Mycoparasitism of *Helminthosporium solani* by *Acremonium strictum*

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ABSTRACT

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Isolates of *Helminthosporium solani*, the causal agent of silver scurf of potato, collected from multiple locations consistently show white sectoring and rings, differential coloration, and reduced sporulation in culture. It has been accepted that this growth pattern is normal for *H. solani* cultures. Scanning electron microscopy confirmed the presence of a contaminating fungus in close association with cultures of *H. solani.* Repeated hyphal tip isolation techniques were used to separate *H. solani* from the fungal contaminant. Resultant pure cultures of *H. solani* were uniformly black in color, without white sectors or rings. The contaminating fungus was identified as *Acremonium strictum*. The purpose of this study was to elucidate the relationship between *A. strictum* and *H. solani*, and evaluate the impact of the fungicolous *A. strictum* on the growth and biology of *H. solani*. In vitro studies demonstrated that *A. strictum* significantly reduced sporulation of *H. solani* isolates from 65 to 35%, spore germination from 53 to 43%, and mycelial growth from 40 to 32% compared with noncontaminated cultures of *H. solani*. These data indicate that *A. strictum* is antagonistic to *H. solani*, and can be considered a mycoparasite. *A. strictum* reduced *H. solani* conidia production on minitubers, thereby reducing inoculum for infection. However, treatment with *A. strictum* does not reduce silver scurf of previously infected tubers. Further studies are warranted to determine the full potential of *A. strictum* as a biological control agent of *H. solanii*-incited silver scurf of stored potato tubers and the most effective manner of use.

Additional keyword: *Cephalosporium*.

Silver scurf of potato (*Solanum tuberosum* L.), caused by the anamorphic fungus *Helminthosporium solani* Durieu & Mont., has caused significant widespread economic losses on all market classes of potato since its introduction into the United States in 1908 (17). It causes superficial blemishes, and the importance of the disease is measured not in terms of yield loss but of quality. Because the disease affects the appearance of the tubers, downgrading can reduce their market value. The disease also can cause a weight loss in storage as severity increases, and sloughing of the periderm can create points of entry for other tuber pathogens (20). Silver scurf is present on tubers before harvest, and the severity increases rapidly on stored tubers (13,14,20).

The major spread of the disease occurs during storage and is influenced by the amount of inoculum and the conditions present in storage (20). Old lesions have less capacity to sporulate than newly formed lesions (13). Conidia from tubers may colonize new tubers or may cause new infection sites on the same tuber. Eventually, a new lesion also will sporulate, rapidly increasing the disease under appropriate environmental conditions (14).

Silver scurf previously has been controlled as a nontarget disease by postharvest application of thiabendazole (TBZ) for Fusarium dry rot caused by *Fusarium sambucinum* Fückel, but development of TBZ resistance in *H. solani* as nontarget populations appears to be the cause, at least in part, of an increase in silver scurf in potato-producing areas in Europe (4,12), the United States (18,21), and Canada (3,15). Silver scurf is not adequately controlled by other methods, such as seed treatment, resistant cultivars, adjusted planting dates, postharvest chemical applications, or other cultural practices (23). Postharvest application of inorganic salts has a suppressive effect on silver scurf, reducing disease severity and *H. solani* sporulation on naturally infected, field-grown tubers after 15 weeks of storage at 10°C (19).

Other strategies may be necessary to provide control of silver scurf. Preliminary trials have shown that repeated treatment of the storage atmosphere with activated chlorine dioxide may reduce the infection and spread of silver scurf in storage. Trials conducted in commercial potato storages have shown that adding chlorine dioxide via humidification systems reduces the number of silver scurf spores and the incidence and severity of the disease after 16 weeks in storage (23).

Biological control agents or antagonistic microorganisms potentially could provide an alternative control of silver scurf. Biological control of postharvest diseases of fruit and vegetables proved feasible in numerous studies, and research has led to many biological control products (26). Chun and Shetty (5) reported some strains of *Pseudomonas corrugata* that can inhibit the mycelial growth of *H. solani* in vitro and reduce disease severity by 28 to 45%. Elson et al. (7) screened microbiota from agricultural soils and tuber samples, and identified some potential biocontrol agents of *H. solani*.

Routine work with *H. solani* cultures in our laboratory usually showed sectoring and contrasting black-and-white sporulation zones accompanied by reduced sporulation and poor growth. Affected colonies appeared to be contaminated with a fungus closely associated with *H. solani*, and preliminary scanning electron microscopy confirmed the presence of two different fungi.

The purpose of this study was to determine the role of this fungicolous fungus on the growth and biology of *H. solani* cultures and elucidate the relationship between the two species. The objectives were to identify the contaminant, axenically culture *H. solani* in order to compare sporulation, spore germination, and radial growth of axenic and contaminated cultures of *H. solani* in

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vitro, and evaluate the potential of the contaminant as a biological control agent of *H. solani*.

