc).

Final disease assessment and soil analysis was undertaken at plant senescence: for disease, a composite sample from 15 randomly chosen areas within the paddock (200 tubers per site) will be assessed for pink rot and other tuber borne diseases. Full soil chemistry tests will also be taken.

Hollow heart mid-crop assessment

Season 1 only. Mid-crop, from selected sites, a minimum of 30 tubers were selected from 10 random plants per treatment. They were cut longitudinally in half and assessed for the presence of necrosis and hollow heart.

Harvest disease and yield assessment – included for field trials only (where Ca treatments were used)

Season 1. Just prior to commercial harvest when the crop was senescing plots were harvested for disease and yield assessment. From each individual treatment plot 10 continuous plants in a row were randomly selected from each of the three replicates. Each plot was double dug with all tubers removed and placed in hessian bags. Total tuber number and weights were recorded and the number of pink rot tubers also recorded. All bags were sub-sampled, with a minimum of 50 tubers then assessed for presence and severity of other tuber diseases including powdery scab, common scab and black scurf. A random selection of 20-50 tubers per individual treatment plot were also assessed for hollow heart as described above.

Season 2. Due to the random and sporadic distribution of pink rot disease assessment methodology was modified to include a greater replicate number per plot assessed. Twenty randomly selected plots (each consisting of 3-4 plants and a minimum of 20 tubers) were hand dug and assessed for visual disease symptoms (mainly pink rot, powdery scab). For pink rot a selection of tubers were cut for confirmation of symptoms.

Site characterisation

Season 1 and 2. All sites were assessed at or just prior to planting to gain an insight into soil quality (See Supplementary Figure 1), soil depth and topography patterns. Assessments were undertaken by field digging in a transect across the paddock to account for maximal variation in one given field. Sites were re-evaluated at or near crop senescence to see if early die back and or pink rot could be associated with soil quality parameters.

Fig. 1. Typical site shown a) at planting, and b) from aerial map (Whelan, Cuckoo site) in 2020/21. Photo taken 7th Nov 2021 by R. Tegg.

Results & Discussion.

Soil chemistry and pink rot pathogen levels – pre-plant:

Season 1. Soil assessments just prior to planting indicated that only one site was positive for *P. erythroseptica* – Vaucluse lower (Table 2). Levels of Ca and pH were variable across the sites examined. Critical low levels for Ca are <1200 and for pH<5.50, shown in red text.

Table 2. Initial pathogen levels, Ca levels and pH across the trial sites just prior to planting.

Season 2. Soil assessments just prior to planting indicated that only one site was positive for *P. erythroseptica* – Whelan upper (Table 2a). Levels of Ca and pH were variable across the sites examined although none were identified at critically low levels (Ca <1200, pH<5.50).

Hollow heart (mid-crop assessment).

Season 1 only. A snapshot assessment of tubers at key trial sites showed that hollow heart was quite prevalent at 2 of the sites assessed, specifically the Lohrey and Wagner site (Table 3, Fig 2). Other sites assessed had a nil - low presence of hollow heart. Nanocal produced a slight reduction in hollow heart at all sites where it was observed, compared to the untreated control.

 $\sqrt{30 - 42}$ tubers were assessed per treatment

Fig 2. Typical cut tubers examined for hollow heart from the a) control, and b) nanocal treated plants from Winnaleah (NE Tas) at approx 11 weeks after planting. Photo taken 23rd Feb 2021 by R. Tegg.

Soil chemistry across the season from non-treated control plots:

Season 1. From all the control plots across all field survey sites there was a significant reduction in pH through the season in 7 of the 11 sites assessed (see red text, Table 4). Calcium levels tended to decrease in some sites (e.g. Elphinstone, Smith) show little change (Perry, Lohrey) or a slight increase (Wagner) which may have been a function of individual site or grower management.

Table 4. Changes in Ca and pH from the 11 sites as measured prior to planting (start) and at harvest (end).

Season 2. From all control plots across eight sites there was a reduction in pH recorded with the most significant being at the Whelan (upper), the South Scottsdale site and the Bridport site (Table 4a). Calcium levels showed variable responses that were grower and site specific.

Table 4a. Changes in Ca and pH from the 8 sites as measured prior to planting (start) and at harvest (end).

Soil chemistry across the season from calcium treated plots:

Season 1. Of the seven sites where calcium treatments were applied calcium levels tended to not vary much between control and treated plots at harvest apart from the Elphinstone and Wagner site where there were some increases in Calcium with the nanocal treatment (Table 5, shown in light green). The calciprill treatment improved Ca levels at the Elphinstone, Van Es and by a small amount at both the Vaucluse sites (Table 5, shown in dark green). The soil pH levels tended to be in the acidic to partly acidic range with nanocal slightly increasing pH at the Vaucluse upper site and the Wagner site. Calciprill increased pH at the Lohrey site and both Vaucluse sites. Through the season the Vaucluse site showed the greatest reduction in pH with initial levels of 6.43 – 6.60 declining by over 0.5 units by the end of the season.

