SURVIVAL OF *HELMINTHOSPORIUM SOLANI* IN SOIL AND *IN VITRO* COLONIZATION OF SENESCENT PLANT TISSUE

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Abstract

Silver scurf, caused by *Helminthosporium solani*, is considered to be a tuberborne disease of potato. The ability of *H. solani* to overwinter in soil was evaluated in field studies at two locations in New York during 1992. Field plots were established at sites where *H. solani* infected potatoes had been produced in 1991. Plants produced from pathogen-free tubers of four cultivars yielded tubers infected with silver scurf at both locations. Up to 61% of tubers were infected in some plots. The ability of *H. solani* to colonize leaf tissue was evaluated in *in vitro* assays. Detached leaves of ten crops were sprayed with a spore suspension of *H. solani* and incubated for 20 days. *H. solani* colonized and sporulated on senescent leaf tissue of alfalfa, sorghum, rye, oats, corn and wheat, and only colonized senescent tissue of rapeseed, red clover and buckwheat. No growth was observed on potato leaf tissue. These results indicate that soil survival and saprophytic ability may be important in the epidemiology of silver scurf of potato.

Compendio

La costra plateada, causada por *Helminthosporium solani*, es considerada una enfermedad de la papa que se origina en el tubérculo. En 1992, se evaluó en estudios de campo en dos localidades de Nueva York, la capacidad de *H. solani* de invernar en el suelo. Se establecieron parcelas de campo en lugares donde se habían producido papas infectadas con *H. solani* en 1991. Las plantas de cuatro cultivares obtenidas de tubérculos libres del patógeno produjeron, en ambas localidades, tubérculos infectados con la costra plateada. En algunas parcelas fueron infectados hasta 61% de los tubérculos. Se evaluó en ensayos *in vitro* la capacidad de *H. solani* para colonizar el tejido foliar. Se asperjaron con una suspensión de esporas de *H. solani* hojas desprendidas de diez cultivos y se incubaron por 20 días. *H. solani* colonizó y esporuló sobre el tejido de hojas senescentes de alfalfa, sorgo, centeno, avena, maíz y trigo y sólo colonizó, tejido senescente de colza, trébol rojo y trigo sarraceno. No se observó crecimiento sobre el tejido foliar de papa. Estos resultados indican que la sobrevivencia en el suelo y la capacidad saprofítica pueden ser importantes en la epidemiología de la costra plateada de la papa.

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Introduction

Silver scurf, caused by *Helminthosporium solani* is a common and economically important disease of potato tubers. The superficial brown or silvery gray lesions can reduce the marketability of tubers that are grown for tablestock and increase water loss during storage (1, 10, 12). The development of thiabendazole (TBZ) resistance in *H. solani* (4, 9, 11, 13) is associated with the increased economic importance of silver scurf. This benzimidazole fungicide is used routinely as a post harvest treatment for control of fusarium dry rot in the United States, and previously provided nontarget control of silver scurf.

The seed tuber has been considered the primary source of inoculum (5, 6), and infected seed tubers are frequently found in commercial seed lots in the United States (11). *H. solani* sporulates on the surface of periderm lesions, however the mechanism by which daughter tubers are infected is not known. The pathogen can overwinter on infected tubers and therefore the control of volunteer plants is an important disease management strategy (5, 10).

Studies by Santerre (14) in Canada and Jellis and Taylor (6) in England indicate that overwintering soilborne inoculum does not play an important role in the epidemiology of silver scurf. However, observations made on foundation seed farms indicate that overwintering soilborne inoculum may be an important component of the disease cycle in some locations. Potatoes are propagated on foundation farms from *in vitro* plantlets, with an intermediate greenhouse crop. Silver scurf symptoms have been observed on tubers produced in the field from symptomless, greenhouse-produced tubers. This suggests that infection of tubers occurs from soilborne inoculum, and that this pathogen is surviving in the soil apart from tuber tissue.

No alternate hosts are reported in the literature and, although *H. solani* infects tuber periderm, it cannot infect other parts of the potato plant (2). Burke (2) demonstrated that sweet potatoes, turnips, beets, parsnips and carrots were not hosts for *H. solani.* Kamara and Huguelet (7) tested 22 plant species in nine families, but no infection by *H. solani* was observed. However, studies on the saprophytic ability of *H. solani* have not been done.

Data presented here demonstrate that *H. solani* can overwinter in soil in New York. *In vitro* colonization of senescent leaf tissue of a number of crops by *H. solani* was also observed, and suggests a mechanism for survival of this pathogen.

