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Aerial dispersal of *Spongospora subterranea sp. f. subterranea*, the causal agent of potato powdery scab

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Abstract Potato powdery scab caused by *Spongospora subterranea subsp . subterranea* (Sss) causes extensive damage to the quality and marketability of tubers. Disease outbreaks in potatoes grown in virgin soils in south Israel, lead us to the hypothesis that wind-driven inoculum may also be a source of new infections. Wind and ground traps (13 of each type) were positioned near contaminated commercial potato fields with a history of powdery scab in two plots during 2013–14 ('Nave 5' and 'Nave 89'). Quantification of pathogen density in soil/dust was carried out by DNA extraction and qPCR analysis. In 'Nave 5' plot, 58 and 45% (December and January, respectively) and 75 and 50% of the ground and wind traps, respectively, were Sss-positive, with no significant differences in Sss concentrations. In 'Nave 89' plot, the percentage of Sss-positive traps increased from 31% and 18% in the ground and wind traps, respectively, in February, to 100% in both trap types, in April, with no significant differences. Evaluation of the dispersal distance of Sss inoculum from contaminated fields was examined in soil samples taken from the top layer of the ground in the uncultivated area adjacent to the contaminated commercial potato fields with a history of powdery scab, in two sites ('Nave 5' and 'Shalom 7') during 2016. All soil samples, taken from uncultivated areas near the infested fields in various distances of up to 750 m, were Sss positive. This study demonstrated that Sss can be dispersed by wind, particularly in an intensive potato production region where contaminated fields exist.

Keywords *Solanum tuberosum* · Soilborne pathogen · Wind trap · Ground trap · qPCR

Introduction

Powdery scab (PS) of potato caused by the soil- and seed-borne plasmodiophorid pathogen *Spongospora subterranea* f. sp. *subterranea* (Sss), is an important disease throughout temperate potato-producing areas and also hot and dry areas where irrigation is applied (Tsror et al. 2019a, 2019b; Tsror et al. 2020; Johnson and Cummings 2015). The disease may cause extensive losses and reduces the quality and marketability of seed and ware tubers worldwide (Harrison et al. 1997; Merz and Falloon 2009; Tsror et al. 2016, 2019a; Tsror et al. 2020). The pathogen persists in the soil for many years in the form of agglomerations of resting spores termed sporosori, which are highly resistant to environmental stresses (Harrison et al. 1997; Merz and Falloon 2009) and also through infection cycles on

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alternative hosts (Falloon 2008; Tsror et al. 2019b). Sporosori are produced in potato tuber lesions and in root galls (Falloon et al. 2011; Merz 2008). Root infections have the potential to reduce plant growth and yield (Falloon et al. 2016). The major inoculum source of the pathogen is contaminated soil (Lees et al. 2008), but, the infected seed is the most significant means for short- and long-distance disease spread (Merz and Falloon 2009). Recent outbreaks of the disease in Israel in potatoes grown for the first time in fields that were never cultivated before (virgin soils) indicate additional sources that may contribute to the severe infestations, among these are symptomless or latently infected seed tubers, contaminated manure from domestic animals fed with tubers infected with PS and infested soil adhering to machinery (Lees et al. 2008; Falloon 2008; Merz and Falloon 2009; Tegg et al. 2016).

The dispersal of plant pathogens is a pivotal epidemiological process. The wind is a main route of pathogen dispersion (Aylor 1978; Firester et al. 2018). Many fungal plant pathogens undergo wind assisted dispersal, thus making them difficult to monitor and contain as inoculum can spread quickly to large areas. For effective disease management, understanding the mode of dispersal is crucial. Soilborne pathogens in comparison to wind-blown aerial spores tend to be relatively static and can only be dispersed to other parts of a field or to new locations when soil particles and crop debris are blown by strong winds (West 2012). Bacteria and viruses can be carried on relatively large soil particles and plant debris, blown hundreds of meters to disperse them around fields and into new fields (Peccia and Hernandez 2006). For example, urediospores of *Puccinia graminis f.sp. tritici* are wind-transported across continents (Meyer et al. 2017). Even microsclerotia of pathogens, such as *Verticillium longisporum,* have been reported to be blown up to distances of hundreds of meters (West 2014). Our hypothesis was that wind-driven inoculum of Sss may also be the source for new infections occuring in virgin soils. The objective of the current study was to determine if Sss can potentially be disseminated by the wind**.**

Fig. 1 Examples of the wind (all three photos) and ground (lower photo) traps used in the study

Materials and methods

Description of the wind and ground traps

In the current study wind and ground traps were used (Fig. 1). The wind traps were built using a 35 cm long pipe, 5.27 cm diameter, which was horizontally welded to a 150 cm long pole. The pipe was only open from one side, which was facing the predominant wind direction. A rectangle of 20 × 7 cm was cut off at the top of the pipe and covered with a 50-μ mesh to enable wind circulation. Plastic boxes of 12 cm diameter and 7 cm height filled with glass beads were used as ground traps. Each ground trap was buried in the soil at 5 cm depth, with 2 cm above ground level open.

