Canola Agronomic Research Program (CARP)
FINAL REPORT

Project Title: Managing small patches of clubroot infestation in canola fields

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Dr. Bruce Gossen	AAFC Saskatoon	Principal Investigator
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Dr. Mary Ruth McDonald	University of Guelph	Co-Investigator: clubroot management, supervise research associate and graduate students
Dr. Tim Dumonceaux	AAFC Saskatoon	Co-Investigator: Molecular pathology

Project Start Date: April 1, 2019 Project Completion Date: September 30, 2023
Reporting Period: April 1, 2019 September 30, 2023

CARP Project Number: 2019.06

Instructions: This Final Project Report shall be completed and submitted on or about March 31st of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project.. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition, a Final Extension Report is due upon completion of the project, maximum 2-3 pages, to be used for publication on the Funders' websites and in the *Canola Digest*. Content will be used in extension material, for consumers and/or industry. Include an Executive Summary, brief project description, key findings and conclusions (with a summary graph/table or supporting image for the project), translation of key findings into best management practices and/or relevance to the canola sector and future research, and funding acknowledgment as determined in the grant award letter. The Final Extension Report is intended to support messaging to all audiences. Information needs to be clear, concise and in "grower-friendly" language.

Please include the funding acknowledgements of the specific CARP funders and other funders outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of completion & status of activity (please check one)

Date of completion: _____

☐ **Ahead of Schedule** ☐ **On Schedule** ☐ **Behind Schedule** **X** ☐ **Completed**

Comments:

2. Summary - Maximum of one page. This must include project objectives, results, and conclusions for use on the Funders' websites.

Clubroot, caused by *Plasmodiophora brassicae*, is a major constraint to canola production in Canada and is spreading rapidly into new fields in many regions. The objective of this project was to develop best management practices for managing the spread of clubroot from small patches in newly infested fields. However, the results could also be used to manage hot spots of new pathotypes in fields where the pathogen is already established. Genetic resistance has been a highly effective strategy for clubroot management. Unfortunately, canola breeders have not been able to keep pace with the rapid breakdown of resistance caused by the development of virulent new clubroot pathotypes, so producers need alternatives for situations where genetic resistance is not available. The current study examined the effect of liming, grass cover crops and their interaction under controlled conditions and in small-plot trials conducted in commercial fields over 4-5 years. One of the first outputs of the study was an improved method to quantify the numbers of resting spores in soil using a molecular approach known as digital drop PCR (ddPCR). This approach was less expensive, more accurate and more robust that previous methods, so it was used throughout the study. Short-term lab studies showed that the concentration of resting spores in infested soil declined dramatically over just 8 weeks and that the presence of either grass or cereal crops produced a small incremental reduction in spore numbers. The field studies also showed that the concentration of resting spores declined rapidly over the first couple of years after a susceptible crop on the Canadian Prairies. Application of lime did not reduce spore numbers relative to the untreated control. Similarly, the effects of a grass cover crop or wheat observed under controlled conditions were not consistently detectable in the field trials, likely because the variability of spore concentration within each field site was so large. In summary, none of the treatments consistently reduced spore levels, which remained at levels sufficiently high to cause complete crop failure even after a four-year interval without an infested crop at four of five sites. That being said, breakdown of genetic resistance to clubroot is much more likely when spore concentration is high. This result strongly supports the recommendation for a minimum 2–3 year break (longer is better) between canola crops wherever clubroot is present to allow for reduction in spore numbers in the field. It also confirmed that growers should always select resistant cultivars for use in infested fields, because high numbers of clubroot spores were almost always still present after 5 years of non-host crops. In the absence of effective techniques to reduce spores numbers, the recommendation to use grass cover crops to manage clubroot patches was also supported. Perennial grass sod growing on a clubroot patch holds the infested soil in place. This minimizes movement of infested soil by wind and water erosion as well as the risk of contaminating farming machinery and other vehicles driven across the infested patch, and so minimize the movement of spores to new sites within the field or among fields. Scientific papers describing the ddPCR technique and the effect of various crops on resting spore survival under controlled conditions have been published and another on the field trials is in preparation.

3. Introduction – Brief project background, rationale, and objectives.

Clubroot, caused by *Plasmodiophora brassicae*, is a major threat to canola production on the Canadian prairies. Since the initial report of clubroot in 12 canola fields outside of Edmonton, Alberta in 2003, it has spread across Alberta and in 2018 was identified at sites across Saskatchewan and Manitoba. Until recently, resting spore populations were thought to decline steadily over many years so there was little point in using crop rotation for

clubroot management. Improvements in assessments of spore populations in soil using molecular methods have shown that a high proportion of resting spores die in the first two years after a susceptible crop, but the remaining spores persist for many years. Therefore, a crop rotation that includes a 2-year break from susceptible crops can substantially reduce spore concentration in soil. However, the concentration of spores remaining in a clubroot hotspot after a two- to three-year rotation is often hundreds or thousands of times higher than that required to produce severe disease in a susceptible host. This indicates that strategies to further reduce inoculum pressure in infested fields are needed.

Establishing a stand of perennial sod-forming grass on a patch of a field infested with clubroot should minimize movement of infested soil to other parts of the field and to other fields. Also, at least one perennial grass crop (perennial ryegrass) is thought to stimulate germination of the resting spores of *P. brassicae* because infection of its root hairs by *P. brassicae* has been documented. Fortunately, root hair infection does not progress to production of new resting spores, so ryegrass is considered a non-host. Stimulation of resting spore germination without production of new spores should reduce the spore concentration in soil, and high spore concentration is associated with both severe symptoms and breakdown of resistance. Establishing a grass cover crop on an infested patch of a field might reduce spore numbers in soil, especially if it continues over time. However, the rate of reduction and the relative efficacy of other crops to stimulate spore germination is not known. Similarly, application of high rates of lime to reduce acidity has been shown to reduce clubroot incidence and severity, but the effect of these changes on spore survival are not known. Addition of lime is another management approach that might affect spread of clubroot; application of lime is known to raise soil pH and make the field less conducive for clubroot infection. However, its effect on spore numbers over time is not know. Also, severe clubroot can also occur in fields where pH is already neutral or sightly alkaline, so increasing pH further is likely of limited value. However, high levels of calcium have also been shown to reduce clubroot, so addition of gypsum, which supplies calcium and magnesium, could also have an effect on clubroot in these situations.

The objective of this project was to provide canola producers with information on practical approaches to managing small patches of clubroot to minimize the risk of spread of clubroot from small patches in newly infested fields. However, the results are also applicable to managing hot spots of new pathotypes in fields where the pathogen is already established.

4a. Methods – Include approaches, experimental design, methodology, materials, sites, etc.

ddPCR quantification

A detailed description of the methods for developing a ddPCR method for quantifying resting spores of *P. brassicae* from soil is available in the published paper (Wen et al. 2020). Briefly, DNA was extracted from a suspension of resting spores in a dilution series produced using standard methods. A cell-lysis protocol at this stage was found to substantially improve DNA yield relative to the standard method. Primers used in the evaluation were based on previous studies, with only minor changes to add suitable dyes for ddPCR assessment.

To compare ddPCR with qPCR for quantifying the inoculum of *P. brassicae* in different soil types, top-soil (10-cm deep) samples were collected at one field in the Brown, Dark Brown and Black Soil Zones of the Canadian Prairies, respectively, near Swift Current, Outlook, and Melfort, Saskatchewan. The soil samples were spiked with serial dilutions of spore dilutions with intact resting spores, dried and then assessed using standard protocols for qPCR and a comparable standard for ddPCR. Three replicates were prepared independently for each inoculum concentration of each soil type. Two technical replicates were used for each sample tested. The experiment was conducted twice for ddPCR but three times for qPCR due to strong inhibition of DNA amplification with black-soil samples. The inhibition was removed by a 10-fold dilution of DNA samples. The variance from repeated experiments was homogeneous based on Bartlett's Test (Little and Hills 1978), so the data were pooled for analysis.

Effect of subsequent crop

A detailed description of the methods for assessing the effect of subsequent crops on resting spore survival is available in the published paper (Drury et al. 2022). Controlled environment studies were conducted at the University of Guelph. The crops evaluated were: smooth bromegrass (*Bromus inermis* L.), three cultivars of perennial ryegrass and meadow bromegrass, spring wheat (*Triticum aestivum* L.) barley (*Hordeum vulgare* L.), soybean (*Glycine max* L.) and field pea (*Pisum sativum* L.). These crops are common rotation crops in Ontario (soybean and barley) or the Prairie provinces (spring wheat, field pea). The clubroot-susceptible Shanghai pak choi (*B. rapa* var. *chinensis*) and a no-plant (bare soil) control were also included in both studies.

The growth medium was 2:1:1 by volume of mineral field soil (pH 6.3, organic matter 2.4%) from Simcoe, ON, noncalcareous coarse sand and soil-less mix (L4A Sunshine Mix, Sun Gro Horticulture, MA). Sand and soil-less mix were added to maintain soil texture. The soil for each replicate was prepared separately. The project was conducted as two separate studies, on focused on grasses and the other on field crops. The inoculum was prepared using standard methods. Each replicate was inoculated separately, with a target of 5×10^5 spores g^{-1} of soil which was chosen as it represents moderate clubroot infestation.

Plastic cups were filled with ~400 g of soil spiked with a known concentration of resting spores from a single source. Twenty seeds were planted in each cup and thinned to 10 seedlings. The grasses were seeded just below the soil surface. All crops were seeded at a depth of 2.5 cm. The cups were placed in 20 cm × 16 cm × 10 cm plastic containers and watered from the bottom with tap water adjusted to pH 6.5 with commercial white vinegar.

Pak choi was harvested at 6 weeks after seeding to avoid decay of clubs and loss of resting spores back into the soil. Clubroot incidence was determined as the percentage of plants with clubroot symptoms. A disease severity index (DSI) was calculated using a standard formula. At 8 weeks after seeding, plants of the other treatments were removed from their pots. Soil from each pot was collected and resting spore concentration was quantified using standard qPCR methods (ddPCR was not available at this research site).

The Shapiro-Wilk test and scatter plots of the residuals were used to evaluate the normality of the data. Spore concentration was analyzed based on a lognormal distribution. The spore concentration of the pak choi control was excluded from statistical analyses of crop treatments because it was expected to increase over time rather than decrease. Variance analyses of the grass crop and field crop studies found that the two runs could be pooled for both studies. Dry root weight per pot among crop species was analyzed based on a normal distribution using Tukey's test for means separation. Pearson correlations were used to examine the relationship between the concentration of resting spores and root weight among all crops and within each crop species using PROC CORR. Studentized residuals identified one outlier in the data.

Field trials

A recipe approach to managing small patches of clubroot, which has been discussed at numerous meetings with agronomists and growers (e.g., Gossen et al. 2018), seeks to combine the benefits of crop rotation and liming with soil stabilization using perennial grasses to minimize or even stop the spread of resting spores from infested patches of a field to clean portions of the field. Initially, a test site was established in a heavily infested field near Spruce Grove, AB (just outside Edmonton) in 2018 with separate funding. In that trial, lime (hydrated lime, standard lime and a mixture) was applied with a target pH of 7.5 in late spring of 2018 and compared with a dense seeding of perennial ryegrass. However, additional sites were needed to permit comparison of results across soil types and conditions (including initial pH), climatic zones, and initial levels of infestation. The study was targeted at small 'hot spots' where clubroot has been identified in commercial fields in Saskatchewan and Manitoba.

Two sites with recent clubroot epidemics were identified in Saskatchewan (North Battleford, Cutknife) and two in Manitoba (Kaleida, Carman). Each site was treated with combinations of hydrated lime, standard lime and perennial ryegrass in the spring of 2019 (target pH 7.5) and monitored over time to assess the impact and

interaction of the treatment factors (liming and grass cover) on resting spore concentration. Additional treatments (e.g., wheat rather than ryegrass, solarization, application of boron) were assessed at some sites. Plot size and the number of replicates were customized to the size and topography of the location. Resting spore populations were monitored using ddPCR analysis of soil samples collected from each plot in fall each year (occasionally also in spring) using a soil corer, based on bulked soil cores from each plot. Soil pH was also monitored in the soil samples.

The layout of the sites in Saskatchewan consisted of eight treatments in a RCBD with four replicates. The treatments were 1) grass mix only, 2) grass + standard lime, 3) grass + hydrated lime, 4) grass + 1.5 X hydrated, 5) grass + gypsum (North Battleford) or grass + 50:50 std: hydrated (Cutknife), 6) wheat/barley, 7) solarization – 2 weeks under totally impermeable film (TIF) in late spring/early summer of 2019, chemfallow in 2020, barley in 2021 and 2022), and 8) bare soil control. Each plot was 3 m x 3 m, with a 1 m space between plots. The site near Cutknife, SK had a starting soil pH of 5.5 and resting spore concentration of 2.3×10^8 g^{-1} soil. It received 9.25 kg per plot (10.3 t/ha) of standard lime, and 8.0 kg per plot (8.9 t/ha) of hydrated lime (1.5X = 12.0 kg or 13.3 t/ha. The site near North Battleford SK (irrigated) had an starting soil pH of 7.2 and resting spore concentration of 1.1×10^8 g^{-1} soil. It received 1.95 kg per plot (2.2 t/ha) of standard lime, 1.44 kg per plot (1.6 t/ha) of hydrated lime (1.5X = 2.16 kg or 2.4 t/ha), or 1.04 kg (1.2 t/ha) of gypsum.