Materials And Methods

Soil Survival: Field Trials

Field trials were established in 1992 at two locations where *H. solani*-infected potato tubers had been produced in 1991. Location 1 was at

Freeville, NY, on a Howard Gravel soil; location 2 was at Ithaca, NY, on a Longford Channery Silt Loam soil. The trial at location 1 was planted 9 June 1992 and the trial at location 2 was planted 11 June 1992. Seed tubers, produced from tissue culture plantlets in the greenhouse and free of *H. solani*, were planted at both locations. The potato cultivars Norchip, Norland, Red Norland and Dark Red Norland were planted in natural soil, natural soil amended with *H. solani* inoculum, and microplots containing a sterilized peat and vermiculite potting mix. Plots consisted of two rows, with ten plants per row, and were hand planted. Treatments were replicated four times, in a completely randomized design, at each location.

Inoculated control plots were planted in natural soil amended with *H. solani* inoculum. Four replicate tubers of each cultivar were planted and inoculum was added before covering the tubers with soil. Uninoculated control plots were planted in microplots containing a sterilized peat and vermiculite potting mix using two replicate tubers per cultivar. Plots were fertilized, irrigated and treated with foliar fungicides and insecticides according to standard commercial practices.

Inoculum was prepared in a 1:1 mixture of vermiculite and oat grains. The oat grains were soaked in water for 24 hr, mixed with vermiculite, and sterilized for 1 hr on three consecutive days. The medium was inoculated with agar plugs from sporulating colonies of several *H. solani* isolates (3SS-5, 4SS-5, 6SS-1, 7SS-3, 10SS-1 and 15SS-1T1). These isolates had been collected from New York and characterized (11). The inoculum was incubated at 22 - 25 C in the dark for 4 wk, and shaken repeatedly to redistribute the fungus. Colonization of the mixture was confirmed by subsequently placing grains on V8 agar and observing pathogen growth.

All tubers were harvested on 18 September 1992 at location 1 and on 15 September 1992 at location 2, and stored in low (<40%) relative humidity at 4 C. The tubers were washed, surface sterilized and incubated in individual cell packs for 20 days in high (95-100%) relative humidity at 25 C. Silver scurf incidence (percent tubers infected) was measured and disease severity (percent surface area covered with sporulating lesions) on infected tubers was estimated.

Growth on Senescent Leaf Tissue: In Vitro Assays

Greenhouse-grown, washed leaves of oats, rye, wheat, corn, sorghum, alfalfa, red clover, buckwheat, rapeseed and potato were cut into small pieces (1 - 2 cm^2), surface sterilized and placed in sterile petri plates lined with glass fiber filters. The leaves were sprayed until run-off with a *H. solani* spore suspension (10^5 spores/ml), the petri plates sealed with parafilm, and incubated at high (> 95%) relative humidity at 25 C in the dark. Inoculum was prepared from 4-week-old colonies of seven *H. solani* isolates (3-SS3, NK4A, HSMN02, HSME27, HSND25, HSNB16, 16-SS1) (11, 13). The sporulating

cultures were flooded with sterile distilled water, the surface of the colony was scrapped to release the spores, and the suspension was filtered through two layers of sterilized cheesecloth. The spores were washed twice by centrifugation at 3,000 rpm for 10 min, and resuspended in sterile water.

After 20 days incubation, the tissue was observed at 64X, and scored for the presence of mycelium and sporulation. Tissue in which typical *H. solani* conidiophores and conidia were not observed, but mycelium could be observed, was plated on V8 agar amended with 100 μg/ml penicillin and ampicillin. The tissue was rinsed with sterile water to remove spores before transferring it to agar plates. The tissue was observed for sporulation 3 to 5 days after transfer. *In vitro* assays were repeated three times.

Results

Soil Survival: Field Trials

Tubers produced from pathogen-free tubers became infected with *H. solani* at both locations (Tables 1 and 2). Disease incidence was higher on Norchip than on the other three cultivars at both locations. At location 1, 5-25% of the tubers harvested from natural soil were infected with *H. solani*, while 16-68% and 0% of the tubers harvested from infested and sterile soil were infected. Disease incidence was greater at location 2, where 18-61%, 3-50% and 0% of the tubers harvested from natural, infested and sterile soil were infected. Cultivar did not have a significant ($p<0.05$) effect on disease severity at either location. Soil infestation did not significantly increase severity of disease at location 1 (12.7% and 16.6% mean surface area infected for tubers harvested from natural and infested soil, respectively),

TABLE 1.—*Incidence and severity [a] of silver scurf on four potato cultivars planted in natural and infested soil [b] at Freeville, NY during 1992.*

	Natural soil			Infested soil		
Cultivar	N[c]	Incidence	Severity	N	Incidence	Severity
Norchip	140	25	14.6 ± 9.9	56	68	18.1 ± 15.4
Norland	148	10	9.1 ± 6.5	66	16	12.7 ± 6.4
Red Norland	153	8	10.1 ± 7.6	66	21	14.3 ± 11.0
Dark Red Norland	142	5	18.5 ± 14.6	72	24	16.5 ± 10.4
Means	145	12	12.7 + 3.1	65	32	16.6 + 3.5

[a]Silver scurf incidence was measured as percentage of tubers infected, disease severity was estimated as percent surface area covered with sporulating lesions.