Site description and experiments design

The study was performed in the northwestern Negev region in Israel which is the major potato production region in the country (Fig. 2). The soil type in the experimental area is sandy with 0.3% organic matter (6% clay, 11% silt, and 83% sand) with pH 8.0. The climate of the region is semi-arid, with a mean annual precipitation of 250 mm, subjected to substantial yearly variation, and all potato fields are sprinkler irrigated. The strongest prevailing winds are from west–southwest, although sporadic changes in wind direction occur throughout the day (Krasnov et al. 2016; Firester et al. 2018). Potatoes are grown once in a 4-year crop rotation with peanuts and wheat. The study was carried out in close proximity to three

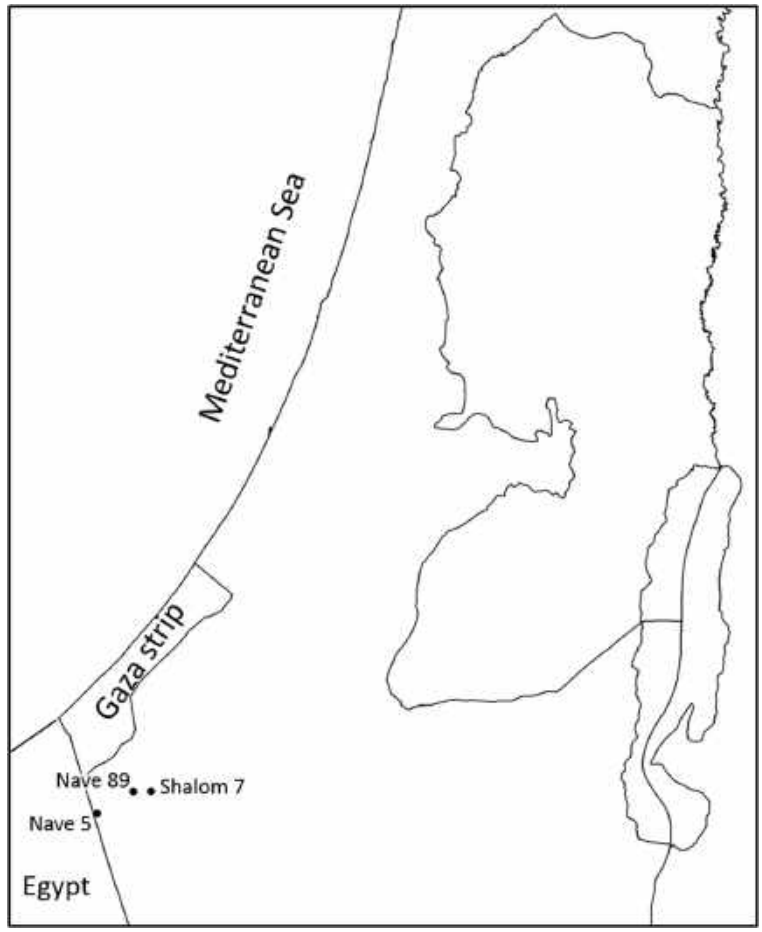

Fig. 2 locations of the three studied plots

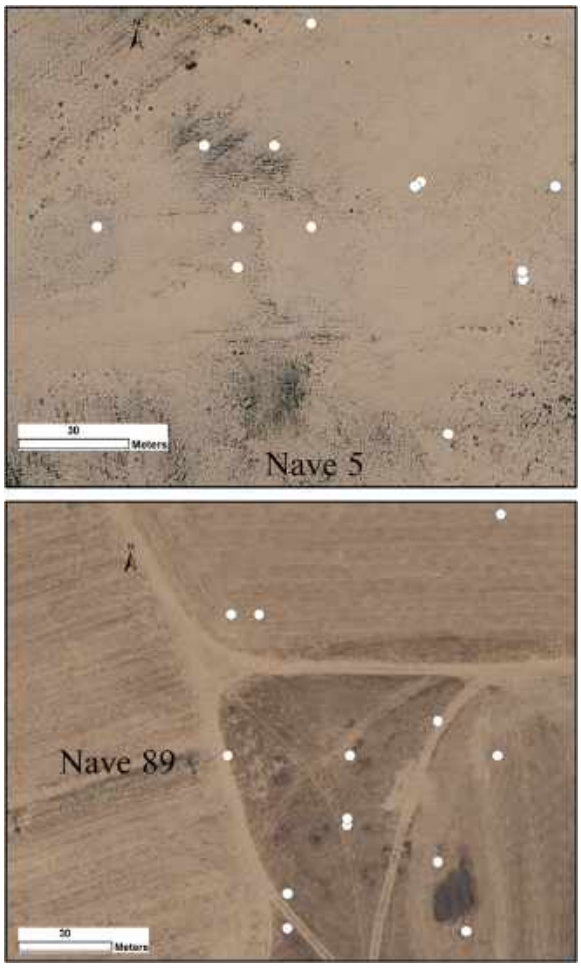

Fig. 3 Locations of the ground and wind traps near the 'Nave 89' and 'Nave 5' plots. Traps marked with white circles

contaminated commercial potato fields with a history of PS: 'Nave 5', 'Nave 89' and 'Shalom 7'. The experiments were conducted during 2013–14 in two different sites, located northern and eastern to the contaminated fields, 'Nave 5' and 'Nave 89', respectively (Fig. 3). In both sites, a total of 13 pairs of wind and ground traps were positioned and distributed in an area of 3000 square meters. Near the 'Nave 5' plot, the traps were positioned on December 22, 2013; sand and dust were collected on December 31, 2013, and on January 9, 2014 (9 and 18 days after trap setup, respectively). Near the 'Nave 89' plot, the traps were positioned on February 3, 2014; sand and dust were collected on February 13 and 25, March 11 and April 6, 2014 (10, 22, 36 and 62 days after trap setup, respectively).