The standard lime and hydrated lime treatments were surface applied, then incorporated with a rototiller to ~ 7.5 cm in May of 2019. A grass mixture of 38% creeping red fescue (common), 42% timothy cv. Promesse, 10% smooth brome cv. Carlton, 10% perennial ryegrass cv. Lactal was seeded at 11.2 kg/ha on the grass plots and border with 22 cm row spacing. Wheat cv. AAC Jatharia was seeded in 2019 at 112 kg/ha and 22 cm row spacing. In 2020, no wheat or barley was seeded due to Covid-19 restrictions and the weeds were advanced before site maintenance could be initiated. Barley was seeded in place of wheat in 2021 and 2022 at 112 kg/ha and 22 cm row spacing.

Three soil samples per plot taken to 10 cm depth in late spring and again after harvest each year. The samples for each plot were bulked and air dried, then pH was measured after adding 20 ml of distilled water to 10 g soil and agitating for 20 min. Soil DNA was purified from 0.25 g of soil using the E.Z.N.A soil DNA kit Omega bio-tech (VWR). The concentration of resting spores of *P. brassicae* was quantified using the ddPCR protocol described previously.

After harvest in the final year of the study (2022), soil samples from the surface to 10 cm depth were collected from each plot and used in a bioassay. The soil was mixed at 3:1 soil :peat by volume to minimize soil compaction associated with disturbance and placed in large plastic pots, with one pot per plot. About 20 seeds of a clubroot-susceptible canola cultivar were planted per pt and thinned to 10 seedlings per pot after about 1 week. At six weeks after planting, the plants were uprooted, assessed for clubroot severity and a DSI value for each plot was calculated as previously described.

In Manitoba, each site consisted of eight treatments in a RCBD with three replicates. This small number of replicates was chosen because the patches were small and spore concentration was likely to be low and highly variable outside the patch. The treatments were 1) grass mix only, 2) grass + standard lime (0.5 T/ha), 3) grass + hydrated lime (0.5 T/ha), 4) hydrated lime alone, 5) grass + gypsum (0.5 T/ha), 6) wheat (2019), soybean (2020), barley (2021) and flax (2022), 7) solarization for4 wk under TIF in 2019, then chemfallowed, and 8) bare soil control. Each plot was 6 m x 2 m. The site near Kaleida, MB had an starting soil pH of 5.9 and resting spore concentration of 5.3×10^6 g^{-1} soil. The site near Carman, MB had an starting soil pH of 6.2 and resting spore concentration of 1.4×10^5 g^{-1} soil with substantial variation in spore concentration across the study area. Soil samples to 10 cm depth were taken each fall after harvest.

For each site, analysis was conducted separately for each sampling date using ANOVA in PROC GLM in SAS. No combined analysis across sites was attempted because the initial spore numbers and soil type were so different at each site.

4b. Major changes from original plan should be cited and the reason(s) for the change should be specified.

In the final year of the study, the grass treatments were to be broken, and the entire study area planted to a susceptible cultivar of canola. After 5-6 weeks, clubroot severity was to be assessed using standard techniques and then all of the plants removed so as not to increase the concentration of clubroot spores in the study area. In Saskatchewan, the grower at North Battleford seeded the main area of clubroot infestation, including the area surrounding the study, to a grass cover crop in 2019. The plan to break the grass plots in the study would have left the grower cooperator with a weedy mess in subsequent years and a potential hot spot of clubroot inoculum. Instead, soil was collected from each plot for assessment in a bioassay under controlled conditions. Similarly, soil from the site at Cutknife was sampled in the spring of 2022. This will provide comparable assessments from each site. The same approach was adopted for the sites in Manitoba, but unfortunately the soil samples collected were too small to be used for bioassays. This approach allowed us to extend the duration of the field trials by an additional year, which was very important because the spore concentration in year 2 from several of the sites were much higher than expected – the clear pattern of response over time provided by an additional year of data provided more confidence that these high levels were artifacts rather than genuine results.

Also, resources from this study were used to extend the duration of the initial field trial conducted near Spruce Grove, AB for an additional year. That study was also terminated in 2022. To assess the effect of treatment on clubroot severity in the trial at Spruce Grove and the two sites in Saskatchewan, a susceptible canola cultivar was seeded into pots of soil and soil-less mix (80: 20) and clubroot symptoms were assessed at 6 weeks after seeding based on standard assessments. The plants were grown in containment to ensure that the pathogen did not contaminate materials destined for field trials. Soil samples from North Battleford and Cutknife will be assessed when space becomes available in containment.

5. Results – Present and discuss project results, including data, graphs, models, maps, design, and technology development.

ddPCR quantification

The number of copies of *P. brassicae* genomic DNA detected in suspensions increased as the resting spore concentration increased from 10^2 to 10^8 spores/mL (Fig.1). The ddPCR estimates of spore concentration generally matched the expected number of resting spores in suspensions between 10^3 and 10^7 spores/mL, but the accuracy was reduced at 10^2 and 10^8 spores/mL levels due to apparent overestimation against the theoretical numbers based on the dilution (Fig.1). Estimates of resting spore concentration based on ddPCR analysis of samples spiked with *P. brassicae* suspensions in a dilution series from 1×10^2 to 1×10^7 resting spores/mL showed an excellent match with expected values (Fig. 2).

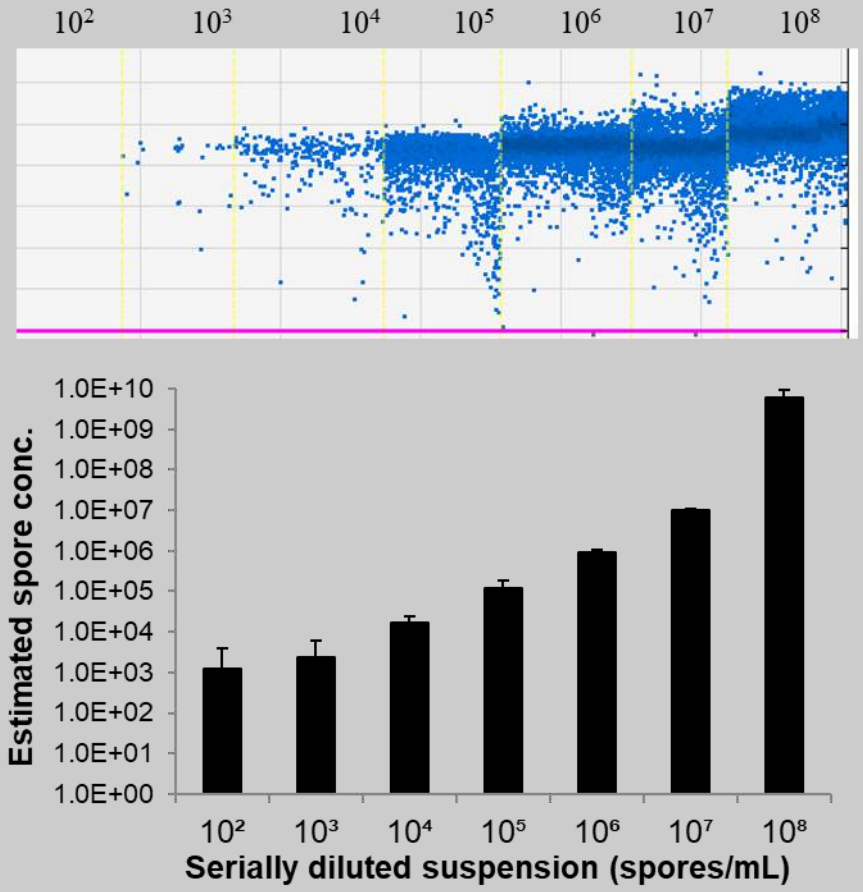


Fig. 1. Quantification of *P. brassicae* resting spores in suspension with ddPCR. **A**) PCR amplification with each blue dot representing a droplet containing FAM-labelled DNA fragments. **B**) Estimated concentration of resting spores for each serial dilution of spore suspension.

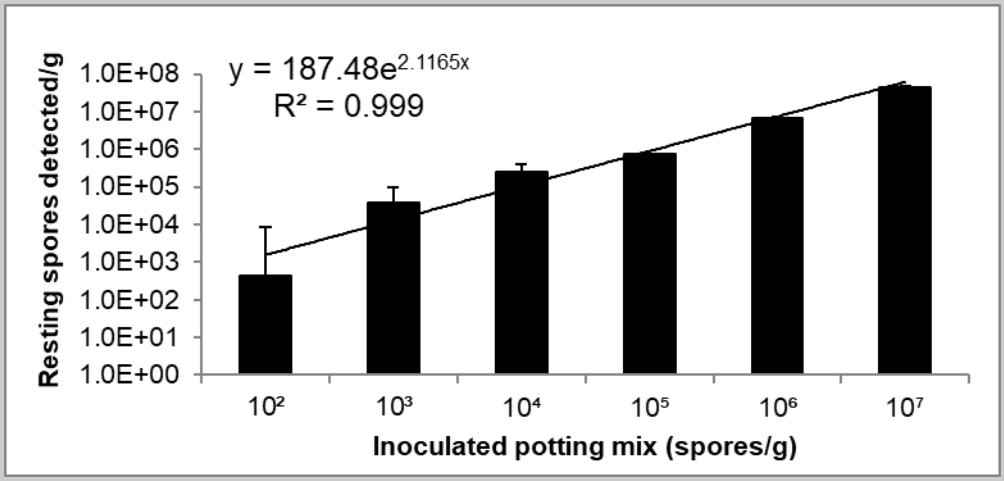


Fig. 2. Estimates made using ddPCR of resting spores of *Plasmodiophora brassicae* in soil-less potting mix spiked with resting spores.

No differences were observed on ddPCR estimates of resting-spore concentration among spiked samples of different soil types (P >0.05) carrying 10^3 to 10^7 spores/g soil. qPCR often displayed lower resting spores on the same soil sample, especially when the inoculum level was at < 10^6 spores/g (Fig. 3). Initially, DNA amplification was inhibited completely for samples from the Black Soil Zone.

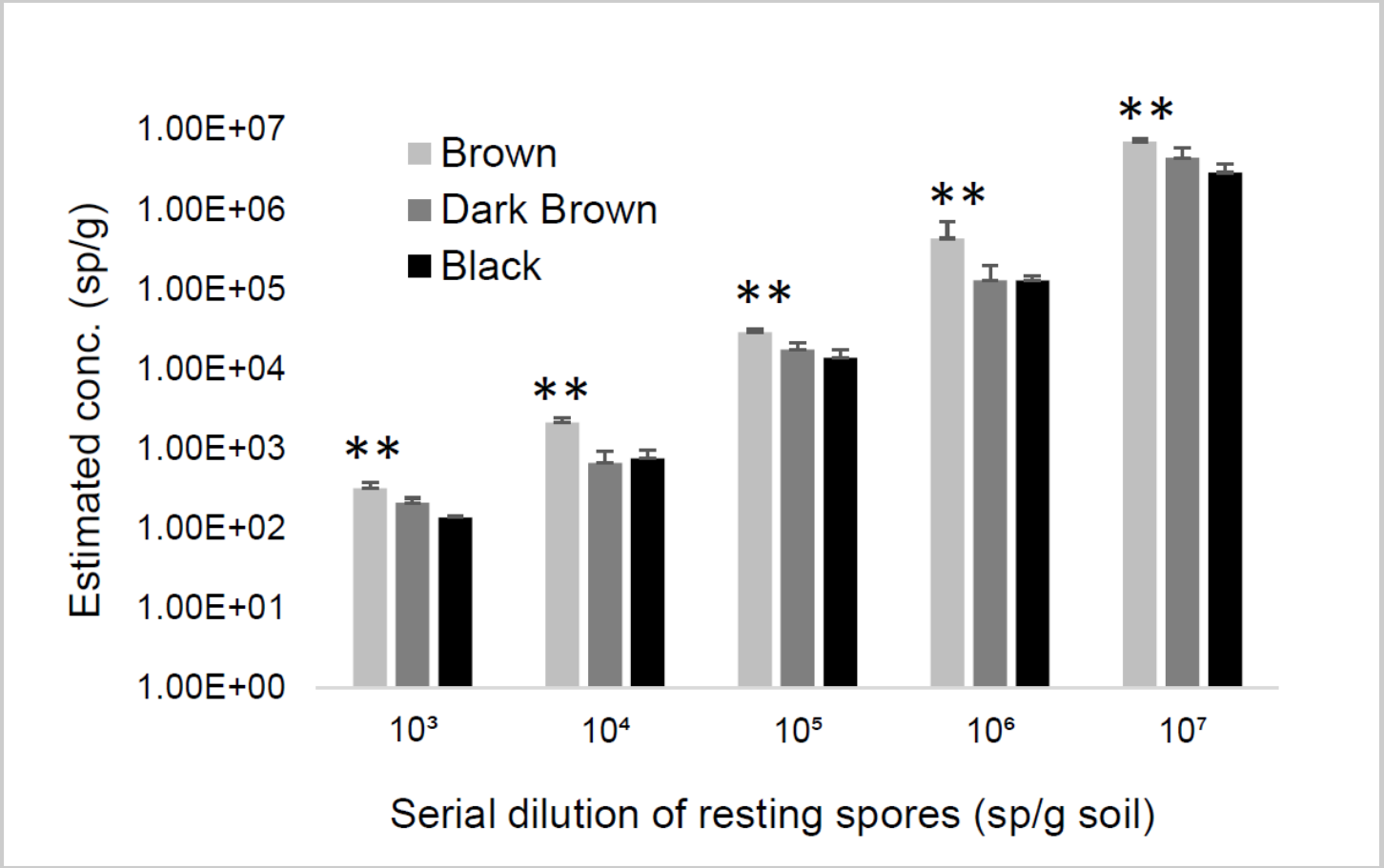


Fig. 3. Comparison of quantification of resting spores of *P. brassicae* using ddPCR from three soil samples representing the main soil types on the Canadian Prairies.

