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Chloropicrin Soil Fumigation Reduces *Spongospora subterranea* Soil Inoculum Levels but Does Not Control Powdery Scab Disease on Roots and Tubers of Potato

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Abstract The effect of chloropicrin fumigation on the soil populations of *Spongospora subterranea* and the development of powdery scab, formation of root galls and tuber yield was investigated in seven field trials conducted in Minnesota and North Dakota. Sixteen potato cultivars, with different levels of susceptibility to disease on roots and tubers, were planted in plots treated with chloropicrin at rates ranging from zero to 201.8 kg a.i. ha^{-1}. The amount of *S. subterranea* DNA in soil was determined using qPCR. Bioassays were conducted to further assess the effect of chloropicrin fumigation on root colonization by *S. subterranea* in two potato cultivars with contrasting disease susceptibility. In the field, chloropicrin applied at rates between 70.1 to 201.8 kg a.i. ha^{-1} significantly decreased *S. subterranea* initial inoculum in soil but increased the amount of disease observed on roots and tubers of susceptible cultivars. The effect of increasing disease was confirmed in controlled conditions experiments. Although the amount of *S. subterranea* DNA in roots of bioassay plants increased with increasing chloropicrin rates, it remained similar among potato cultivars. Chloropicrin fumigation significantly increased tuber yield which in cultivars such as Shepody and Umatilla Russet were associated with the amount root galls ($r = 0.30$; $P < 0.03$). Results of these studies contradict earlier reports on the use of chloropicrin fumigation for the control of powdery scab. Factors other than inoculum level, such as environmental conditions that affect inoculum efficiency and host susceptibility, may be significant contributors to the development of powdery scab and root gall formation.

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Resumen El efecto de la fumigación del suelo con cloropicrina sobre la concentración del inóculo de *Spongospora subterranea* en suelo y el desarrollo subsecuente de sarna polvorienta en tubérculos, agallas radicales y rendimiento a cosecha fue investigado en siete ensayos de campo llevados a cabo en Minnesota y Dakota del Norte. Diesiceis cultivares de papa con diferentes niveles de susceptibilidad a la enfermedad en tubérculos y raíces fueron sembrados en parcelas tratadas con cloropicrina en dosis comprendidas entre cero y 201.8 kg i.a. ha^{-1}. La cantidad de ADN de *S. subterranea* en suelo fue determinada usando qPCR. Se llevaron a cabo bioensayos con la finalidad de evaluar el efecto de la fumigación con cloropicrina sobre la colonización por *S. subterranea* en raíces de dos cultivares de papa con niveles contrastantes de susceptibilidad a la enfermedad. En campo, la aplicación de cloropicrina en dosis entre 70.1 a 201.8 kg i.a. ha^{-1} disminuyó significativamente la cantidad de inóculo inicial de *S. subterranea* en suelo, pero incrementó la cantidad de enfermedad observada en raíces y tubérculos de cultivares susceptibles. El efecto de incremento de enfermedad fue confirmado bajo condiciones controladas. Aunque las cantidades de ADN de *S. subterranea* en raíces de plantas del bioensayo aumentó con la dosis de cloropicrina, permaneció similar entre cultivares. La fumigación con cloropicrina aumentó significativamente el rendimiento a cosecha, el cual se observó asociado a la cantidad de agallas radicales en cultivares como Shepody y Umatilla Russet ($r = 0.30$; $P < 0.03$). Los resultados de este estudio contradicen reportes previos sobre el uso de cloropicrina en el manejo de la sarna polvorienta. Factores distintos al nivel de inoculo, tales como las condiciones del ambiente que afectan la eficiencia del mismo y la susceptibilidad del hospedante podrían contribuir significativamente en el desarrollo de la enfermedad.

Keywords Plasmodiophorids · Disease management · *Solanum tuberosrum* · Soil-borne · Tuber-borne

Introduction

The obligate biotroph *Spongospora subterranea* (Wallr.) Lagerh. (Cercozoa, Phytomyxea) causes powdery scab and root gall formation on potato (*Solanum tuberosrum* L.) and is the vector of the *Potato mop-top virus* (Jones and Harrison 1969; Harrison et al. 1997; Neuhauser et al. 2010). The pathogen overwinters forming resting spore aggregates (sporosori) (Braselton 2001) from which zoospores germinate to further infect potato roots, stolons and tubers (Harrison et al. 1997; Merz and Falloon 2009). The development of tuber symptoms (powdery scab) represents the main detrimental effect of disease caused by *S. subterranea* since it can drastically reduce the quality and marketability of the harvested crop (Braithwaite et al. 1994).

Although the tuber phase of the disease has been more extensively studied, a number of reports demonstrate the negative effect of *S. subterranea* root infection on host growth and water and nutrient uptake (Falloon et al. 2016; Gilchrist et al. 2011; Hernandez Maldonado et al. 2013; Lister et al. 2004). Under controlled conditions, *S. subterranea* can potentially reduce total tuber yield across a range of inoculum concentrations (Shah et al. 2004, 2012). However, in a four-year field study in which the cultivars Shepody and Umatilla Russet were grown in pathogen-free and *S. subterranea* naturally infested soils, no evidence of a negative effect on tuber yield due to root gall formation and powdery scab on tubers was observed (Johnson and Cummings 2015). Whether reduction in tuber yield may result as consequence of *S. subterranea* infection and development, the resulting marketable tuber yield will closely relate to the incidence and severity of powdery scab (Braithwaite et al. 1994).

Root galls and tuber lesions are filled with masses of sporosori contributing to an increase in soil inoculum levels and the spread of the pathogen to other locations (Brierley et al. 2009; Merz 2008). In a series of shadehouse experiments, a curvilinear relationship was observed between the severity scores of powdery scab and the inoculum amount ranging from 25 to 10^5 sporosori ml soil^{-1}. However, at inoculum concentrations <1000 sporosori · ml soil^{-1} no clear relationship was observed (Shah et al. 2012). In addition, no significant differences in the severity of powdery scab and root gall formation were observed in artificially infested pot experiments in which *S. subterranea* inoculum concentration ranged from 5 to 50 sporosori ml soil^{-1} (van de Graaf et al. 2005, 2007). In the field, evidence of a significant association between the initial inoculum level (0 to 1.4×10^4 sporosori g^{-1} soil) and powdery scab incidence was observed for 17 potato fields in the United States (Qu et al. 2006). Nonetheless, these results are in contrast with a number of studies conducted in naturally infested soils reporting a weak or non-existent relationship between the initial inoculum amount and the resulting degree of powdery scab (Merz et al. 2012; Nakayama et al. 2007; Shah et al. 2014). Unlike results observed on initial inoculum and its relationship to disease expression, a significant association between the level of soil infection potential (i.e. infecting zoospores) and powdery scab severity was observed when soil samples of 27 potato fields in Japan were assessed (Nakayama et al. 2007). Collectively, these results suggest that conditions other than the initial inoculum level can influence the resulting degree of disease development (Burnett 1991). Nevertheless, a lower amount of initial inoculum might decrease the chances for the pathogen to infect and disease to occur in the field (Brierley et al. 2013; Tegg et al. 2015).

The distribution of *S. subterranea* is ubiquitous (Harrison et al. 1997; Merz and Falloon 2009). The pathogen is found causing disease in temperate potato producing areas as well as in hot weather climates where production is conducted at high altitudes or under irrigation (Harrison et al. 1997; Merz and Falloon 2009; Wale 2000). *S. subterranea* resting spores are resistant to environmental stresses allowing the pathogen to survive in soil and on tubers for long periods of time (> 10 years; Braselton 2001; Sparrow et al. 2015). In addition, the pathogen is capable to infect several solanaceous and non-solanaceous plant species (Falloon 2008; Iftikhar and Ahmad 2005; Neuhauser et al. 2014; Qu and Christ 2006; Shah et al. 2010). The above-mentioned factors make the direct control of *S. subterranea* difficult to achieve.

Strategies implemented in order to minimize the risk of powdery scab and root gall formation have commonly focused on the reduction of primary inoculum and conditions that favor disease development (Braithwaite et al. 1994; Falloon et al. 1996; Hughes 1980; Thangavel et al. 2015). Included among these approaches are cultural practices such as irrigation withhold and crop rotation (Falloon 2008) as well as soil and seed application of chemical compounds such as flusufalmide and fluazinam (Falloon 2008). However, no single approach offers effective suppression of disease caused by *S. subterranea*. A management strategy that integrates multiple practices for disease control is likely to increase the chances of successful disease suppression (Falloon 2008). Such approach requires the evaluation of methods of control that can be incorporated in a management plan.

Recent studies have shown promising results with the application of chloropicrin for the management of powdery scab (Tsror 2014; Tsror et al. 2009; Tsror et al. 2016) in which disease reduction was in most instances accompanied by an increase in tuber yield. However, the efficacy of soil fumigation at reducing pathogen inoculum and disease expression may be influenced by several factors, including pathogen soil inoculum level, fumigant motility in soil, temperature and depth of application and host susceptibility in roots and tubers (Ajwa et al. 2002; de Boer and Theodore 1997; Falloon 2008; Lembright 1990; Merz and Falloon 2009). Therefore, the

evaluation of chloropicrin soil fumigation under cropping condition of North Dakota and Minnesota is necessary. The objective of this study was to investigate the effect of chloropicrin soil fumigation on the soil populations of *S. subterranea* and its associated effect on the development of powdery scab on tubers, root galls and total tuber yield.

Materials and Methods

Soil Sampling

In order to assess the effect of chloropicrin fumigation rates on soil populations of *S. subterranea*, soil samples were collected from fumigated plots at pre- (October–November) and post-fumigation (June–July) with exception of the experiments conducted in 2011, for which only post-fumigation soil samples were collected. In 2011, post-fumigation sampling was performed at nine days after planting (DAP) and at 50 DAP during 2012 and 2013. Soil samples collected during 2011 were obtained at arbitrary sampling points (>10 sites) within each fumigated plot. During 2012 and 2013, a total of 48 sampling points were flagged at pre-fumigation to form a 12 × 4 or a 24 × 2 (sampling points x row) grid, each running parallel to the direction the soil fumigant was applied. In total, 12 sampling points were located between the two middle rows of each fumigated. In all conducted experiments, compound samples (approx. 0.5 l) were obtained by bulking ten soil subsamples obtained at arbitrary points within one-meter diameter area around each flagged site. Sampling was performed using a soil auger at two depths (0 to 0.1 m and 0.1 to 0.2 m). All collected samples were air-dried at 25 °C for seven days in the dark and kept at 4 °C until use.