Fig. 4 Locations of soil sampling in the wind dispersal experiments. Traps marked with white circles

In order to evaluate the distance of spread of Sss inoculum from a contaminated field, soil samples were taken in 2016, from the top layer of the ground in the uncultivated area adjacent to two contaminated commercial potato fields with a history of PS, 'Nave 5' and 'Shalom 7' (Fig. 4). Samples near the 'Shalom 7' field, were taken from the uncultivated area, positioned east of the field, at 0, 30, 60, 120, 180, 200, 260 and 320 m from the field border. Near 'Nave 5' field, samples were taken from the uncultivated area, positioned south of the field, at 60, 120, 210, 300, 390, 480, 570, 660, 750 and 770 m from the field border. Three subsamples were taken from each sampling point.

Quantification of the pathogen density in traps and in the soil

Sand and dust were collected from the wind and ground traps into paper bags, kept at ambient temperature in the lab until analyzed. The sand and dust were filtered through a 50-μ sieve. A duplicate of 0.25 g from each

Table 1 Studied plots and traps locations

Plot name	Latitude	longitude	Sss inoculum (ng DNA per g soil)	# of Traps/ samples	Traps/sampling location
Wind and ground traps experiments					
Nave 89	31.176	34.33	5.32	13	East of the plot
Nave 5	31.159	34.302	6.94	13	North of the plot
Wind dispersal experiment					
Nave 5	31.159	34.302	6.94	10	East of the plot
Shalom 7	31.197	34.406	2.60	8	South of the plot

Sss = *Spongospora subterranea* f. sp. *subterranea*

sample was used for DNA extraction and quantitative real-time PCR assay (qPCR) analysis. Levels of Sss (ngDNA per g soil) were determined using a qPCR assay (van de Graaf et al. 2003), in the sand and dust collected in the traps and also from soil samples taken from the top 10 cm of soil. DNA extractions were performed on duplicate 0.25 g soil samples with a GeneMATRIX Soil Purification Kit (EURx, Poland). Duplicate samples from each DNA extraction were analyzed using a qPCR assay. Inoculum level was expressed as ng DNA per g soil. The inoculum levels in the three plots, 'Nave 5', 'Nave 89', 'Shalom 7', were checked prior to the experiments. Samples were taken from the top 10–20 cm of soil (from at least 100 points in a W-shape across the selected plot to give a total of approximately 1 kg) (Brierley et al. 2009). DNA extraction followed by qPCR assays were done as previously described. Sss inoculum levels in the three plots are presented as ng DNA per g soil in Table 1.

Statistical analysis

For visualizing the DNA concentration in the ground and wind traps, boxplots were created using the function *ggboxplot* from the package 'ggpubr' (Kassambara 2018). Mapping was done in ArcMap 10.5 (ESRI, Redlands, CA).

Results

Sss levels in the wind and ground traps

Although a total of 13 pairs of wind and ground traps were positioned in both plots, the number of traps with captured dust or soil varied among sampling dates and types of traps. In the 'Nave 5' plot, the percentage of ground traps with identified Sss DNA (out of the traps with captured dust or soil)

Table 2 Number of ground and wind traps with captured soil and Sss DNA at different sampling dates

Plot /	Number of ground traps *		Number of wind traps *	
sampling date	traps with captured soil	traps with Sss DNA	traps with captured soil	traps with Sss DNA
Nave 5				
31.12.2013	12	7	9	6
9.1.2014	11	5	10	5
Nave 89				
13.2.14	13	4	11	2
25.2.14	13	13	10	10
11.3.14	–	–	13	13
6.4.14	9	9	13	13

Sss = *Spongospora subterranea* f. sp. *subterranea*

*In both plots, a total of 13 pairs of wind and ground traps were positioned and distributed in an area of 3000 square meters