The current study demonstrated that ddPCR can provide sensitive and accurate estimates of *P. brassicae* resting spores ($\leq 10^7$) in suspension, in soil-less potting mix, and in infested field soils that differed in texture and pH, without the problems of inhibitors in soil that are routinely encountered in qPCR assessments, which was the standard approach for quantification of resting spores of *P. brassicae* from soil. ddPCR provides robust estimates of resting-spore concentrations in different soil types found commonly on the Canadian Prairies and was more versatile for quantification of *P. brassicae* resting spores in different soil types compared to qPCR.

Effect of subsequent crop

Severe clubroot symptoms (disease severity index of 100%) developed on roots of the pak choi but not on any other crop. At 8 weeks after initiation of the study, the concentration of resting spores in the bare soil control had decreased by 27-87% compared to pre-plant levels. All of the grasses and field crops assessed further reduced the survival of resting spores, except for soybean, which had no effect (Fig. 4). Perennial ryegrass had the highest root dry weight per pot compared to the other crops, field pea had the lowest, and the other crop species were intermediate, but there was no relationship between root biomass and reduction of resting spores. Wheat provided the numerically largest reduction in resting spore concentration, but root hairs of wheat were only rarely infected with *P. brassicae*, while root hairs of ryegrass were often heavily infected. This indicated that root biomass and root hair infection were not associated with reduction in resting spores. There was no difference in spore numbers between the PMA- treated spores (only live spores are assessed) and water-treated spores and no interaction between these factors in any study, so the PMA and water-treated samples were treated as subsamples.

This portion of the study demonstrated that three grass species and several common field crops reduced resting spore concentrations relative to a bare soil control. The high concentration and special variability of resting spores expected at sites with severe infestations (Ingram and Tommerup, 1972) makes detection of even massive reductions in spore concentration based on clubroot symptoms highly unlikely. The moderate inoculum levels used in the current study, together with rigorous mixing of inoculum into the soil and molecular assessment of spore concentration, may have allowed reductions in spore numbers to be detected.

We had hypothesised that crops with more extensive root systems would induce a larger reduction in spore numbers because the larger root system would access a larger volume of soil and release greater quantities of root exudates, which would in turn stimulate more spore germination. This hypothesis was not supported. The data supporting that conclusion are presented in detail in the published paper (Drury et al. 2022).

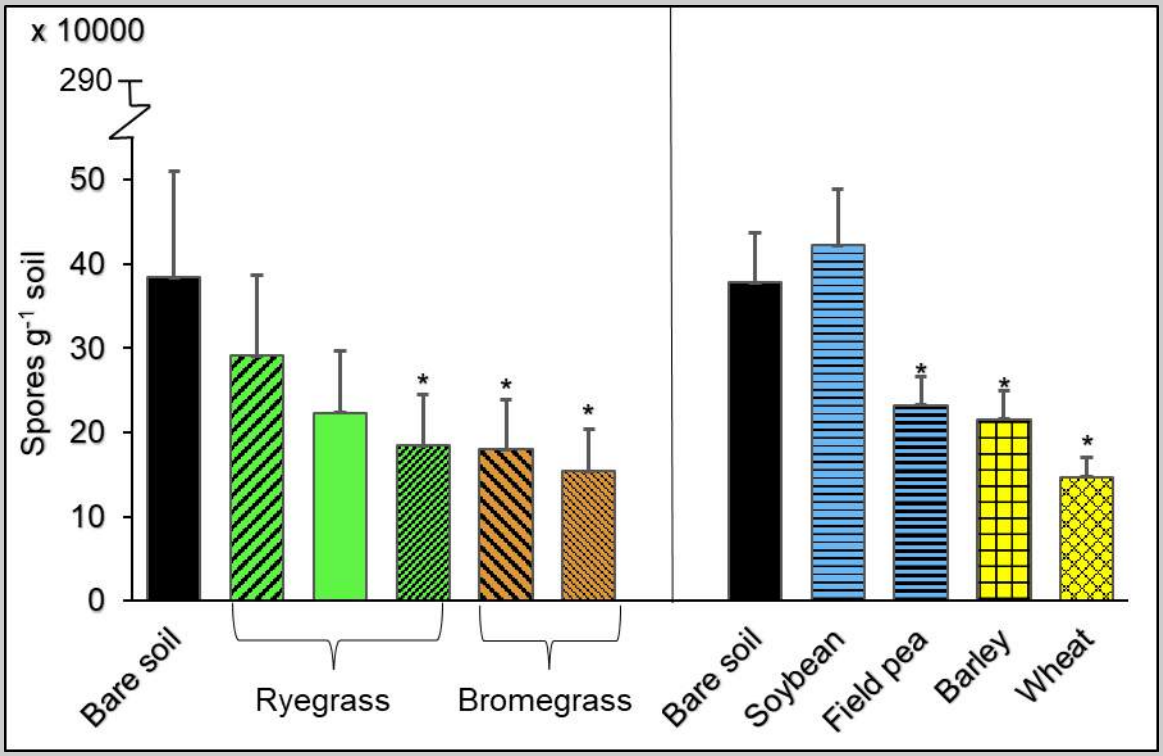


Fig. 4. Dense plantings of perennial grasses, spring cereals and even field pea quickly reduce the concentration of resting spores of *Plasmodiophora brassicae* (clubroot) in soil in a study under controlled conditions.

Field trials

The most important result of the long-term field study to examine resting spore survival, which consisted of four sites situated across a range of environmental regions and soil types, was to confirm that the concentration of resting spores of *P. brassicae* declined dramatically in the initial years after a susceptible crop. However, the treatments (liming, grassing, gypsum) did not consistently have a measurable effect relative to the bare soil control. In fact, the control was often at or near the bottom of the mean spore concentrations in the studies (Figs. 5, 6, 7 and 8).

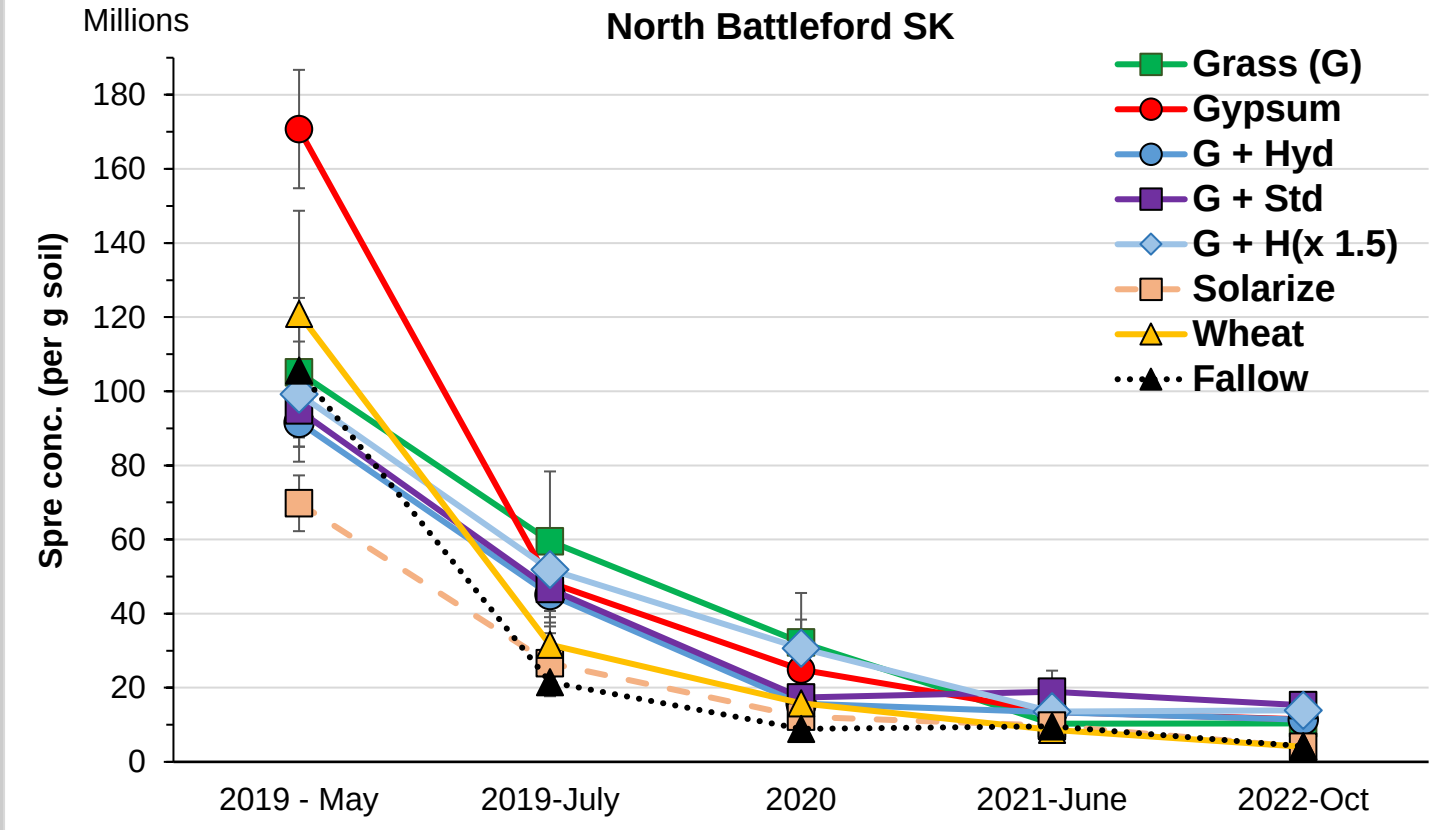


Fig. 5. Effect of grass, lime and other select treatments on survival of resting spores at an irrigated site with sandy soil and near neutral pH near North Battleford, SK from 2019 to 2022.

At the irrigated site on sandy soil near North Battleford, SK, resting spore concentration in the plot area varied from about 70 million spores per g of dry soil to over 170 million spores, with a mean of about 100 million spores per g in the May of 2019 when the study was established, but had already declined to about 45 millin by July of 2019 and about 20 million by July of 2020 (Fig. 5).

At the rain-fed site on heavy claynear Cutknife, SK, spore concentration per g of dry soil ranged from slightly less than 100 million spores to almost 400 million spores when the study was established in May 2029. The tragectory of recuction in spore numbers wasn't nearly as consistent in 2020 as at North Battleford, with numbers ranging from les than 50 mioon to almost 300 million, but by the spring of 2021 numbers had fallen to between 50 and 100 spores per g and dropped only slightly from that level over the subsequent year (Fig. 6).

