Hairy Nightshade Is an Alternative Host of *Spongospora subterranea*, the Potato Powdery Scab Pathogen

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Introduction

Powdery scab of potato tubers is caused by *Spongospora subterranea* (Wallr.) Lagerh f. sp. *subterranea*. Potato (*Solanum tuberosum*) cultivars that are susceptible to the disease demonstrate galls on the roots and stolons and lesions on the tubers (Harrison et al. 1997). Infected potato tubers and infested soils are means of disseminating the powdery scab pathogen, which can remain viable and infectious for many years (Kole 1954), and may acquire and transmit the *Potato mop-top virus* (Jones and Harrison 1969; Qu and Christ 2006).

Spongospora subterranea is a plant pathogenic protozoan (Braselton 2001, Corliss 1994), classified in the phylum *Plasmodiophoromycota* (Alexopoulos et al. 1996). The pathogen is characterized by: 1) obligate parasitism, 2) the formation of plasmodia inside host roots and tubers, 3) bi-flagellate zoospores, 4) infectious activity at temperatures between 10 to 20°C under wet conditions, and 5) the formation of conglomerations of resting spores in sporeballs (Karling 1981; Braselton 1995). The sporeballs aggregate on the roots as galls and in the tuber lesions (Harrison et al. 1997; Merz 2008). The disease gets its name, powdery scab, from the appearance of the lesions. The life cycle of the pathogen initiates from the sporeballs that are the primary source of inoculum and may be tuber or soil-borne. In the presence of a host and favorable environmental conditions, primary zoospores emerge from the sporeballs to infect the root hairs. Following infection, a plasmodium develops within the root, and cleaves into segments that develop into zoosporangia, releasing secondary zoospores. The secondary zoospores continue to infect roots and developing tubers. Eventually, root galls and tuber lesions are produced. It has been suggested that *S. subterranea* acts as a polycyclic pathogen and secondary zoospores are released from the root galls or through the roots from zoosporangia increasing inoculum in the soil. Also, it is suggested that, during the life cycle of the pathogen, sporeballs development in root galls or tuber lesions occurs at the same time with secondary zoospore infection (Alexopoulos et al. 1996; Harrison et al. 1997; Merz 2008).

Hairy nightshade (*Solanum sarrachoides*) is an annual weed, which is wide spread in North America, and is especially abundant on irrigated lands in the western USA (Ogg and Rogers 1989). At low crop infestation densities (2-5 plants per meter row) it can produce over 45,000 seeds per plant, and at high crop infestation densities (20-50 plants per meter row) it can produce over 300,000 seeds per plant. Hairy nightshade competes with potato plants for water and nutrients (Alvarez and Hutchinson 2005, Boydston et al. 2008a, Eberlein et al. 1992) and having a physiology similar to that of potato, it is very difficult to control with herbicides in potato fields. A very important aspect of hairy nightshade in potato production is its tendency to host numerous potato nematodes, insect pests, viruses and disease causing agents (Alvarz and Sinivasan 2005, Alvarez and Hutchinson 2005, Boydston et al. 2008a, Boydston et al. 2008b). Being so, it

maintains their populations in the absence of potato, and serves as a source of inoculum in their presence.

Spongospora subterranea has been reported to have a wide host range (Jones and Harrison 1969, Harrison and Jones 1970, Jones and Harrison 1972, Ansersen et al 2002). Galls similar to those of *S. subterranea* on potato roots were observed on roots of hairy nightshade (Boydston 2006). These galls were observed annually on hairy nightshade plants grown in commercial potato fields in Washington State with history of *S. subterranea* root galls and powdery scab on tubers. When these galls were ground, sporeballs were observed (Rick Boydston-unpublished data). The present study tested the hypothesis that the root galls observed on hairy nightshade were the outcome of infection by *S. subterranea*. The objectives of the present study were to identify the causal agent of the root galls on hairy nightshade; and if it was *S. subterranea* to reinoculate potato and hairy nightshade with sporeballs from both potato and hairy nightshade to confirm cross infection.

Materials and Methods

Potato plants of the powdery scab susceptible cultivars Shepody, Umatilla Russet or Russet Burbank were propagated using stem cuttings originating from disease-free, nuclear tubers that were produced from tissue cultured plants. The stem cuttings consisting of one or two nodes were dipped in rooting hormone. The plantlets were incubated at room temperature (21-25ºC) under fluorescent light until roots developed (approximately 2 weeks), and were then moved to the greenhouse.