MATERIALS AND METHODS

Isolates. For the isolation of *H. solani*, silver scurf-infected tubers were surface sterilized with 0.5% sodium hypochlorite for 10 min and incubated in a humid chamber at room temperature. After 5 to 7 days of incubation, conidia were picked from the tuber surface with a sterile needle and spread on potato dextrose agar (PDA) medium acidified with 0.015% lactic acid. After 24 to 48 h, germinated single spores were transferred to modified V8 agar medium (mod V8) consisting of 1.5% V8 juice, 8% agar (Difco Agar), and 1.5 g of calcium carbonate per liter, adjusted to pH 6.8. The isolates were transferred as mycelial plugs to an ultrafreezer at –78°C for long-term storage.

The *H. solani* isolates used in this study originated from tubers with silver scurf received from cooperators in the states of Alaska, Nebraska, and Washington in the United States, and the province of New Brunswick in Canada. The isolates used in the study were HSAK03 (Alaska), HSNE4A (Nebraska) HSNB16 (New Brunswick), and HSWS04 (Washington).

All isolates used in this study were contaminated originally, as evidenced by sectors or rings of white mycelium within the colonies (Fig. 1). Preliminary scanning electron microscopy confirmed the presence of a fungus in close association with *H. solani* (Fig. 2A). Consecutive hyphal tip isolation was used to separate the contaminant from the *H. solani* and produce pure cultures of *H. solani*. After transfer, the plates were incubated at 20°C in the dark. The transfers were made before the colony reached 1 cm in diameter to facilitate the hyphal tip isolation. This procedure was repeated several times until a culture of *H. solani* free of the contaminant was produced (Fig. 2B). However, it was not possible to obtain an axenic culture of the contaminant, because only in the presence of *H. solani*, even as a small piece, was the fungus able to grow.

The contaminant originally was identified as a *Cephalosporium* sp. by Dr. Neil Anderson, University of Minnesota, based on colony growth and conidia morphology (Fig. 3), and subsequently identified as *Cephalosporium acremonium* Corda (Sukapure & Thirum.) (R. W. Stack, *personal communication*). The genus *Cephalosporium* has been reclassified to *Acremonium*, and the proposed name of this fungus is presently *Acremonium strictum* W. Gams.

A matching set of four *H. solani* isolates naturally infected with and freed from *A. strictum* was prepared by repeated hyphal tip culture as previously described. The isolates were grown in plastic petri dishes containing modified V8 medium. Inoculated plates were incubated in continual darkness for 4 weeks in a temperature-controlled growth chamber at 20°C in order to induce sporulation.

Effect of *A. strictum* on sporulation of *H. solani*. The number of spores produced by each *H. solani* isolate with and without *A. strictum* was estimated as density per unit area. A spore suspension was prepared by dipping a 1-cm-diameter disk from a 30-day-old culture of *H. solani* in 10 ml of distilled water and stirring with a sterile glass rod to dislodge conidia from the plug. The concentration of the conidial suspension was determined using a hemacytometer chamber. Three plates were used per isolate, each plate being one replication. Ten readings were made per replication. The experiment was performed three times. Analysis of variance for a randomized complete design (RCD) was performed as described by Goldstein (9).

Effect of *A. strictum* on germination of *H. solani* at various temperatures and relative humidities. The effect of *A. strictum* on germination of conidia was determined by incubating conidia from cultures of each isolate with and without *A. strictum* at