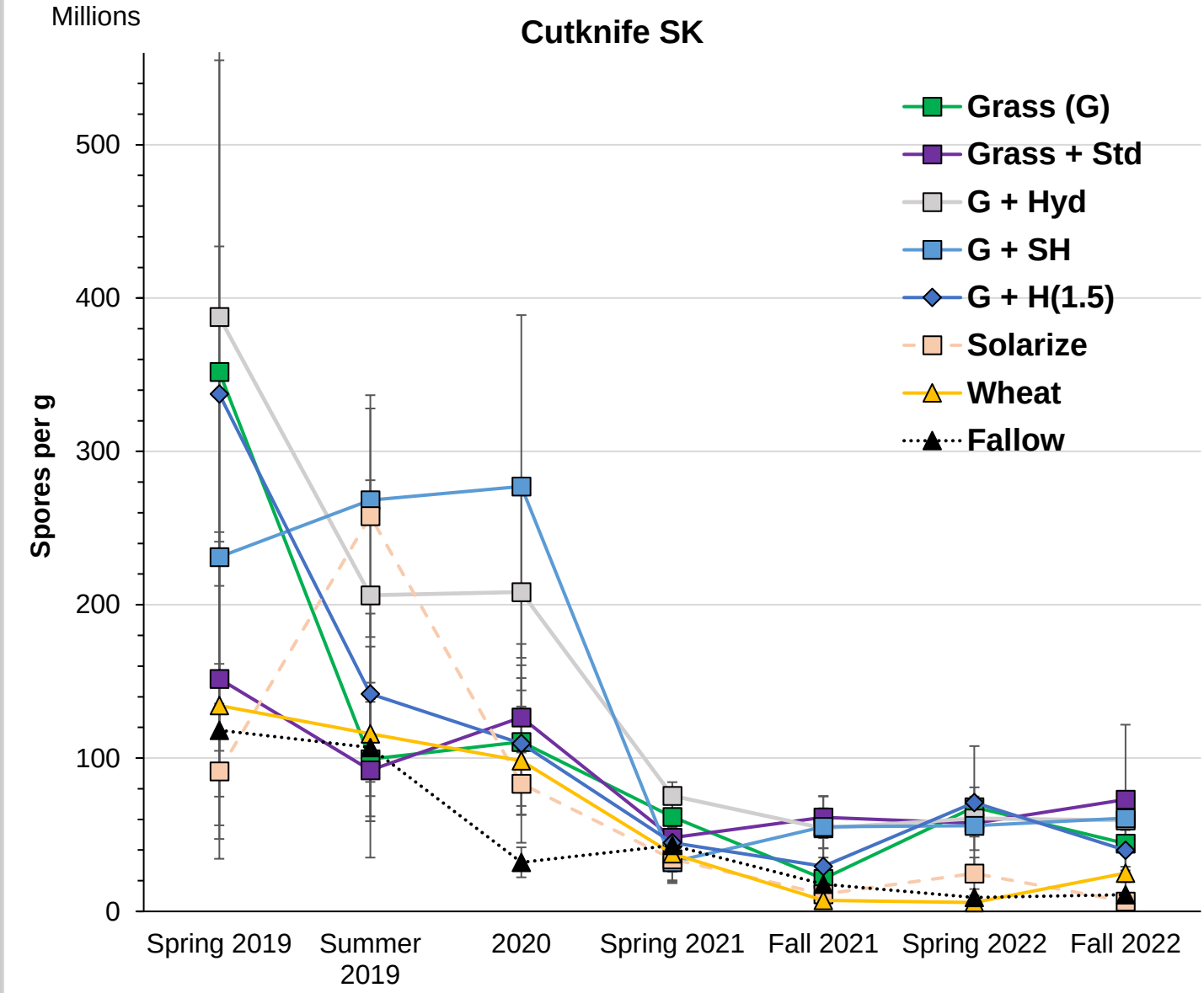


Fig. 6. Effect of grass, lime and other select treatments on survival of resting spores at an site with clay soil and acidic pH near Cutknife, SK from 2019 to 2022.

At the site at Carman MB, spore numbers were substantially lower than at the two sites in Saskatchewan, with a range of concentration among the treatents when the study was initiated in 2019 from about 20,000 spores per g to 600,000 spores, with a neam of less than 100,000 spores. Spore numbers appeared to increase in 2020 in several treatments, but increase of spores in the absence of susceptible hosts is not possible for this pathogen. However, it is possible that some spore increase occurred on volunteer canola plants or susceptible weeds before they could be removed. Another possibility is problems with the assessment of spore numbers for this site in 2020. Whatever the cause, this anomoly disappeared in 2021 and 2022, where spore numbers stabalized at levels at or below 10,000 spores per g of dry soil (Fig. 7).

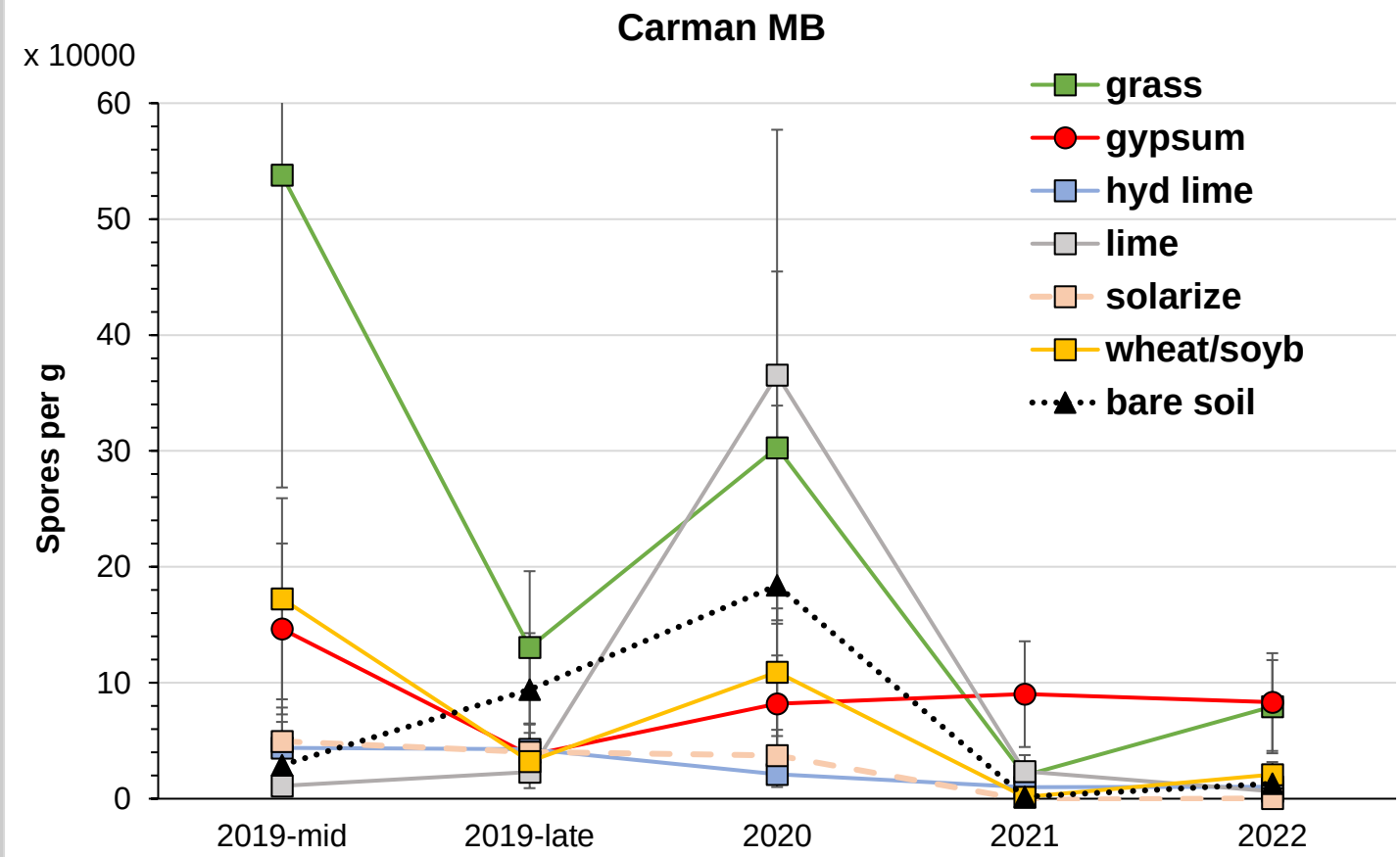


Fig. 7. Effect of grass, lime and other select treatments on survival of resting spores at an site with clay soil and slightly acidic pH and comparitively low initial ascospore levels near Carman, MB from 2019 to 2022.

At the site at Kaleida in Manitoba, spore numbers also increased dramatically and unexpectedly in the fall of 2019 (the year the trial was initiated), but then came back down and remained stable over the remainder of the study. This, taken together with the increase in 2020 at Carman, indicates that there were issues with the analysis of samples at these early sampling dates, rather than that the pathogen actually increased after iinitiation of the trial.

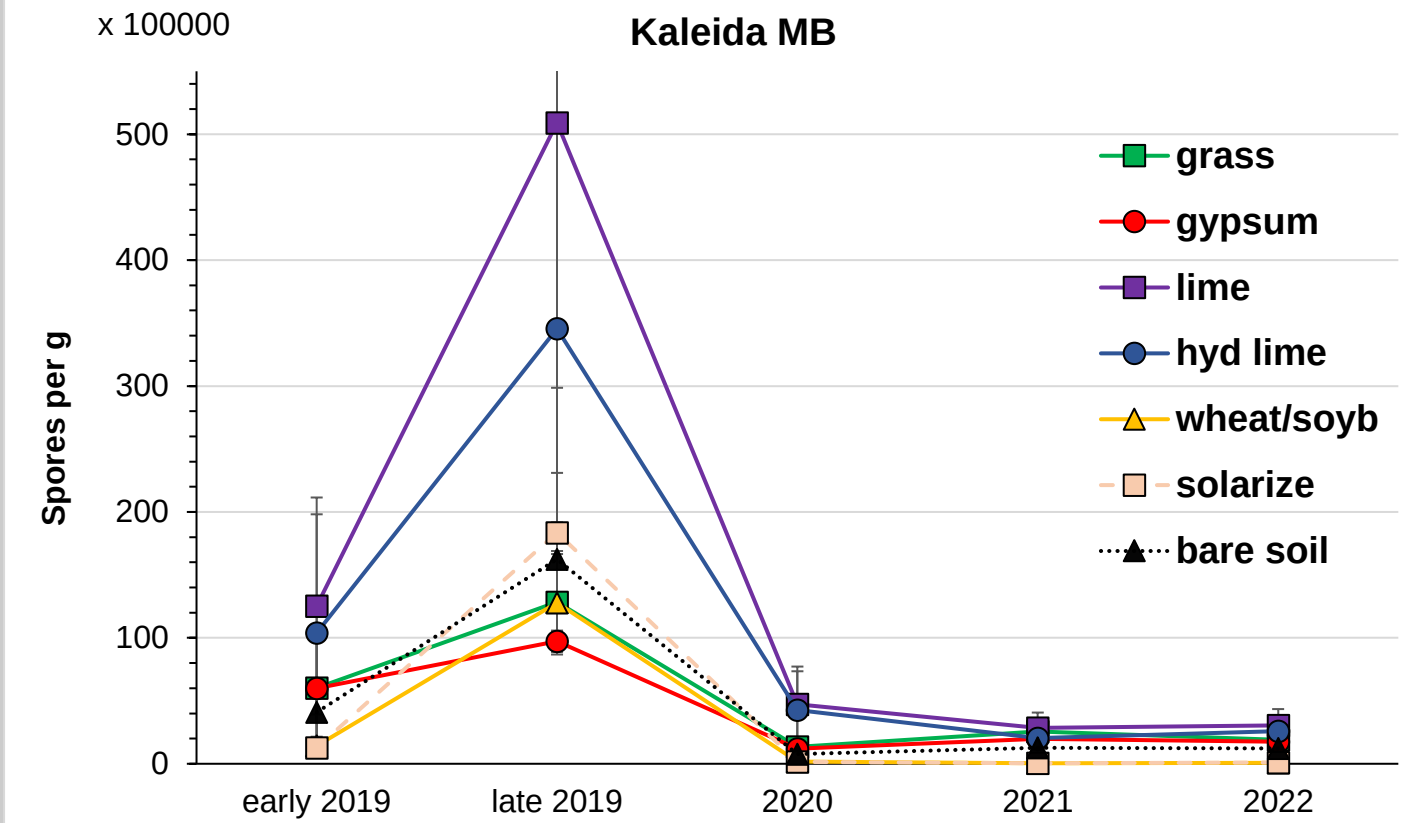


Fig. 8. Effect of grass, lime and other select treatments on survival of resting spores at an site with clay soil and slightly acidic pH and comparitively low initial asspore levels near Kaleida, MB from 2019 to 2022.

The soil pH at North Battleford was neutral to slightly alkaline. Treatment with standard lime or hydrated lime increased it slightly, so that it was consistently over 7.5 (Fig. 8). At Cutknife, the initial soil pH was about 5.5., and treatment with standard lime or hydrated lime increased it to around 7.0 (Fig. 10). The initial soil pH at Carman was about 6.5 (Fig. 11) and about 6.0 at Kaleida (Fig. 12). Treatment with standard or hydrated lime increased soil pH at both sites in Manitoba to be consistently over pH 7.5 for the duration of the trial. However, soil pH did not have a consistent impact on survival of resting spores at any site.