Hairy nightshade seed was extracted from locally collected berries and treated with 1500 ppm of gibberillic acid for 48 hrs prior to germinating at 29ºC in the dark. Seedlings in the cotyledon stage were transplanted into 4 L plastic pots containing methyl bromide fumigated (0.3 kg/m^3) loamy sand soil composed of 84% sand, 10% silt and 6% clay (Brown et. al 2006). The hairy nightshade and potato plantlets were grown to an average height of 15 cm before inoculating.

Potato inoculum was prepared from infected potato tuber lesions and root Hairy nightshade inoculum was prepared from root galls collected from hairy nightshade plants grown in a commercial potato field near Moses Lake, Washington during 2007 and 2008.

The hypothesis and objectives were tested in five independent experiments in half liter plastic pots filled with Sun-Shine potting mix and arranged in a completely randomized design with 3 to 8 replications per treatment. The treatments included potato and hairy nightshade plants inoculated or not inoculated with one of each inoculum source (potato or hairy nightshade). The trials were 2-3 months in duration, and were conducted in a growth chamber with a constant temperature of 15ºC and under continuous light. The soil was kept moist by irrigating to field capacity every 2 days.

Roots were visually assessed for presence or absence of root galls with the aid of a magnifying glass at the end of each trial, immediately after harvest. To compare between the number of sporeballs produced in root galls from potato and hairy nightshade, ten freshly harvested galls from plants grown in the growth chamber, and six dried root galls from plants collected in the field were removed at random. Since numbers of sporeballs can vary due to gall age, the root galls were removed from plants that were growing side by side in the same field, and were harvested at the same time; and from plants that were inoculated at the same time, grown in the growth chamber under the same conditions and harvested at the same time. The galls were weighed, and then macerated in 1 mL of water using a mortar and pestle, and the average number of

sporeballs was quantified with a hemacytometer per 1 g of root gall. All photographs were recorded with Canon EOS Digital Rebel XT camera. Photographs under the dissecting microscope were recorded at 60x-310x magnification.

Spongospora subterranea-specific PCR was performed to determine the presence or absence of the pathogen in: 1) root galls from field collected potato and hairy nightshade; 2) root galls from artificially inoculated potato and hairy nightshade; and 3) asymptomatic (without galls) roots of both hosts from artificially inoculated plants. Nucleic acid was extracted from root galls and root tissue using the method reported by Crosslin et al. (2006).

Statistical analysis of all data was carried out in SAS (Version 9.1, SAS Institute, Carry, NC). The relationship between the source of inoculum (potato or hairy nightshade), the type of host plant (potato or hairy nightshade) and the presence or absence of root galls was analyzed using Proc GENMOD. The association between visual assessment of root galls and PCR outcome was carried out as a correlation for dichotomous nominal-scale data using Proc FREQ. Differences in sporeballs production were analyzed as continuous data using Proc GLM, and means were separated with student's t-test LSD. All inferences were conducted at 5% significant level (Zar 1996).

Results

Root galls were recorded on potato and hairy nightshade. A statistically significant ($P<0.05$) interaction was recorded between the source of inoculum and the host in relation to the frequency of plants with root galls. More ($P<0.05$) potato plants had root galls when the inoculum originated from potato than from hairy nightshade (Table 1). The frequencies of hairy nightshade plants with root gall were similar ($P>0.05$) regardless of the inoculum source.

The visual and the PCR assessments significantly correlated ($P<0.0001$; Phi Coefficient = 0.69). The visual assessment of root galls, and the PCR assessment of the presence of *S. subterranea* corresponded in 34 of 40 samples (Table 2). Among the samples that the PCR and the visual assessments were incongruent, 5 of 40 were negative visual assessments, but positive PCR outcomes; and only 1 of 40 was a positive visual assessment, but a negative PCR outcome (Table 2).

The numbers of sporeballs produced in galls developing on potato were greater ($P<0.05$) than the numbers of sporeballs produced in galls developing on hairy nightshade (Table 3). The galls did not differ in weight (Table 3). Figures 1 and 3 represent root galls from potato and hairy nightshade plants, which were growing side by side in the same field, and were harvested at the same time (3-4 months into the growing season). Figures 2 and 4 represent galls on potato and hairy nightshade plants that were inoculated at the same time, grown in the growth chamber under the same conditions and harvested at the same time. The galls developing on potato in the field (Figure 1) or the growth chamber (Figure 2) had a milky color when freshly harvested, and had the usual *S. subterranea* gall structure (blackberry or raspberry-like structure). On the other hand, the galls developing on hairy nightshade in the field (Figure 3) were light tan in color and had a round, smooth and bulkier structure then the galls on potato. The *S. subterranea* galls developing on hairy nightshade in the growth chamber (Figure 4) were somewhat elongated, relatively smooth, and dark tan to brown (necrotic) in color when freshly harvested.