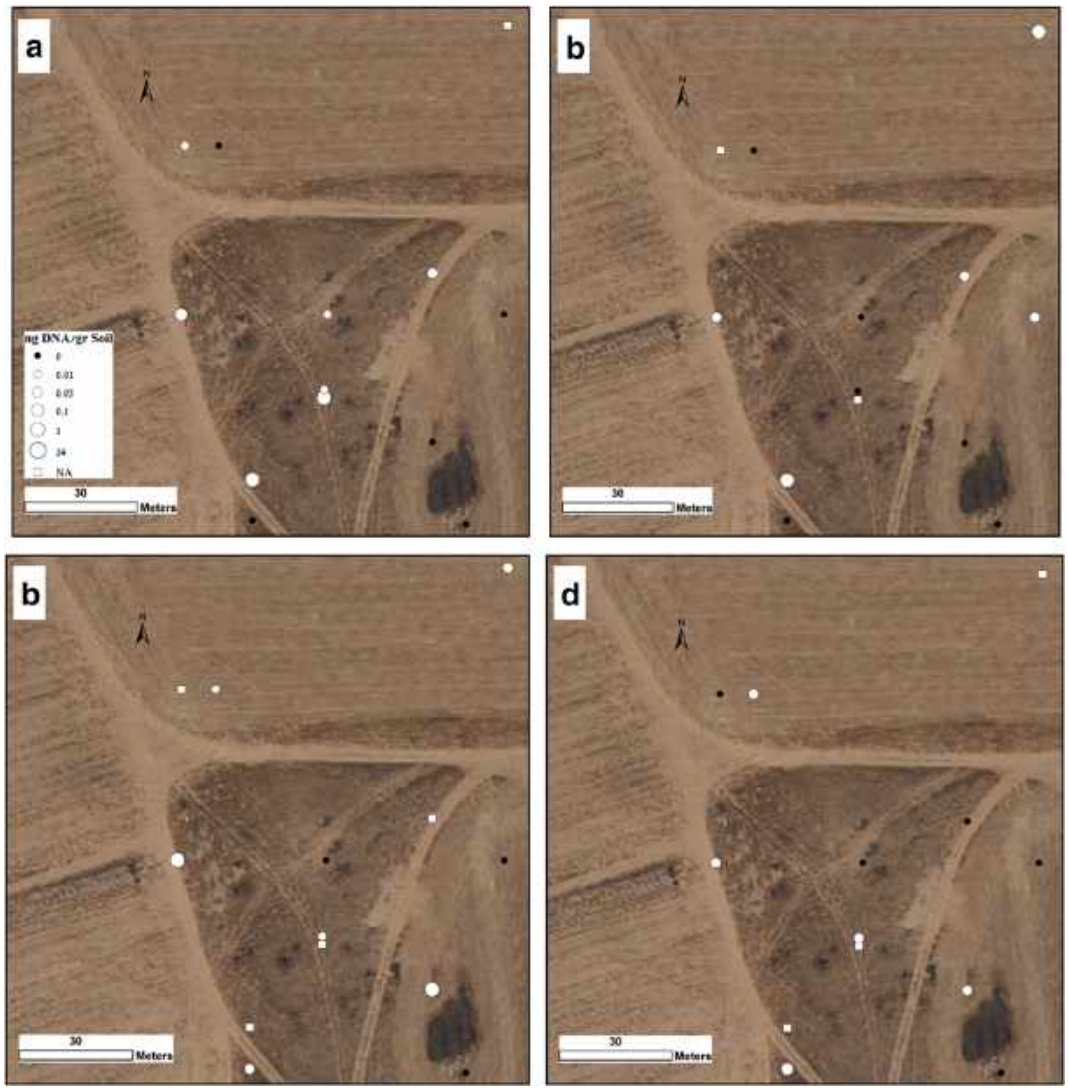

Fig. 5 Sss DNA concentration in the 13 traps in 'Nave 5'. Ground traps: A- December 12 and C- January 1. Wind traps: B- December 12 and D- January 1

ranged between 45% (five out of 11 traps in January) and 58% (seven out of 12 traps in December) (Table 2). The percentage of wind traps with Sss DNA in traps with captured dust or soil was higher and ranged between 50% (five out of 10 traps in January) and 67% (six out of 9 traps in December). However, the average Sss DNA concentrations did not vary significantly in December and January in either the ground or wind traps (Figs. 5, 6, and 7). The percentage of traps with identified Sss DNA in traps with captured dust or soil in 'Nave 89' plot in early February, increased from 31% (four out of 13 traps) and 18% (two out of 11 traps) to 100% (nine out of nine ground traps and 13 out of 13 wind traps) in April in both trap types (Table 2). There were no statistically significant differences in Sss DNA concentrations in either the ground or wind traps (Figs. 6 and 8). The DNA catchment was not uniform and varied considerably in both ground and wind traps (Figs. 5 and 6).

Assessment of wind dispersal of Sss

All soil samples taken from the uncultivated areas, at various distances from potato fields with a history of PS, in two plots, were contaminated with Sss (Figs. 9 and