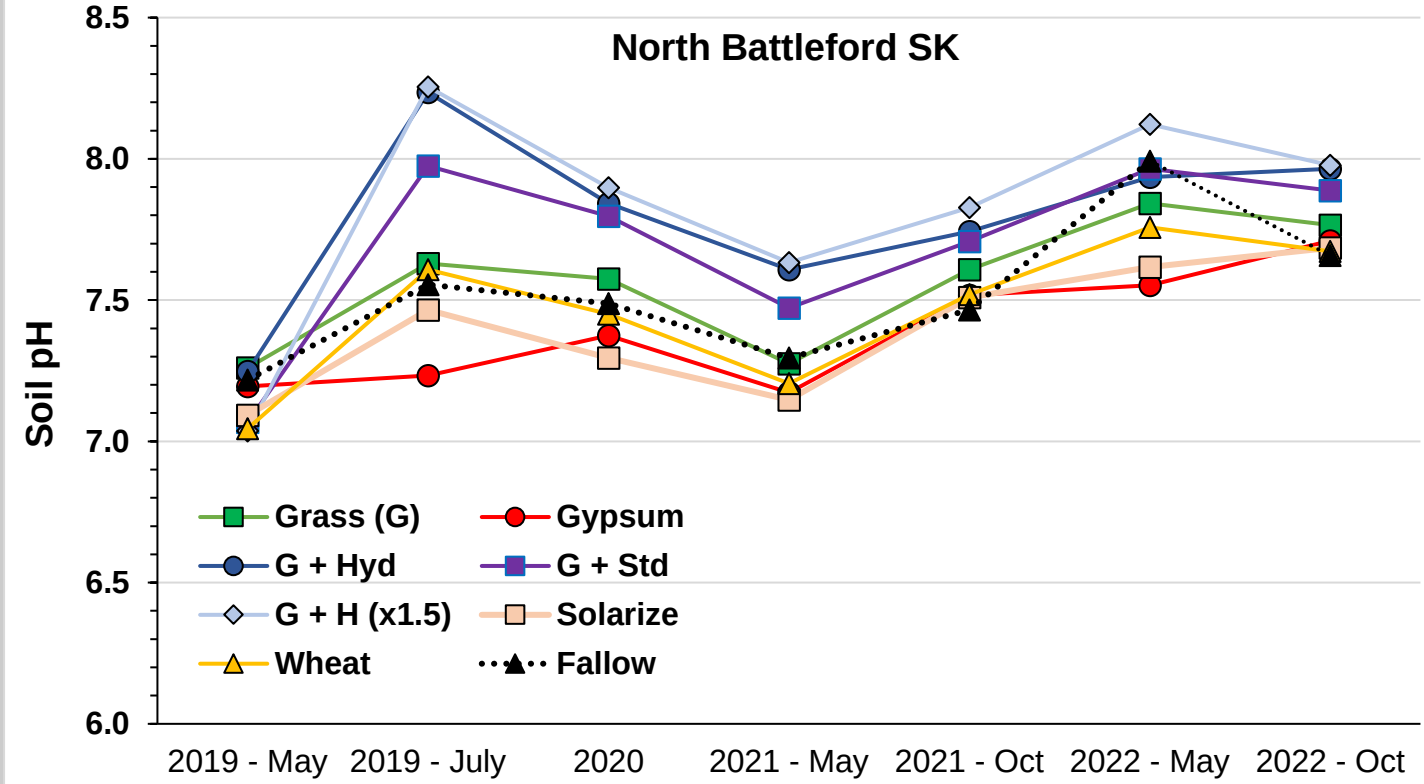


Fig. 9. Effect of grass, lime and other select treatments on soil pH at an site with sandy soil and neutral pH near Noth Battelford, SK from 2019 to 2022.

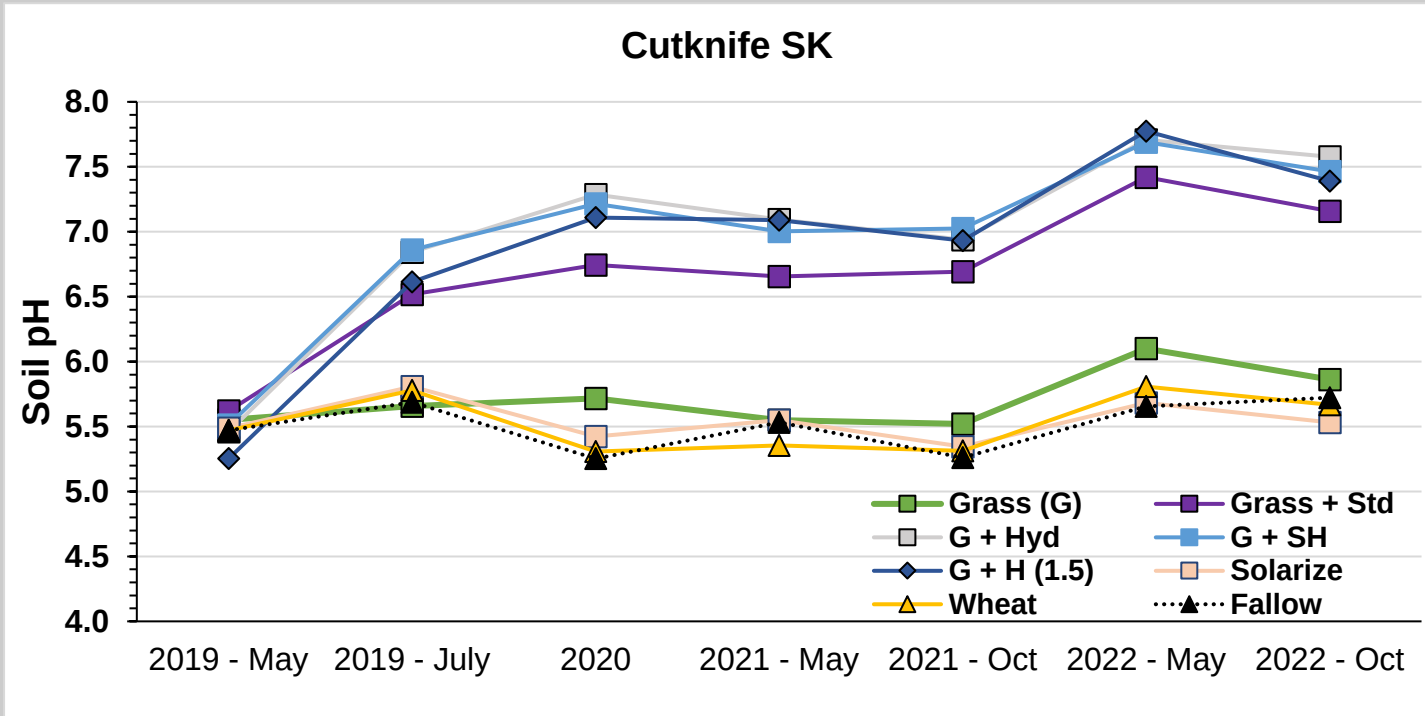


Fig. 10. Effect of grass, lime and other select treatments on soil pH at an site with clay soil and acidic pH near Cutknife, SK from 2019 to 2022.

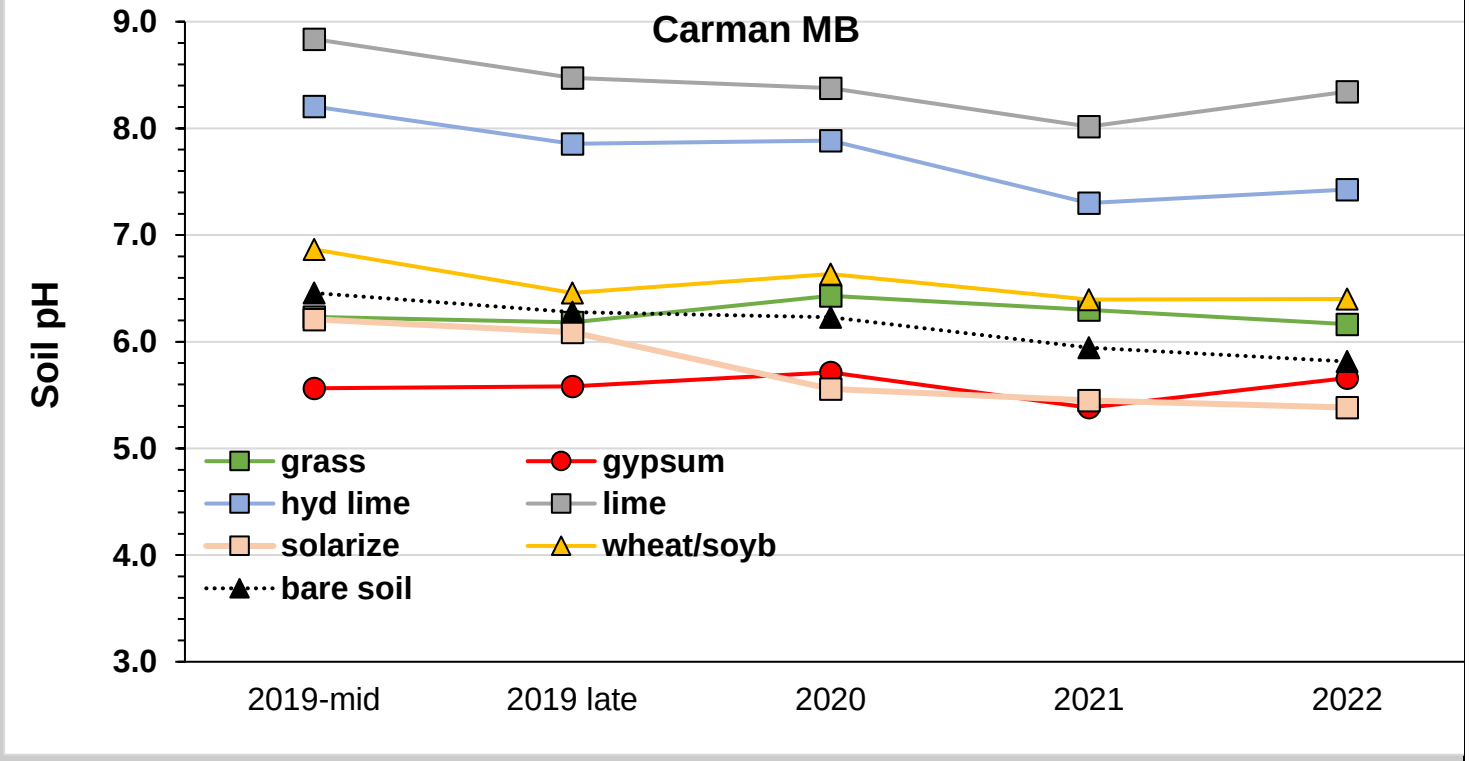


Fig. 11. Effect of grass, lime and other select treatments on soil pH at an site with clay soil and slightly acidic pH and comparitively low initial asspore levels near Carman, MB from 2019 to 2022.

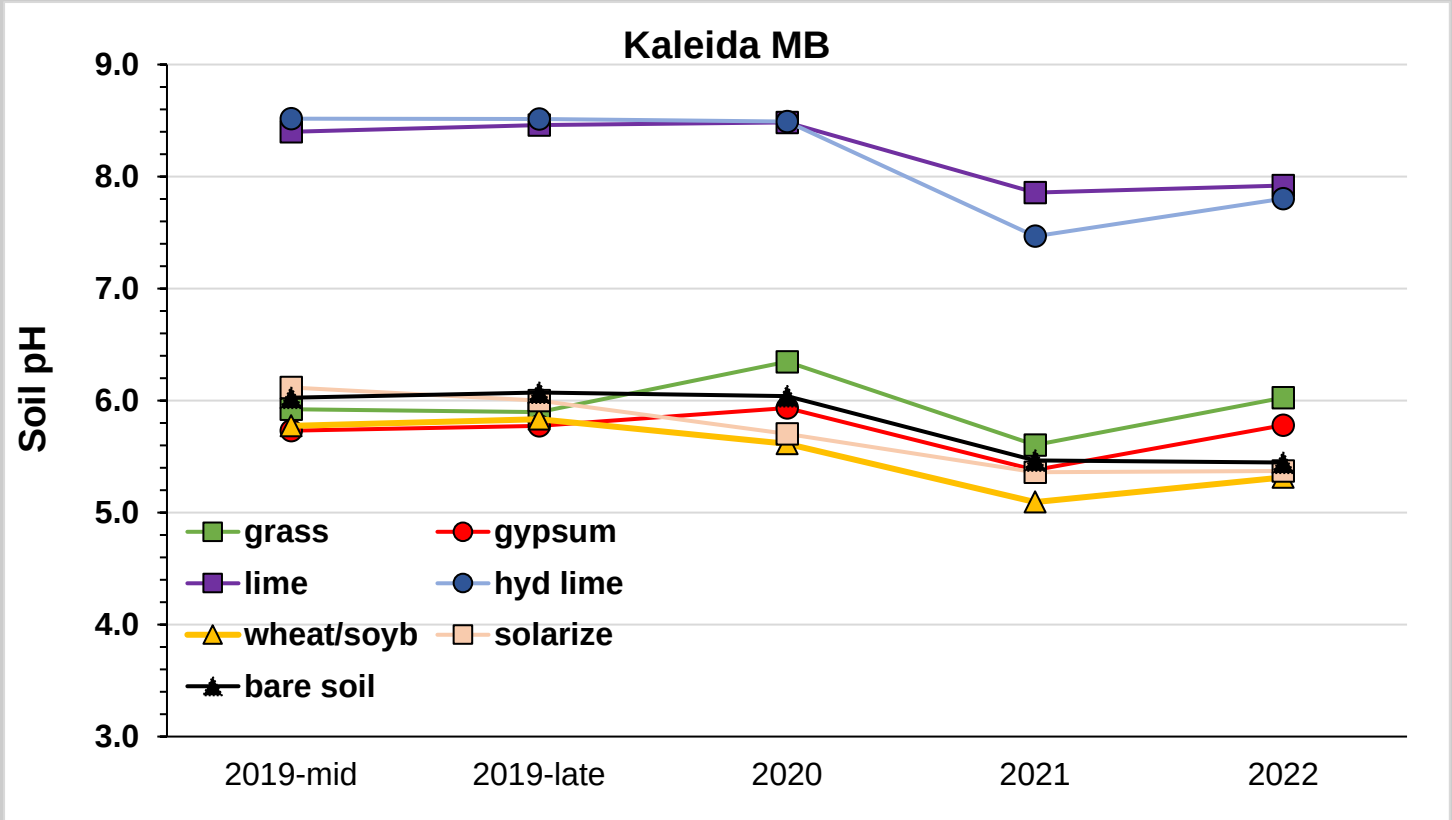


Fig. 12. Effect of grass, lime and other select treatments on soil pH at an site with clay soil and slightly acidic pH and high initial asspore levels near Kaleida, MB from 2019 to 2022.