Table 5. Ca levels and pH – measures at planting date and at harvest (with/without nanocal/calciprill) at the seven trial sites in season 2020/21.

Season 2. At the one site where Calcium and/or alternative treatments were applied (Whelan – upper) CaNO3 treatments were able to maintain higher pH than when treated with Platinum or Nanocal (Table 5a).

Table 5a. Ca levels and pH – measures at planting date and at harvest (with/without Calcium and/or alternative treatments) at Whelan upper site in season 2021/22.

Pathogen proliferation

Season 1. Whilst *P. erythroseptica* was only identified at one site at planting, periodic soil assessment was able to detect the build up of *P. erythroseptica* across the majority of sites. Pathogen build up was more related to topography than treatment impact and was quite sporadic. The site where the pink rot pathogen was first detected was at the Van Es site where it was detected in early December (Fig. 3).

Season 2. Similar to season 1 the pathogen was only detected at one site at planting with detections increasing from the majority of sites as the season progressed (Fig. 3).

Fig 3. Pathogen (*P. erythroseptica*) levels detected from soil through the growing season at multiple sites across two separate seasons; top – 2020/21, bottom 2021/22.

Harvest assessments

Pink rot disease – across each site.

Season 1. Pink rot disease was sporadic across the sites and treatments with the range of pink rot percentages across the whole site quite large, best typified by the Whelan site that had some plots with 0% and one plot 40% pink rot incidence (Table 6). Those where pink rot were considered a significant issue by the grower and/or field officer are shown by red coloured text, indicating that 8 of the 11 sites had some issues with pink rot in the 2020/21 season.

Table 6. Description of pink rot and percentage ranges across each whole site (2020/21)

Season 2. Pink rot disease was assessed using slightly different methodology in Year 2 but identified similar trends to Year 1, with all sites recording some pink rot, with 4 of the 8 sites classed as having moderate pink rot disease symptoms – shown in red (Table 6a). In those cases the crops were not generally suitable for storage due to percentages of pink rot tubers present. It should be noted that some of the sites with only minor pink rot were also not considered suitable for storage, particularly when taking into account relatively high incidence of other rots i.e., Bridport site.

Table 6a. Description of pink rot and percentage ranges across each whole site (2021/22)

Calcium treatment impacts on disease and yield.

Season 1.Taking into account the site variability and sporadic nature of the disease, as reflected in the site differences above (Table 6), the presentation of the impact of calcium treatments on disease and yield is shown below.

NW sites: Firstly the impact of nanocal on pink rot disease and hollow heart is shown from the three trial sites in the NW region, Elphinstone, Lohrey and Van Es (Table 7, Fig 4). Across these three sites there were variable results with nil disease at Elphinstone's and up to 12% incidence at Van Es. Nanocal was able to reduce pink rot at the Van Es site.

When looking at the impact of calcium treatments on yield there were variable results which resulted in no significant yield impacts (Table 8). Of note, the nanocal treatment at the Lohrey site, increased yield (shown in green) but not significantly.

Table 7. Impact of calcium treatment (nanocal) on pink rot and hollow heart at three trial sites in NW Tasmania – Season 2020-21

*Paired comparison shows a significant reduction in symptom incidence at P<0.10.

Fig 4. Typical cut tubers examined for hollow heart and pink rot from control plants from Sisters Creek (Van Es) at harvest. Photo taken 4th April 2021 by R. Tegg.

Table 8. Impact of calcium treatments (nanocal/calciprill) on yield (kg's from 10 plants) at NW Tasmania – Season 2020-21

Vaucluse (Northern midlands sites):

Two sites (bottom – lower area; top crest area) in a large commercial field at Vaucluse produced variable but high levels of pink rot (18.4% - bottom; 10.1% top, Table 10). Analysis of calcium treatments showed that both nanocal and Ozcal reduced pink rot compared to the control, although not by a statistically significant difference (Table 9). Both calcium treatments increased the average tuber size and total tuber weight, although this was not statistically significant. It should be noted that test strips were parallel to each other although the Ozcal treatment was positioned 50 m to one side (Fig 5), so may of also partly contributed to the higher yields in these plots. Of the other diseases assessed (powdery scab and common scab) levels were very high with every tuber infected (Fig 6). Comparison of disease severities showed that common scab was greater, although not statistically different, from the Ozcal plots.

Fig 5. Overview of trial sites and treatments at Vaucluse in the 2020-21 season. Photo taken 10th Feb 2021 by R. Tegg.

Table 9. Impact of calcium treatments on yield (kg's from 10 plants) and disease from Vaucluse, Northern Midlands – Season 2020-21. Results are averaged from two sites (with 3 replications per treatment) within the commercial field.

