Co-Inoculations of Potato Plants with *Spongospora subterranea* (Powdery Scab), *Pratylenchus penetrans* (Root-Lesion Nematode) and *Meloidogyne chitwoodi* (Columbia Root-Knot Nematode)

Nadav Nitzan, Neusa Guera, Ekaterini Riga and Chuck Brown
First and fourth authors: USDA-ARS, Prosser, WA 99350. Second and third authors: Washington State University, IAREC, Prosser, WA 99350

Introduction

Spongospora subterranea (Wallr.) Lagerh f. sp. *subterranea* is a major concern for potato production in the Columbia Basin of central Washington and north-central Oregon (Nitzan et al. 2008). The pathogen is an obligate parasitic protozoan that causes powdery scab on potato tubers (Alexopoulos 1996, Braselton 1995, Braselton 2001), and develops galls on the roots and stolons of the plants (Harrison 1997, Fallon 1995, Fallon 1996, Merz et al 2000). The disease on the tubers causes powdery scab lesions that impair appearance and reduce tuber quality (Harrison 1997, Fallon 1995, Fallon 1996, Merz et al 2000). Infection of the roots may cause young plants to wilt and die (Lawrence & Mckenzie, 1981), and was suggested to disturb nutrient and water uptake, affecting photosynthetic translocation to the forming tubers, reducing yields in weight (Fallon 1996).

The Columbia root-knot nematode *Meloidogyne chitwoodi* Golden et al. is a serious pest of potato in the USA, and it is prevalent in the Pacific Northwest (Brodie et al 1993; MacGuidwin 2008). *Meloidogyne chitwoodi* is a sedentary endoparasite in which the female remains permanently attached to the root and tuber tissues at the feeding site once feeding is initiated. Plants infected with the nematode may demonstrate poor shoot growth, foliar chlorosis, small tubers and internal tuber browning. Roots invaded by the pest demonstrate galls at the feeding sites, and tubers demonstrate sub-epidermal swellings (bumps) and discoloration of the flesh (Brodie et al 1993; MacGuidwin 2008). The damage caused to tubers is unacceptable by the fresh market and the French-fry processing industries, resulting in price reduction penalties or rejection of harvested tubers (Brown et al. 1991).

The root lesion nematode *Pratylenchus penetrans* is a cosmopolitan plant parasitic nematode. In potatoes, lesion nematodes interact with the fungus *Verticillium dahliae* (Kleb.) causing potato early dying syndrome (Brodie et al 1993; MacGuidwin 2008). This nematode is a migratory endoparasite that inhabits the roots, but can move freely between roots and soil. Symptoms associated with this lesion nematode are mild discoloration to necrosis on the entire root system, stunting, delayed canopy closure, reduction of foliar growth and reduction in tuber yield and quality (Brodie et al 1993; MacGuidwin 2008).

The potato cultivars that are the most extensively grown in the Pacific Northwest are Russet potatoes (Schreiber 2006), which are relatively resistant to tuber infection by *S. subterranea* (Nitzan et al 2008). However, their roots can become severely infected by *S. subterranean* during the growing season demonstrating abundant root galls (Nitzan et al 2008). Potatoes grown in the Columbia Basin of Washington State in fields with a history of *S. subterranea,* had reduced numbers of large (>280 g) tubers needed for processing, resulting in the reduction of the useable potato yield. In addition, processing cultivars such as Russet Burbank are no longer commercially produced in fields where high severities of *S. subterranea* root galls occur annually (Nitzan et al. 2008). The potato industry of Washington State is concerned with damage to roots caused by *S. subterranea* and its potential to reduce yield weight

in tonnage, and affect tuber size and quality. To broaden our knowledge regarding factors that trigger *S. subterranean* damage to potato, the present study tested the hypothesis that *S. subterranea* can synergistically interact with the root-lesion nematode *Pratylenchus penetrans,* and/or the root-knot nematode *Meloidogyne chitwoodi* and reduce potato health.