In the trial at Spruce Grove, Alberta, resting spore numbers dropped precipitously after the first winter, but then continued to decline more slowly over the remaining two years of the study (Fig. 13). The only exception was that the estimates of spore numbers increased dramaticlly in the grass treatment in 2019, but then declined precipitously in subsequent assessments such that they were similar to the other treatments in the last two years of the study. The increase in the grass treatment was likely caused by root hair infection of the grass cover crop; this would result in a dramatic increase in the pathogen DNA in the root hairs, but would not result in production of new resting spores because perennial ryegrass is not a true host of *P. brassicae*, so it cannot complete its life cycle in this grass (or any other).

The initial soil pH at the Spruce Grove site was about 5.7. The initial treatment with lime raised mean pH to about 6.7 and a second application raised it to around 7.0 (Fig. 14). However, the soil pH had already dropped near to or below 7.0 in the plots treated with standard or hydrated lime. This indicated that high rates of lime application would likely be required to suppress clubroot infection of a susceptible canola crop the following year. As with the other sites, soil pH did not appear to affect the survival of resting spores.

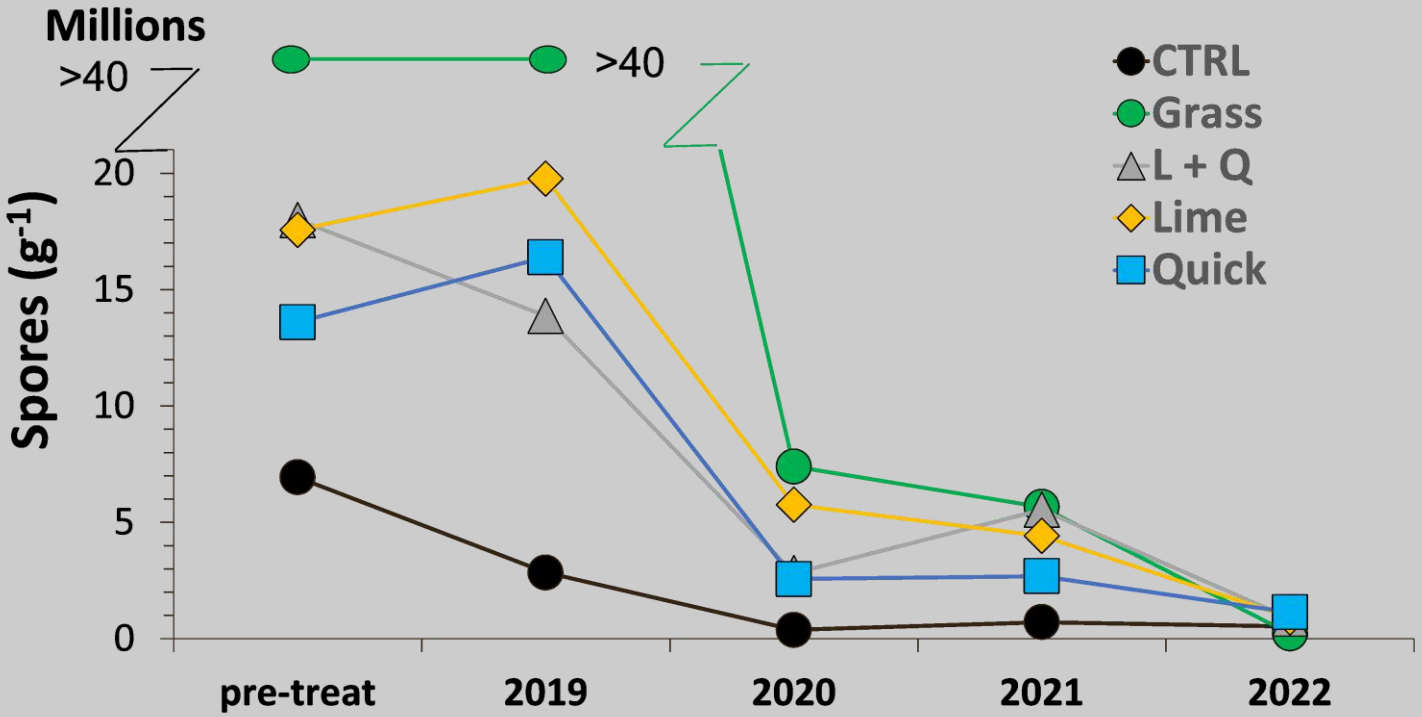


Fig. 13. Effect of grass, standard lime (L), hydrated lime (Q) and other selected treatments on survival of resting spores at an site with loam soil and acidic pH and high initial asspore leves near Spruce Grove, AB from 2019 to 2022.

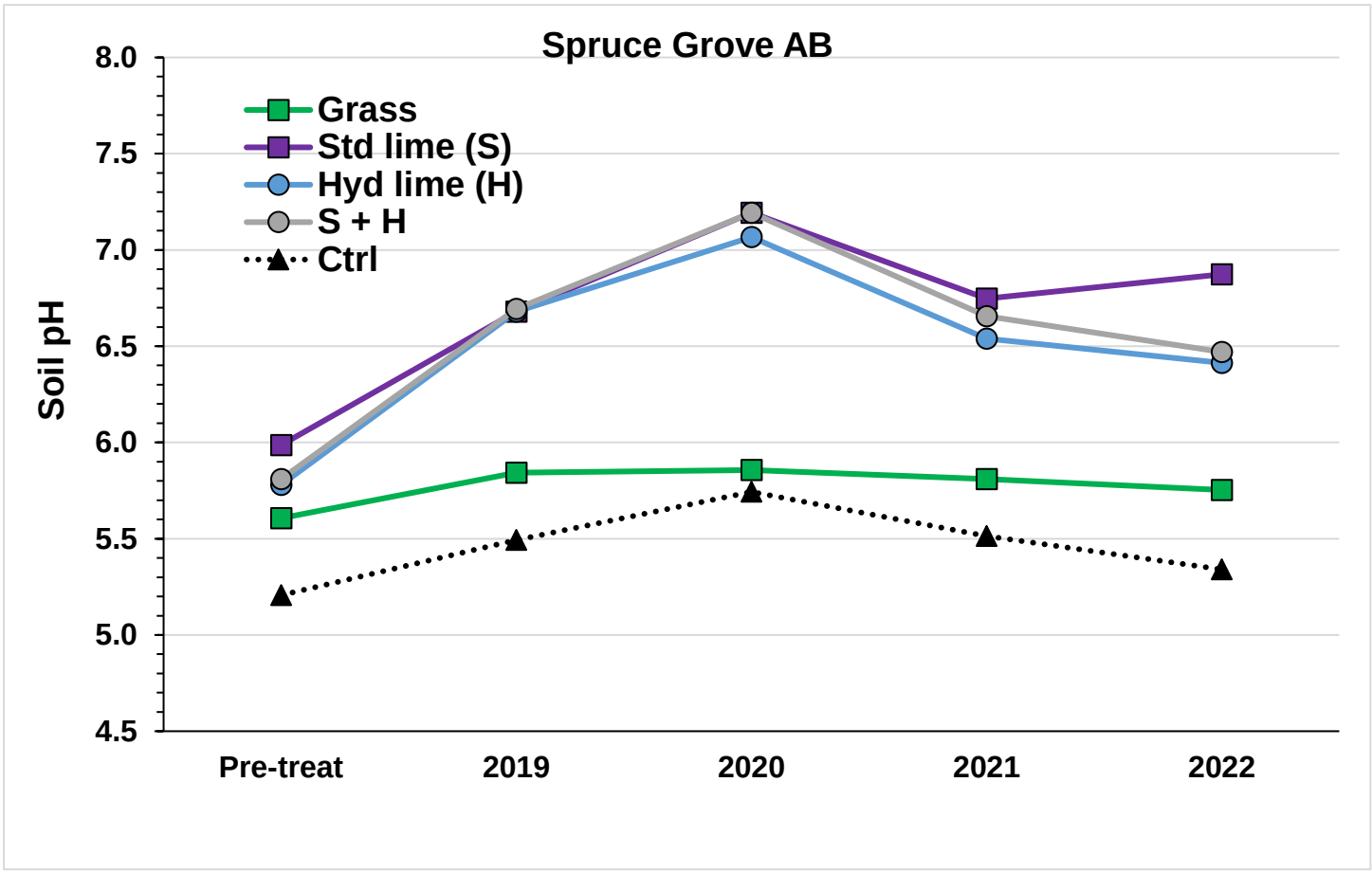
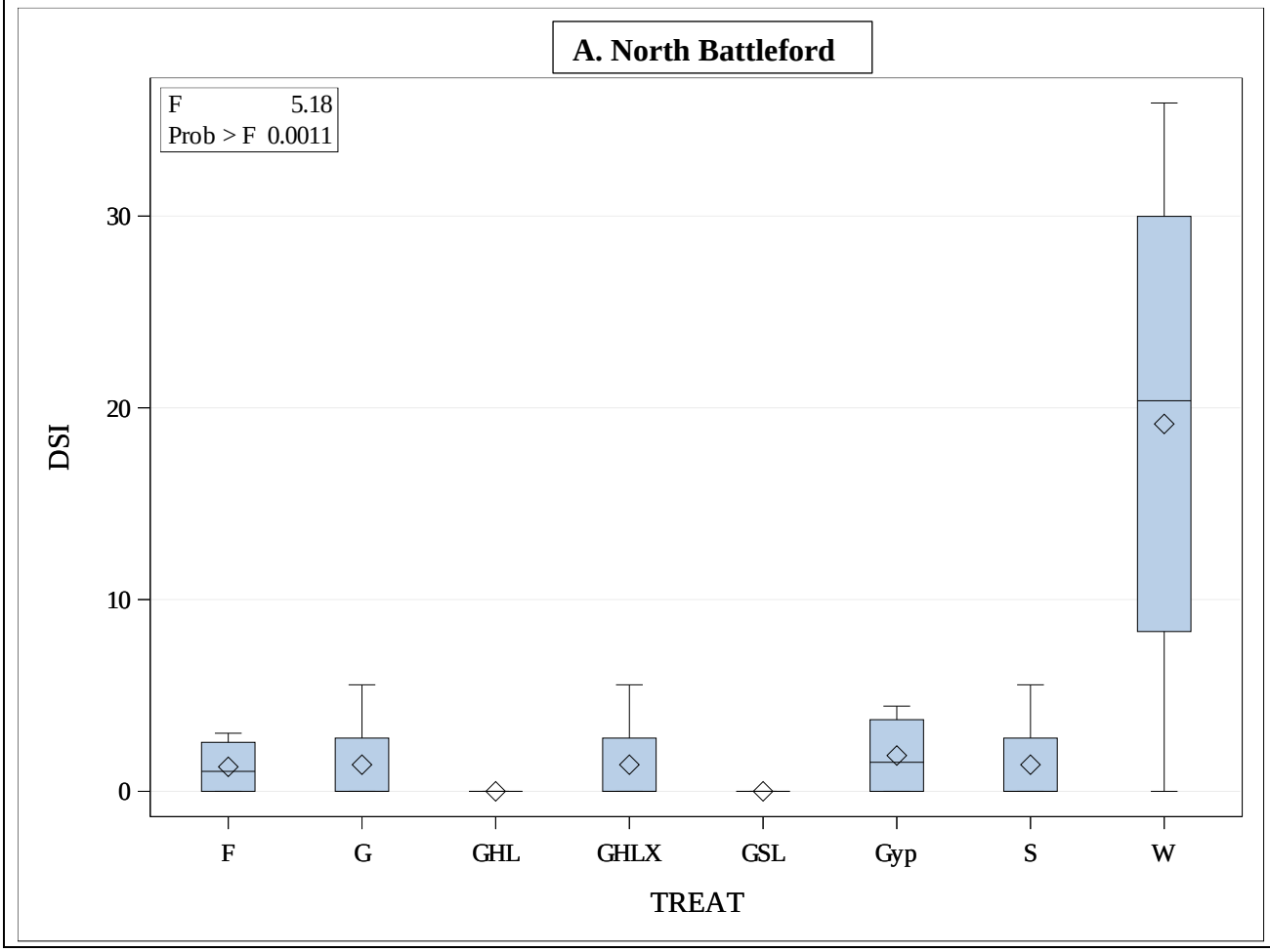


Fig. 14. Effect of grass, lime and other select treatments on soil pH at an site with loam soil and acidic pH and high initial asspore leves near Spruce Grove, AB from 2019 to 2022.

Although infection by *P. brassicae* can occur from only a single resting spore, infection of canola under field conditions is thought to be inconsistent until there are 50,000 spores or more per g of dry soil, even under conducive conditions. In the current study, the concentration of spores in soil was only this low in the final year of the study at one site (Carman, MB), where the initial spore level in the study was only 250,000 spores per g. At the other sites, spore concentration in soil was at least 10^6 per g. Several recent studies (Fox et al. 2021, McDonald unpublished) have demonstrated that when spore levels are low to moderate (10^5 or even 10^6), soil pH above 7.2 can substantially reduce or even eliminate clubroot infection. At the site at North Battleford in Saskatchewan and the two sites in Manitoba, we expected that high levels of clubroot would have been eliminated by the lime treatments, which increased soil pH to well above the pH 7.2 critical level.