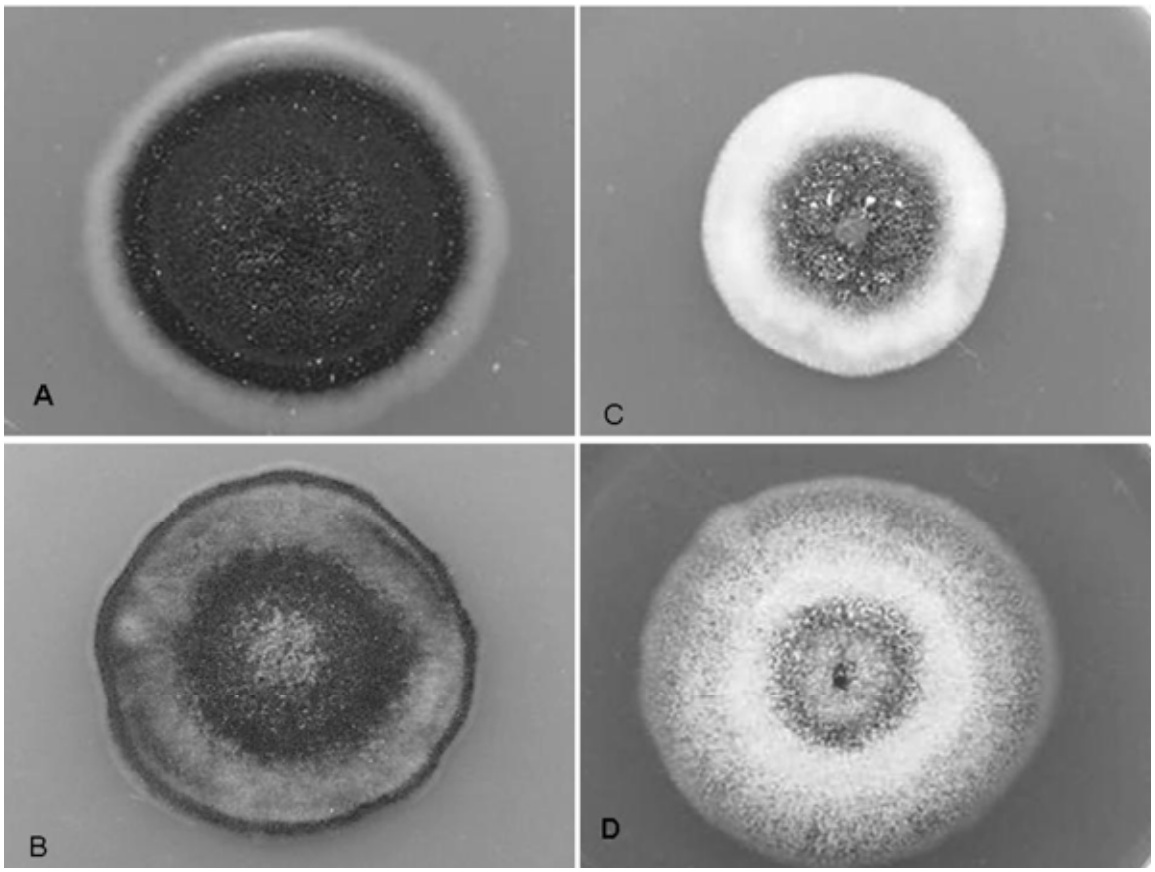

Fig. 1. *Helminthosporium solani* cultures with and without *Acremonium strictum*. **A,** Colony free of *A. strictum*. **B, C, and D,** Colonies with *A. strictum* showing sectors and rings of white growth characteristic of contaminated *H. solani* cultures.

different temperatures and relative humidities. Conidia were collected from a 30-day-old culture grown as previously described. Conidia were dislodged from the conidiophores by flooding the plates with distilled water and gently scraping with a flame-sterilized glass slide. Two drops of Tween 20 were added per 100 ml of conidial suspension to disperse conidia and prevent clumping. The suspension was adjusted to 2×10^4 conidia ml^{-1} to provide a minimum of 100 conidia per 10 µl.

Using the technique described by Harris et al. (10), as modified by Alderman and Beute (1), water agar amended with NaCl at varying concentrations was used to produce different relative humidities for spore germination studies. A 20-µl droplet of the conidial suspension was placed on each of four microscope cover glasses using a micropipette (Pipetman P-20; Rannin Instrument Co.) and air dried until half of the droplet was evaporated to aid in relative humidity (RH) equilibration. The four cover glasses then were placed in a petri dish containing 40 ml of water agar amended with NaCl and sealed with Parafilm. The RH of the air in the sealed dish is related to the NaCl molality according to the values given by Lang (16). The relative humidities tested were 100, 99, 98, and 95%, which were obtained by amending the agar with 0, 0.3, 0.6, and 1.5 M NaCl, respectively. The RH chambers were conditioned at the desired temperature at least 12 h prior to introduction of the conidia. The effect of *A. strictum* on germination of *H. solani* at various temperatures at each RH was determined by placing the RH chambers in controlled temperature incubators at 5, 10, 15, 20, and 25°C.

Spore germination was evaluated 48 h after conidia were placed in the RH chamber. The cover glass from each RH chamber was inverted on a drop of cotton blue in lactophenol on a glass slide to stop germination and growth and to preserve the spores and germ tubes for examination. The percentage of germinated conidia was determined by observing 50 conidia at random in each cover glass using a microscope at ×10. A conidium was considered to have germinated if the germ tube length was at least one-half the length of the spore (2). Each experiment was performed twice, with two replications per treatment and four readings per replication per treatment (200 conidia observed/replication).

An analysis of variance was performed for an RCD in a four-by-five-by-four factorial arrangement as described by Steel and Torrie (24). Bartlett's test for homogeneity of variance was performed to determine whether experiments could be combined for analysis. Temperature, RH, and mycoparasite were considered as fixed effects and the *H. solani* isolate as a random effect.

Effect of *A. strictum* on radial growth of *H. solani.* To determine the effect of *A. strictum* on vegetative growth of *H. solani*, a 5-mm-diameter agar disk from a 30-day-old culture of each isolate with and without *A. strictum* was transferred to mod V8 medium. The radial growth was determined at 20°C because previous in vitro studies had shown this temperature to be the optimum for vegetative growth of *H. solani* (*unpublished data*). After 30 days of incubation in the dark, growth was evaluated by making two perpendicular measurements directly across the colonies and averaging the diameters. Four replications were made per isolate, and the experiment was repeated three times. An analysis of variance was done using the general linear model procedure of SAS (22).

Effect of *A. strictum* on infection of potato tubers by *H. solani.* To measure the potential of *A. strictum* as a biological control agent, two experiments were conducted to examine the effect of *A. strictum* on infection of potato tubers by *H. solani.* A pure culture of *H. solani,* isolate HSNE4A, was used in this experiment because it had the highest sporulation and germination rates of the four isolates evaluated in this study. The isolate was grown on mod V8 at 20°C for 30 days. *H. solani* conidia were harvested and the concentration of the conidia suspension was adjusted to 2×10^4 conidia ml^{-1}.