Materials and Methods

The hypothesis was tested separately for each nematode species in two repeated trials. Co-inoculations with both nematodes were not carried out. The susceptible industry standard cultivar Russet Burbank was used in all trials (Nitzan et al. 2008). The experiments were arranged in a completely randomized design with five replications per treatment, which were as follows: 1) *S. subterranea* + *P. penetrans*, or *M. chitwoodi*; 2) *S. subterranea* alone; 3) *P. penetrans*, or *M. chitwoodi* alone; and 4) non-inoculated control plants. The trials were conducted in a controlled environment growth chamber with the soil kept at a temperature of 15ºC, and the plants were exposed to continuous fluorescent light. The plants were irrigated every second day and the soil was kept moist to promote *S. subterranea* infection. A soil mixture composed of 84% sand, 10% silt, and 6% clay that was previously treated with methyl bromide (0.3 kg/m^3) was used (Brown et. al 2006). This soil mix had a similar composition as the majority of the soils found in commercial potato fields in the Columbia Basin. The pots used in the trials were 0.5 liter in volume and filled with 643g of moist soil mix. Each pot received 2 grams of Osmocot® (18-6-12 N-P-K; Scotts®) at planting, and no additional fertilizer was added during the trials.

Potato plants of the cultivar Russet Burbank were propagated from disease-free nuclear tubers that were produced from tissue cultured plants. When the plants reached a height of approximately 20 cm the stems were cut into segments and were dipped in a rooting hormone. The stem cuttings were transferred into new flats in cells filled with Sun Shine potting mix #1. These stem cuttings were incubated in room temperature (21-25ºC) under fluorescent light for two weeks to allow root development, and were then moved to the greenhouse for two more weeks to harden before used.

Inoculum of *S. subterranea* was prepared from field grown infected potato tubers that were washed in warm water with soap and surface dried at room temperature. The infected epidermis was peeled and the peels were surface sterilized in 10% bleach for 20 min. and washed well in tap water. The peels were dried at room temperature and macerated into a fine powder using a coffee grinder. The inoculum was stored at 15ºC until used.

Inoculum of *P. penetrans* was prepared by extraction of nematodes from roots of mint plants maintained at the greenhouse of Washington State University, IAREC, Prosser, WA. Infested mint roots were washed well with tap water and were cut into 5 cm long sections. Then they were placed in a plastic bag, partially covered in tap water, and incubated at room temperature (21-25º) for 7 days. Following incubation, additional water was added to the plastic bag, and the bag was shaken by hand. The content was filtered through a 35 mesh screen nested over a 400 mesh screen and the nematodes were collected into flasks. Inoculum of *M. chitwoodi* (race 1) was prepared from cultures maintained on tomato (*Lycopersicum escelantum L.*) at the greenhouse of Washington State University, IAREC, Prosser, WA (Riga et al 2008). The roots were washed well and surface sterilized with 10% bleach for 10 min. Then, the roots were shaken for 10 min. in 100 ml of water to extract the eggs. The egg solution was filtered through a 35 mesh screen nested over a 400 mesh screen, and the eggs were collected in flasks. All nematodes and eggs were quantified per 1 mL of water with the aid of a StereoZoom®7

microscope (Bausch & Lomb, zoom range 1.0x – 7.0x, Boyle Instruments, P.O. Box 574, Gig Harbor, WA 98335, 206-858-8155).

Table 1 summarizes the planting, inoculations and harvesting dates of the trials. The potato plants were inoculated with 0.01 g of *S. subterranea* inoculum that was thoroughly mixed in the soil at planting. Immediately afterwards the plants were watered to field capacity and moved to the growth chamber. The nematodes were added two weeks later to the assigned replications at a concentration of 2 larvae or eggs per 1 g of soil, for *P. penetrans* and *M. chitwoodi*, respectively. The two weeks delay was carried out in order to synchronize the infection of the roots with *S. subterranea* and the nematodes. Two weeks is the typical time it takes the zoospores of *S. subterranea* to germinate from the sporosori and infect the roots under optimal conditions (Merz et al 2004). Since *S. subterranea* is an obligate parasite and can not be recovered into a pure culture, and since the source of its inoculum was from field grown potato tubers, the plants were treated during the duration of the trials with azoxystrobin (Quadris, Syngenta Crop Protection) according to the label to control certain seed-borne pathogens that could have been present on the surface of the potato tubers during the preparation of the inoculum.