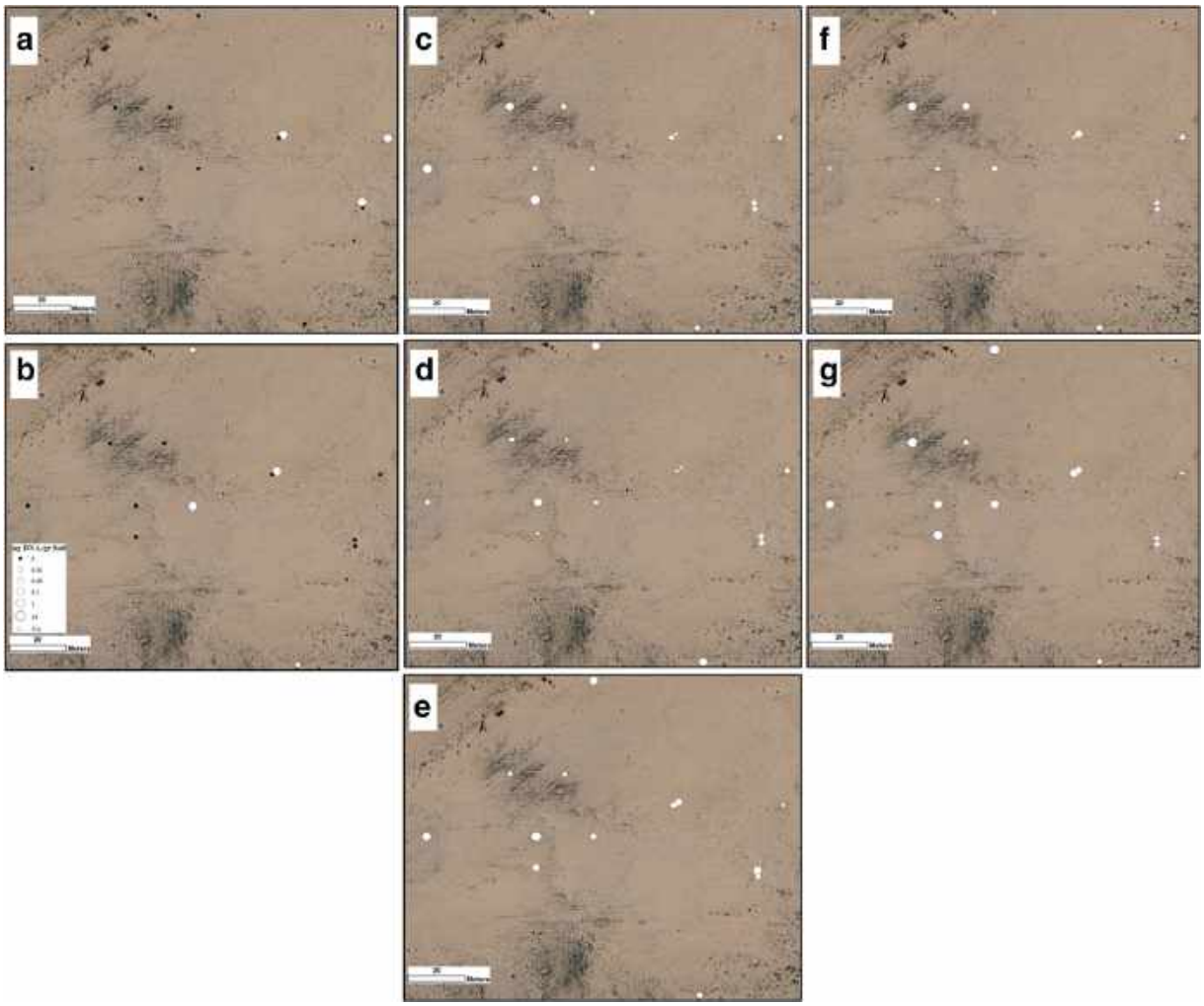

Fig. 6 Sss DNA concentration in the 13 traps in 'Nave 89'. Ground traps: A- February 13; C- February 25 and F- April 6. Wind traps- B February 13; D- February 25; E- March 11 and G- April 6

10). Pathogen levels in the 'Nave 5' plot were higher than in the 'Shalom 7' plot, with averages of 0.203 ± 0.1389 and 0.0236 ± 0.0144 ngDNA per g soil in 'Nave 5' and 'Shalom 7', respectively. In the 'Nave 5' plot, in the samples taken at a distance of 750 m, an anomalous high Sss concentration was obtained because one replicate out of the three had an exceptionally high concentration. This sample was omitted from the analysis.

Discussion

This study demonstrated that Sss can be dispersed by the wind, particularly in an intensive potato production region where contaminated fields exist. The pathogen was detected in both wind and ground traps. Sporosori have the potential to spread from Sss-infested plots to nearby virgin soils or fields that have been fumigated with metam sodium or chloropicrin. The top layer of dry soil can also be disseminated over large distances by wind, especially with sandy soils, which prevail in the north-western Negev region where potatoes are mainly produced in Israel. Dust storms are a common phenomenon in arid and semi-arid areas, and their impacts on both physical and human environments are of great interest; dust storms in Israel are more typical in the winter and spring (Krasnov et al. 2016). As the soil type in the study area is light sand with low clay content, wind-driven movement of substantial amounts of cropping soils is expected. This may be less frequent