The culture of *A. strictum* was grown in association with a small starter plug of *H. solani* isolate HSNE4A, the original source of that *A. strictum*, on mod V8 medium for 30 days. The *A. strictum* spores were harvested by flooding the plates with sterile distilled water, gently scraping the mycelium and spores from the medium using a sterile glass slide, and pouring the water/spore mixture through consecutive sievings of progressively smaller screens (60 to 160 mesh) to separate the *H. solani* conidia from

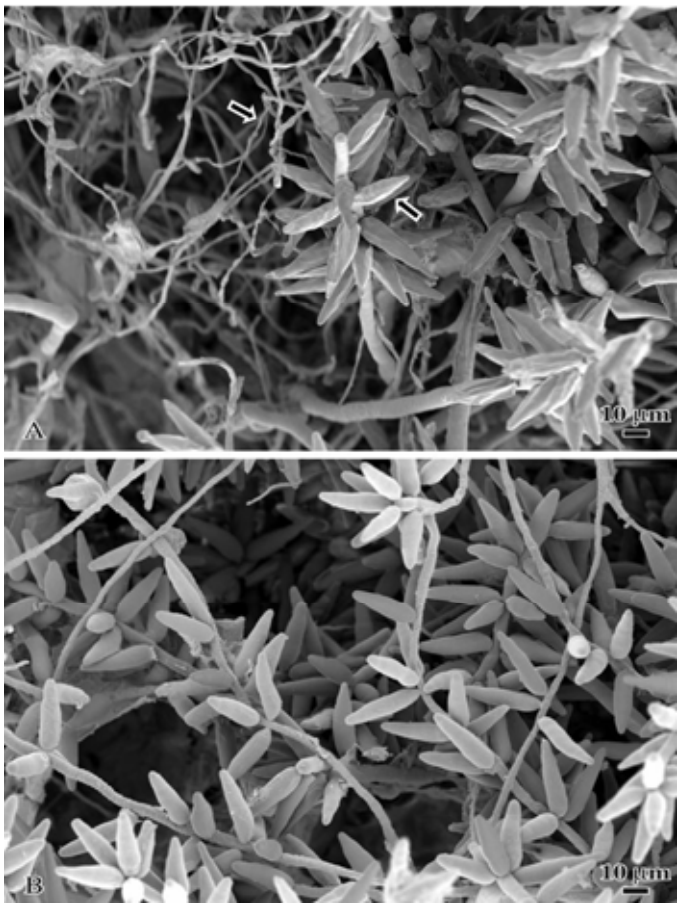

Fig. 2. Scanning electron micrographs of *Helminthosporium solani* colonies **A,** with and **B,** without *Acremonium strictum.* Upper arrow denotes hyphae of *A. strictum* and the lower arrow denotes shriveled *H. solani* conidia.

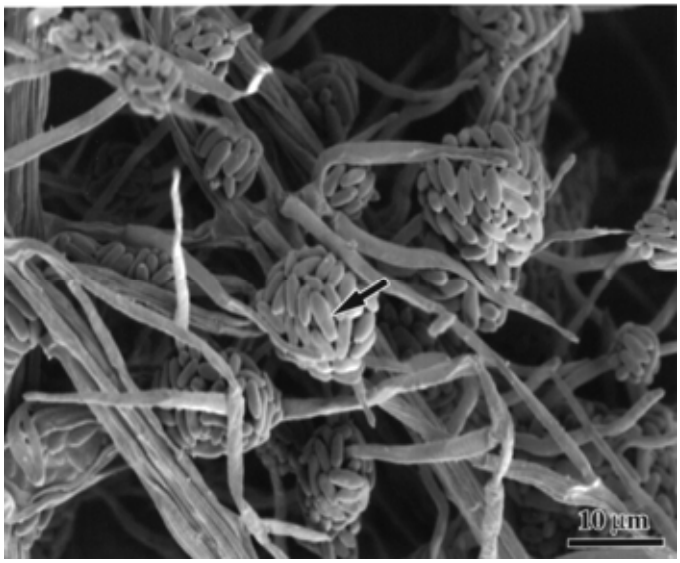

Fig. 3. Scanning electron micrograph of *Acremonium strictum*; arrow denotes conidia in head; (magnification ×500).

the mixed spore solution. *H. solani* conidia were removed in larger meshes, and *A. strictum* conidia were collected in the final 160-mesh screen. The spore concentration was determined by preparing a solution with a turbidity reading of 0.08 to 0.1 optical density units (OD), followed by serial dilution. The serial dilution was plated on water agar and colonies counted after 24 h. A standard curve was prepared and used to estimate inoculum concentration. The spore concentration was adjusted to 2×10^5 and 2×10^7 spores ml^{-1} as low and high rates, respectively.