Site position	Av	Total	Total	Pink rot (%	Common	Powdery
	tuber	tuber	tuber	incidence)	scab (av.	scab (av.
	wt (g)	No.	wt (kg)		Severity)	Severity)
					$0-6$	$0 - 6$
Top site	166	65	10.91	10.1	3.36	2.83
Bottom site	180	65	11.59	18.4	3.88	2.68
LSD	ns	ns	ns	ns	ns	ns
F prob	0.398	0.593	0.093	0.098	0.133	0.724

Table 10. Impact of site position in field on potato production (per 10 plants) at Vaucluse

Fig 6. Typical tubers from the Transformer control treatment at Vaucluse – showing significant common and powdery scab symptoms. Photo taken 12th April 2021 by R. Tegg.

NE sites (Scottsdale and surrounds):

The impact of nanocal on pink rot disease, hollow heart and yield is shown from the two trial sites in the NE region (Table 11). Yield was not impacted by calcium treatment, nor was hollow heart which tended to be site specific and a lot higher at the Wagner site. Pink rot was very low at the Wagner site and was highly sporadic at the Whelan site, where nanocal tended to increase pink rot, but only at P<0.10.

Table 11. Impact of calcium treatments on yield (kg's from 10 plants) and disease

*Paired comparison shows a significant reduction in symptom incidence at P<0.10.

Other tuber diseases

Other tubers diseases such as powdery scab are shown at 4 sites although there was no significant relationship between applied calcium and powdery scab percentage incidence or severity (Table 12).

Table 12. Impact of calcium treatment (nanocal and /or calciprill) on powdery scab incidence (%) and severity at 4 trial sites in Tasmania – Season 2020-21

Worth noting was the presence of black scurf at the Wagner site where nanocal was shown to reduce the percentage incidence of the disease, although this was not quite statistically significant (P = 0.110, Table 13).

Table 13. Impact of calcium treatment (nanocal) on black scurf incidence (%) at Winnaleah in NW Tasmania – Season 2020-21

Season 2. One site (Whelan – upper) was examined for the impacts of calcium treatments and alternative fertiliser sources on pink rot and other rots (Table 13a). Overall there was low to moderate disease with all plots showing some infection but no statistical differences between the treatment plots. This was consistent with the results from the six sites in Season 1.

Table 13a. Impact of calcium treatments (nanocal) and alternate fertiliser sources (Calcium Nitrate, Platinum Plus) on pink rot at Whelan (upper site) NE Tasmania – Season 2021-22

Key observational findings from the sites including topography and soil quality assessments:

Season 1. Legerwood (NE) – Observational work showed compacted areas within the Smith paddock (Fig 7) that were more prone to pink rot. Also prevalent were mixed infections and diseases with the presence of pink rot, Spongospora galling and stem Sclerotinia.

Figure 7. Image of Smith field at Legerwood (top) showing zone along bottom of paddock that was compacted and expressing significantly more pink rot infection than the higher non-compacted area of the field. Images below show typical pink rot symptoms, and mixed symptoms (pink rot, Spongospora galling and stem Sclerotinia). Photo taken 10th Mar 2021 by R. Tegg.

Legerwood (NE) – the Coates site below resulted in 40-50 % losses attributable to pink rot and was characterised by early die back (Figure 8) and many senesced spots across the whole paddock. This site was also characterised by a low soil pH at harvest (5.37 – See Table 4).

Figure 8. Image of Coates field at Ledgerwood (top) showing area of paddock that was assessed with early die back observed across the paddock (bottom and upslope). Digging these areas revealed significant pink rot (bottom two images). Photo taken 10th Mar 2021 by R. Tegg.

General assessment of soil quality, depth and topography was used to understand the landscape patterns and relationship with crop performance. There was variability across most sites typical of Tasmanian topography (Supplementary Table 1). At the Lohrey site (Table 14, Fig 9) there was distinct variation in topsoil depth and soil structure score through a transect up the paddock. The headland gate area was compacted and scored the poorest soil structure and was associated with poorer quality crop growth.

Table 14. Site assessment characteristics of the Lohrey site at Sisters Creek, NW coast, season 2020- 21.

Fig 9. Visual image of the Lohrey site at Sisters Creek, NW coast, season 2020-21 showing variability across a field. Photo taken 28th Mar 2021 by R. Tegg.

Analysis of a nearby site, Van Es showed interesting topography and soil depth changes that was partly associated with earlier and greater pink rot disease expression (Fig 10). Where soil depth was less and slope greater, dieback and pink rot occurred in those regions.

Fig 10. Visual image of the Van Es at Sisters Creek, NW coast, season 2020-21. Images taken Nov 2020 to Mar 2021 by Ed Blanchard.