Disease measurements were recorded immediately after harvest. The entire volume of the soil from each replication/pot was separated from the plants and collected in plastic bags and stored at 15ºC. The roots were washed in tap water to remove soil deposits, and were immediately blotted with a linen towel to remove excess water and weighed for fresh root weight. The severity of *S. subterranea* root galls was quantified using a semi-quantitative scale of 0 to 8, where 0 = no galls, 2 = 1 gall, 4 = 3 galls, 6 = 10 galls, 8 ≥ 10 galls (Merz et al. 2004). Root rot severity was scored using a 0 to 3 visual scale, where: 0 = no rot, 1 = low (yellow to 30% of the root with light brownish root discoloration), 2 = medium (light brown to 30% of the root with dark brown discoloration), 3 = high (more than 50% of the root with dark brown discoloration).

Immediately after scoring disease, the roots were wrapped in moist paper towel to prevent them from drying, and placed in plastic bags in 4ºC and were assessed for nematode colonization. *Pratylenchus penetrans* was extracted from roots by placing the roots in plastic bags, partially covered in tap water, and incubating at room temperature (21-25º) for 7 days. Following incubation, the bag was shaken and the content was filtered through a 35 mesh sieve onto a 400 mesh sieve on which the nematodes were collected. *P. penetrans* was extracted from soil using the sugar flotation technique (Jenkins 1964). *Meloidogyne chitwoodi* was evaluated by staining the roots with acid fuchsin using the technique described by Byrd et al. (1983). Root colonization was expressed as numbers of *P. penetrans* larvae, or *M. chitwoodi* females and 2nd stage juveniles per 1 g of fresh root weight. *Pratylenchus penetrans* in the soil was expressed as total number of larvae per pot.

Data were analyzed in SAS (SAS Institute, Carry, NC) using the general linear models procedure (Proc GLM) at a 5% significance level. The trials represented a randomized complete design and the statistical model used was response = trial + treatment + trial*treatment. Trial and treatment were analyzed as fixed effects, and the responses of interest were (i) root galls severity, (ii) root rot severity, (iii) fresh and dry root weights, and (iv) nematode colonization of root and soil. In the lack of interaction between trial and treatment the data of the trials were analyzed jointly. The severity of *S. subterranea* root galls and the severity of root rot were recorded on a category bases and were analyzed non-parametrically as ordinal data using the Kruskal-Wallis analysis of variance by ranks (Zar, 1996). Fresh and dry root weights and nematode colonization

were analyzed as continuous data, and in the lack of a normal distribution transformations were carried out with Log (response + 1). If normality was not achieved by transformation, the data was analyzed non-parametrically using the Kruskal-Wallis analysis of variance by ranks.

Results and Discussion

The majority of the potato cultivars grown in the Columbia Basin of Washington State and North-Central Oregon have russet skin (Schreiber 2006). These cultivars usually are not susceptible to tuber infection by *Spongospora subterranea*; however, their roots can become severely infected demonstrating high numbers of root galls (Nitzan et al. 2008). Therefore, this study focused on root infection. The co-inoculation with *Pratylenchus penetrans* and with *Meloidogyne chitwoodi* did not significantly (P>0.05) increase the number of *S. subterranea* galls on the roots (Tables 2 & 3). In addition, fresh and dry root weights were not reduced by the co-inoculations and did not differ (P>0.05) from the non-inoculated control plants (Tables 2 & 3). However, root rot severity was increased in the presence of *S. subterranea* regardless of the presence or absence of either nematode species (Table 4 and Fig. 1). Recording root rot due to *S. subterranea* supported the previous report by Eraslan and Turhan (1989), indicating that this pathogen can affect potato health by increasing root rot, which is usually associated with reduction of yield.