Experiment I: minitubers. Sixty greenhouse-grown minitubers of the potato cv. Red Norland (Valley Tissue Culture, Halstad, MN) were immersed in either a high (2×10^7) or low (2×10^5) suspension of *A. strictum* spores for 3 min or in water alone. After the minitubers were air dried, they were inoculated with *H. solani* (isolate HSNE4A) by spraying 20 times with a solution of *H. solani* at 2×10^4 conidia ml^{-1} using a hand-held plastic sprayer with an average discharge of 0.8 ml (total volume applied = 16 ml per 60 tubers).

The minitubers were incubated 30, 60, and 90 days in darkness at 15°C in a humid chamber consisting of a plastic box lined with moist paper towels. Twenty tubers were removed at random at each date and rated using sporulation as indicator of the amount of disease. The experiment was performed twice with three replications per treatment. The number of conidia produced and percent germination of those conidia in each treatment was determined by collecting conidia from each tuber.

To determine the number of spores, tubers were washed by individually placing each tuber into a 250-ml beaker containing 100 ml of distilled water plus 150 µl of Tween 20 and shaken vigorously to remove conidia from silver scurf lesions. The washing solution was transferred to a 50-ml centrifuge tube and centrifuged at 10,000 × *g* for 5 min and the resulting pellet resuspended in 50 ml of water. The concentration of *H. solani* conidia was determined by using a hemacytometer chamber. Ten readings were made for each replication.

To determine percent germination, three cover glasses/treatment/replication containing two 10-µl droplets of the conidia solution washed from the tubers were placed in a petri dish containing 40 ml of water agar to create 100% RH, and the dish was sealed with Parafilm. The plates were incubated at 20°C and 100% RH because previous in vitro studies had shown these to be the optimum condition for conidia germination. After 48 h, germination was evaluated by inverting each cover glass in a drop of 0.1% cotton blue lactophenol stain (0.1 g of cotton blue in 100 ml of anhydrous lactophenol) on a glass slide. Percent germination was determined as previously described by observing 100 conidia per cover glass.

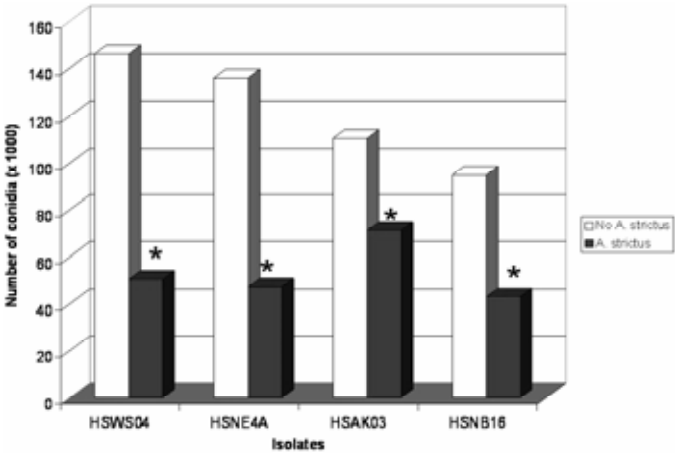

Fig. 4. Effect of *Acremonium strictum* on the number of conidia produced by four *Helminthosporium solani* isolates. The * indicates the value is significantly different from the paired value ($P = 0.05$).

Experiment II: field tubers. Freshly harvested tubers of the potato cv. NorDonna (Kendall Hill Farms, North Carolina) were used to determine the effect of the *A. strictum* on tubers naturally infected with *H. solani* and showing silver scurf symptoms The tubers were separated into three categories based on visual estimation of percent severity of silver scurf—low (<20%), medium (20 to 50%), and high (>50%)—prior to treatment. Forty tubers of each category were dipped in a suspension of *A. strictum* conidia at either a low (2×10^5 spores ml^{-1}) or high (2×10^7 spores ml^{-1}) concentration. Forty tubers were dipped in water alone and used as an untreated control. After inoculation, the tubers were placed in plastic boxes lined with moist paper towels to maintain high humidity (>90%) and incubated at 15°C in the dark.

For each treatment and date, 20 tubers were removed at random 60 and 90 days after treatment. Infection was estimated by visually counting the numbers of sporulating lesions of silver scurf using a stereoscope (magnification ×2.5). The experiment was performed two times, with three replications per treatment.

RESULTS

Effect of *A. strictum* on sporulation of *H. solani*. Analysis of variance indicated a significant ($P = 0.05$) effect of *A. strictum* on the sporulation of *H. solani* isolates. In the same analysis, a significant difference was observed in the number of conidia produced by each isolate of *H. solani* (Fig. 4). The average number of conidia produced across all *H. solani* isolates without the *A. strictum* was 12.0×10^4 conidia ml^{-1}, compared with 5.3×10^4 conidia ml^{-1} for the same isolates with *A. strictum*. The number of conidia produced by the isolates without *A. strictum* ranged from 9.4×10^4 to 14.6×10^4 conidia ml^{-1} and for the isolates with *A. strictum* from 4.4×10^4 to 7.1×10^4 (Fig. 4). In the presence of *A. strictum*, the average reduction in number of spores produced by the isolates ranged from 35.6 to 65.3%. The sporulation of *H. solani* isolates was affected in different degrees by the presence of *A. strictum*; isolates HSWS04 and HSNE4A had the greatest reduction in sporulation; and isolate HSAK03 had the least reduction in the number of conidia produced (Fig. 4).