Season 2:

Cuckoo (lower marsh site) – This site was characterised as putatively not suitable for planting due to water pooling and or boggy situation (Fig. 11). The soil quality assessments prior to planting indicated a very poor soil structure within the boggy component of this field with ratings of 2 (See Supplementary Table 1) and a distinct odour of sulfurous gases suggestive of anaerobic conditions. That this site recorded significant pink rot (see Table 6a) is not suprising and indicates the importance of topography and site selection.

Figure 11. Impacts of topography and water pooling on pink rot development at Cuckoo, NE Tas. Shown is the water build up and early senescence (die back) in parts of the crop in Feb (left image) and the almost complete die back in April. Photos taken 16th Feb 2022 and 6th April 2022 by R. Tegg. **North Scottsdale (Gully site)** – this gully site had areas of different topography from run off to run on with the majority of pink rot occurring at the bottom of the slope where water tended to pool. In the base area 5 of the 20 plots were infected while in the hill area only one of the 20 plots recorded pink rot infection (Fig 12). Where pink rot infection was recorded on the hill it was related to a change in row architecture or orientation that encouraged water pooling area in that area (see section below).

Hill (1/20 plots with pink rot)

Base $(5/20)$ plots with pink rot)

Preplant

Harvest

Figure 12. Impacts of topography on pink rot development. Image of sites at North Scottsdale preplant and at harvest. Hill area (blue circles) shows an area of run off less prone to pink rot in contrast to the base (red circle) that was more prone to early die back and tuber pink rot. Photos taken 13th Oct 2021 and 15th Mar 2022 by R. Tegg.

Legerwood site – this site recorded moderate levels of pink rot which tended to be slightly related to depressions in the field but also to soil structure within those specific depressions which were of slightly poorer structural quality (ratings of 6) compared to the rest of the paddock (ratings of 7 and 8) – see Supp Table 1. Another observational finding from this paddock and a range of others across the seasons was the presence of inconsistent or different hill/mould heights which can occur on sloping ground or when tractor set-up is not optimised. Digging of moulds of different heights showed that pink rot was a lot more common in the shallower moulds compared to the standard or taller moulds indicating that just subtle differences may contribute to the presence or the absence of pink rot (Fig. 13).

Figure 13. Variability in mound/hill/mould height observed across the two seasons may be contributing to pink rot

Row orientation and how rows may transect each other was also identified as a factor that can contibute to water pooling at the presence of pink rot (Fig. 14). This can occur on slopes in gullys and is also very common in headland areas where a change in row orientation occurs.

Rows 90° to each other

Figure 14. There is a greater chance of pink rot where rows intersect each other at 90 degrees which can result in water pooling and favourable conditions for pink rot. Image is from 15th March at North Scottsdale taken by R. Tegg.

CONCLUDING REMARKS

The results above suggest that Calcium amendments are not a silver bullet for pink rot disease control. Other agronomic and crop performance benefits, although not a focus of this study were identified at some sites and suggests a benefit of calcium application. The topography and site (soil characteristics) provided very good insight into pink rot patterns. Poor soil structure and reduced topsoil depth were often associated with a greater likelihood of pink rot occurring. Additionally, presence of other soilborne pathogens and/or physical damage of plants by wind, tractor was also associated with a greater presence of pink rot. Row orientation (frequent intersecting mounds) and reduced mound depth were also observed to encourage pink rot.

Pathogen detection early in the season generally failed to identify the environmentally and sporadic *P. erythroseptica* pathogen, with levels only becoming detectable mid-season, this makes risk assessment difficult. Insights into other physical soil factors are worthy of further investigation either using direct measurement or other tools (EM38 mapping).

Supplementary Figure 1. Overview of the soil quality scorecard used for soil structural assessments. (Source: soilquality.org.au/factsheets/soil-structure)

Supplementary Table 1. Overview of some key physical soil properties of the 19 sites assessed over the 2020/21 and 2021/22 seasons.

2020/21 SEASON

2021/22 SEASON

GRID TRIAL SA

Summary

As part of a prior historical study within PT15008 pink rot was recorded from multiple points within a dedicated grid trial in South Australia. This work presented here included the specific analysis of soil chemistry at harvest (with a focus on soil pH and Ca) from the 39 plots to establish if a relationship between pink rot occurrence and soil chemistry occurred at this trial site. While pink rot was associated with wet areas where other rot pathogens were also present, analysis showed that the higher pH (>7 (H₂O) at harvest was more likely to see the presence of tubers expressing pink rot disease. Calcium levels weren't as obviously linked with pink rot disease. This work on a sandy loam provides some preliminary evidence that an increased pH does not negate pink rot disease expression, within the constraints of this specific trial site.

Materials and Methods (background to historical trial)

Grid trials were conducted at four centre pivots in South Australia planted with Innovator potatoes. These trials were not targeting pink rot, but growers identified that pink rot may be a risk in these paddocks. *Phytophthora* EDC DNA levels were below detection in all soil samples taken at planting and on peel of seed planted in assessment plots (PT15008).