The colonization of roots or soil by *P. penetrans*, and of roots by *M. chitwoodi* was not significantly (P>0.05) increased in the presence of *S. subterranea* (Tables 2 & 3). Nevertheless, there was approximately a two-fold reduction in the numbers of *P. penetrans* larvae in the roots and the soil in the presence of *S. subterranea* (Table 2), suggesting a possible antagonistic relationship. Furthermore, in the presence of *S. subterranea* there was a two-fold increase in the numbers of 2nd stage juveniles of *M. chitwoodi* in the roots (Table 3). Synergistic interactions between plant pathogens and nematode pests causing disease complexes have been reported before in potato. For example, *Ralstonia solanacearum* bacterial wilt tends to be more severe in the presence of the southern root knot nematode *Meloidogyne incognita* than in its absence (MacGuidwin 2008). Possibly, the most well-known complex is the synergistic interaction between *V. dahliae* and *P. penetrans*, which enhances potato early dying (Martin 1982). The results of the present study suggested a possible interaction between *S. subterranea* and *M. chitwoodi*, or *P. penetrans*. However, unlike the two interactions mentioned above, the results did not point towards a disease complex that affected potato health. Yet, the trend towards a two-fold increase in the number of *M. chitwoodi* juveniles that was recorded should not be overlooked, as it could have economic importance under commercial potato production.

References

Alexopoulos, C.J., Mims, C.W., Blackwell, M. 1996. Phylum *Plasmodiophoromycota*. Endoparasitic slime molds. Chapter 26. Pp 75. In: Introductory Mycology, 4th edition, John Wieley & Sons, Inc.

Braselton JP. 1995: Current status of the Plasmodiophorids. *Critical Reviews in Microbiology* 21: 263-275.

Braselton JP. 2001. Plasmodiphoromycota. In The Mycota VII Part A Systematics and Evolution. DJ McLaughan, EG McLaughlin & PA Lemke. Springer-Verlag, Berlin and Heidelberg: 81-91.

Brown, C.R., Mojtahedi, H., and Santo, G.S. 1991. Resistance to Columbia root-knot nematode in *Solanum* ssr and in hybrids of *S. hougasii* with tetraploid cultivated potato. *American Potato Journal* 68:445-452.

Brown C.R., Mojtahedi, H., James, S., Novy, R.G., and Love, S. 2006. Development and evaluation of potato breeding lines with introgressed resistance to Columbia root-knot nematode (*Meloidogyne chitwoodi*). *American Journal of Potato Research*. 83: 1-8.

Brodie, B.B., Evans, K. and Franco, J. 1993. Nematode parasites of potato. In: Plant parasitic nematodes in temperate agriculture. Evans, K., Trudgill, D.L. and Webster, J.M. Eds. CAB International, University Press, Cambridge, UK. ISBN:0851988083.

Byrd, D.W., Jr., Kirkpatrick, T. and Barker, K.R. 1983. An improved technique for clearing and staining plant tissue for detection of nematodes. *Journal of Nematology* 15:142-143.

Eraslan, F., and Turhan, G. 1989. Studies on powdery scab of potato with special regard to the reaction of certain potato cultivars and clones. *Zeitschrift fur pflanzen-krankheiten und pflanzenschutz* 96:353-360.

Fallon, R. E., Viljanen-Rollinson, L. H., Coles, G.D., Poff, J.D. 1995. Disease severity keys for powdery and downy mildews of pea, and powdery scab of potato. *New Zealand Journal of Crop and Horticultural Science* 23: 31-37.

Fallon, R.E., Wallace, A.R., Braithwaite, M., Genet, R.A., Nott, H.M., Fletcher, J.D., Braam, W.F. 1996. Assessment of seed tuber, in-furrow, and foliar chemical treatments for control of powdery scab (*Spongospora subterranea f.sp. subterranea*) of potato. *New Zealand journal of crop and horticulture science* 24: 341-353.

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

Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease reporter* 48:692.

Lawrence, C.H., and McKenzie, A. R. 1981. Powdery scab. In: Hooker, W.J. ed. Compendium of potato diseases. St. Paul, Minnesota: The American Phytopathological Society.