Discussion

The results of the present study supported the hypothesis that the galls observed on roots of hairy nightshade plants in commercial potato fields were the outcome of infection by *Spongospora subterranea* f.sp. *subterranea* (*S. subterranea*.). The results indicated that *S. subterranea* can infect and complete its life cycle on hairy nightshade and produce a new generation of sporeballs that are infectious on both potato and hairy nightshade.

The results of the artificial inoculations indicated that potato derived inoculum had a greater pathogenicity than hairy nightshade derived inoculum on potato, but not on hairy nightshade. However, the pathogenicity of potato derived inoculum was similar on both hosts; and also the pathogenicity of hairy nightshade inoculum was similar on both hosts. This indicated that *S. subterranea* could be transmitted to potatoes regardless of the source of inoculum. Therefore, the elimination of hairy nightshade from potato fields is highly desirable as a strategy for preventing *S. subterranea* inoculum buildup.

The limited production of sporeballs on hairy nightshade could explain the low frequencies of root galls that developed on potato plants artificially inoculated with hairy nightshade inoculum compared to potato inoculum. The limited production of sporeballs, regardless of the source of inoculum, may indicate the presence of unknown resistance factors in this host, or that the pathogen is in the process of adapting, overcoming this putative resistance. *S. subterranea* can infect a variety of plant species, other than potato, producing zoosporangia and root galls (Qu and Christ, 2006). However, knowledge of the production of root galls containing sporeballs was limited to yellow mustard (*Brassica campestris* L.), oat (*Avena sative* L.), and tomato (*Lycopersicon esculentum* Mill.) (Qu and Christ, 2006). Information is lacking regarding production of sporeballs on these three plant species under cropping systems or *in vitro*, and their ability to re-infect potato.

The *S. subterranea* specific PCR confirmed the presence of the pathogen in 34 of 40 root samples that were visually recorded with galls. Only 1 of 40 root samples was visually recorded to have galls, but was not confirmed as such by PCR. This outcome indicated that the root galls on hairy nightshade were distinct from healthy roots and could be correctly identified visually. Five of the 40 root samples testing positive for *S. subterranea* using PCR were visually asymptomatic, lacking galls. This phenomenon has been previously recorded by Qu and Christ (2006); and recently, Van de Graff et al. (2007) reported that potato plants of the cultivar Estima, which were inoculated with a range of sporeballs concentrations, were infected, but root galls did not develop.

In the present study 44% of the hairy nightshade plants that were artificially inoculated with potato or hairy nightshade derived *S. subterranea* inocula developed root galls. Additionally, 31% of the hairy nightshade plants that were artificially inoculated with hairy nightshade derived inoculum developed root galls. This is a very important agronomic observation as each of these plants had numerous galls, which contained as much as 93 sporeballs per gram (Table 3). Potato fields in the Columbia Basin of Washington State range from 32 to 81 hectares. Since hairy nightshade is a common weed, each field may contain many hairy nightshade plants producing large quantities of *S. subterranea* sporeballs. The findings of the present study indicated that hairy nightshade has the potential to sustain viable *S. subterranea* inoculum in the absence of potato, providing an inoculum source, which can be infectious and damaging to potato.

Literature Cited

Alexopoulos, C.J., Mims, C.W. Blackwell, M. 1996. Phylum *Plasmodiophoromycota*. Endoparasitic slime molds. In Introductory Mycology, 4th ed., 751-758 John Wiley & Sons, Inc.

Andersen B.A.B, Nicolaisen, M., and Nielsen, S.L. 2002. Alternative hosts for potato mop-top virus, genus Pomovirus and its vector Spongospora subterranea f.sp. subterranea. *Potato Research* 45: 37-43

Alvarez, J.M. and Sinivasan, R. 2005. Evaluation of hairy nightshade as an inoculum source for aphid-mediated transmission of potato leafroll virus. *Journal of Economic Entomology* 98:1101-1108.

Alvarez, J.M. and Hutchinson, P.J.S. 2005. Hairy nightshade management in potatoes. Managing hairy nightshade to reduce potato viruses and insect vectors. *Outlooks on Pest Management. DOI: 10.1564/16dec03*.

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.

Braselton JP (1995): Current status of the Plasmodiophorids. *Critical Reviews in Microbiology* 21(4): 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.