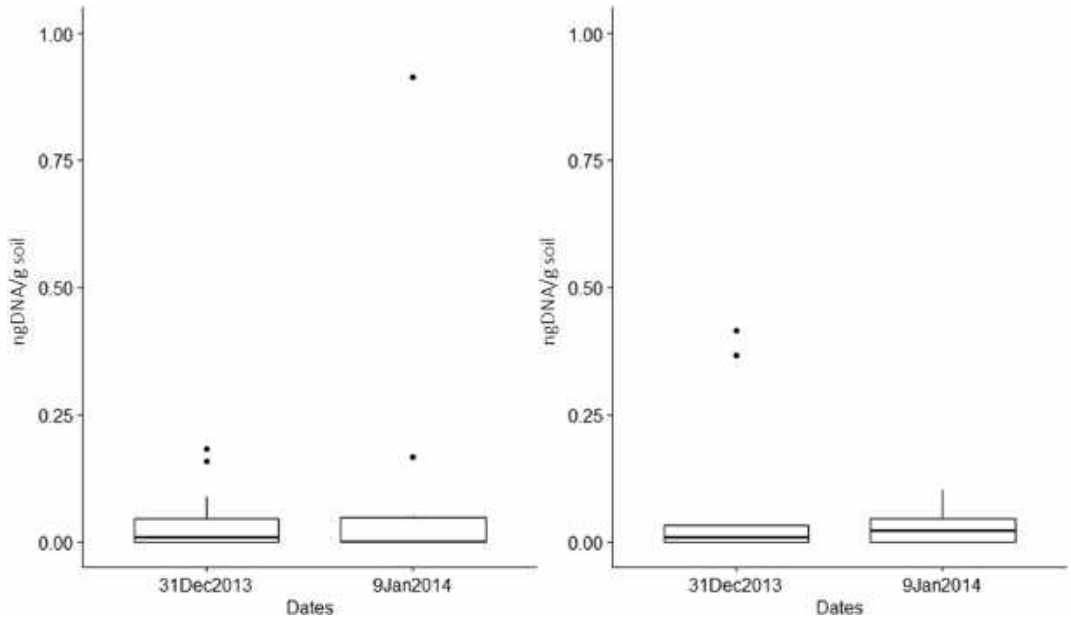

Fig. 7 Box plots presenting the Sss DNA concentrations in the ground (left) and wind (right) traps in different dates in plot 'Nave 5'. The solid and bold black line denotes the median, and the upper and lower limits of the box denote the 75% and 25% percentiles, respectively. The whiskers denote the interquartile range (1.5 × IQR)

in other intensively cropped production regions having different weather conditions and different soil types, and thus further work should be performed to examine the relative role of wind-blown inoculum.

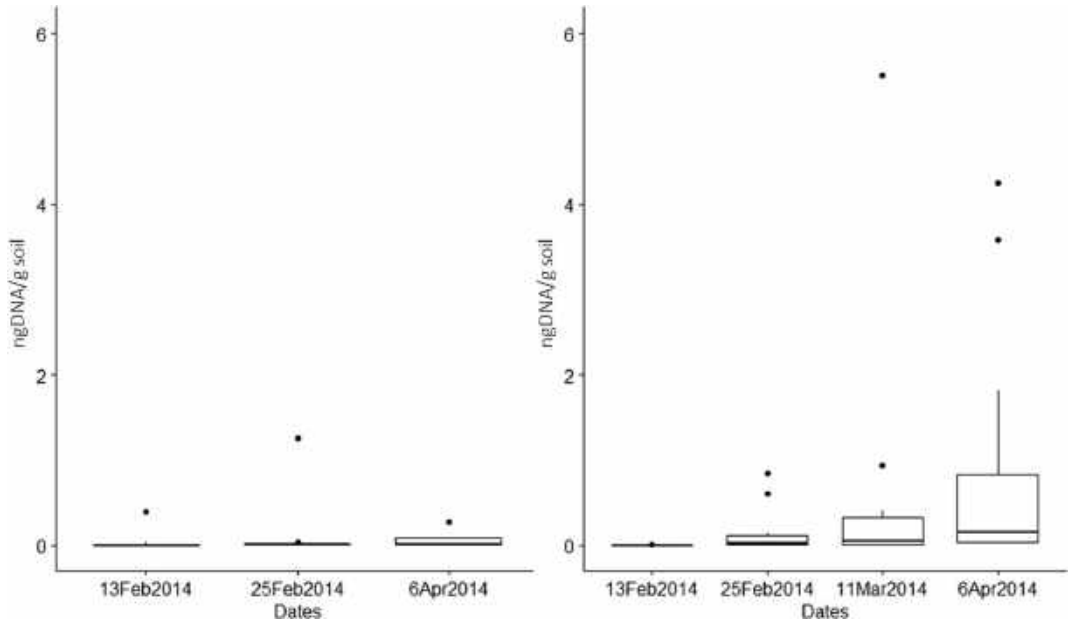

Fig. 8 Box plots presenting the Sss DNA concentrations in the ground (left) and wind (right) traps in different dates in plot 'Nave 89'. The solid and bold black line denotes the median, and the upper and lower limits of the box denote the 75% and 25% percentiles, respectively. The whiskers denote the interquartile range (1.5 × IQR)

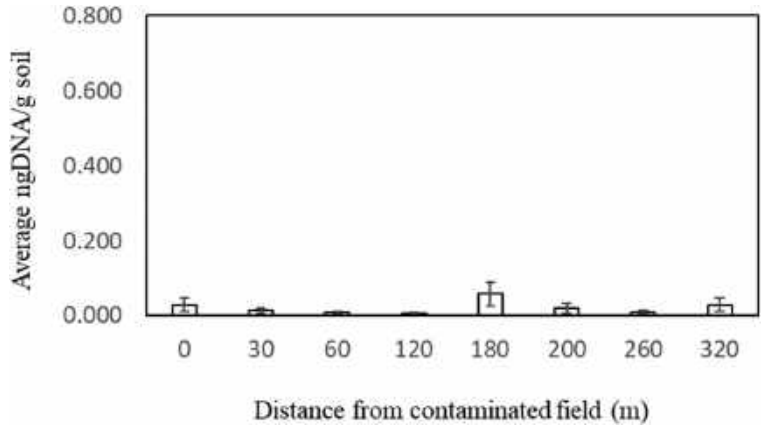

Fig. 9 Average Sss DNA concentrations in soil samples at increasing distance from 'Shalom 7' plot