Effect of *A. strictum* on germination of *H. solani* at various temperatures and relative humidities. The germination of the *H. solani* conidia was significantly affected by the presence of *A. strictum*. The analysis of variance indicated a significant ($P < 0.05$) effect of *A. strictum*, temperature, RH, isolate, and all interactions on conidia germination (Table 1). In these trials, the optimum germination across all isolates with and without the *A. strictum* treatment and across all relative humidities was obtained at 20°C, and germination was reduced to near zero at 5°C (Table 2). The percent germination was significantly highest at 100% RH for all temperatures across all isolates with and without the *A. strictum*, and germination was reduced to near zero at 95% RH (Table 2). Therefore, the effect of *A. strictum* on conidia germination is only presented for data obtained at 100% RH and at 20°C, the optimum conditions for conidial germination.

TABLE 1. Analysis of variance for the effect of temperature, relative humidity, and *Acremonium strictum* on germination of *Helminthosporium solani* isolates

Source of	Degree of	Mean square	*F* value[z]
Isolate	3	5,003.7	181.2**
Temperature	4	38,417.1	6852.6**
Relative humidity	3	99,466.6	71.8**
A. strictum	1	59,568.6	256.6**
Isolate–*A. strictum*	3	224.3	14.4**
Isolate–temperature	12	2,196.4	285.6**
Isolate–relative humidity	9	1,385.1	284.0**
Error	954	25.53	…
Corrected total	1,279	…	…

[z] Asterisks (**) = significant at $P = 0.05$.

A highly significant isolate–*A. strictum* interaction was detected. In a combined analysis, the mean values for the percent germination of *H. solani* conidia with and without *A. strictum* across all temperatures and humidities was 11.2 and 28.4%, respectively (Table 2). A significant difference in the percent germination was observed among isolates. The percentage of germination of *H. solani* isolates without *A. strictum* ranged from 81.0 to 92.5%, and for the same isolates with *A. strictum*, the percent germination varied between 30.0 and 45.5% at 100% RH and 20°C (Table 3). The percent reduction of sporulation due to the presence of *A. strictum* ranged from 50.8 to 64.1% (Table 3). Isolate HSWS04 had a significantly greater percent germination in pure culture even when contaminated with *A. strictum*, compared with the other isolates. Isolate HSAK03 appeared to be affected to a higher degree by the presence of *A. strictum* contamination compared with the other isolates (Table 3). There was no significant difference in sporulation of isolates HSNE4A and HSNB16 either with or without *A. strictum* (Table 3).

Effect of *A. strictum* on radial growth of *H. solani.* The effect of *A. strictum* on radial growth was determined at 20°C, the temperature that was shown to be the optimum for germination of *H. solani* isolates. The analysis of variance indicated a significant negative effect of *A. strictum* on the radial growth across all *H. solani* isolates. The presence of the *A. strictum* significantly reduced the radial growth of isolates HSAK03, HSNE4A, HSWS04, but the radial growth of isolate HSNB16 was not affected by the presence of *A. strictum* (Fig. 5).

Effect of *A. strictum* on infection of potato tubers by *H. solani.* *Experiment I: minitubers. Sporulation.* Neither Bartlett's test nor Levine's test for homogeneity indicated that the variance between the two trials for conidia production on inoculated minitubers was homogenous; therefore, each trial was analyzed separately. The analysis of variance indicated a significant ($P < 0.05$) effect of *A. strictum* on the conidia production for both trials. The presence of *A. strictum* reduced the number of conidia produced by isolates of *H. solani* compared with the same isolates without *A. strictum* at both concentrations (Table 4). In both trials, the lower concentration (2×10^5 spores ml^{-1}) of the *A. strictum* had a greater effect on reducing conidia production than the higher concentration (2×10^7 spores ml^{-1}) of *A. strictum*, but both concentrations of *A. strictum* significantly reduced the number of *H. solani* spores in both trials compared with the untreated control (Table 4).

The analysis of variance also indicated a significant ($P < 0.05$) effect of the incubation period and the number of conidia produced by *H. solani* on inoculated minitubers. In both trials, the lowest number of conidia was produced during the first 30 days of incubation (Table 5). There was a significant positive relationship between the numbers of conidia produced and the length of incubation, with the highest conidia production at 90 days of incubation in both experiments across all treatments (Table 5). The interaction of treatments (no *A. strictum*, *A. strictum* low, and *A. strictum* high) and time (30, 60, and 90 days) showed significant differences among treatments (Table 5).

Experiment I: minitubers. Germination. Germination of *H. solani* spores recovered from inoculated minitubers was significantly reduced when the inoculated minitubers also were inoculated with *A. strictum* spores at both low and high concentrations (Table 6). The analysis of variance indicated a nonsignificant effect of the incubation period on germination of *H. solani* conidia in a combined analysis. However, germination was significantly reduced when the tubers were treated with *A. strictum* spores at both low and high concentration, compared with the untreated control for each period of incubation (Table 6). The low concentration of *A. strictum* (2×10^{-5} spores/ml) was the most effective in reducing germination of *H. solani* in all incubation periods (Table 6).