Boydston, R. 2006. Potato disease, nematode, and insect problems worsened by hairy nightshade. In Proceedings of the 45th Washington State Potato Conference, 1-4. Published by the Washington State Potato Commission, www.potatoes.com.

Boydston, R.A., Hutchinson, P.J.S., Bellinder, R.R. 2008a. Managing weeds. In Potato Health Management, 2nd Ed., 223-225. The American Phytopathological Society, St. Paul, MN.

Boydston, R.A., Mojtahedi, H., Crosslin, J.M., Brown, C.R., and Anderson, T. 2008b. Effect of hairy nightshade (*Solanum sarrachoides*) presence on potato nematode, disease, and insect pests. *Weed Science* 56:151-154.

Corliss JO (1994): An interim utilitarian ("user-friendly") hierarchical classification and characterization of the Protists. *Acta Protozoologica* **33**: 1-51.

Crosslin, J.M., Vandemark, G.J., and Munyaneza, J.E. 2006. Development of a real-time, quantitative PCR for detection of the Columbia Basin potato purple top phytoplasma in plants and beet leafhoppers. *Plant Disease* 90: 663-667.

Eberlein C. V., M. J. Guttieri, and W. C. Schaffers. 1992. Hairy nightshade (*Solanum sarrachoides*) control in potatoes (*Solanum tuberosum*) with bentazon plus additives. *Weed Technol*. 6:85-90.

Harrison, B.D. and Jones, R.A.C. 1970. Host range and some properties of potato mop-top virus. *Annals of applied Biology* 65: 393-402

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

Jones, R.A.C., and Harrison, B.D. 1969. The behaviour of potato mop top virus in soil, and evidence for its transmission by *Spongospora subterranea* (Wallr.) Lagerh. *Annals of Applied Biology* 63:1-17.

Jones, R.A.C and Harrison, B.D. 1972. Ecological studies on potato mop-top virus in Scotland. *Annals of applied Biology* 71: 47-57

Kole, A.P. 1954. A contribution to the knowledge of *Spongospora subterranea* (Wallr.) Legerh., the cause of powdery scab of potatoes. *Tijdschr Planten* 60:1-65.
Karling JS (1981): Woronina leptolegniae n. sp., a plasmodiophorid parasite of Leptolegnia. Nova Hedwigia 37: 17-24
Merz, U. 2008. Powdery scab of potato – Occurrence, life cycle and epidemiology. *American Journal of Potato Research* 85:241-246.
Ogg, A.G. and Rogers, B.S. 1989. Taxonomy, distribution, biology, and control of black nighshade (*Solanum nigrum*) and related species in the United States and Canada. *Reviews of Weed Science* 4:25-58.
Qu, X., and Christ, B.J. 2006. The host range of *Spongospora subterranea* f.sp. *subterranea* in the United States. *American Journal of Potato Research* 83:343-347.
Van de Graaf, P., Wale, S.J., Lees, A.K. 2007. Factors affecting the incidence and severity of *Spongospora subterranea* infection and galling in potato roots. *Plant Pathology* 56:1005-1013.
Zar, Jerrold H. 1996. Biostatistical Analysis, 3rd ed. Published by Prentice Hall. NJ. U.S.A.

Table 1. Contingency table summarizing the outcomes of five growth chamber trials, which tested the relationship between numbers of potato or hairy nightshade plants with root galls and the source of *Spongospora subterranea* inoculum.

Source of inoculum	Proportion (%) of plants with galls on roots on the roots		
	Potato	Hairy nightshade	*P* [a]
Potato	83 a A	52 a A	0.08
Hairy nightshade	10 a B	31 a A	0.1
P [b]	0.0005	0.2	

Capital letters represent statistical differences within each column. Lower-case letters represent statistical differences within each row. [a] Statistical significance probabilities within row; [b] Statistical significance probabilities within column.

Table 3. Mean gall weight and number of sporeballs per root gall on potato and hairy nightshade plants that were artificially inoculated with inoculum from potato tuber lesions and grown in the growth chamber, or collected from the field [a].

Host	Mean root galls weight (g)		Number of sporeballs (sporeballs/g) [b]	
	Artificially inoculated [c]	Field collected [c]	Artificially inoculated	Field collected
Potato	0.004 a	0.003 a	1522 a	1775 a
Hairy nightshade	0.003 a	0.003 a	93 b	76 b

Different lower case letters within a column represent statistically significant differences ($P<0.05$) between potato and hairy nightshade.