S. subterranea Molecular Quantification from Soil and Host Tissue

DNA Extraction from Soil and Root Tissue

Soil samples were sieved to a fine grain (<5 mm) and DNA was extracted from 0.25 g (in triplicate) using the PowerSoil® DNA isolation kit (MoBio, Carlsbad, CA) according to manufacturer's instruction with a few modifications. Sample homogenization and cell lysis was achieved using a FastPrep® instrument (MP Biomedicals, Santa Ana, California) at speed 5.5 for 40 s and an additional 15 min on a horizontal vortex platform at medium speed. An additional 2 min of incubation was added to step 20. The extracted DNA was eluted in 100 μl of solution C6 (10 mM Tris, pH 8.0). For root DNA extractions, 10 mg of freeze-dried tissue were employed. The PowerPlant Pro® extraction kit (MoBio) was used following manufacturer's instruction with few modifications. Sample homogenization and cell lysis was achieved using a FastPrep® instrument at speed 5.0 for 30s, and an additional 10 min of horizontal vortex agitation. The DNA extracts were incubated for 2 min in the Spin Filter prior to elution in 100 μl of solution PD7 (10 mM Tris, pH 8.0).

Artificially Infested Soils

S. subterranea sporosori were obtained from heavily infected tubers of the cv. Shepody (Merz 1989). A sample of the sporosori retained between the 45 to 150 μm mesh sieves was used to estimate the mean number of resting spores per sporosori and the average sporosori volume (Falloon et al. 2011). The sample size (± 5%) was determined as $= (t^2)\frac{(S^2)}{(d^2)}\left(\bar{x}^2\right)$, with $t_{n-1;\frac{\alpha}{2}}$ (n = sampled population; $\alpha = 0.05$), and where d is half of the total length of the confidence interval of the mean ($\bar{x}$) expressed as a proportion (Neher and Campbell 1997). A total of three soil samples [A: organic matter (OM) 4.2%, clay 40%, pH 7.9; B: OM 4.2%, clay 30.9%, pH 7.6; C: OM 2.5%, clay 34.9%, pH 7.3] previously tested as pathogen-free (below), were inoculated with a sporosori suspension to a final concentration of 10^6 resting spores g^{-1} soil (d. w.). DNA was extracted from the infested soil samples and tenfold serial dilutions were prepared in order to obtain concentrations equivalents to 10^5, 10^4, 10^3, 10^2 and 10^1 resting spores g^{-1} soil.

Real-Time PCR and Pathogen Quantification from Soil and Host Tissue

The *S. subterranea* specific primer/probe set SsTQF1 (5 - CCG GCA GACCCA AAA CC -3), SsTQR1 (5 - CGG GCG TCACCCTTC A-3) and TaqMan® probe SsTQP1 (5 FAM - CAGACA ATC GCA CCC AGG TTC TCA TG-3 ; van de Graaf et al. 2003) were used in this research. The real-time quantitative PCR (qPCR) reactions were performed using a Stratagene Mx3005P® qPCR System (Agilent Technologies, Santa Clara, CA) in which each reaction consisted of 2 μl of sample DNA (five-fold diluted for soil samples) and 23 μl of reaction mix [1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM each primer, 0.1 μM of the probe and one unit GoTaq® DNA polymerase (Promega, Madison, WI)]. The qPCR thermal conditions used consisted of an initial cycle at 98 °C for two min, followed by 40 cycles of 98 °C for 10s and 58 °C for 45 s.

Absolute quantification of the pathogen was performed using a standard curve containing different amounts of a plasmid carrying the *S. subterranea* ITS gene. The target DNA (63 bp) was amplified using the SsTQF1/SsTQR1 primer set, and cloned using the pGEM®-T Easy Vector System (Promega) according manufacturer's instructions. The correct insert was verified by PCR and DNA sequencing, followed by plasmid purification using the Plasmid Mini Kit (QIAGEN)

and spectrophotometric quantification (260 nm). A series of tenfold dilutions of the plasmid-DNA, with concentrations ranging from 1×10^{-2} to 1×10^{-8} ng plasmid-DNA μl^{-1}, was analyzed in triplicates. The resulting Ct values were plotted against the amount of plasmid-DNA to construct a standard curve. This procedure was also used to create a standard curve between the amounts of plasmid-DNA and *S. subterranea* resting spores · g^{-1} soil, using the artificially infested soils A, B and C (above) and taking in account the initial soil sample weight and the amount of soil DNA in each qPCR reaction. The efficiency of each assay was calculated as $E = [(10^{-1/\text{slope}}) - 1] \times 100$; across qPCRs E was kept between 90 and 110%. The regression coefficient (r^2) of each assay was kept at $r^2 > 0.99$. Among assays, the standard curves were compared using the heterogeneity of regression coefficients test ($\alpha = 0.05$; Burns et al. 2005; Bustin et al. 2009). Soil and root tissue samples were tested in three individual assays in which triplicate reactions were included for each sample and standard concentration; additionally, a non-template reaction was included in each plate. Results were expressed as picograms (pg) of plasmid-DNA g^{-1} root and femtograms (fg) plasmid-DNA g^{-1} soil. Prior to analysis, data on pg plasmid-DNA g^{-1} root were log-transformed; data on plasmid-DNA g^{-1} soil were square-transformed.

Field Experiments

Trial Location and Experimental Design

A total of seven large-scale field experiments were conducted from 2011 to 2013 in five irrigated commercial fields in North Dakota and Minnesota (Table 1). Fields were selected based on history of previous powdery scab presence, soil type and soil pH. The trials were aimed to assess the effect of chloropicrin soil fumigation rate on the development of powdery scab, root gall formation, and tuber-yield using a randomized split-plot design with four replicates with exception of the trial conducted at Larimore, ND in 2011, which utilized a randomized strip-plot design with four replicates. The whole plot (or horizontal factor) was represented by the fumigant rate which ranged from zero (non-treated) to 201.8 kg a.i. ha^{-1} (Table 1).

From 2011 to 2013, chloropicrin fumigant [Pic Plus Fumigant™ = Chloropicrin (CCl_3NO_2) – 85.5% active ingredient, TriEst AG Group Inc., Greenville, NC] was applied in-row (i.e. fumigant application in parallel strips or bands) to a depth of 0.3 m using a standard, commercial shank injector system. Alternatively, broadcast (i.e. uniform fumigant application to an entire field or soil bed) applications were conducted in 2011 using a commercial coulter rig (Table 1). Soil fumigations, with exception of the trial conducted at McCanna, ND, during 2011, were carried out during the fall (Table 1) preceding each cropping season (May to September). Spring soil fumigation at McCanna during 2011 was conducted on May 26, followed by late planting on June 20. During fumigation procedures, soil temperatures to a depth of 0.1 m ranged between 3.2 and 18.9 °C (Table 1). Fumigated plots remained uncovered until planting and a buffer area (approx. 15 m) was left between fumigated plots in order to reach the fumigation rate required for each soil treatment.

A set of potato cultivars ranging in susceptibility to powdery scab and root gall formation (Houser and Davidson 2010) were randomly assigned to each split-plot (vertical factors). Each subplot was separated by a 0.9 m buffer area. Certified seed-tubers (60 to 100 g) were employed in all trials. These tubers were visually inspected for powdery scab symptoms throughout seed preparation procedures. In the field, each seed-tuber was planted 0.3 m apart and 0.1 m depth; the distances between rows was 0.9 m. Soil texture among experimental field ranged from sand to sandy loam and loamy sand (Table 1). Crop management and pest and diseases control was conducted by the growers according to recommendations developed for the area.

Root Gall Formation Assessment

Three plants were carefully removed from the soil at 60, 75 and 90 DAP. In experiments where main plots consisted of three and four rows, plants were arbitrarily sampled in row one (i.e. first row to be planted), whereas sampling in six row plots was performed in row two. Large soil particles were removed by gently shaking the plant root system. Galls on roots were counted using a magnifying glass (1.75X) and the number of galls per plant averaged for each plot. The area under disease progress curve (AUDPC) for each plot was calculated as AUDPC $= \sum_{j=1}^{n_j-1} \left(\frac{Y_j+Y_{j+1}}{2}\right)\left(t_{j+1}-t_j\right)$ (Madden et al. 2007). AUDPC data were log-transformed prior analysis.

Powdery Scab Assessment

At harvest, all tubers over 40 g were collected and stored for approximately six weeks at 12 °C *prior* to disease evaluations, tubers were washed and dried overnight at room temperature. A total of 100 tubers were evaluated per plot; when this amount was not available, all existing tubers were evaluated. Disease incidence was determined as the percentage of symptomatic tubers in a sample. Disease severity was estimated on each side of the tuber using an increasing percentage graphic scale (Falloon et al. 1995), and then averaged. The mean disease severity was calculated as $\left[\sum \frac{(nN_n)}{5N_0}\right]$ *100, where n is the disease index (0 = no disease, 1 = one pustule to 2.0%, 2 = 2.1 to 5.0%, 3 = 5.1 to 10.0%, 4 = 10.1 to 25%, 5 = > 25% (Houser and Davidson 2010; Nakayama et al. 2007), N_n is the number of tubers with disease symptoms at level "n" and N_0 is the total number of tubers evaluated. A powdery scab index was

Table 1 Potato cultivars and chloropicrin rates evaluated in field trials conducted in Minnesota and North Dakota between 2011 and 2013