The interaction of treatments (no *A. strictum, A. strictum* low, and *A. strictum* high) and time (30, 60, and 90 days) did not show significant differences in conidia germination over time for any treatment (data not shown).

Experiment II: field-grown tubers. After 90 days of incubation, the tubers were decayed by soft rot and could not be evaluated for *H. solani* infection. Therefore, only data from the 60-day incubation were evaluated. Analysis of variance indicated that *A. strictum* did not have a significant effect on reducing *H. solani* sporulation in naturally infected tubers after 60 days of incubation. None of the interactions between the treatment with A. *strictum* (high and low level of inoculum) and level of silver scurf disease in the tubers (low, medium, and high) were significant (data not shown). It appears that *A. strictum* does not have the ability to eliminate postharvest disease of field-grown tubers already infected with *H. solani*.

TABLE 2. Effect of temperature, relative humidity, and *Acremonium strictum* on the germination of *Helminthosporium solani* conidia in a combined analysis

Variable	Mean values (%)[z]
Temperature (°C)	
25	27.9 a
20	30.6 b
15	19.6 c
10	11.3 d
5	0.6 e
Relative humidity (%)	
100	40.7 a
99	22.4 b
98	8.0 c
95	0.8 d
A. strictum	
Not present	28.4 a
Present	11.2 b

[z] Means followed by the same letter are not significantly different ($P = 0.05$).

TABLE 3. Effect of *Acremonium strictum* on conidia germination (%) of *Helminthosporium solani* isolates at 20°C

	Isolate[y]			
Treatment	HSWS04	HSNE4A	HSAK03	HSNB16
No *A. strictum*	92.5	81.0	83.5	82.0
With *A. strictum*	45.5*	37.5*	30.0*	37.5*
Reduction (%)[z]	50.8	53.7	64.1	54.3

[y] Column means with an asterisk are significantly different from the paired value ($P = 0.05$).
[z] Reduction of germination.

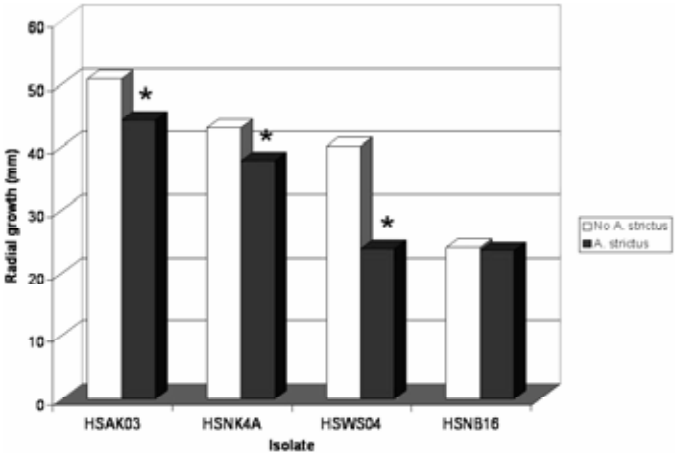

Fig. 5. Effect of *Acremonium strictum* on radial growth of *Helminthosporium solani* isolates at 20°C and 100% relative humidity on modified V8 medium. Means with an * are significantly different from the paired value ($P = 0.05$).

TABLE 4. Mean values for the number of conidia produced by *Helminthosporium solani* isolates on potato minitubers with different concentrations of *Acremonium strictum*

Treatment[z]	Trial 1		Trial 2	
	Mean value (conidia ml^{-1})	Spore reduction (%)	Mean value (conidia ml^{-1})	Spore reduction (%)
Untreated	30.7×10^3	0.0	14.9×10^4	0.0
A. strictum low	23.2×10^3	25.8	6.8×10^4	54.4
A. strictum high	25.7×10^3	16.1	8.9×10^4	40.3

[z] Low and high treatments = 2×10^5 and 2×10^7 conidia ml^{-1}, respectively; least significant difference ($P = 0.05$) = 1,601 and 2,476 for trials 1 and 2, respectively.

TABLE 5. Number of conidia produced by *Helminthosporium solani* on minitubers inoculated with high and concentrations of *Acremonium strictum* after different incubation times

Treatment[y]	Time (days)[z]	Mean value	
		Trial 1	Trial 2
Control	30	8.0×10^3	4.7×10^3
Control	60	42.7×10^3	197.1×10^3
Control	90	41.7×10^3	244.4×10^3
$LSD_{P0.05}$	…	3,578	56,956
A. strictum low	30	5.4×10^3	2.5×10^3
A. strictum low	60	30.0×10^3	65.6×10^3
A. strictum low	90	34.3×10^3	135.8×10^3
$LSD_{P0.05}$	…	4,072.1	43,163
A. strictum high	30	4.1×10^3	2.3×10^3
A. strictum high	60	36.3×10^3	87.2×10^3
A. strictum high	90	36.5×10^3	178.1×10^3
$LSD_{P0.05}$	…	3,904.2	20,239

[y] Low and high treatments = 2×10^5 and 2×10^7 conidia ml^{-1}, respectively; least significant difference ($P = 0.05$).
[z] Period of incubation.