Table 2. Visual and PCR assessments for the presence or absence of *Spongospora subterranea* in the roots of potato and hairy nightshade (HNS)

Sample [a]	Host [b]	Source of inoculum [c]	Visual assessment [d]	PCR assessment [e]
1	HNS	HNS	+	+
2	HNS	HNS	+	+
3	HNS	HNS	+	+
4	HNS	HNS	+	+
5	HNS	HNS	-	-
6	HNS	Potato	+	+
7	HNS	Potato	+	+
8	HNS	Potato	+	+
9	HNS	Potato	+	+
10	HNS	Potato	+	+
11	HNS	Potato	+	+
12	HNS	Potato	-	+
13	HNS	Potato	+	+
14	HNS	Potato	+	+
15	HNS	Potato	+	+
16	HNS	Potato	+	+
17	HNS	Potato	-	+
18	HNS	Potato	+	+
19	Potato	HNS	+	-
20	Potato	HNS	-	-
21	Potato	HNS	-	-
22	Potato	HNS	-	-
23	Potato	HNS	-	-
24	Potato	HNS	-	+
25	Potato	HNS	-	-
26	Potato	HNS	-	-
27	Potato	HNS	-	+
28	Potato	HNS	-	-
29	Potato	HNS	-	-
30	Potato	Potato	+	+
31	Potato	Potato	+	+
32	Potato	Potato	+	+
33	Potato	Potato	+	+
34	Potato	Potato	+	+
35	Potato	Potato	-	+
36	Potato	Field	+	+
37	HNS	Field	+	+
38	Potato	Potato	+	+
39	HNS	Healthy root	-	-
40	Potato	Healthy root	-	-

[a] Samples 1 through 35 were artificially inoculated and grown at 15ºC in a growth chamber. Sample 36 was a powdery scab lesion from an infected tuber. Sample 37 were root gall-like structures from field grown hairy nightshade (HNS) plants. Sample 38 was a powdery scab root gall used as positive control. Samples 39 and 40 were roots of disease free HNS plants grown from true seed of HNS, and of potato plants grown in tissue cultures. [b] HNS = hairy nightshade; potato = plants of the cultivars Shepody, Umatilla Russet or Russet Burbank. [c] HNS inoculum from field collected root gall-like structures. Potato inoculum from powdery scab lesions from field grown potato tubers. [d] Visual assessment: (+) and (-) indicate the presence or absence of root galls, or root gall-like structures, respectively. [e] PCR assessment: (+) and (-) indicate that the expected band was present or absent, respectively, after agarose gel electrophoresis.

Figure 1. Galls on roots of a potato plant (cultivar not recorded) grown in a commercial field where populations of *S. subterranea* were high. Photograph courtesy of Dr. Dennis Johnson, Dept. of Plant Pathology,Washington State University, Pullman.

Figure 2. Galls on roots of a potato plant (cultivar Russet Burbank) that was artificially inoculated with *Spongospora subterranea* sporeballs from potato.

Figure 3. Galls on roots of hairy nightshade grown in a commercial field where populations of *Spongospora subterranea* were high.

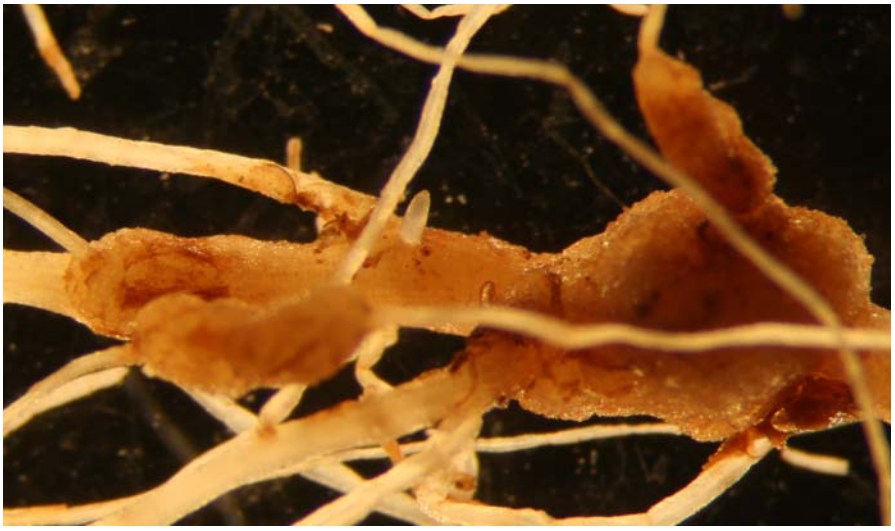

Figure 4. Galls on roots of a hairy nightshade plant that was artificially inoculated with *Spongospora subterranea* sporeballs from potato.