Year	Location	Soil texture / pH	Fumigation date / Soil temperature (C)	Planting / Harvest date	In-row fumigant rate (kg a.i. ha^{-1})	Split-plot	Cultivars
2011	Perham, MN	Sand / 7.4	October, 2010 / n. a.	May 10 / September 15	0 (non-treated) 74.7[a] 149.4[a]	3 rows of 9 m long each	Alpine Russet, Bannock Russet, Dakota Trailblazer, Ivory Crisp, Kennebec, Lamoka, Nicolet, Ranger Russet, Red LaSoda, Red Norland, Red Pontiac, Russet Burbank, Russet Norkotah, Shepody, Umatilla Russet, Yukon Gold
	Larimore, ND	Sandy loam / 6.7	October, 2010 / n. a.	May 17 / September 27	0 74.7[a] 149.4[a]	3 rows of 9 m long each	Alpine Russet, Bannock Russet, Dakota Trailblazer, Ranger Russet, Russet Burbank, Russet Norkotah, Shepody, Umatilla Russet
	McCanna, ND	Sandy loam / 5.6	May 26, 2011 / 10.6	June 20 / October 3	0 29.2[a] 58.3[a] 112.1 154.1 196.1	3 rows of 4.5 m long each	Alpine Russet, Bannock Russet, Dakota Trailblazer, Ivory Crisp, Kennebec, Ranger Russet, Red LaSoda, Red Norland, Red Pontiac, Russet Burbank, Russet Norkotah, Shepody, Umatilla Russet, Yukon Gold
2012	Osage, MN	Sandy loam / 6.1	October 8, 2011 / 18.9	May 2 / September 3	0 112.1 154.1 196.1	4 rows of 4.5 m long each	Dakota Trailblazer, Ivory Crisp, Kennebec, Ranger Russet, Red LaSoda, Red Pontiac, Russet Burbank, Shepody, Umatilla Russet, Yukon Gold
	McCanna, MN	Sandy loam /5.6	October 11, 2011 / 14.6	April 25 / September 6			
2013	Perham, MN	Loamy sandy /5.8	October 24, 2012 / 8.2	May 24 / September 25	0 70.1 140.1 201.8	6 row of 9 m long each	Red Norland, Russet Burbank, Shepody
	Park Rapids, MN	Sand / 7.2	November 1, 2012 / 3.2	May 27 / September 27			

[a] Broadcast rather than applied in-row, in-row equivalent presented

n.a. Not available

obtained by multiplying the mean disease severity by mean disease incidence of each plot (Houser and Davidson 2010). Powdery scab index data were log-transformed prior to analysis.

Tuber Yield Assessment

Total tuber yield was obtained at harvest (approximately 115 to 120 DAP). In those plots consisting of three and four rows, harvest was performed in rows two and three respectively. For plots consisting of six rows, harvest was conducted in row five. Complete rows were harvested and tuber yield from each row converted to metric tons per hectare for analysis.

Bioassay

During 2013, two bioassays were conducted using chloropicrin fumigated soil at four rates (0, 70.1, 140.1 and 201.8 kg a.i. · ha^{-1}). Large soil samples (10 kg) were collected from two fields located near Perham and Park Rapids, MN. A single sampling point was positioned at the center of each main plot replicate (fumigation rate). In total, four soil samples (0 to 0.2 m depth) were collected for each fumigation treatment and bulked. Large debris masses and crop residues were removed using a sieve (0.25 cm mesh); then, 300 g of soil were added to black plastic cups (350 ml capacity) with drain holes in the bottom. The cups were placed into a growth chamber and maintained at 15 °C in dark for 7 days. After the incubation period, one sprouted disease-free mini-tuber was planted in each cup. Potato cultivars Dakota Trailblazer, very resistant to powdery scab on tubers and root gall formation and Shepody (very susceptible) were selected for this portion of the research (Bittara et al. 2016). Plants were grown at 17/15 °C (day/night) with 16 h of cool white fluorescent lighting. The soil water content was inspected on a daily basis and kept at field capacity by watering at periodic intervals. After planting, predesignated plants were carefully harvested weekly during a five-week period. In total, the experiments consisted of 96 treatment combinations comprising two potato cultivars, two locations, four chloropicrin rates and 6 sampling dates. Four replicates of these combinations were arranged in a 6 × 16 grid using a randomized block design where each row of 16 cups represented a replicate. The root system of each harvested plant was washed free of soil using a soft brush under tap water and inspected for the presence of root galls. Whole root systems were freeze-dried, weighed and ground to a fine powder and kept at 4 °C until use (Hernandez Maldonado et al. 2013). The experiment was performed twice and the observed root galls were expressed as number of galls · g^{-1} dry weight.

Statistical Analysis

Statistical analysis was carried out using SAS 9.3 (SAS Institute, Cary, NC). Bartlett's test was used to assess the homogeneity of variances and the Wilk-Shapiro test was used to assess the normality of the residuals. Potato cultivars evaluated in at least two trial locations were ranked based on their susceptibility to powdery scab and root gall formation (Bittara et al., 2016). Regression analysis was conducted between the amounts of *S. subterranea* plasmid-DNA and number of resting spores in artificially infested soils. A preliminary analysis of the results was performed in order to determine differences in the amount of pathogen DNA between soil sampled strata. These results were combined as no significant differences were observed. The analysis of 2012 and 2013 experiments was performed using ANCOVA where the on level of *S. subterranea* plasmid-DNA in soil at pre-fumigation was used as a covariate. Data from field experiments conducted during 2011 were analyzed individually with ANOVA; and a combined analysis was performed with data from 2012 and 2013 experiments. Spearman's rank correlation was used to assess the degree of linear association among evaluated parameters. Data obtained in bioassays were combined and analyzed with ANOVA for which plants presenting no galls during weekly evaluations were excluded from the analysis to avoid underestimation of the variation (Hernandez Maldonado et al. 2013). Treatment means were compared using a protected LSD and contrasts ($\alpha = 5\%$).

Results

Spongospora subterranea Quantification in Soil

Regression analysis on the quantity of *S. subterranea* plasmid-DNA and the number of spiked resting spores resulted in a linear relationship ($r^2 = 0.992$, $P < 0.001$; Fig. 1a). The detection limit of the qPCR assay varied among artificially infested soils and pathogen levels of 0.25 sporosori · g^{-1} soil (equivalent to 500 resting spores · g^{-1} soil) were detected in three of six spiked soils. Pathogen levels of 2.5 sporosori · g^{-1} soil (5.0×10^3 resting spores · g^{-1} soil) were detected for all spiked soils.

The amount of *S. subterranea* in non-treated soil samples collected at pre- and post-fumigation was assessed among trial locations, for which significant differences were observed ($P < 0.001$; Fig. 1b). *S. subterranea* was detected in all collected soil samples with the highest amount of pathogen DNA detected near Larimore, ND, and Perham, MN. At these locations, the average level of pathogen in the soil were 97.7 (2.0×10^5 resting spores · g^{-1} soil) and 90.6 sporosori · g^{-1} soil (1.8×10^5 resting spores · g^{-1} soil) respectively (Fig. 1b). Across trial locations, the average amount of pathogen in soil in non-treated plots was 74.3 sporosori · g^{-1} soil (1.5×10^5 resting spores · g^{-1}), while the highest level observed was 563.6 sporosori · g^{-1} soil (1.1×10^6 resting spores g^{-1}) near Park Rapids, MN, in 2013 (Fig. 1b).

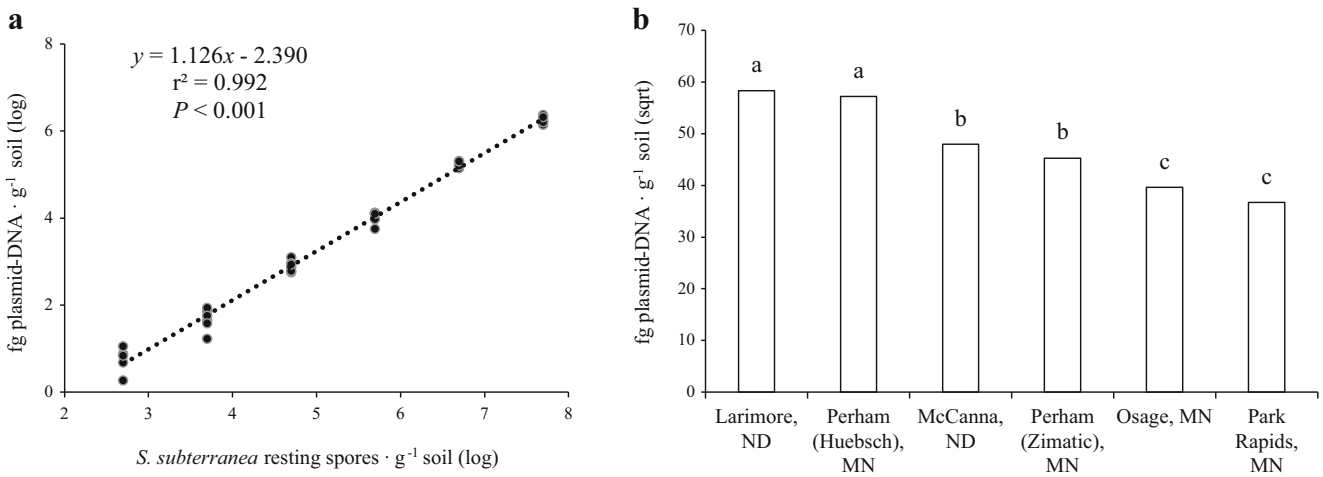

Fig. 1 *Spongospora subterranea* inoculum quantification in soil. **a** Linear regression between femtograms (fg) of *S. subterranea* plasmid-DNA · g^{-1} soil and number of resting spores in artificially infested soils (n = 36). Means from three individual PCR assays. **b** Mean fg of *S. subterranea* plasmid-DNA · g^{-1} soil from six trial locations in Minnesota and North Dakota in non-treated plots. Means labeled with the same letter were not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$)

Effect of Chloropicrin Fumigation Rates on *S. subterranea* Soil Levels

During 2011, soil samples were collected at post-fumigation only. Near Larimore, ND chloropicrin fumigation at rate of rate of 149.4 kg a.i. · ha^{-1} significantly decreased the amount of *S. subterranea* plasmid-DNA in soil ($P = 0.014$), resulting in a reduction was of 18.6% compared to the non-treated control (Fig. 2a). In contrast, near Perham, MN, a significant reduction of *S. subterranea* plasmid-DNA in soil (10.8%) was observed at the rate of 74.7 kg a.i. · ha^{-1} when compared to the non-treated control (Fig. 2a). In 2012, a significant reduction of *S. subterranea* plasmid-DNA in soil was observed near McCanna, ND ($P < 0.001$), and Osage, MN ($P < 0.001$; Fig. 2b). Near McCanna, ND, the highest reduction in pathogen DNA in soil was observed with fumigant rate of 196.1 kg a.i. · ha^{-1}. This fumigant rate reduced *S. subterranea* inoculum by 32.6% compared to the non-treated control. Near Osage, MN, all applied fumigant rates resulted in significant reduction of *S. subterranea* plasmid-DNA in soil (Fig. 2b). When compared to the non-treated control, the percentage of reduction among fumigant rates ranged from 11.5% at 112.1 kg a.i. ha^{-1} to 14.2% at 196.1 kg a.i. ha^{-1}. During 2013, significant differences were observed among chloropicrin rates near Park Rapids ($P < 0.001$) and Perham, MN ($P < 0.001$) for the amount of pathogen plasmid-DNA in soil. At these locations, all chloropicrin rates resulted in a significant reduction of pathogen soil level compared to the non-treated control (Fig. 2c). Near Park Rapids, MN, reductions of *S. subterranea* soil inoculum ranged from 17.7% at 70.1 kg a.i. · ha^{-1} to 34.4% at 140.1 kg a.i. · ha^{-1}. Near Perham, MN, inoculum reduction ranged from 15.9% at 70.1 kg a.i. · ha^{-1} to 30.9% at 140.1 kg a.i. · ha^{-1}.