TABLE 6. Effect of *Acremonium strictum* on germination of *Helminthosporium solani* conidia from inoculated minitubers in a combined analysis

Treatment[z]	Mean value	Germination (%)
Untreated	21.6	86.3
A. strictum low	16.7	66.8
A. strictum high	18.3	73.38

[z] Low and high treatments = 2×10^5 and 2×10^7 conidia ml^{-1}, respectively; least significant difference ($P = 0.05$) = 1.6.

DISCUSSION

Examination of *H. solani* colony growth from our extensive collection and isolates from other locations, including such diverse areas as Idaho, British Columbia, New Brunswick, New York, Washington, and Ireland, have shown consistent sectoring and black-and-white growth coloration. It has been accepted that this black-and-white growth pattern is normal for *H. solani*. In addition, reports from colleagues, and our experience, indicate that it is often difficult to collect large numbers of spores for study because of the paucity of spores produced in culture. This observation led to a preliminary hypothesis that perhaps a mixture of fungi in *H. solani* cultures existed that would explain both the unusual growth and lack of sporulation. Initial examination of cultures did indeed demonstrate that two fungi were present in *H. solani* cultures. Removal of the white fungus, which turned out to be *A. strictum,* resulted in a pure culture of *H. solani* that was black, without the white rings or sectors. This indicated that all cultures in our collection and those of other researchers may be universally contaminated. This appears to be a consistent phenomenon regardless of the source of *H. solani* cultures. It is interesting to speculate whether the *A. strictum* is an inherent part of the *H. solani* pathosystem or merely a consistent laboratory contaminant that is biologically interesting or potentially useful as a biological control. Regardless, the presence of *A. strictum* in cultures of *H. solani* complicates any laboratory study of *H. solani.*

A. strictum is a cosmopolitan fungus in plant debris, soil, and water (6) and has various fungicolous relationships, including commensalism (11), and mycoparasitism of many saprophytic and plant-pathogenic fungi (8). However, it has not been reported previously to be a mycoparasite of *H. solani. A. strictum* is tightly linked to, and partially dependent on, *H. solani* in culture. It is difficult to separate the two organisms, and we were not able to successfully culture *A. strictum* axenically even after multiple attempts. In this pathosystem, it seems to be dependant on *H. solani* for its survival and requires some *H. solani*, no matter how little, for it to grow in culture. *H. solani* growth, sporulation, and germination are reduced in the presence of *A. strictum*. In this regard, the relationship appears to be antagonism by *A. strictum,* which is surviving at the expense of *H. solani*. *A. strictum* appears unaffected by the relationship. We do not know whether *A. strictum* actually parasitizes *H. solani* or simply grows in close association. Scanning electron microscope examination shows shriveled and shrunken conidia of *H. solani* when contaminated by *A. strictum*, apparently due to either direct parasitism or some toxic metabolite (Fig. 2). Recently, the corn endophyte *Acremonium zeae* (Gams) has been shown to produce pyrrocidines that are active against *Aspergillus flavus* and *F. verticillioides* (25). It is unknown whether *Acremonium strictum* produces similar pyrrocidines. The exact mechanism of inhibition and the specific type of fungicolous relationship remain unknown. This can be the subject of future studies.

One purpose of this study was to determine whether the contaminant fungus *A. strictum* was responsible for the lack of sporulation of *H. solani* cultures observed by many researchers. This study shows that the naturally occurring antagonistic *A. strictum* has a significant effect on the biology of *H. solani* in vitro. *A. strictum* not only reduces sporulation but also spore germination and mycelial growth. It appears from these data that differences exist among *H. solani* isolates in their ability to produce conidia, and this ability is reduced in every isolate by the presence of *A. strictum*. These sporulation differences likely are due to genetic variability, but the genetics of this organism have not been studied extensively. It also reduced sporulation of disease-free potato minitubers that were inoculated with *H. solani*, but did not reduce disease of previously infected tubers at harvest. This makes it a potential candidate for use as a biological control agent. However, this study had limited experiments testing the potential of *A. strictum* as biological agent.

Potato tubers are stored under conditions that are ideal for biological control (cool temperatures, high humidity, closed systems, air movement, and large numbers); therefore, it is conceivable that introduction of *A. strictum* at the beginning of the storage season could help reduce or delay silver scurf spread and development. Earlier studies by our group demonstrated that *H. solani* sporulates and spreads to other tubers in storage, resulting in an increase in disease during storage. The same air-handling system that spreads spores in stored potato tubers could be used to introduce and spread *A. strictum*. It may, however, be effective when used in conjunction with other management practices, such as reduced RH (90 to 95%), postharvest fungicides (thiabendazole and chlorine dioxide), food preservative salt (K propionate), or resistant cultivars. More work is needed to determine the full potential of *A. strictum* as a biological control agent of stored potato tubers under commercial conditions.

Further investigations into the biology of *A. strictum*, nutritional requirements, mechanism of parasitism, toxin production, and the identity of *A. strictum* strains with the potential to colonize tubers infected with silver scurf in long-term storage are recommended.

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