Two paddocks had challenging topography that resulted in wet and dry areas developing. In one paddock plots were not located in wet areas; however, additional testing of roots and tubers from wet areas did not find any symptoms of pink rot. In the other paddock, pink rot developed in 9 of 39 plots tested, of which 8 were in confirmed wet areas. No pink rot was observed on tubers or detected in the peel of tubers from the other 30 plots grown under favourable to slightly dry moisture conditions. Productivity was compromised and rots were observed in the plots located in wet spots, irrespective of whether *Phytophthora* spp. were present. Other pathogens such as *Pythium* spp. also cause rots in these conditions. Impact of waterlogged soils resulting in unfavorable growing conditions and development of pink rot on yield are shown in Figure 1 (from PT15008).

Figure 1: Relationship between infection level by *Phytophthora erythroseptica*, *P. drechsleri*, *P. cryptogea* and yield (left) and incidence of pink rot and yield (right) of potatoes from plots at grid trial site A in South Australia. Plots that developed pink rot were in low wet areas of the paddock (from PT15008)

Materials and Methods (soil data and pink rot relationship)

Due to pink rot presence in multiple spots within a grid trial setup it was decided to undertake soil chemistry analysis of the 39 plots and determine whether any relationship between pink rot presence and soil chemistry (specifically pH and Calcium levels) was present. Graphical depiction was used to look for any correlation or relationship between the parameters assessed.

Results and Discussion

Utilising the specific soil chemistry results from the trial plots the plots comparing pink rot with soil pH (Figure 2a) and soil Calcium (Figure 2b) are shown. Only one plot that had a soil pH <7.0 recorded symptoms of pink rot. In contrast the other 8 plots that recorded pink rot symptoms all had a soil pH >7.0. This preliminary evidence suggests that a higher pH is not reducing pink rot in contrast to the hydroponic studies conducted by Benson (Benson et al., 2009a and 2009b).

From a soil Calcium perspective (Figure 2b) there was no clear linkage between soil Ca and pink rot disease symptoms expressed. All Calcium levels recorded were above critical minimum stipulated levels (<1200 mg/kg Ca) that can negatively impact plant growth in potatoes. The preliminary evidence from this study suggests that elevated Calcium levels do not reduce pink rot disease which again partly contradicts the findings of Benson (Benson et al., 2009a and 2009b).

Figure 2: Relationship between incidence of pink rot and **A)** soil pH – water, or **B)** soil Calcium, of potatoes from plots at grid trial site A in South Australia.

Pot trials – 2021 (Year 1) and 2022 (Year 2) Updated January 9 2023

Summary

Pink rot is one of the most important soil borne and storage diseases of potato (*Solanum tuberosum*) caused by the fungal pathogen *Phytophthora erythroseptica*. Pink rot can lead to economic loss of the tubers, reduce the grade quality of seed tubers and lead to storage losses. Pink rot predominantly occurs in key production areas of Tasmania, specifically the NW and NE regions. Limited evidence in USA and Tasmania have shown that increasing soil pH and application of Calcium can reduce pink rot disease development, but further investigation is required. Russet Burbank potatoes, the key processing cultivar in Tasmania, were grown in pot trials with treatments applied including calcium amendments (OzCal, gypsum, nanocal), less acidifying fertilizers (CaNO3, Platinum Plus) across two soil types (ferrosol and sandy loam), with soil pH and chemistry, and pathogen development monitored across the duration of the trial. Disease outcomes were measured as impact on stem dieback early season and as pink rot tuber disease at plant senescence.

Year 1. Results indicated that high levels of nutrient supplement, calcium products were required to raise pH levels into the reported efficacious range >7-7.5. Specifically, 4 and 17.5 tonne/ha of OzCal was required to elevate pH levels to the desired range in the organic loam and ferrosol soils, respectively. All gypsum and OzCal treatments significantly elevated calcium levels. Pathogen levels measured within the pots were reflective of amounts added with greater inoculum added resulting in higher detectable levels of *P. erythroseptica*. Levels were not impacted via calcium treatments. However levels of *P. erythroseptica* did decline through the experiment, possibly due to cool soils, resulting in the need to top up inoculum levels half way through the experiment. Early season die-back of shoots/stolons was observed randomly across treatments with no observable benefit of the calcium products. There was little pathogen remaining in the soil at harvest and associated with this very little tuber disease to differentiate treatment differences.

Year 2. This experiment took on board the learnings from Year 1 and utilised higher pathogen levels with sequential pathogen application. It also increased glasshouse temperatures to approximately 23 degrees C which resulted in *Phytophthora* symptoms, both in early growth stages (stolon shoot browning) and at later stages (pink rot tuber disease). Calcium treatments (OzCal, gypsum, Nanocal) failed to reduce or negate disease symptoms. Additionally, less acidifying fertiliser treatments (CaNO3, Platinum Plus) also did not negate disease symptoms. Year 2 trials indicated that under relatively high disease pressure the applied treatments were ineffective for pink rot control.