[b]No disease was observed on tubers harvested from microplots containing a sterilized peat-vermiculite mix.

[c]Number of tubers rated.

TABLE 2.—*Incidence and severity [a] of silver scurf on four potato cultivars planted in natural and infested soil [b] at Ithaca, NY during 1992.*

	Natural soil			Infested soil		
Cultivar	N[c]	Incidence	Severity	N	Incidence	Severity
Norchip	176	61	12.6 ± 8.2	60	50	22.3 ± 14.4
Norland	164	29	13.6 ± 10.4	56	20	6.3 ± 2.3
Red Norland	166	18	9.8 ± 5.6	52	38	13.5 ± 12.4
Dark Red Norland	175	28	10.4 ± 9.7	64	3	7.5 ± 5.0
Means	170	34	11.6 + 2.6	58	28	16.1 + 3.7

[a]Silver scurf incidence was measured as percentage of tubers infected, disease severity was estimated as percent surface area covered with sporulating lesions.
[b]No disease was observed on tubers harvested from microplots containing a sterilized peat-vermiculite mix.
[c]Number of tubers rated.

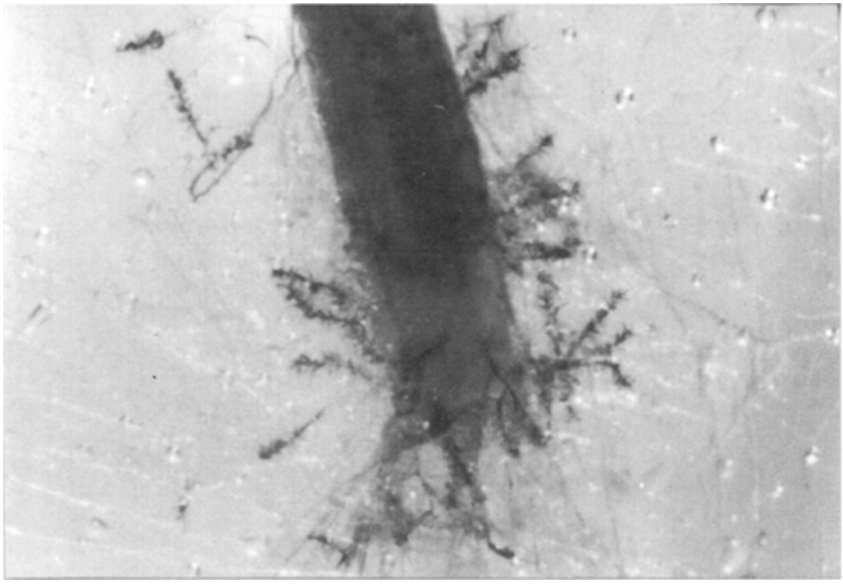

FIG. 1. *Helminthosporium solani* growing and sporulating on senescent alfalfa leaf tissue after 20 days incubation in the dark.

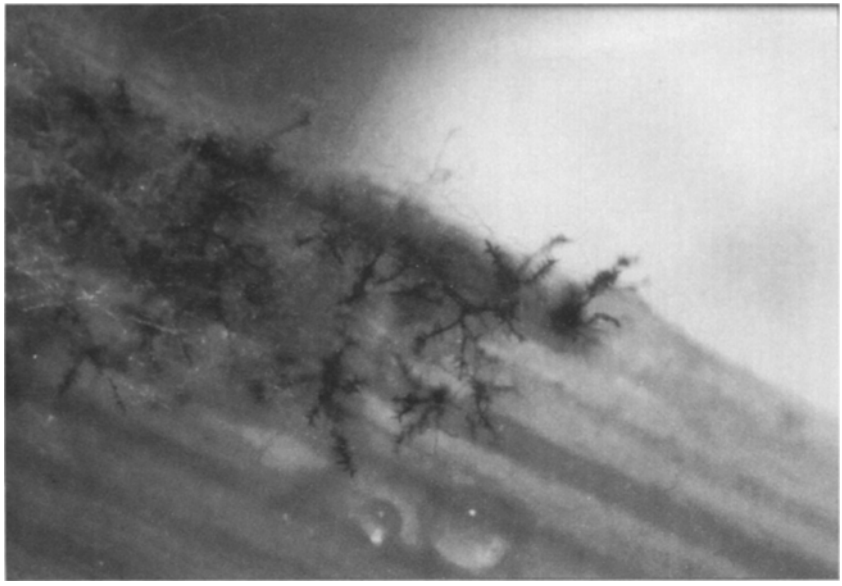

FIG. 2. *Helminthosporium solani* growing and sporulating on senescent rye leaf tissue after 20 days incubation in the dark.