In the bioassays conducted under controlled conditions of field soil collected at the end of the trials, there were no significant effects of treatment and no consistent pattern of response. The DSI of treatments at North Battleford was unexpected low (generally < 5). The low levels of DSI from the study at North Battleford may be the result of difficulties in maintaining a level of free water adequate for infection in this standy soil – we hope to collect fresh soil samples from the site in the spring of 2024 and repeat the study. In contrast, the DSI at Cutknife was high, generally > 80 (Fig. 15), as expected based on the high numbers of spores remaining in the soil at this site. In the bioassay of the site at Spruce Grove, there were also no statistical differences among treatments (Fig. 16). There were, however, large numerical differences among treatments, with treatments that received hydrated lime lower than the grass treatment, as expected because a higher pH from application of lime should reduce symptom severity. However, the control was also numerically low, which was consistent with low numbers of spores in this treatment throughout the study.

The high cost of managing patches of clubroot through application of standard or hydrated lime is an important concerns for growers. The current study only adds to this concern. It confirmed that a soil pH of >7.2 was difficult to achieve in a strongly acidic soil, as demostrated by the need for a repeated application at the Spruce Grove site after application of several tonnes per ha, but would also require repeated application over time to maintain an alkaline pH. Indeed, the soil pH at Spruce Grove had already fallen well below 7.2 (what the literature indicates to be a critical value) only two years after the lime application. Also, lime is costly to transport and difficult to apply (especially hydrated lime, which is as fine as flour). Even if the cost of agricultural lime could be substantially reduced, the cost: benefit ratio is likely to remain low and grower's enthusiasm for and uptake of this approach will be lower still.



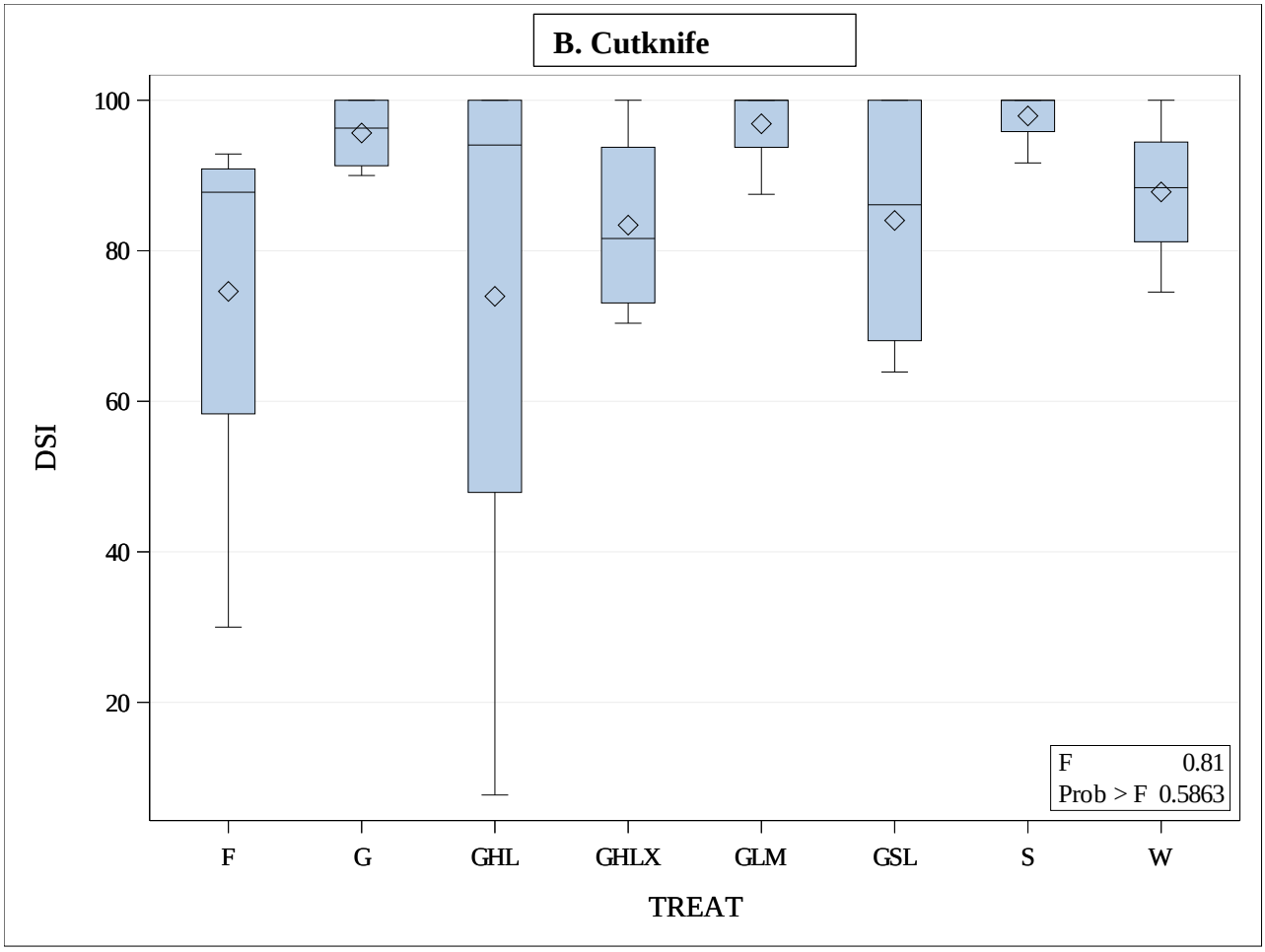


Fig. 15. Clubroot severity (disease severity index, DSI) in a bioassay to assess infection potential at the end of the study (2022) at two sites in Saskatchewan: A) North Battleford and B) Cutknife. The treatments were F (fallow, bare soil), G (grass cover), HL (hydrated lime), HLX (1.5. rate of HL), SL (standard lime), S (solarization), and W (wheat cover crop).

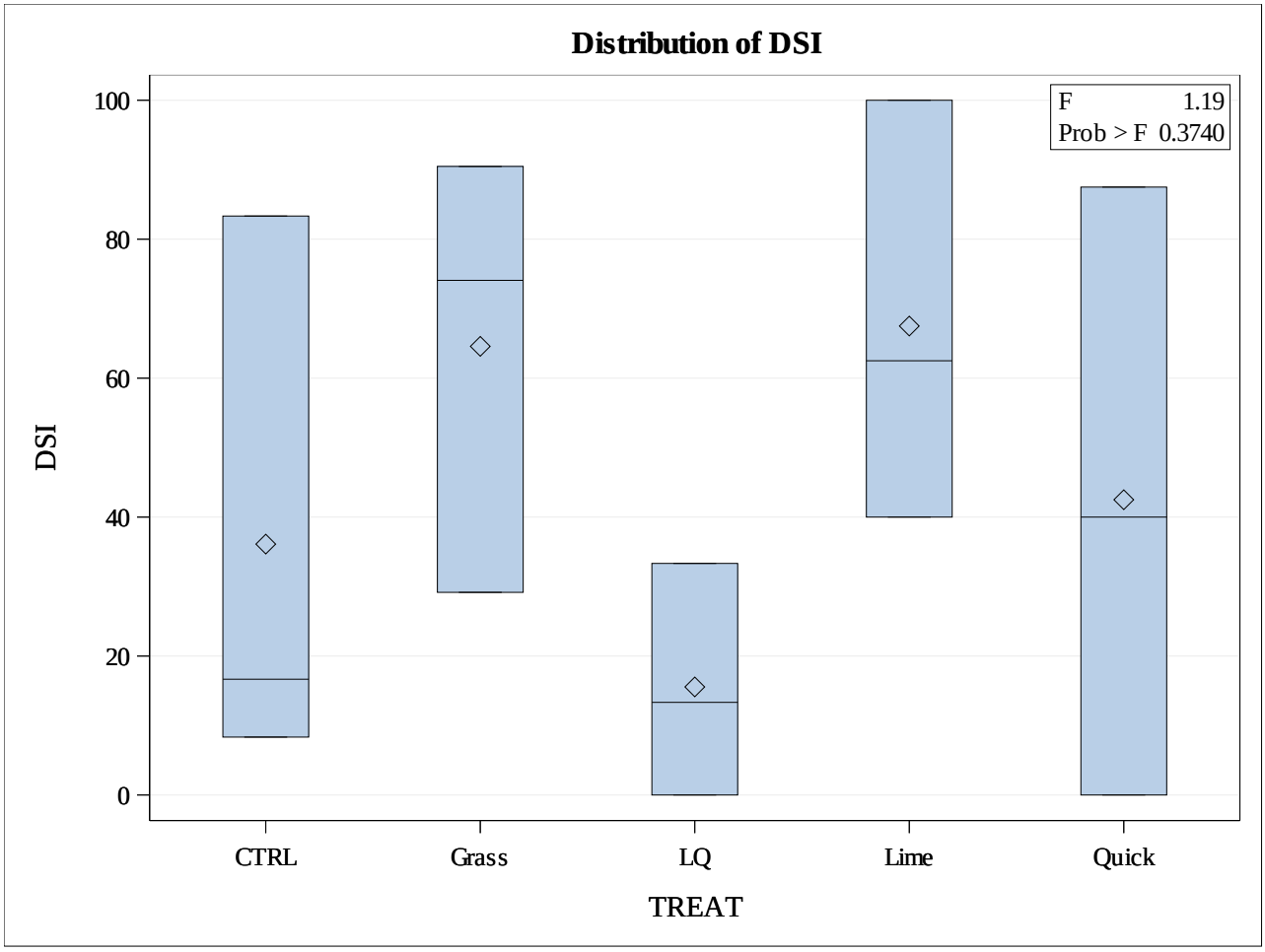


Fig. 16. Clubroot severity (disease severity index, DSI) in a bioassay to assess infection potential at the end of the study (2022) at a study at Spruce Grove. Alberta. The treatments were CTRL (fallow, bare soil), Grass (grass cover), Quick (hydrated lime), Lime (standard lime), and LQ (1/2 rate of Lime and 1/2 rate of of Quick).

Although grass crop did not result in dramatic reductions in resting spore numbers in soil as we had hoped, the recommendation to grow a grass cover crop for several years on a small clubroot patch is still supported. A strong grass cover on the patch would minimize soil transfer onto equipment, so normal equipment operations could resume in the field without risk of moving the disease to other portions of the field or other fields. A patch of green grass would also alert operators of tillage and spray equipment to avoid the infested patch until the grower is ready to break the patch and re-integrate it into normal field operations.

In the current study, one grower / co-operator in Saskatchewan was not yet ready to break the grass cover crop that had been seeded around our research trial in his field. We respected his wishes, so instead of breaking the research plots and seeding each plot to canola, we took large soil samples from the surface to 10 cm depth of each plot and used them to conduct a bioassay of clubroot infection potential under controlled conditions. Since we were obliged to do this at one site in Saskatchewan, we elected to use the same approach at the other site. This allowed us to obtain one additional year of data on spore survival from these sites. The same approach was taken at the sites in Manitoba, except that the soil samples taken were too small to use in a bioassay. As it turned out, the summer of 2022 was extremely hot and dry at all of these sites, such that late-seeded canola on heavily tilled (and therefore dried-out) soil would likely not have provided a good test of clubroot infection from the field plots even if we had chosen to conduct such tests.

The observation that assessments using the new ddPCR technique for estimating resting spore numbers in soil did not provide estimates consistent with initial of final spore numbers from at least two sites in the 2nd year of the study is concerning, because these appear to be artifacts rather than real increases in spore numbers over time. The occurrence of such artifacts indicates that there were problems in DNA extraction and subsequent spore estimation duing this period. However, it isn't possible to go back and redo these assesments. Fortunately, the pattern and consistency of spore estimates stabilized in the 3rd and 4th years of the study, which provides confidence that the patterns observed represent the actual situation.

At several sites, the spore concentration in the fallow treatment was numerically (although not statistically) lower than many other treatments. This result was highly unexpected, but may indicate that some spores were being moved down in the soil, below the level of our sampling, in the fallow treatment. This might occur as a result of moving downward with rainwater or through soil mixing from rototilling to remove weeds. A previous study demonstrated that resting spores of *P. brassicae* do move downward in the soil profile over time (Cranmer et al 2017), but it is not known if spores also move upward with ground water or if spore present deep in the soil profile are even viable.