Across two trials and two separate years which produced negligible to low disease in Year 1 and higher disease in Year 2 the applied calcium treatments were unable to negate *Phytophthora* induced stolon/stem die back or pink rot tuber disease. Whilst treatments were able to significantly alter Calcium levels and pH into the reported putative range the amounts of fertiliser required were very large and not practical. Under the specific conditions of these pot trials it can be concluded that the treatments are not a practical solution for pink rot disease control.

Materials and methods

Experimental Setup:

In this experiment we examined the role of altered pH and calcium levels on pathogen production in the soil and disease development in the planted potato crop. Experiments were conducted in a glasshouse environment (20 ± 3 ^O C) at the New Town Research Laboratories, Tasmania.

Year 1. The experimental setup was a randomised block design consisting of four replicants. Treatments included two types of soils, either a sandy organic loam or Ferrosol soil with basal level of standard potato fertiliser applied. There were seven different Calcium treatments, this included a control with no additional calcium added. Three treatments were OzCal, applied at comparable rates of 4-ton, 17.5 ton, and 35-ton/ha; OzCal supplies both calcium and modifies soil pH. The other three treatments were Gypsum at 4-ton, 10-ton, and 20-ton/ha treatments: gypsum acts as a calcium source without affecting soil pH. There were also three levels of *P. erythroseptica* pathogen added to the soil, three weeks prior to planting, P0 (no pathogen added), P1 (5 grains of inoculated millet added) and P2 (20 grains of inoculated millet added). In all there were a total of 168 individual pots in this experiment all planted to the commercial processing cultivar 'Russet Burbank'.

Year 2. The experimental setup was a randomised block design consisting of six replicants. Treatments included two types of soils, either a sandy loam or Ferrosol soil with basal level of standard potato fertiliser applied. There were nine different treatments, this included a control with no additional calcium added. Two treatments were OzCal, applied at comparable rates of 17.5 ton, and 35-ton/ha. There were two gypsum treatments at 10-ton, and 20-ton/ha. There was a specific nanocal treatment (3L/ha) applied in furrow and on the soil surface at tuber initiation. Three additional treatments were applied to reduce soil acidification through the trial: one was CaNO3 as an alternate N source at planting and top-up; one was CaNO3 at planting but with traditional urea at top-up; the third was Premium Plus, an alternate N basal supply applied at planting This trial utilised one level of *P. erythroseptica* pathogen added to the soil, which included 20 grains of inoculated millet 2 weeks prior to planting, followed by a further 20 grains each at 6 and 12 weeks after emergence. In all there were a total of 108 individual pots all planted to the commercial processing cultivar 'Russet Burbank'.

Pathogen preparation:

Pure isolates of the pathogen *P. erythroseptica* were provided by Simplot Australia Pty Ltd (Melbourne, Australia) and they were maintained and bulked on potato Dextrose Agar (Amyl Media, Dandenong, Australia). They were sub-cultured every 2-3 weeks and grown in a dark culture room maintained at 25^0C .

In order to prepare the inoculum for soil application, 250 grams of millet seed (a highly nutritive substrate) was soaked in 400ml of distilled water in a 2L conical flask. The mixture was stirred and left to sit in room temperature for 1-2 hours so that any contaminants within it would germinate and be eliminated by autoclaving. The media was then autoclaved consecutively for three days at 120°C for 60 minutes to ensure sterility. To this flask agar pieces colonized by the fourteen-day old pure *P. erythroseptica* isolates was added aseptically and mixed. The flask was stoppered with cotton plugs and incubated at 25° C in the dark for fourteen days to ensure complete colonization. During this time the flasks were regularly shaken to break aggregate clods that formed. The millet was placed at a depth of 8cm, lightly mixed into the soil and covered up, following inoculum addition all the pots were lightly watered.

Trial Management:

Individual pots were set up with nutrient treatments and inoculated with various levels of *P. erythroseptica* and gently watered and allowed to settle over a three-week period. After this time initial soil samples were taken for nutrient, pH and pathogen analysis, as described below. One cut tuber piece $(\sim 30g)$ was planted into each pot at 10-12 cm depth and covered over. Pots were irrigated and placed into a randomised block design. The trial was grown under glasshouse conditions (20°C) with additional lighting (16 day:8 night). It should be noted that in Year 1 glasshouse conditions were highly variable, particularly cool at times due to glasshouse malfunction so temperatures were sometimes below 15°C. In Year 2 conditions were maintained at higher temperatures (23°C). Irrigation was by hand and applied once to twice weekly to maintain moist soils. Insecticides were applied every second week to manage insect pests.