Interaction of Chloropicrin Fumigation Rates and Potato Cultivar on the Formation of Root Galls

During 2011, root gall disease development (AUDPC) was determined at Perham, MN and Larimore, ND only. Near Perham, MN no significant chloropicrin rate x potato cultivar interaction was observed ($P = 0.518$). At this trial location, significant differences in root gall disease development (AUDPC) were found among potato cultivars ($P_{\text{main effect}} < 0.001$), but not among fumigant rates ($P_{\text{main effect}} = 0.415$; Fig. 3a). Cultivars such as Bannock Russet, Russet Norkotah and Ranger Russet had the lowest root gall AUDPC, whereas cvs. Ivory Crisp, Russet Burbank and Nicolet had the highest (Table 2). In contrast, a significant chloropicrin rate x potato cultivar interaction was observed near Larimore, ND ($P < 0.001$), where similar fumigation rates were applied to the soil (Fig. 3b). Compared to the non-treated control, root gall AUDPC in cvs. Such as Ranger Russet, Shepody and Umatilla Russet increased significantly with the incremental increase in chloropicrin rate. No galls where observed on roots of cv. Dakota Trailblazer across fumigant rates.

In 2012, no significant chloropicrin rate x potato cultivar interaction was observed ($P = 0.613$); however, significant differences in root gall AUDPC values were observed among potato cultivars ($P_{\text{main effect}} < 0.001$) and among fumigant rates ($P_{\text{main effect}} < 0.001$). Cultivar Dakota Trailblazer had the lowest root gall AUDPC, followed by cvs. Ranger Russet and Yukon Gold; whereas cvs. Red LaSoda and Red Pontiac had the highest number of root galls (Table 2). At this location, all fumigant rates significantly increased root gall AUDPC compared to the non-treated control (Fig. 3c). However, root gall AUDPC did not differ significantly among fumigant rates. The overall increase in root gall AUDPC after chloropicrin soil fumigation ranged from 104 to 179%.

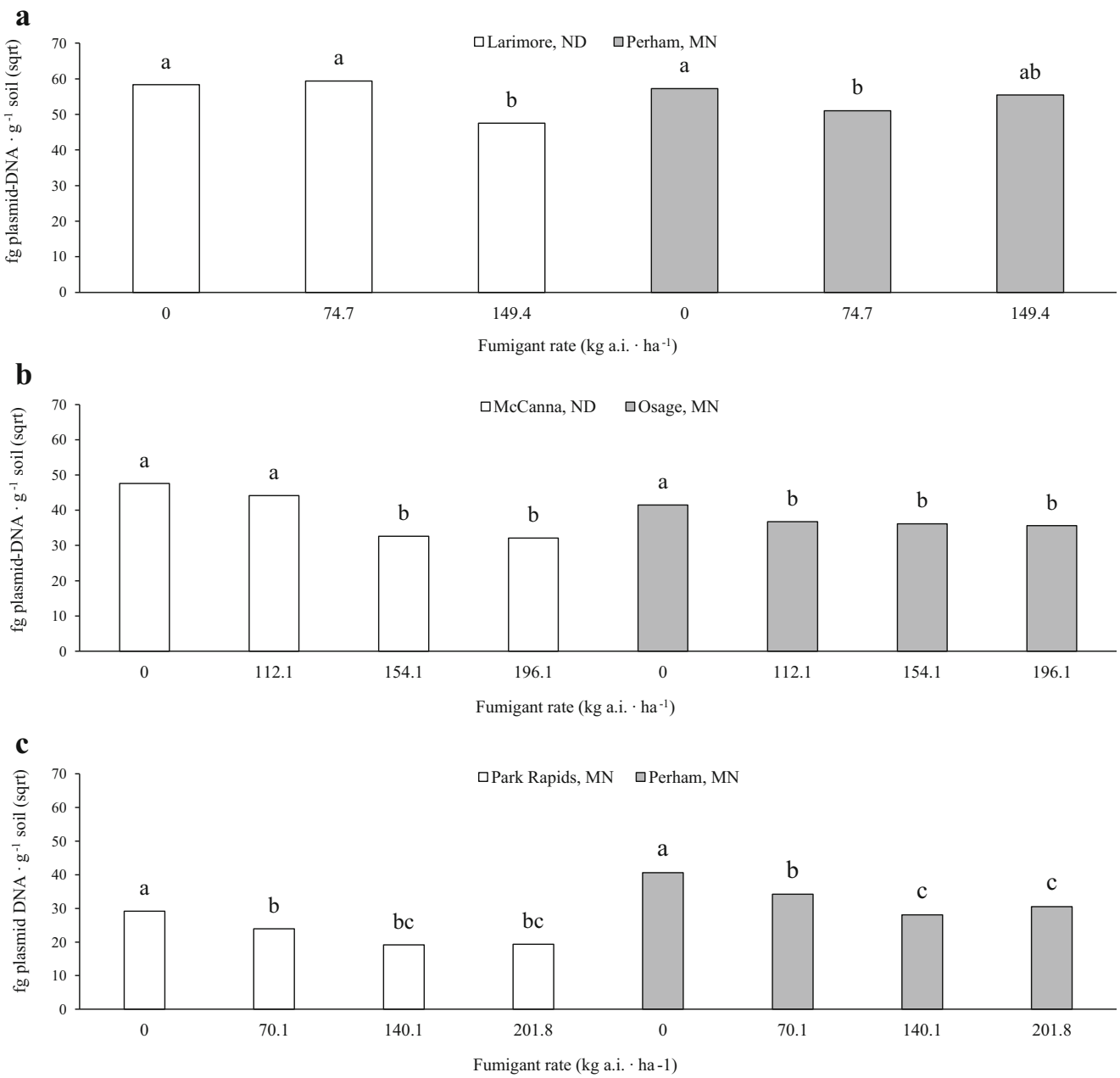

Fig. 2 Effect of chloropicrin rates on *Spongospora subterranea* soil level at post-fumigation in Larimore, ND and Perham, MN in 2011 (**a**), Osage, MN and McCanna, ND in 2012 (**b**), and Perham and Park Rapids, MN in 2013 (**c**). Means from three individual PCR assays. Location means labeled with the same letter were not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$)

In 2013, no significant chloropicrin rate x potato cultivar interaction was detected ($P = 0.981$); however, as observed in previous years, significant differences in root gall AUDPC values were noted among potato cultivars ($P_{\text{main effect}} < 0.001$) and among fumigant rates ($P_{\text{main effect}} < 0.001$). Cultivars Shepody and Red LaSoda developed significantly more root galls than cv. Russet Burbank. Additionally, significantly higher root gall AUDPC values were observed in chloropicrin treated plots compared to the non-treated control. Among fumigant rates, the highest amount of root galls developed at rates of 140.1 kg a.i. · ha^{-1}, while disease on roots observed at 70.1 and 201.8 kg a.i. · ha^{-1} did not differed significantly from each other (Fig. 3d). During this year, the overall increase in root gall AUDPC after chloropicrin soil fumigation ranged from 99 to 356%.

Interaction of Chloropicrin Fumigation Rates and Potato Cultivar on the Expression of Powdery Scab

During 2011, data on powdery scab was collected from all field experiments but only results from McCanna, ND and Perham, MN are presented. Near McCanna, ND no significant chloropicrin rate x potato cultivar interaction was observed