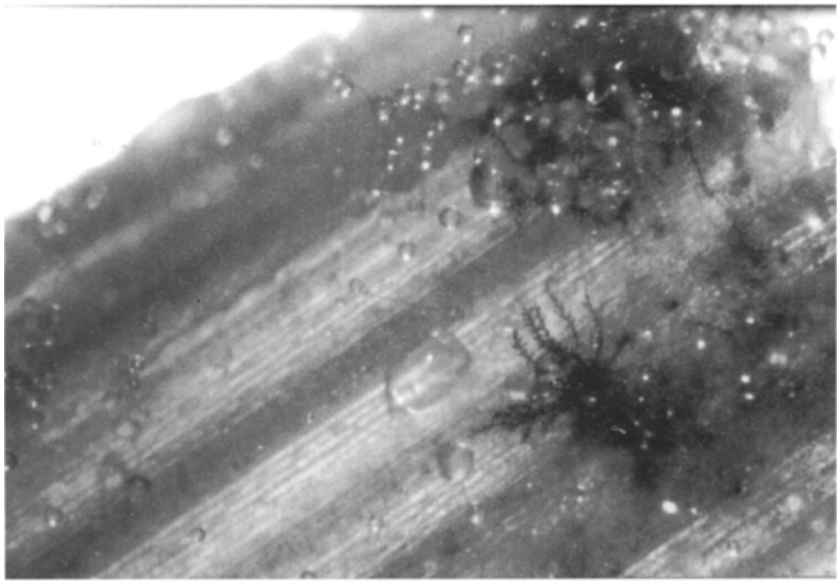

FIG. 3. *Helminthosporium solani* growing and sporulating on senescent wheat leaf tissue after 20 days incubation in the dark.

but it significantly ($p < 0.01$) increased disease severity at Location 2 (11.6% and 16.1% mean surface area infected for tubers harvested from natural and infested soil, respectively).

Growth on Senescent Leaf Issue: In Vitro Assays

Mycelial growth and *H. solani* conidiophores and conidia were observed on senescent tissue of alfalfa, sorghum, rye, oats, corn and wheat after 20 days of incubation in the dark (Figs. 1 - 3). Mycelial colonization was observed on rapeseed, red clover and buckwheat, and *H. solani* sporulated on these leaves within 5 days incubation on V8 agar at 25 C. Colonization of potato leaves was not observed.

Discussion

Studies conducted in Canada (14) and England (6) indicate that seed tubers were the primary source of inoculum, and that soilborne inoculum is not an important component in the epidemiology of silver scurf. Our data demonstrate that *H. solani* can overwinter in soil under field conditions in New York, where up to 61% of the tubers harvested from natural soil were infected from soilborne inoculum. Differences between our results and those reported previously (6, 14), may be due to plant material used to establish plots or to methods used for disease assessment. Previous studies did not use pathogen-free seed tubers (14) or did not incubate tubers after harvest to optimize disease detection (6).

Reports by Burke (2) and Kamara and Huguelet (7) indicate that *H. solani* cannot infect potato tissue, other than the potato tuber periderm, or healthy tissue of other plant species. However, the saprophytic ability of *H. solani* had not been investigated previously. We observed colonization of senescent leaf tissue of a number of crops by *H. solani* in *in vitro* assays. Our observations are consistent with reports that *H. solani* was not a pathogen of other plant species (2, 7), because colonization of leaf tissue was not observed until leaves had begun to senesce.

All of the crops that were tested are commonly planted as rotational or winter cover crops in potato fields, and residues of these crops may support growth and survival of *H. solani.* The lack of growth or sporulation of *H. solani* on potato leaves is surprising, but was consistent in all assays.

Observations made on farms which produce nuclear seed stocks have indicated that *H. solani* must survive in soil for several years between potato crops. All studies conducted to date (2, 7) indicate that *H. solani* does not have alternate hosts. The data presented here demonstrate that significant levels of disease can develop from overwintering inoculum and suggest that saprophytic activity may play a role in between-season survival of this pathogen. Other *Helminthosporium* species are saprophytes on woody plants (3).

Little is known about the ecology of *H. solani.* Because of its slow growth and the lack of a selective medium, it is very difficult to isolate this pathogen from soil or plant tissue, other than potato periderm. Recently, DNA hybridization probes have been developed for *H. solani* (8) and may allow evaluation of its saprophytic activity in soil.

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