pH and nutrient MEASUREMENT:

Levels of soil pH were assessed two times throughout the experimental period. Four randomly selected soil samples (approximately 20g each) were taken from each pot, just below the soil surface, with the sample combined. This soil sample was mixed 1:10 with de ionised water in a beaker, stirred thoroughly and allowed to stand for 15 minutes. One ml of soil solution from the container was placed in the receptacle of the pH meter (LAQUA twin compact pH-11 meter, Horiba Scientific, Japan) and readings were recorded. Measurements were undertaken in house and additionally through the AgVita laboratory (Devonport Tasmania). Soil chemistry was also assessed at pre-planting and at harvest, 50 g samples with methodologies as described above, sent to AgVita for full nutrient assessment (ES22 test).

SOIL PATHOGEN LEVELS:

Year 1. Soil pathogen levels were measured twice throughout the experimental period. Once, just prior to tuber planting and a second time, approximately 6 weeks after planting. For pathogen analysis, selected pots had approximately 50 grams of soil subsampled (from 5 random spots in each pot). Soil samples were dried at 40°C for 3 days prior to being double bagged and posted to a commercial lab for *P. erythroseptica* quantification (SARDI Root testing service;

https://www.pir.sa.gov.au/research/services/molecular_diagnostics/predicta_pt).

Year 2. Levels were not measured in Year 2 due to the usage of only one inoculum level and sequential inoculations.

DISEASE ASSESSMENT:

Underground and emerging stems were examined at or just after plant emergence for early signs of stem die-back induced by Phytophthora. The percentages of stems with dieback and or necrosis was recorded for each pot.

Pink rot disease was assessed at crop senescence by examining each pot and every tuber for the presence of pink rot disease. Scores were provided as to numbers of pots infected and percentages of tubers per pot with pink rot.

Results and Discussion

YEAR 1.

One of the key components was the modification of pH with the calcium amendments with the results shown in Table 1 across the two assessment dates. The organic loam had a naturally higher pH and was able to be altered easier than the ferrosol, with its pH raised into the putative efficacious range >7-7.5, with a lower level of Calcium supplement – 17.5 tonne/ha OzCal. In contrast, for the ferrosol, the required amount of OzCal was 35 tonne/ha OzCal. This would be many times the commercial rates for such a chemical where 1 tonne/ha would be considered a high dose for topdressing. The gypsum rates, as expected, didn't significantly raise the pH, rather it tended to slightly lower the soil pH.

		Ferrosol soil		Organic loam soil		
	Pathogen	Pre-plant pH	Harvest pН	Pre-plant pН	Harvest pН	
Treatments	levels	(H ₂ O)	(H ₂ O)	(H ₂ O)	(H ₂ O)	
Control	P ₀	4.81	4.80	6.26	6.39	
Control	P ₂	4.76	4.83	6.08	6.16	
Ca _{4t}	P ₀	6.14	5.52	6.76	6.67	
Ca 17.5t	P ₀	6.76	6.89	7.11	7.08	
Ca _{35t}	P ₀	6.88	7.28	7.12	7.31	
Gyp $3.8t$	P ₀	5.10	4.99	6.21	6.43	
Gyp $10t$	P ₀	4.75	4.78	5.81	5.84	
Gyp $20t$	P ₀	4.67	4.64	5.78	5.77	

Table 1 Impact of calcium and gypsum treatments on pH levels in two different soil types, ferrosol or organic loam, just prior to potato planting and at potato harvest.

Calcium levels were tested at pre-plant and harvest stage from a variety of selected treatments (Table 2). All calcium treatments (OzCal, gypsum) resulted in significant increases in Calcium levels available in both soil types, with these effects also remaining at harvest. All treatments resulted in Calcium levels being well above the critical low threshold (1200 mg/kg) apart from the ferrosol control treatments that ranged from 995-1156 mg Ca/kg.

Table 2. Calcium levels in two different soil types, ferrosol or organic loam, just prior to potato planting (top table) and at harvest (bottom table).

HARVEST

PATHOGEN LOAD:

Pathogen load, i.e. levels of *P. erythroseptica* DNA, has been measured twice through this experiment, once pre-plant and then \sim 6 weeks after planting (Fig 1). There was a significant difference ($P= 0.03$) in levels detected from the three treatments ($P₀$, $P₁$ and $P₂$) with $P₀$ having negligible pathogen loads, with levels increasing from P1 to P2. There was no significant impact of Calcium addition on pathogen loads. Of note, the pathogen levels declined

significantly between preplant and \sim 6 weeks after planting. This may have been partly attributable to the cool soil conditions during this time of the year (June-July). To increase the chances of pink rot disease in the final crop all the P1 and P2 treatments were topped up with additional pathogen at \sim 10 weeks after planting. Unfortunately, the cool conditions didn't favour pathogen growth with negligible pathogen detected just prior to harvest (data not presented).

preplant 6 weeks

Fig 1. Different pathogen load levels of *P. erythroseptica* either in ferrosol soil or organic loam soils in Calcium and gypsum treated soils. For P1 - 5 grains of inoculated millet was added and for P2 - 20 grains of inoculated millet added.