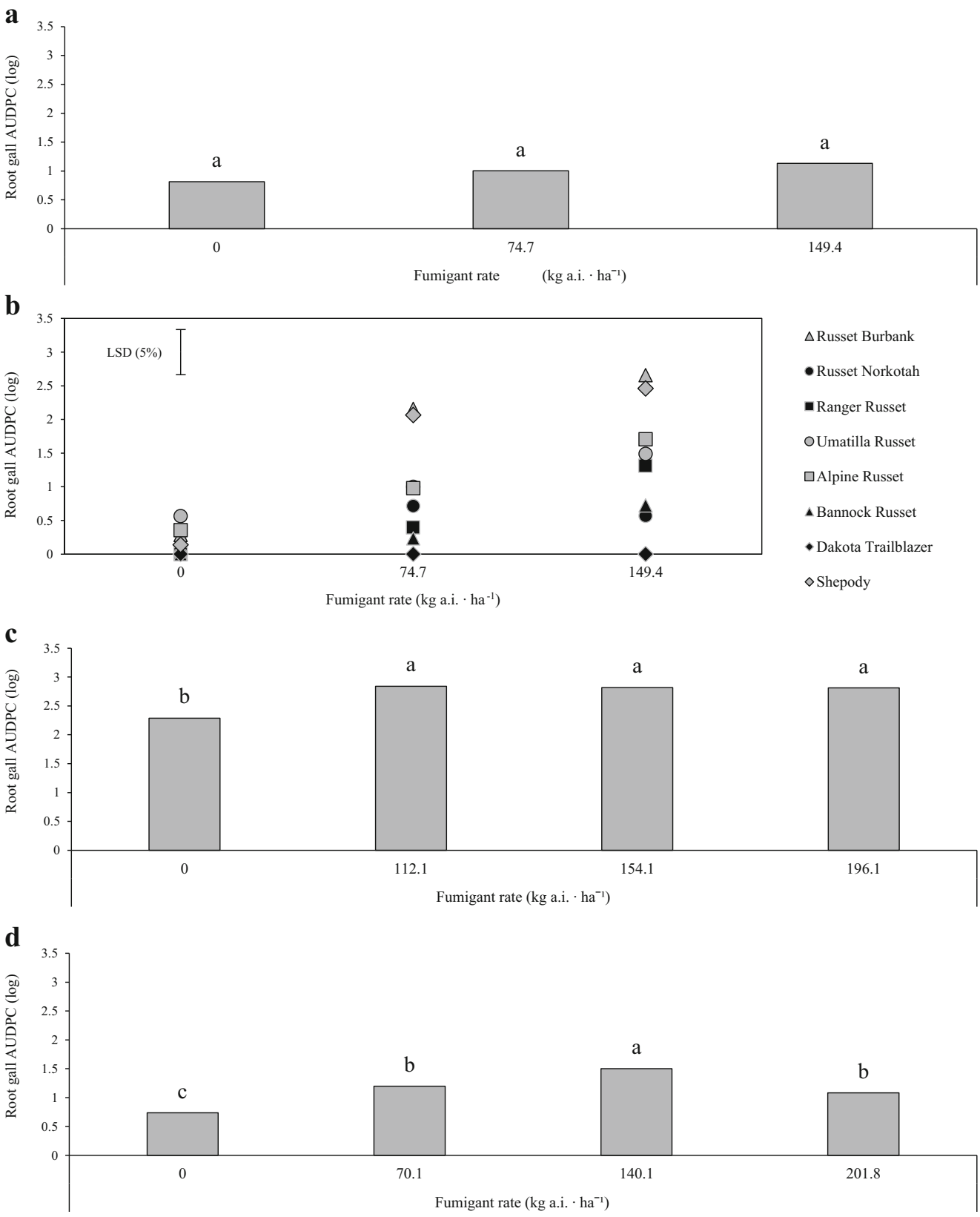

Fig. 3 Effect of chloropicrin soil fumigation on the Area Under Disease Progress Curve (AUDPC) of root gall formation in potato cultivars evaluated at Perham, MN (**a**) and Larimore, ND (**b**) in 2011, Osage, MN and McCanna, ND in 2012 (**c**), and Perham and Park Rapids, MN in 2013 (**d**). AUDPC means for 2012 and 2013 experiments from combined ANOVA. Means labeled with the same letter were not significantly different according to Fisher's protected least significant difference (LSD; $\alpha = 0.05$)

Table 2 Powdery scab and root gall formation (*S. subterranea*) susceptibility ranking of sixteen potato cultivars grown in non-treated naturally infested soils of North Dakota and Minnesota

Cultivar	2011, 2012[a]				2011, 2012, 2013[b]			
	Powdery scabindex	Susceptibility rank[c]	Root gall formation index	Susceptibility rank[c]	Powdery scab index[a]	Susceptibility rank[a]	Root gall formation index[a]	Susceptibility rank[a]
Alpine Russet	0.003	VR	0.070	MR	0.010	VR	0.060	MR
Bannock Russet	0.014	VR	0.033	VR	0.001	VR	0.005	VR
Dakota Trailblazer	0.002	VR	0.014	VR	0.016	VR	0.016	VR
Ivory Crisp	0.366	VS	0.257	VS	0.338	VS	0.449	VS
Kennebec	0.644	VS	0.567	VS	0.625	VS	0.771	VS
Lamoka	0.153	MR	0.438	VS	n/a	n/a	n/a	n/a
Nicolet	0.137	MR	0.457	VS	n/a	n/a	n/a	n/a
Ranger Russet	0.012	VR	0.022	VR	0.070	MR	0.027	VR
Red LaSoda	0.613	VS	0.153	MS	0.916	VS	0.121	MR
Red Norland	0.137	MR	0.071	MR	0.139	MR	0.172	MS
Red Pontiac	0.171	MS	0.328	VS	0.423	VS	0.384	VS
Russet Burbank	0.001	VR	0.169	MS	0.005	VR	0.262	VS
Russet Norkotah	0.002	VR	0.008	VR	0.002	VR	0.002	VR
Shepody	0.468	VS	0.289	VS	0.850	VS	0.437	VS
Umatilla Russet	0.009	VR	0.233	MS	0.001	VR	0.523	VS
Yukon Gold	0.059	VR	0.042	VR	0.058	MR	0.069	MR

[a] Based upon results presented by Bittara et al. (2016)

[b] Results from current study

[c] Potato genotypes evaluated in at least two environments were ranked into four categories according to their root and tuber phase indices using arbitrary cut-off points: very resistant (0 to 0.05), moderately resistant (> 0.05 to 0.15), moderately susceptible (> 0.15 to 0.25) and very susceptible (> 0.25)

n.a. Not applicable

($P = 0.269$). At this location, significant differences were observed among potato cultivars ($P_{\text{main effect}} < 0.001$) but no differences in powder scab development was observed among chloropicrin rates ($P_{\text{main effect}} = 0.109$; Fig. 4a). Potato cvs. Bannock Russet, Umatilla Russet and Yukon Gold had the lowest powdery scab indices, whereas cvs. Shepody, Red LaSoda and Kennebec had the highest (Table 2). Near Perham, MN, a similar scenario was noted. There was no chloropicrin rate x potato cultivar interaction ($P = 0.205$), however significant differences in powdery scab indices were noted among potato cultivars ($P_{\text{main effect}} < 0.001$). No significant differences were observed among chloropicrin rates ($P_{\text{main effect}} = 0.101$; Fig. 4b). Cultivars such as Bannock Russet, Yukon Gold and Red Norland had the lowest disease indices, whereas cvs. Shepody, Red LaSoda and Ivory Crisp had the highest (Table 2).

In 2012, a significant chloropicrin rate x potato cultivar interaction was observed ($P = 0.035$; Fig. 4c). Powdery scab indices in cultivars such as Shepody, Red Pontiac and Kennebec significantly increased with increasing of the fumigant rate compared to the non-treated control. Conversely, in cvs. Dakota Trailblazer, Ranger Russet and Yukon Gold no significant increase of powdery scab indices was observed regardless of the fumigant rate. During this year, the increase in powdery scab indices across cultivar and fumigant rates ranged from 43 to 90% compared to the non-treated control.

During 2013, no significant chloropicrin rate x potato cultivar interaction was observed ($P = 0.263$). Significant differences for powdery scab indices were detected among potato cultivars ($P_{\text{main effect}} < 0.001$) and among fumigant rates ($P_{\text{main effect}} = 0.011$). The highest powdery scab indices were observed on cv. Shepody followed by Red Norland; while on cv. Russet Burbank, few symptomatic tubers were recorded resulting in a low disease index. In addition, chloropicrin soil fumigation significantly increased powdery scab indices compared to the non-treated control, but no differences in disease reduction were observed among fumigant rates (Fig. 4d). Overall, after chloropicrin soil fumigation, powdery scab indices increased from 133 to 315%.

Effect of Chloropicrin Fumigation Rates on Tuber Yield

In 2011, no significant chloropicrin rate x potato cultivar interaction was observed for tuber yield at Perham, MN ($P = 0.457$). Significant differences in tuber yield were observed among cultivars ($P_{\text{main effect}} < 0.001$), but no effect of chloropicrin fumigation was noted ($P_{\text{main effect}} = 0.308$; Fig. 5a). Cultivar Red Pontiac had the highest yield, whereas Yukon Gold had the lowest. Near Larimore, ND, no significant chloropicrin rate x potato cultivar interaction was observed ($P = 0.444$); however, significant differences for tuber yield were noted among cultivars ($P_{\text{main effect}} < 0.001$) and among fumigant rates ($P_{\text{main effect}} = 0.004$; Fig. 5b). Cultivars such Alpine Russet and Russet Norkotah had the highest tuber yield, whereas Bannock Russet had the lowest. Chloropicrin soil fumigation significantly increased tuber yield (average 18%); however, no significant differences were observed between fumigant rates.

In 2012, no significant chloropicrin rate x potato cultivar interaction was observed ($P = 0.406$); however, tuber yield among cultivars varied significantly ($P < 0.001$; Fig. 5c). Tuber yield for cvs. Umatilla Russet and Kennebec were significantly higher than cv. Yukon Gold, but did not differ from cvs. Dakota Trailblazer and Red Pontiac. Additionally, chloropicrin soil fumigation did not significantly affect in tuber yield ($P = 0.443$) compared to the non-treated control.

In 2013, no significant chloropicrin x potato cultivar interaction was observed ($P = 0.612$), however, there were significant differences in tuber yield among potato cultivars ($P = 0.004$; Fig. 5d). During this year, tuber yield for cv. Red Norland was significantly higher than cv. Russet Burbank, but did not differ from cv. Shepody. Furthermore, chloropicrin soil fumigation significantly increased tuber yield ($P = 0.004$) compared to the non-treated control. Nevertheless, no significant variations among chloropicrin rates were observed. Overall, chloropicrin fumigation increased tuber yield between 8 and 14% (Fig. 5d).

Association of *S. subterranea* Initial Inoculum, Chloropicrin Rate, Disease and Yield

A significant negative association between chloropicrin rates and the amount of *S. subterranea* plasmid-DNA in soil at post-fumigation (Sspf) was observed across experiments. The degree of association between these two variables, ranged from −0.268 ($P = 0.033$) for Umatilla Russet to −0.325 for Yukon Gold ($P = 0.019$) and Red LaSoda ($P = 0.019$; Table 3). The association between Sspf and root gall AUDPC was observed negatively associated in most potato cultivars, however, for cv. Russet Burbank such association was positive ($r = 0.421$; $P = <0.001$). Conversely, no significant association was observed between Sspf and powdery scab index. Sspf and tuber yield was observed negatively associated for cv. Red LaSoda ($r = -0.320$; $P = 0.021$).

Chloropicrin fumigation was found positively associated with increasing AUDPC levels in all selected cultivars (r: 0.292 to 0.447; P: 0.001 to 0.011), in contrast, a significant association between chloropicrin fumigation and powdery scab index was observed in cvs. Shepody ($r = 0.362$; $P = 0.001$), Yukon Gold ($r = 0.279$; $P = 0.007$) and Red LaSoda ($r = 0.373$; $P = 0.007$; Table 3). Similarly, chloropicrin fumigation and tuber yield was observed positively associated. The degree of such association ranged from 0.248 for Russet Burbank ($P = 0.015$) to 0.335 for Dakota Trailblazer ($P = 0.007$).