A solarization treatment was included in the study because a series of studies on high-organic matter soils in Ontario has shown that application of TIF covers for only 2 weeks reduce (but do not eliminate) resting spore populations in soil and subsequent clubroot severity in a susceptible cropr. Indeed, a TIF alons is as effective as fumigants + TIF (McDonald et al. 2020; McDonald, unpublished). The mechanism underlying this reduction is not well understood, because measurement of soil temperatures under the tarp indicate that the increase in temperature with this short period of solarization is not sufficient to kill spores of *P. brassicae*. It is possible that the warm soil under the TIF is conducive for other microbes, resulting in biocontrol of clubroot, or that this treatment produces a bried period of anaerobic conditions that is highly deterimental to resting spores. In the current study, the TIF treatments were consistently at the low end of the tretment list in samples collected after treatment and stayed low throughout the remainder of the study. It is possible that a longer interval of coverage with TIF in mid-summer might be even more effective. However, TIF is very expensive, is difficult to apply without specialized equipment and does not completely eliminate the pathogen, but needs to be applied in mid-summer, so an entire cropping season is lost. Therefore, we conclude that this approach likely does not represent a viable alternative for effective clubroot management in canola production on the Canadian Prairies. It does, however, merit more assessment for use in management of clubroot in high-value brassica vegetable production.

Similarly, a treatment with gypsum was included at sites where the soil pH was close to neutral (North Battleford in Saskatchewam and both sites in Manitoba) to assess the effect of increased concentration of calcium in the soil without a change in pH. This represents an attempt to separate the effects of changes in Ca concentration and pH that are both associated with application of lime. As expected, application had no effect on soil ph (Figs. 9, 11 and 12), but also no effect on spore survival.

6. Conclusions and Recommendations – Highlight significant conclusions based on the discussion and analysis provided in the previous section with emphasis on the project objectives specified above; also provide recommendations for the application and adoption of the project results and identify any further research, development, and communication needs, if applicable.

The current study examined the effect of liming, grass cover crops and their interaction on survival of resting spores of *P. brassicae* under controlled conditions and in small plot trials conducted in commercial fields over several years. One important achievement of the study was to develop and validate an improved method of assessing the concentration of resting spores in soil using a molecular approach known as ddPCR. This approach was less expensive, more accurate and more robust that previous methods. The technique was used throughout the course of the current study. Controlled environment studies demonstrated that grass and cereal crops contributed to a small but consistent reduction in resting spore

concentration in infested soil over fairly short time frames. However, the effects of subsequent crops on spore survival were rarely detectable at field sites, likely because the variability of spore concentration in field sites was much larger than the effect of subsequent crop. Finally, the study supported previous report that the concentration of resting spores declined rapidly over time, and demonstrated that this occurred at sites across the Canadian Prairies, regardless of soil type or pH. This observation strongly supports the recommendation for a minimum 2-3 year break between canola crops (+ use of resistant cultivars) wherever clubroot is present. Growing a perennial grass cover crop on clubroot patches did not reduce resting spores, but a grass cover crop holds soil in place and minimizes movement of spores to new sites within or among fields, so the recommendation to use grass cover crops to manage clubroot patches was also supported.

7. Extension and communication activities: (e.g. extension meetings, extension publications, peer-reviewed publications, conference presentations, photos, etc).

Date	Category/Sub-Category/Type	Description	Status
2023/09/24	1.1.6 Abstract - Conference	Gossen, B.D., Sedaghatkish, A., and McDonald, M.R. Balancing selection in *Plasmodiophora brassicae* and its impact on resistance breakdown. Invited presentation at Clubroot Workshop, September 24, 2023 at the 16th Intern. Rapeseed Congress**,** Sydney, Australia.	Published
2023/09/24	1.1.6 Abstract - Conference	McDonald, M.R., Gossen, B.D., Froese, D., and Wigness, M. Survival of *Plasmodiophora brassicae* over time in trials on the Canadian prairies. Presentation 122, 16th Intern. Rapeseed Congress**,** September 24-27, 2023. Sydney, Australia.	Published
2023/09/24	1.1.6 Abstract - Conference	McDonald, M.R., Robson, J., Holy, K., Prapagar, K., and Gossen, B.D. Repeated freezing and thawing reduces viability of resting spores of *Plasmodiophora brassicae.* Poster 305, 16th Intern Rapeseed Congress**,** September 24-27, 2023. Sydney, Australia.	Published
2023/07/29	1.1.5 Abstract - Journal	Gossen, B.D., Froese, D., Wigness, M., and McDonald, M.R. 2024. Survival of *Plasmodiophora brassicae* over time in trials on the Canadian Prairies. Can. J. Plant Pathol. xxx: xxx. (presentation).	In press
2023/07/29	1.1.5 Abstract - Journal	Drury, S.C., Gossen, B.D., and McDonald, M.R. Growing wheat and liming to manage clubroot. Can. J. Plant Pathol. xxx: xxx. (presentation).	In press
2023/07/29	1.1.5 Abstract - Journal	Gossen, B.D., and McDonald, M.R. Decline of *Plasmodiophora brassicae* over time in response to liming or a grass cover crop in a field trial. Can. J. Plant Pathol. 45: 215. (poster).	Published

2023/01/30	3.1.2 Speaker / Presenter	McDonald, M.R., and Gossen, B.D. 2023. Clubroot Research Update. Invited presentation to the Clubroot Steering Committee of the Canola Council of Canada	Invited
2023/01/30	3.1.2 Speaker / Presenter	Gossen, B.D., Froese, D., and McDonald, M.R. Host-pathogen inter-action pillar: Update. Invited presentation to the Clubroot Steering Committee of the Canola Council of Canada.	Invited
2022/12/16	3.1.2 Speaker / Presenter	Gossen, B.D. 2022. Managing clubroot patches. Invited presentation to Nutrien agronomy staff, Saskatoon, SK.	Invited
2022/12/09	3.1.1 Event Organizer	Gossen, B.D., and McDonald, M.R. 2022. Learning to live with clubroot. Invited presentation at CAP program update meeting organized by the Canola Council of Canada as part of Canola Week, Saskatoon, SK.	Invited
2022/11/29	1.1.5 Abstract - Journal	Drury, S., Gossen, B.D., and McDonald, M.R. 2022. Reduction of resting spores of Plasmodiophora brassicae with wheat and lime. Phytopathology 112 S3: 195.	Published
2022/11/17	3.1.2 Speaker / Presenter	McDonald, M.R., and Gossen, B.D. 2022. Update on clubroot of canola. Invited presentation (McDonald) to the Ontario Canola Growers Assoc., Virtual.	Invited
2022/07/26	3.1.2 Speaker / Presenter	Gossen, B.D. 2022. Issues in forage seed production: Silvertop, root rot of clover and clubroot of canola. Invited presentation at the summer meeting of the Saskatchewan Forage Seed Association, Merrick SK,	Invited
2022/06/09	1.1.1 Article/Paper	Drury, S.C., Sedaghatkish, A., Gossen, B.D., McDonald, M.R. (2022). Grasses and field crops reduce the concentration of resting spores of Plasmodiophora brassicae in soil under controlled conditions. Plant Pathology, [online] 71(8), 1793-1800. http://dx.doi.org/10.1111/ppa.13601	Published
2022/02/28	3.1.2 Speaker / Presenter	Gossen, B.D., and McDonald, M.R. 2022. Update on clubroot research: February 2022. Invited presentation to the Saskatchewan Clubroot Initiative. On-line,	Invited
2021/02/22	3.1.2 Speaker / Presenter	Gossen, B.D., and McDonald, M.R. 2021. Clubroot research update: 2021. Invited presentation at the annual meeting of the Saskatchewan Clubroot Initiative Committee. February 22, 2021. (Virtual).	Invited
2021/02/19	3.1.2 Speaker / Presenter	McDonald, M.R., Gossen, B.D., Al-Daoud, F., Kasinathan, H., Drury, S., and Sedaghatkish, A. 2021. Challenges in the management of clubroot of canola. Invited presentation (McDonald) to the Professional Pest Management Association of British Columbia.	Invited

2021/02/02	3.1.2 Speaker / Presenter	Gossen, B.D., and McDonald, M.R. 2021. Update from the Outback 2021. Invited presentation at the annual meeting of the disease subcommittee of the Western Canola Recommending Committee.	Invited
2019/07/30	1.1.6 Abstract - Conference	Sedaghatkish, A., Gossen, B.D., and McDonald, M.R. 2019. Several grass crops reduce resting spores of Plasmodiophora brassicae in soil. Proc. Plant Canada 2019, pg. 294. PC2019 Program & Proceedings (plantcanada.ca).	Published
2019/07/30	1.1.6 Abstract - Conference	McDonald, M.R., and Gossen, B.D. 2019. Billions, trillions and quadrillions: The challenge of managing clubroot on canola and Brassica vegetables. Proc. Plant Canada 2019, pg. 58. PC2019 Program & Proceedings (plantcanada.ca) (Invited plenary presentation).	Published
2019/06/29	1.1.8 Poster - Conference	Sedaghatkish, A., Gossen, B.D., and McDonald, M.R. 2019. Grass cover crops reduce the concentration of Plasmodiophora brassicae resting spores in soil under controlled conditions. Proc. Intern Rapeseed Congress, Berlin, Germany, June 2019.	Published
2019/06/29	1.1.5 Abstract - Journal	Orchard, D., Gossen, B.D., MacDonald, M.R., and Strelkov, S.E. 2019. Practical solutions for managing clubroot (*Plasmodiophora brassicae*) on canola in western Canada. Can. J. Plant Pathol. 41: 158–159.	Published
2019/05/31	1.1.1 Article/Paper	Gossen, B.D., Al-Daoud, F., Dumonceaux, T., Dalton, J.A., Peng, G., Pageau, D., McDonald, M.R. (2019). Comparison of techniques for estimation of resting spores of Plasmodiophora brassicae in soil. Plant Pathology, [online] 68(5), 954-961. http://dx.doi.org/10.1111/ppa.13007	Published

8. Acknowledgements – Include actions taken to acknowledge support by the Funders.

The funders of this project have been identified and acknowledged in every paper, presentation and poster arising from this project, including at events such as Canola Week, meetings of the Clubroot Steering Committee of the Canola Council of Canada and the Saskatchewan Clubroot Initiative of the Province of Saskatchewan. As a result of this extensive extension work, information from the project has been made available to canola researchers, agronomists and extension specialists across the Prairie region.

Also, we thank M. Wigness and many summer students for technical assistance, the producers who provided the field sites, and the graduate students (S. Drury and Dr. A. Sedaghatkish) who contributed to the studies.

9. Literature Cited

Cranmer, T.J., Gossen, B.D., Al-Daoud, F., Deora, A., and McDonald, M.R. 2017. Vertical distribution of resting spores of *Plasmodiophora brassicae* in soil. Eur. J. Plant Pathol. 149(2): 435-442. DOI 10.1007/s10658-017-1193-x.

Drury, S., Sedaghatkish, A., Gossen. B.D and McDonald, M.R. 2022. Grasses and field crops reduce the

concentration of resting spores of *Plasmodiophora brassicae* in soil. Plant Pathol. . 71: 1793–1800. https://doi.org/10.1111/ppa.13601.

Fox, N.M., Hwang, S.F., Manolii, V.P., Turnbull, G., and Strelkov, S.E. 2021. Evaluation of lime products for clubroot (*Plasmodiophora brassicae*) management in canola. Can. J. Plant Pathol. DOI**:** 10.1080/07060661.2021.1940590

Gossen, B.D., Sedaghatkish, A., Hwang, S.F., and McDonald, M.R. 2018. A recipe for managing small patches of infestation of clubroot in canola. Proc. International Clubroot Workshop, Edmonton, AB, Aug 7-9, 2018.

McDonald, M.R., Gossen, B.D., Robson, J., and Al-Daoud, F. 2020. Interaction of solarization, fumigation and totally impermeable film for the management of clubroot (*Plasmodiophora brassicae*) on brassica crops. Acta Hortic. 1270: 153–160. Proc. IX International Symposium on Soil and Substrate Disinfestation Eds.: A. Gamliel et al. DOI 10.17660/ActaHortic.2020.1270.17.

Wen, R., Lee, J., Chu, M., Tonu, N., Dumonceaux, T., Gossen, B.D., Yu, F., and Peng, G. 2020. Quantification of *Plasmodiophora brassicae* resting spores in soil using droplet digital PCR (ddPCR). Plant Dis. 104: 1188–1194. doi:10.1094/PDIS-03-19-0584-RE.

10. Other Administrative Aspects: HQP personnel (PhD and/or MSc students) trained and involved; equipment bought; project materials developed

Three graduate students at the University of Guelph have been directly involved in the project; Sarah Drury (MSc) and Afsaneh Sedaghatkish (PhD) conducted the assessments of spore survival under controlled conditions, and Kirsten Holy (MSc) is currently studying spore movement in soil and spore survival in response to freeze / thaw cycles. Also, Shauna Chesney (MSc) was indirectly involved via studies of TIF to reduce clubroot on canola and brassica vegetables.

11. Appendices - If necessary, include any materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications.

12. Financial (to be provided to CCC)

a. Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to the Funders' Funding.
b. Explanation of variances from budget which are greater than 10%.
c. Invoice

13. Final Report Posting Do you consent to a version of this Final Report (with sensitive information removed) to be posted on the funder's website?	Yes.
14. Research Abstract Posting Do you consent to the 2-3 Research Abstract submitted with this Final Report to be posted on the funders and the Canola Council of Canada's website?	Yes.

Please send an electronic copy of this completed document to:

Ellen McNabb
Research Administrator
Canola Council of Canada
400 – 167 Lombard Ave.
Winnipeg, MB R3B 0T6

Phone: (204) 982-2110
Fax: (204) 942-1841
E-Mail: mcnabbe@canolacouncil.org