DISEASE

Shoot emergence (die back). Early in the season visual die back associated with pink rot infection of underground stems/stolons was visually observed randomly across the trial.

There was no relationship with applied treatments and most pots were able to send out new shoots and re-grow. There was a higher proportion of die back shoots observed in the organic loam compared to the ferrosol. In the pot environment the organic loam had poorer structure and water holding capacity than the ferrosol.

Pink rot. No significant pink rot was observed in the final tubers harvested so no treatment impact could be assessed at harvest.

YEAR 2.

Consistent with the results in year 1 the loam soil had a naturally higher pH than the ferrosol (Table 3). In contrast to Year 1 the ferrosol was amended into the efficacious pH range $(7 -$ 7.5) with 17.5 tonne/ha OzCal. The gypsum rates, as expected, didn't significantly raise the pH, rather it tended to slightly lower the soil pH. consistent with the Year 1 results. The nanocal treatment tended to provide a slightly higher pH at harvest from both soil types while both the CaNO3 treatments in both soil types raised the pH both at pre-plant and at harvest. The results from Premium Plus suggested no impact on soil pH compared to the control treatment.

Table 3 Impact of calcium, gypsum and other fertiliser treatments on pH levels in two different soil types, ferrosol or sandy loam, just prior to potato planting and at potato harvest.

Calcium levels tended to be lower from the ferrosol soil compared to the sandy loam soil (Table 4). All calcium treatments (OzCal, gypsum) resulted in significant increases in Calcium levels available in both soil types, with these effects also remaining at harvest. Nanocal slightly increased calcium levels, at harvest, in both the ferrosol and sandy loam. Both CaNO3 treatments in both soil types also provided additional calcium compared with the control treatment. The premium plus treatment had negligible impact on calcium levels.

Table 4. Calcium levels in two different soil types, ferrosol or sandy loam, just prior to potato planting (top table) and at harvest (bottom table).

PREPLANT

HARVEST

PATHOGEN LOAD:

Pathogen load, i.e. levels of *P. erythroseptica* DNA, was not measured in this Year 2 pot trial as only one inoculation level was applied across all pots. Incorporation of pathogen at multiple application dates (pre-plant), 6 and 12 weeks after emergence, combined with increased growing temperatures in this trial $(23 \pm 3^{\circ}C)$ ensured pathogen presence and pink rot infection.

DISEASE

Shoot emergence (die back). Early in the season visual die back associated with pink rot infection of underground stems/stolons was visually observed randomly across the trial (Table 5). This was more obvious in the sandy loam soil where some pots showed obvious dieback (Fig 2). There was no relationship with applied treatments and most pots were able to send out new shoots and re-grow. There was a higher proportion of die back shoots observed in the sandy loam compared to the ferrosol, consistent with year 1 pot trial results. *Pink rot.* Pink rot was observed in the final tubers harvested (Table 5, Fig. 3) However, like the dieback data there was no consistent treatment benefit with all treatments comparable to the untreated control.

Fig 2. Impact of early season infection of emerging R. Burbank shoots by *P. erythroseptica* in a sandy loam soil with OzCal 17.5 ton/ha treatment. Shown is one infected necrotic stem. Photo taken 3rd Aug 2022 by R. Tegg.

Fig 3. Pink rot tuber disease on one of three R. Burbank tubers at harvest. Soils were treated with 20 ton/ha gypsum and inoculated with *P. erythroseptica*. Photo taken 2nd Dec 2022 by R. Tegg.

CONCLUSIONS

These two pot trials provide an insight into not only measuring the impacts of given treatments on pink rot disease but also improved methodology relating to providing reliable disease outcomes in pot trials with an environmentally sensitive pathogen, *Phytophthora erythroseptica*. Methodology for understanding soilborne disease development in pot trials can be complex with the smaller pot soil environment more heavily influenced by environmental (temperature, moisture) factors than the more robust and larger field soil environment. Optimising conditions for both pathogen survival and disease development was a component of this work as the cooler environmental conditions in Year 1 resulted in significant pathogen decline and only minimal disease, primarily early stem/stolon blackening, but negligible pink rot tuber disease.

The assessment of materials that provide additional calcium (with and without associated pH changes) indicated that very large quantities of product would be required to ameliorate soils into the efficacious pH ranges reportedly required (Benson et al. 2009), particularly for the highly buffered ferrosols. This may make such applications uneconomical. Additionally, that these products were unable to reduce disease symptoms (*Phytophthora* induced stem dieback and pink rot tuber disease) indicates that under these trial conditions the treatments were not effective.