In cultivars Umatilla Russet ($r = 0.43$; $P = 0.001$) and Dakota Trailblazer ($r = 0.44$; $P = 0.001$) a significant positive association between tuber yield and root gall AUDPC was observed

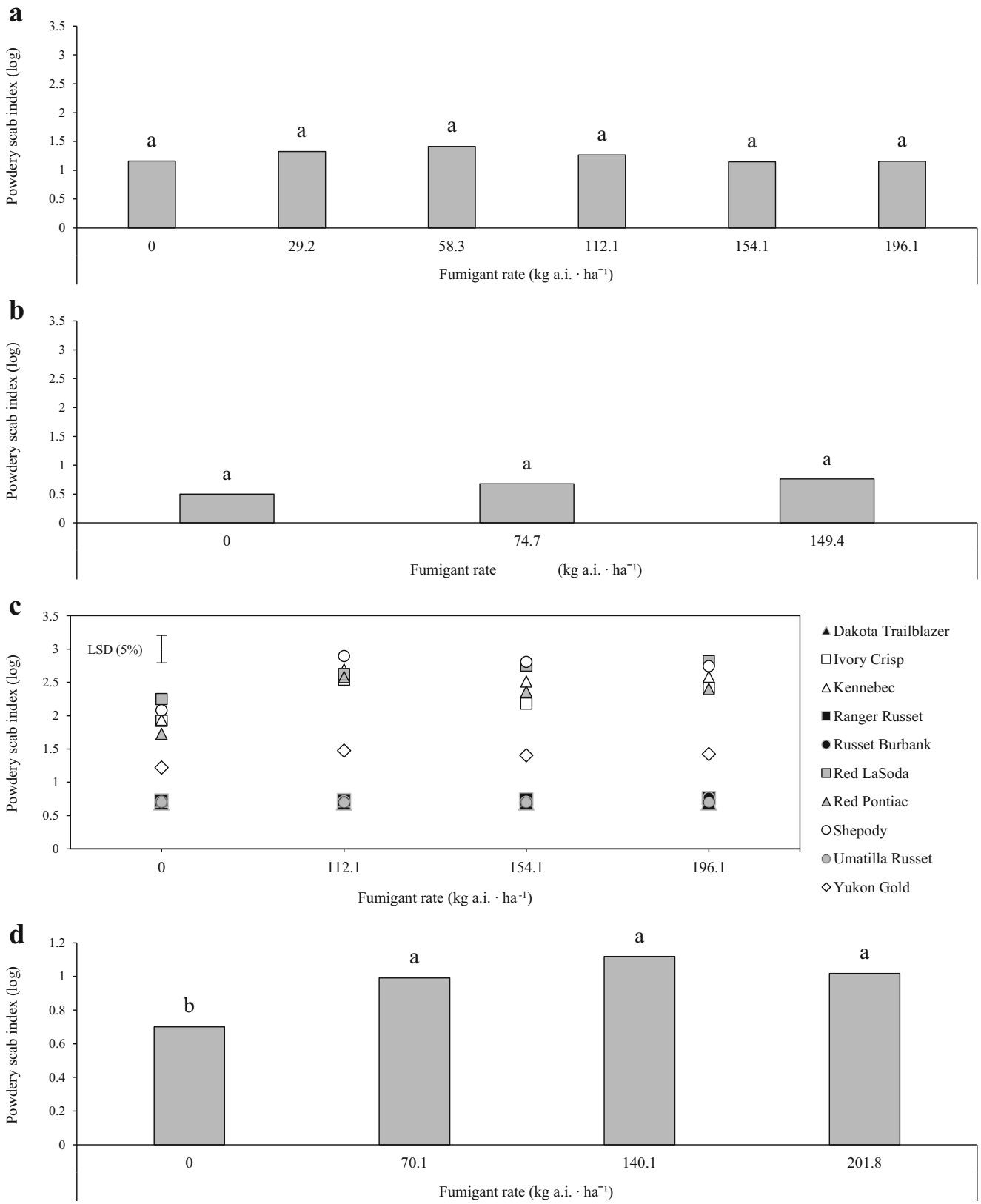

Fig. 4 Effect of chloropicrin soil fumigation on powdery scab indices in potato cultivars evaluated at McCanna, ND (**a**) and Perham, MN (**b**) in 2011, Osage, MN and McCanna, ND (**c**) in 2012, and Perham and Park Rapids, MN in 2013 (**d**). Powdery scab index means for 2012 and 2013 experiments from combined ANOVA. Means labeled with the same letter were not significantly different according to Fisher's protected least significant difference (LSD; $\alpha = 0.05$)

(Table 3). The relationship between AUDPC and powdery scab index was observed significantly associated (r: 0.29 to 0.75; *P*: <0.001 to 0.005), however, this relationship was not significant in powdery scab resistant cvs. Dakota Trailblazer ($r = -0.134$; $P = 0.289$) and Umatilla Russet ($r = 0.04$; $P = 0.771$).

Bioassays

Quantification of S. subterranea in Roots

No significant four-way interaction (potato cultivar x location x chloropicrin rate x sampling date) was observed ($P = 0.580$) for the quantification of *S. subterranea* in potato roots using bioassay. However, a significant potato cultivar x location x sampling date interaction ($P = 0.004$) was observed for the amount of *S. subterranea* plasmid-DNA quantified in root tissue (Fig. 6a). *S. subterranea* infection was detected in both cultivars and locations one week after the start of the experiments (data not shown). The highest amount of pathogen plasmid-DNA in potato roots was observed at week 3 in plants grown in soil from Perham, MN followed by a second lower peak occurring at week 5. In potato plants grown in soil from Park Rapids, MN the highest amount of *S. subterranea* plasmid-DNA in roots was observed at week 6. Between these two locations, the largest differences in pathogen quantity were noted in week 3, but at week 6, no differences were observed despite variations in pathogen plasmid-DNA levels observed in previous sampling dates (Fig. 6a).

In the analysis of main effects, no significant differences ($P_{\text{main effect}} = 0.653$) between the amount of *S. subterranea* plasmid-DNA in roots of cultivars Dakota Trailblazer and Shepody were observed (Fig. 6b). In contrast, the amount of pathogen plasmid-DNA detected in potato roots differed significantly among chloropicrin rates ($P_{\text{main effect}} < 0.001$; Fig. 6b). Compared to the non-treated control, the levels of pathogen plasmid-DNA in potato roots increased from 1.4 at 70.1 kg a.i. · ha^{-1} to 4.3 times at 201.8 kg a.i. · ha^{-1}.

Root Gall Formation

Root galls on cv. Shepody were first observed in chloropicrin treated soil five weeks after planting compared to six weeks in cv. Dakota Trailblazer (Fig. 6c). However, no significant four-way interaction was observed ($P = 0.203$). A significant potato cultivar x location x sampling date interaction was detected ($P = 0.015$), with the highest number of galls · g^{-1} root observed on cv. Shepody grown in soil from Perham, MN six weeks after planting. No differences in number of root galls were observed on cv. Dakota Trailblazer despite location and sampling date (Fig. 6c). Nevertheless, the analysis of main effects revealed significant differences in the number of galls · g^{-1} root between cultivars ($P_{\text{main effect}} < 0.001$) and among chloropicrin rates ($P_{\text{main effect}} < 0.001$; Fig. 6d). Across potato cultivars, Shepody yielded 8 times more root galls than cv. Dakota Trailblazer. Across fumigant rates, the amount of galls observed with chloropicrin rates of 140.1 kg a.i. · ha^{-1} and 201.8 kg a.i. · ha^{-1} were significantly higher than the non-treated control. Fumigation with chloropicrin increased the number of root galls by 18 and 22 times at rates of 140.1 kg a.i. · ha^{-1} and 201.8 kg a.i. · ha^{-1}, respectively.

The number of galls · g^{-1} root observed across chloropicrin rates on cv. Shepody were significantly associated with the mean amount of *S. subterranea* plasmid-DNA detected in root tissue from week 2 to week 5 ($r = 0.426$ to 0.636; $P < 0.001$ to 0.016). The highest degree of association was observed at week 5 ($r = 0.636$; $P < 0.001$), closely followed by week 3 ($r = 0.607$; $P < 0.001$). However, although root gall formation occurred in cv. Dakota Trailblazer, no significant association between disease and the amount of pathogen plasmid-DNA in roots was observed ($P > 0.05$).

Discussion

To our knowledge, this study represents the first report of large-scale field experiments aimed at evaluating the effect chloropicrin soil fumigation on *Spongospora subterranea* soil inoculum levels and its concomitant effect on disease expression in the United States.

A number of factors including pathogen inoculum level and distribution in soil are likely to influence the occurrence powdery scab and root gall formation in the field (Brierley et al. 2013; Qu et al. 2006; Shah et al. 2014; Sparrow et al. 2015). Our results indicate mean levels of *S. subterranea* inoculum in non-fumigated plots equivalent to 74 sporosori g^{-1} · soil across locations and the maximum level of inoculum near Park Rapids, MN (564 sporosori · g^{-1} soil). Previous reports using similar molecular approaches, indicated maximum levels of inoculum of 105, 148 and 1.4×10^4 sporosori g^{-1} soil in Japan, the UK and the US respectively (Brierley et al. 2013; Nakayama et al. 2007; Qu et al. 2006). Despite the aforementioned differences, chloropicrin fumigation was efficacious at reducing *S. subterranea* soil inoculum at rates ranging from 70.1 to 201.8.1 kg a.i. · ha^{-1} based on qPCR assays. At these rates, the reduction of pathogen inoculum ranged from 10.8% to 34.4% compared to the non-treated control; however, fumigation efficiency varied largely among trial locations and fumigant rates. For example, the highest chloropicrin rate applied in 2012 (196.1 kg a.i. ha^{-1}) resulted in an inoculum reduction of 32.6% near McCanna, ND; whereas near Osage, MN, the inoculum decrease was 14.2%. Alternatively, the lowest fumigant rate applied in-row during 2013 experiments (70.1 kg a.i. ha^{-1}) yielded a significant inoculum reduction near Perham (15.9%) and Park Rapids, MN (17.7%), but in 2011, a similar chloropicrin rate (74.7 kg a.i. ha^{-1}) applied broadcast resulted in significant reductions near Perham, MN (10.8%) but remained

similar to the non-treated control near Larimore, ND. In most cases, the inoculum reduction observed at the highest chloropicrin rates applied (i.e. 196.1 and 201.8.1 kg a.i. ha^{-1}) did not differ significantly from the observed at rates ranging from 70.1 to 154.1 kg a.i. ha^{-1}. The primary mechanism by which soil injected fumigants move though the soil profile is vapor diffusion (Ajwa et al. 2002). Several factors, including inoculum level, soil texture and temperature, are involved in the efficacy of this movement therefore affecting its efficacy of control (Ajwa et al. 2002; Lembright 1990). Although fumigations described in this research were performed at soil temperatures commonly used in the area of study and EPA recommendations (Gudmestad et al. 2007; Pasche et al. 2014; Taylor et al. 2005), the reduction of soil inoculum ranged from 15.9 to 34.4% when chloropicrin was applied at soil temperatures ranging from 3.2 to 14.6 °C. However, at Osage, MN where soil fumigation was performed at 18.9 °C, reduction efficiency ranged from 11.5 to 14.2%. Chloropicrin among other soil fumigant has a low vapor pressure (18 mmHg at 20 °C) and high boiling point (110 °C). Fumigants with these characteristics are more dependent on the method of delivery into the soil but also meteorological conditions (Ajwa et al. 2002; Lembright 1990). It is likely that soil temperature at the moment of fumigant application had influenced the observed results.