Merz, U. 2000. Powdery scab. Research in Switzerland. In: Merz, U., Lees, A.K., edt. Proceedings of the first European Powdery Scab workshop, SAC, Aberdeen, Scotland, 20-22 July, 67-71.

Merz, U., Martinez, V. and Schw¨arzel, R. 2004. The potential for the rapid screening of potato cultivars (*Solanum tuberosum*) for resistance to powdery scab (*Spongospora*

subterranea) using a laboratory bioassay. *European Journal of Plant Pathology* 110: 71–77, 2004.

MacGuidwin, A.E. 2008. Managing diseases caused by nematodes. In: Potato Health Management. Johnson D.A. ed. Second Ed. APS Press. St. Paul, MN. ISBN:978-0-89054-353-5.

Martin, M.J., Riedel, R.M. and Rowe, R.C. 1982. *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Ohio. *Phytopathology* 72:640-644.

Nitzan, N., Cummings, T. F., Johnson, D. A., Miller, J. S., Batchelor, D. L., Olsen, C., Quick, R. A., and Brown, C. R. 2008. Resistance to root galling caused by the powdery scab pathogen *Spongospora subterranea* in potato. *Plant Disease* 92:1643-1649.

Riga, E., Lacey, L.A., Guerra, N. 2008. Muscodor Albus, a potential bio-control agent against plant-parasitic nematodes of economically important vegetable crops in Washington State, USA. *Biological Control* 45: 380-385.

Schreiber, A. 2006. Potato tuber-worm: a potential new pest of potato in Washington storages. *Potato Progress* 6 (13): 3. Published by the Washington State Potato Commission. www.potatoes.com.

Zar, Jerrold H. 1996. Biostatistical Analysis, 3rd ed. Published by Prentice Hall. NJ. U.S.A.

Table 1. Planting, inoculation and harvest dates of Russet Burbank potato plants that were inoculated with *Spongospora subterranea*, and the nematodes *Pratylenchus penetrans* and/or *Meloidogyne chitwoodi* in two repeated growth chamber trials in 2008.

Trial [a]	Planting date	Inoculation with *S. subterranea*	Inoculation with *P. penetrans*	Inoculation with *M. chitwoodi*	Harvesting date
Sss x Pp – 1	July 16	July 16	July 29	--	Sept. 23
Sss x Pp – 2	Aug. 6	Aug. 6	Aug. 20	--	Oct. 14
Sss x Mc – 1	Aug. 6	Aug. 6	--	Aug. 20	Oct. 16
Sss x Mc - 2	Aug. 7	Aug. 7	--	Aug. 21	Nov. 4

[a] Sss = *Spongospora subterranea*; Pp = *Pratylenchus penetrans*; Mc = *Meloidogyne chitwoodi*; 1 = trial 1; 2 = trial 2 (repeated trial)

Table 2. Root gall and root rot severity, fresh and dry root weights and number of *Pratylenchus penetrans* nematodes in roots and soil of Russet Burbank potato plants inoculated or not inoculated with *Spongospora subterranea* and/or *P. penetrans* in two repeated growth chamber trials in 2008.

Treatment [a]	Root galls (0-8)[b]	Root rot (0-3) [c]		Fresh root Weight (g)	Dry root weight (g)	*P. penetrans* in root [d]	*P. penetrans* in soil [e]
		Trial 1	Trial 2				
Sss + / Pp +	6.5 a	0.75 a	1.6	1.1	0.03	591 a	28 a
Sss + / Pp -	5.5 a	0.56 b	2.0	1.4	0.04	0 b	0 b
Sss - / Pp +	0 b	0.5 b	1.0	1.3	0.04	965 a	65 a
Non-inoculated control plants	0 b	0 c	1.4	1.1	0.02	0 b	0 b

Different lower case letters with in a column represent significant statistical differences among the treatments at 5% significance level.
[a] Sss + = inoculated with *Spongospora subterranea*; Sss - = not inoculated with *S. subterranea*; Pp + = inoculated with *Pratylenchus penetrans*; Pp - = not inoculated with *P. penetrans*.
[b] Root galls severity was scored on 0-8 scale, where 0 = no galls, 2 = 1 gall, 4 = 3 galls, 6 = 10 galls, 8 ≥ 10 galls (Merz et al. 2004).
[c] Root rot severity was scored using a 0 to 3 visual scale, where 0 = no rot, 1 = low (yellow to 30% of the root with light brownish root discoloration), 2 = medium (light brown to 30% of the root with dark brown discoloration), 3 = high (more than 50% of the root with dark brown discoloration)
[d] Number of *P. penetrans* larvae per gram of fresh root weight.
[e] Number of *P. penetrans* larvae per pot (643g of moist soil mix).