Fungal dispersion over short distances is common (Peay et al. 2010). However, fungal spores can be disseminated in dust by air currents from meters to hundreds of kilometers depending on air turbulence, wind velocity, UV radiation, and desiccation tolerance until they are deposited or washed out by rain (Aylor 2003). Overall fungal pathogen community composition in dust is more strongly linked to environmental conditions (in particular soil pH, precipitation, and frost) than to potential agricultural hosts and agricultural practices (Dietzel et al. 2019). Soil-borne and dust-dispersed pathogens play a critical role in shaping plant community diversity and composition because they are able to mediate plant species' coexistence through trade-offs between competitive ability and pathogen defense (Mordecai 2011; Bever et al. 2015). Field measurements in blowouts during storm events indicated that the drifted sand contained plant pathogenic fungi and plant-parasitic nematodes (de Rooij-van der Goes et al. 1997). Recently, the feasibility of combining qPCR and spore trapping for the investigation of the spatio-temporal distribution of *Mycosphaerella graminicola* was shown (Duvivier et al. 2013). Rogers et al. (2009) extracted *Sclerotinia sclerotiorum* DNA from a wax-coated plastic tape from a Hirst-Burkard trap using ITS2 based primers and a SYBR-Green based assay. Carisse et al. (2009) collected conidia of the airborne onion pathogen *Botrytis squamosa* with the help of rotating-arm samplers and quantified the amount of DNA showing that qPCR was as reliable as microscopy data contributing to a more advanced disease risk management. The findings of the current study on Sss soil contamination in the uncultivated area near infested potato fields (with a history of PS and verified to Sss presence in soil

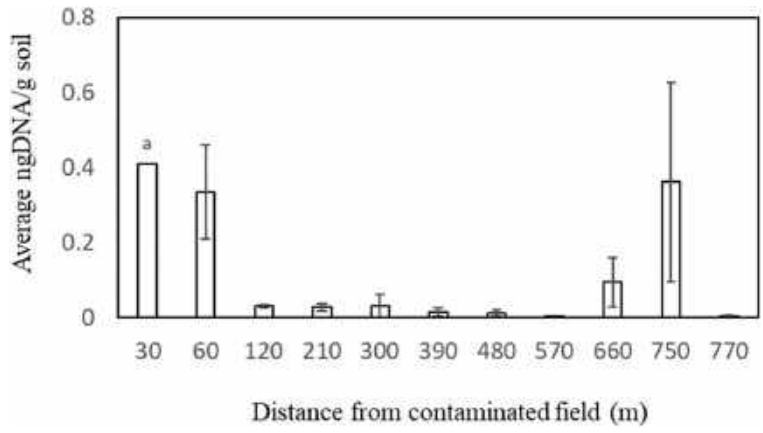

Fig. 10 Average Sss DNA concentrations in soil samples at increasing distance from 'Nave 5' plot. a- denote a single sample that was collected three weeks prior to the sampling of the other samples

by qPCR analysis, Table 1), further support the hypothesis on wind-dispersal of Sss. In future work, more significant distances should be measured in order to learn the extent of wind dispersal of Sss. To our best knowledge, this is the first report on dissemination of Sss the causal agent of PS by wind. Taking into account that seed-borne inoculum can drive an epidemic irrespective of soil-inoculum levels, further work in comparing the relative importance of inoculum sources in the contamination of virgin field sites is required.

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Authors' contributions Contribution of the authors in the preparation of this manuscript: Conceptualization [Tsror (Lahkim)]; Methodology and sampling [Lebiush, Hazanovsky, Erlich]; Analysis [Tsror (Lahkim), Blank]; Writing - original draft preparation, review and editing [Tsror (Lahkim), Blank]; Funding acquisition [Tsror (Lahkim)]; Supervision [Tsror (Lahkim)]. All the authors have consented to submission thereof to the European Journal of Plant Pathology.

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interests.

Human or animals participants The authors declare that this research did not involve human participants or animals.

References

Aylor, D.E. (1978). Dispersal in time and space: Aerial pathogens, in: Plant disease. An Advanced Treatise: How Disease Develops in Populations, JG Horsfall and EB Cowling Eds. Academic Press, New York, USA, pp. 159–180.

Aylor, D. E. (2003). Spread of plant disease on a continental scale: Role of aerial dispersal of pathogens. *Ecology, 84*, 1989–1997. https://doi.org/10.1890/01-0619.

Bever, J. D., Mangan, S. A., & Alexander, H. M. (2015). Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics, 46*, 305–325. https://doi.org/10.1146/annurev-ecolsys-112414-054306.

Brierley, J. L., Stewart, J. A., & Lees, A. K. (2009). Quantifying potato pathogen DNA in soil. *Applied Soil Ecology, 41*, 234–238.

Carisse, O., Tremblay, D. M., Levesque, C. A., Gindro, K., Ward, P., & Houde, A. (2009). Development of a TaqMan real-time PCR assay for quantification of airborne conidia of *Botrytis squamosa* and management of botrytis leaf blight of onion. *Phytopathology, 99*(11), 1273–1280.

de Rooij-van der Goes, P. C. E. M., van Dijk, C., van der Putten, W. H., & Jungerius, P. D. (1997). Effects of sand movement by wind on nematodes and soil-borne Fungi in coastal Foredunes. *Journal of Coastal Conservation, 3*, 133–142.

Dietzel, K., Valle, D., Fierer, N., U'Ren, J. M., & Barberán, A. (2019). Geographical distribution of fungal plant pathogens in dust across the United States. *Frontiers in Ecology and Evolution, 7*, 304. https://doi.org/10.3389/fevo.2019.00304.

Duvivier, M., Dedeurwaerder, G., de Proft, M., Moreau, J. M., & Legreve, A. (2013). Real-time PCR quantification and spatio-temporal distribution of airborne inoculum of *Mycosphaerella graminicola* in Belgium. *European Journal of Plant Pathology, 137*, 325–341.