The management of disease caused by *S. subterranea* represents a challenge. Intrinsic factors to the pathogen such as polycyclic nature and persistence and distribution in soil may interact with environment conditions and the degree of host susceptibility in roots and tubers (Brierley et al. 2013; Hughes 1980; Nitzan et al. 2008; Sparrow et al. 2015) and may influence the success and reproducibility of chemical approaches for disease suppression (Davidson and Houser 2009; Falloon 2008; Zink et al. 2004). Results presented in this research demonstrate that powdery scab and root gall formation were significantly increased when chloropicrin was applied to the soil at rates ranging from 70.1 to 201.8 kg a.i. · ha^{-1}. The effect of increasing disease on tubers ranged from 43 to 315% and was mainly observed on smooth-skinned potato cultivars (e.g. Red LaSoda, Shepody), while the effect of increasing disease on roots ranged from 99 to 356% and involved cultivars of all skin types. To reinforce these observations, a similar effect of increasing powdery scab was recorded when chloropicrin was applied in combination with 1,3-dichloropropene (1,3-D; Bittara et al. 2016). Nevertheless, our results are contrast with overseas reports indicating the effectivity of chloropicrin (Telopic™ = 61% 1-3D; 35% chloropicrin) either alone or in combination with plastic covers, in suppressing powdery scab with rates ranging from 70 to 175 kg a.i. ha^{-1} (Tsror 2014; Tsror et al. 2009; Tsror et al. 2016). It remains unclear the factors affecting the process of infection and disease development that might have account for the differences observed between our results and previous reports. However, an increase of powdery scab was reported after soil applications with mancozeb at rates of 2.3 kg ha^{-1} (Falloon et al. 1996). In that study, the authors suggested a plausible effect of the fungicide rate in prolonging the susceptibility of the host or offering a sub-lethal effect on the pathogen population in soil.

Fig. 5 Effect of chloropicrin soil fumigation on tuber yield of potato cultivars evaluated at Perham, MN (**a**) and Larimore, ND (**b**) in 2011, Osage, MN and McCanna, ND in 2012 (**c**), and Perham and Park Rapids, MN in 2013 (**d**). Tuber yield means for 2012 and 2013 experiments from combined ANOVA. Means labeled with the same letter were not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$)

The effect of chloropicrin on non-target soil microorganisms includes ammonium- and nitrite-oxidizing bacteria (Stromberger et al. 2005; Tanaka et al. 2003). Both ammonium and nitrate are reported to decrease the amount of root gall formation caused by *S. subterranea* in pot experiments (Falloon et al. 2009) which suggests that nitrogen applications are likely to increase disease expression due to its effect on host rather than on the pathogen (Falloon et al. 2009; Shah et al. 2014). Although nitrogen fertilizer was supplied to plants in field experiments, an increased disease effect for the amount of root galls developed was further observed in bioassays for which pathogen free tubers and no nitrogen amendments were employed. The role played by soil microorganisms in the events leading to disease development is a topic that requires further investigation. However, their potential use in the management of disease caused by *S. subterranea* has been demonstrated (Nakayama et al. 2013; Nielsen and Larsen 2004).

A singular aspect of this research was the similarity in *S. subterranea* DNA content observed in roots of cvs. Shepody and Dakota Trailblazer, two genotypes with marked differences in susceptibility to root gall formation and powdery scab in tubers (Bittara et al. 2016). These similarities were noted despite chloropicrin rate and location, and at the same time, contrast the differences in amount of galls developed on each potato cultivar during the bioassays. Similar growth rates based on *S. subterranea* DNA and zoosporangia development were reported occurring between Gladiator (very resistant to powdery scab) and Iwa (very susceptible) and Desiree (moderately susceptible) and Russet Burbank (moderately resistant) (Hernandez Maldonado et al. 2013; Thangavel et al. 2015). *S. subterranea* is able to infect a number of plant species across a wide range of families (Iftikhar and Ahmad 2005; Neuhauser et al. 2014; Qu and Christ 2006; Shah et al. 2010) however, the production of resting spores

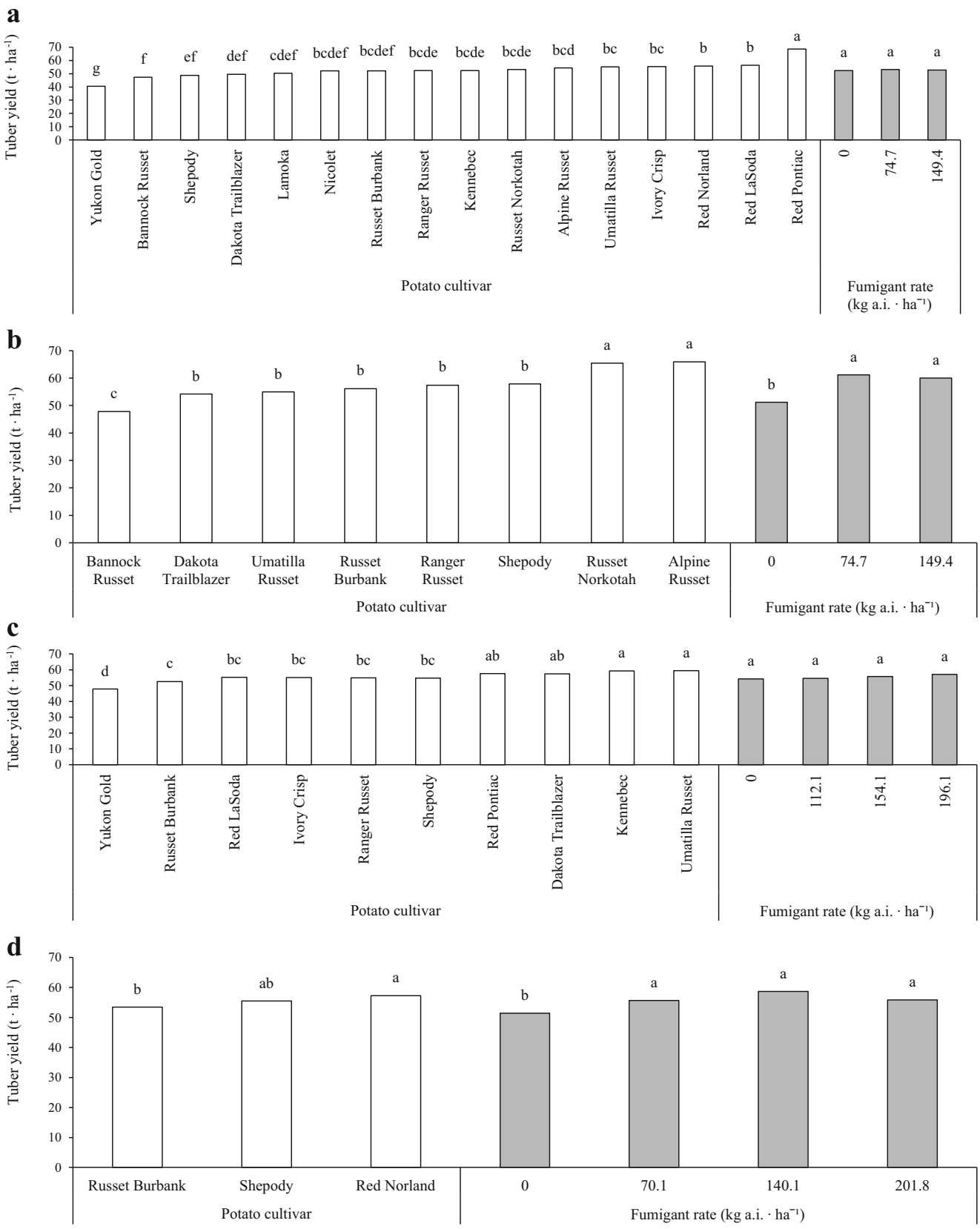
a
Tuber yield (t · ha-1)
g f ef def cdef bcdef bcdef bcde bcde bcde bcd bc bc b b a a a a
Yukon Gold
Bannock Russet
Shepody
Dakota Trailblazer
Lamoka
Nicolet
Russet Burbank
Ranger Russet
Kennebec
Russet Norkotah
Alpine Russet
Umatilla Russet
Ivory Crisp
Red Norland
Red LaSoda
Red Pontiac
0
74.7
149.4
Potato cultivar
Fumigant rate (kg a.i. · ha⁻¹)
b
Tuber yield (t · ha-1)
c b b b b b a a b a a
Bannock Russet
Dakota Trailblazer
Umatilla Russet
Russet Burbank
Ranger Russet
Shepody
Russet Norkotah
Alpine Russet
0
74.7
149.4
Potato cultivar
Fumigant rate (kg a.i. · ha⁻¹)
c
Tuber yield (t · ha-1)
d c bc bc bc bc ab ab a a a a a a
Yukon Gold
Russet Burbank
Red LaSoda
Ivory Crisp
Ranger Russet
Shepody
Red Pontiac
Dakota Trailblazer
Kennebec
Umatilla Russet
0
112.1
154.1
196.1
Potato cultivar
Fumigant rate (kg a.i. · ha⁻¹)
d
Tuber yield (t · ha-1)
b ab a b a a a
Russet Burbank
Shepody
Red Norland
0
70.1
140.1
201.8
Potato cultivar
Fumigant rate (kg a.i. · ha⁻¹)