Table 3. Root gall and root rot severity, fresh root weight and number of *Meloidogyne chitwoodi* nematodes on Russet Burbank potato plants inoculated or not inoculated with *Spongospora subterranea* and/or *M. chitwoodi* in two repeated growth chamber trials in 2008.

Treatment [a]	Galls (0-8) [b]	Root rot (0-3) [c]	Fresh root weight (g)	No. of *M. chitwoodi* females	No. of *M. chitwoodi* J2	Total nematodes
Sss + / Mc +	7.2 a	1.6 b	1.5	141 a	147 a	288 a
Sss + / Mc -	6.8 a	2.75 a	0.8	0 b	0 b	0 b
Sss - / Mc +	0.7 b	0.7 c	1.9	121 a	69 a	190 a
Non-inoculated control plants	0.9 b	1.1 c	1.5	0 b	0 b	0 b

Different lower case letters with in a column represent significant statistical differences among the treatments at 5% significance level.

[a] Sss + = inoculated with *Spongospora subterranea*; Sss - = not inoculated with *S. subterranea*; Mc + = inoculated with *Meloidogyne .chitwoodi*; Mc - = not inoculated with *M. chitwoodi*.

[b] Root galls severity was scored on 0-8 scale, where 0 = no galls, 2 = 1 gall, 4 = 3 galls, 6 = 10 galls, 8 ≥ 10 galls (Merz et al. 2004).

[c] Root rot severity was scored using a 0 to 3 visual scale, where 0 = no rot, 1 = low (yellow to 30% of the root with light brownish root discoloration), 2 = medium (light brown to 30% of the root with dark brown discoloration), 3 = high (more than 50% of the root with dark brown discoloration)

[d] Number of *M. chitwoodi* females per gram of fresh root weight.

[e] Number of *M. chitwoodi* J2 juveniles per gram of fresh root weight.

Table 4. Comparison of root rot severity on Russet Burbank potato plants inoculated with *Spongospora subterranea* or not inoculated with *S. subterranea* across all trials conducted in the growth chamber in 2008.

Treatment comparison for root rot severity (0 – 3 scale) [a]			
S. subterranea present alone without nematodes (Sss+/Pp- and Sss+/Mc-) [b]		Non-inoculated control plants	*P* [c]
2.0	vs.	0.8	0.0011
S. subterranea present with or without nematodes (Sss+/Pp+, Sss+/Pp-, Sss+/Mc+ and Sss+/Mc-)		*S. subterranea* not present (Sss-/Pp+, Sss-/Mc+ and non inoculated control plants)	
1.7	vs.	0.84	<0.0001

[a] Root rot severity was scored using a 0 to 3 visual scale, where 0 = no rot, 1 = low (yellow to 30% of the root with light brownish root discoloration), 2 = medium (light brown to 30% of the root with dark brown discoloration), 3 = high (more than 50% of the root with dark brown discoloration).

[b] Sss + = inoculated with *Spongospora subterranea*; Sss - = not inoculated with *S. subterranea*; Pp + = inoculated with *Pratylenchus penetrans*; Pp - = not inoculated with *P. penetrans*; Mc + = inoculated with *Meloidogyne .chitwoodi*; Mc - = not inoculated with *M. chitwoodi*.

[c] *P* value of ANOVA for treatment comparison.

Figure 1. Plants inoculated with *Spongospora subterranea* demonstrating root rot (1st and 2nd from the left) in comparison to non-inoculated control plants (1st and 2nd on from the right).