Falloon, R. E. (2008). Control of powdery scab of potato, towards integrated disease management. *American Journal of Potato Research, 85*, 253–260.

Falloon, R. E., Merz, U., Butler, R. C., Curtin, D., Lister, R. A., & Thomas, S. M. (2016). Root infection of potato by *Spongospora subterranea*: Knowledge review and evidence for decreased plant productivity. *Plant Pathology, 65*, 422–434.

Falloon, R., Merz, U., Lister, R. A., Wallace, A. R., & Hayes, S. P. (2011). Morphological enumeration of resting spores in sporosori of the plant pathogen Spongospora subterranea. *Acta Protozoologica, 50*, 121–132.

Firester, B., Shtienberg, D., & Blank, L. (2018). Modeling the spatio-temporal dynamics of *Phytophthora infestans* at a regional scale. *Plant Pathology, 67*, 1552–1561.

Harrison, J. G., Searle, R. J., & Williams, N. A. (1997). Powdery scab disease of potato - a review. *Plant Pathology, 46*, 1–25.

Johnson, D. A., & Cummings, T. F. (2015). Effect of powdery scab root galls on yield of potato. *Plant Disease, 99*, 1396–1403.

Kassambara, A. (2018). Ggpubr: 'ggplot2' based publication ready plots. R package version 0.2. https://CRAN.R-project.org/package=ggpubr

Krasnov, H., Kloog, I., Friger, M., & Katra, I. (2016). The spatio-temporal distribution of particulate matter during natural dust episodes at an urban scale. *PLoS One, 11*(8), e0160800. https://doi.org/10.1371/journal.pone.0160800.

Lees, A. K., van de Graaf, P., & Wale, S. J. (2008). The identification and detection of *Spongospora subterranea* and factors affecting infection and disease. *American Journal of Potato Research, 85*, 247–252.

Merz, U. (2008). Powdery scab of potato – Occurrence, life cycle and epidemiology. *American Journal of Potato Research, 85*, 241–246.

Merz, U., & Falloon, R. E. (2009). Review: Powdery scab of potato - increased knowledge of pathogen biology and disease epidemiology for effective disease management. *Potato Research, 52*, 17–37.

Meyer, M., Cox, J. A., Hitchings, M. D. T., Burgin, L., Hort, M. C., Hodson, D. P., & Gilligan, C. A. (2017). Quantifying airborne dispersal routes of pathogens over continents to safeguard global wheat supply. *Nature Plants, 3*, 780–786. https://doi.org/10.1038/s41477-017-0017-5.

Mordecai, E. A. (2011). Pathogen impacts on plant communities: Unifying theory, concepts, and empirical work. *Ecological Monographs, 81*, 429–441. https://doi.org/10.1890/10-2241.1.

Peay, K. G., Garbelotto, M., & Bruns, T. D. (2010). Evidence of dispersal limitation in soil microorganisms: Isolation reduces species richness on mycorrhizal tree islands. *Ecology, 91*, 3631–3640. https://doi.org/10.1890/09-2237.1.

Peccia, J., & Hernandez, M. (2006). Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. *Atmospheric Environment, 40*, 3941–3961.

Rogers, S. L., Atkins, S. D., & West, J. S. (2009). Detection and quantification of airborne inoculum of *Sclerotinia sclerotiorum* using quantitative PCR. *Plant Pathology, 58*(2), 324–331.

Tegg, R. S., Thangavel, T., Balendres, M. A., & Wilson, C. R. (2016). Grading seed potato lots to remove tubers with powdery scab damage may not eliminate the pathogen threat. *American Journal of Potato Research, 93*, 231–238.

Tsror, L., Erlich, O., Hazanovsky, M., & Lebiush, S. (2019a). Control of potato powdery scab *(Spongospora subterranea)* in Israel with chloropicrin, metam sodium or fluazinam. *Crop Protection, 124*, 104836. https://doi.org/10.1016/j.cropro.2019.05.030.

Tsror, L., Shapira, R., Erlich, O., Hazanovsky, M., & Lebiush, S. (2019b). Characterization of weeds and rotational crops as alternative hosts of *Spongospora subterranea*, the causal agent of powdery scab in Israel. *Plant Pathology., 69*, 294–301. https://doi.org/10.1111/ppa.13117.

Tsror, (Lahkim) L., Lebiush, S., Hazanovky, M., Erlich, O. (2020). Control of potato powdery scab caused by Spongospora subterranea by foliage cover and soil application of chemicals under field conditions with naturally infested soil. *Plant Pathology*, https://doi.org/10.1111/ppa.13193.

Tsror, L., Rosenberg, A., Erlich, O., & Lebiush, S. (2016). Epidemiological aspects and control of potato powdery scab. *American Journal of Potato Research, 93*, 144–145.

van de Graaf, P., Lees, A., Cullen, D., & Duncan, J. (2003). Detection and quantification of *Spongospora subterranea* in soil, water and plant tissue samples using real-time PCR. *European Journal of Plant Pathology, 109*, 589–597.

West, J. S. (2012). Aerobiology and air sampling in plant pathology. *Alergologia Immunologia, 9*, 80–81.

West, J. S. (2014). *Plant Pathogen Dispersal*. Chichester: Wiley. https://doi.org/10.1002/9780470015902.a0021272.