Table 3 Spearman rank correlation among chloropicrin rates, *S. subterranea* soil inoculum level at post-fumigation, disease on tubers and roots and tuber yield in field experiment conducted in Minnesota and North Dakota from 2011 to 2013

Cultivar			Chloropicrin rate	Tuber yield	Sspf	AUDPC	Cultivar			Chloropicrin rate	Tuber yield	Sspf	AUDPC	Cultivar			Chloropicrin rate	Tuber yield	Sspf	AUDPC
Shepody (n = 96)	Tuber yield	r[d]	0.302				Yukon Gold (n = 52)	Tuber yield	r	0.212				Red LaSoda (n = 52)	Tuber yield	r	-0.166			
		P[e]	0.003						*P*	0.131						*P*	0.238			
	Sspf[a]	r	-0.170	–0.182				Sspf	r	-0.325	0.027				Sspf	r	-0.325	0.303		
		P	0.093	0.076					*P*	0.019	0.851					*P*	0.019	0.029		
	AUDPC[b]	r	0.328	0.229	0.193			AUDPC	r	0.354	0.501	–0.377			AUDPC	r	0.447	-0.176	-0.320	
		P	0.001	0.025	0.061				*P*	0.011	0.001	0.018				*P*	0.010	0.211	0.021	
	PS index[c]	r	0.362	0.404	-0.111	0.684		PS index	r	0.279	0.711	–0.199	0.749		PS index	r	0.373	0.132	-0.020	0.594
		P	0.001	0.001	0.281	<0.001			*P*	0.045	<0.001	0.155	<0.001			*P*	0.007	0.349	0.900	<0.001
Dakota Trailblazer (n = 64)	Tuber yield	r	0.335				Russet Burbank (n = 96)	Tuber yield	r	0.248				Umatilla Russet (n = 64)	Tuber yield	r	0.305			
		P	0.007						*P*	0.015						*P*	0.015			
	Sspf	r	-0.270	-0.223				Sspf	r	-0.201	-0.109				Sspf	r	-0.268	-0.057		
		P	0.033	0.077					*P*	0.052	0.291					*P*	0.033	0.653		
	AUDPC	r	0.409	0.437	–0.278			AUDPC	r	0.292	-0.034	0.421			AUDPC	r	0.345	0.426	-0.200	
		P	0.001	0.001	0.027				*P*	0.004	0.742	<0.001				*P*	0.005	0.001	0.122	
	PS index	r	-0.090	-0.142	0.053	-0.134		PS index	r	0.183	0.021	0.103	0.289		PS index	r	0.118	0.098	-0.100	0.037
		P	0.495	0.262	0.675	0.289			*P*	0.076	0.846	0.322	0.005			*P*	0.354	0.442	0.451	0.771

[a] Femtograms of *Subterranea subterranea* plasmid-DNA · g^{-1} soil at post-fumigation

[b] Area Under Disease Progress Curve for root gall formation

[c] Powdery scab index (disease severity x disease incidence)

[d] Spearman's coefficient

[e] *P*-value

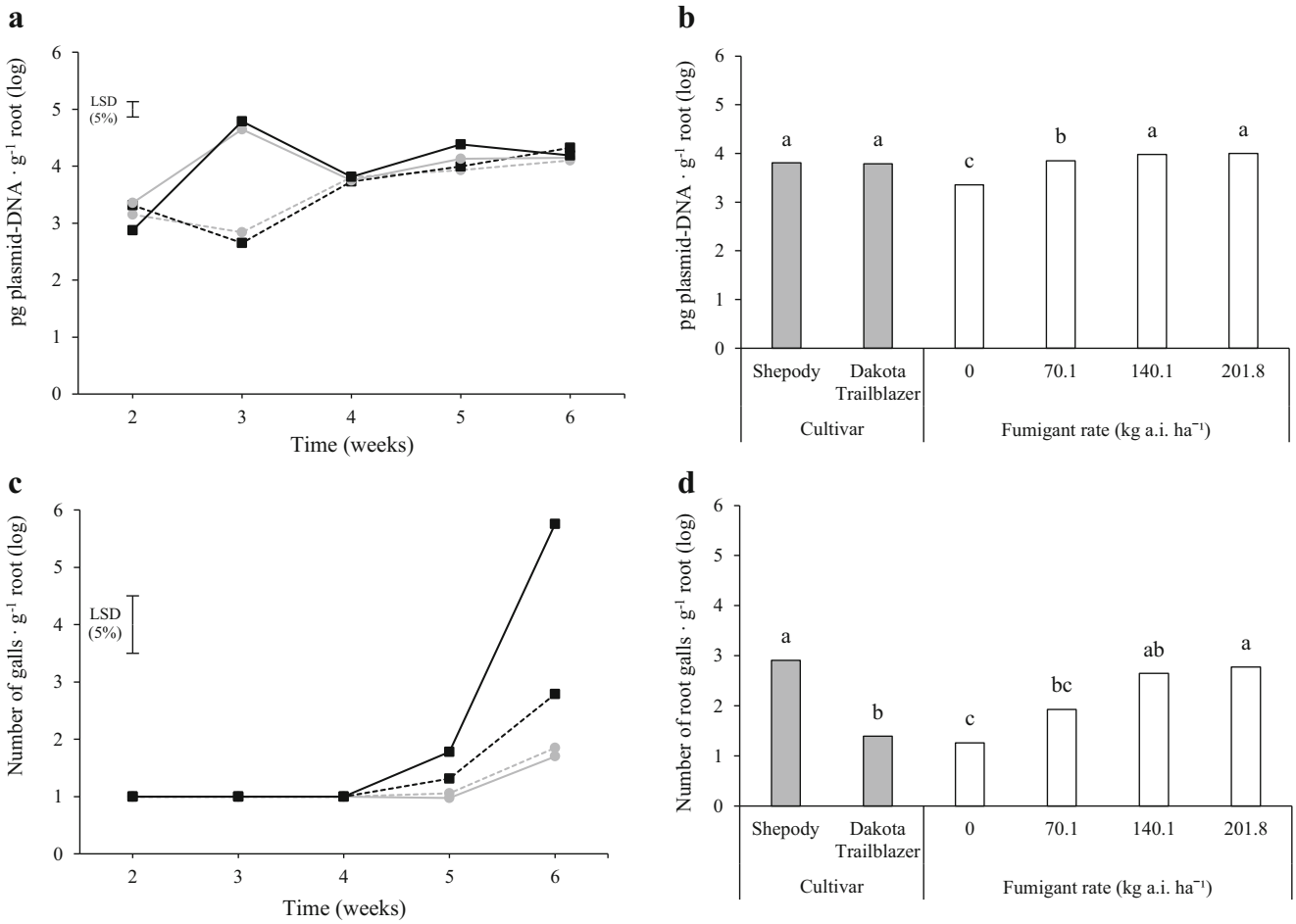

Fig. 6 Effect of chloropicrin soil fumigation on *Spongospora subterranea* root infection progress and disease development on roots of two potato cultivars. **a** Mean picograms (pg) of *S. subterranea* plasmid-DNA g^{-1} root in cv. Shepody (*black square*) and Dakota Trailblazer (*gray circle*) grown in chloropicrin-treated naturally infested soil from Park Rapids, MN (*dashed lines*) and Perham, MN (*continuous line*). **b** Mean pg of *S. subterranea* plasmid-DNA · g^{-1} root by potato cultivar and fumigant rate. Means (**a** and **b**) from three individual PCR assays across two replicated trials. Main effect means labeled with the same letter were not significantly different according to Fisher's protected least significant difference (LSD; $\alpha = 0.05$). **c** Mean number of root galls · g^{-1} root in cv. Shepody and Dakota Trailblazer grown in chloropicrin-treated naturally infested soil from Park Rapids, MN and Perham, MN. **d** Mean number of root galls · g^{-1} root by potato cultivar and fumigant rate. Means (**b** and **d**) from five sampling dates across two replicated trials. Main effect means from two replicated trials. Means labeled with the same letter were not significantly different according LSD ($\alpha = 0.05$)

and development of symptoms by the pathogen is limited to a narrow number of plant species (Qu and Christ 2006). It remains a matter of speculation regarding the factors inherent to the pathogen and host responsible for disease induction, however, observations presented in this research are in agreement with previous reports suggesting that defense factors acting against the sporangial phase of the pathogen may differ from those controlling symptom development (sporogenic phase; Hernandez Maldonado et al. 2013).

Chloropicrin soil fumigation resulted in an increase of tuber yield in most field trials. Yield increase after chloropicrin applications in crops such as strawberry and tomatoes have been related to the reduction of soil-borne pathogens (Sydorovych et al. 2006, 2008). Similarly, chloropicrin has been demonstrated to be effective in controlling wilts of potato caused by *Verticillium dahliae* Kleb. and *Colletotrichum coccodes* (Wallr.) Hughes (Gudmestad et al. 2007). Although potato tuber yield was not negatively affected by root gall formation in cvs. Shepody and Umatilla Russet (Johnson and Cummings 2015), a significant association between root gall formation and tuber weight was observed. Our results indicate a similar association in cvs. Shepody and Umatilla Russet, suggesting that development of lesions on tubers and roots by the pathogen are closely involved with the metabolism of factors required for host growth and tuber development (Johnson and Cummings 2015).

In summary, the results described in this research show that chloropicrin fumigation, effectively reduced the amount *S. subterranea* in soil but was not efficacious in the control of powdery scab disease on tubers and roots. Further research will be required in order to determine whether control strategies aimed at the sporangial stage of the pathogen, rather than

initial inoculum, are efficacious in suppressing the disease. The selection of resistant cultivars (Bittara et al. 2016), however, can play an important role in disease management and is highly recommended. Additionally, results presented in this research raise the question of the importance of the involvement of biotic and abiotic factors in the development of powdery scab and root gall epidemics, an aspect of *S. subterranea* epidemiology relatively unexplored.

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