Persistence and Efficacy of Thiabendazole on Potatoes for Control of Silver Scurf

Sunny Y. Szeto,*,† Vippen Joshi,† Patricia M. Price,† and Jim Holley‡

Agriculture Canada Research Station, 6660 Northwest Marine Drive, Vancouver, British Columbia, Canada V6T 1X2, and Alberta Special Crops and Horticultural Research Centre, Alberta Agriculture, Brooks, Alberta, Canada T0J 0J0

Potato samples were taken randomly from four commercial storages 250–265 days after treatment with thiabendazole at the recommended rate of 40 g of ai/ton for control of silver scurf (*Helminthosporium solani*). The mean concentrations of surface residues varied greatly among the samples, ranging from 1.02 ± 1.46 to 28.6 ± 5.4 ppm (n = 10). There was no direct correlation between the occurrence and the severity of the disease with the levels of surface residues on the potatoes. The temperature and relative humidity of the storages appeared to be important. In potatoes treated with thiabendazole using proper technique, at $^1/_2$, 1, 2, and 4 times the recommended rate and stored at 7 °C with 95% relative humidity, the surface residue decreased by 57–67% in about 84 days and persisted thereafter for 154 days (34 weeks total). Colonies of *H. solani*, isolated from infected potatoes, consisted of strains that were susceptible, tolerant, or even resistant to thiabendazole. The existence of mixed populations of the pathogen may be the cause of inconsistent control of the disease with this chemical in the recent past.

INTRODUCTION

Silver scurf is a disease of potato that is caused by the fungus *Helminthosporium solani* Dur. & Mont. The fungus produces round brownish lesions that often have a silvery sheen. Initially the lesions are superficial and harmless, but when the potatoes are stored, these lesions increase in number and size and other organisms may invade and colonize the infected areas (Jellis and Taylor, 1977; Hide and Adams, 1980). The lesions then thicken to form a tough leathery rind, often referred to as "elephant hide". Symptoms are most noticeable on non-Russet and colored cultivars such as cv. Red La Soda. Severe infection may completely obscure the pigmentation of tubers, reducing their market value, and excessive fresh weight losses occur as a result of increased permeability of the infected skin to water vapor (Jellis and Taylor, 1974). Potatoes with elephant hide are difficult to peel in commercial chipping plants. Chips made from potatoes with silver scurf have brown burnt edges which are unsightly and not acceptable for chipping. A number of companies in British Columbia and Alberta have experienced serious difficulties in packaging their products because of this disease during the past 2–5 years.

Thiabendazole is an effective contact fungicide for control of silver scurf (Cayley *et al.*, 1979; Hide *et al.*, 1980) and currently registered in Canada for use on stored potatoes. The recommended application rate for Mertect Flowable Liquid (450 g of thiabendazole/L) is 8 L of the formulated product/170 L of water (0.047 L of product/L of water); and 2 L of the suspension is to be applied to 1 ton of potatoes (i.e., 40 g of ai/ton). Exact methods for calibration and application with different sprayers to ensure thorough spray coverage are not specified in official instructions in British Columbia and Alberta. Silver scurf has increased to unacceptably high levels in the past 2–5 years in spite of the fact that chipping contracts specify that all potatoes are to be treated with the fungicide thiabendazole. Usually, potatoes treated with thiabendazole do not show any symptoms of silver scurf infection in storage, for 3–4 months after harvest. Severe symptoms begin to occur in late January or early February, and the infection increases dramatically in subsequent months, soon rendering the potatoes unmarketable. One or more of three possible reasons are responsible for the sharp increase of this disease: (1) thiabendazole is not being applied properly; (2) the surface residues do not last for the storage period of 8–10 months; or (3) *H. solani* has developed resistance to the fungicide. We conducted a 3-year study to determine possible causes of failure of thiabendazole in controlling the disease. Our findings are reported here.

MATERIALS AND METHODS

Survey of Stored Potatoes. Surveys were conducted in 1989 and 1990 to determine surface residues of thiabendazole and its control of silver scurf. In 1989 and 1990 six representative samples of potatoes (cultivars Norchips, Elite 1, Elite 3, and Russett Burbank) were taken from four storages, one in British Columbia (sample 1) and the others in Alberta (samples 2–6). Samples 2–4 were harvested from the same field and then stored in the fall of 1989 in two separate bins, one for samples 2 and 3 and the other for sample 4. Sample 1 consisted of four tubers which were treated on October 1, 1988, and they were analyzed for surface residues 255 days after treatment on June 12, 1989. Samples 2–6 consisted of 10 tubers per sample which were treated in September 1989, and all were analyzed for surface residues 250–265 days after treatment in June 1990. An extra four tubers were taken concurrently with sample 1 to form sample 1A for the determination of tissue residues after the tubers were washed under running tap water to remove dirt from the surface.

At the time of sampling, the growers were asked to provide as much information on thiabendazole application as possible. The fungicide was applied with power sprayers, except for samples 2 and 3 for which a hand sprayer was used to ensure thorough coverage. None of the growers were able to describe how their sprayers were calibrated or how the coverage of spray was evaluated. On the basis of the information provided by the growers, the spray concentration (liters of product per liter of water) and spray volume (liters of spray per ton of potatoes) were estimated to be, respectively, 0.047 and 2 for sample 1,

† Agriculture Canada.
‡ Alberta Agriculture.

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0.094 and 4 for samples 2 and 3, 0.094 and 0.2 for sample 4, 0.088 and 2 for sample 5, and 0.018 and 1.25 for sample 6.

All potato samples including sample 1A were inspected for symptoms of silver scurf. On the basis of the observations, the levels of infection were ranked in increasing order of severity as none (0% of the surface area infected), little (<5% of the surface area infected), some (5–15% of the surface area infected), moderate (15–50% of the surface area infected), or extensive (>50% of the surface area infected). Both samples 1 and 1A were combined as one sample in ranking the levels of infection.

Persistence of Thiabendazole Surface Residues. A study was conducted to determine the persistence of thiabendazole surface residues in 1990. Seeds of cv. Red La Soda were provided by a commercial grower in British Columbia. Uncut seeds (average 50–60 g) were grown in a silt loam soil (Abbotsford soil series, classification Orthic Hummo-Ferric Podzol, pH 5.76; 5.1% organic matter content, 39.6% sand, 54.1% silt, 6.3% clay) at the Research Substation of Agriculture Canada in Abbotsford, BC, on May 15, 1990. The crop was harvested on September 26; 200 potatoes (157 ± 56 g) were visually inspected for infection by silver scurf, and none was detected. They were treated on October 1 with thiabendazole at $^1/_2$, 1, 2, and 4 times the recommended rate before storage (50 potatoes/rate). The fungicide was applied with a mist bottle which was calibrated to deliver 4 mL of suspension/kg of potatoes to ensure complete coverage of the whole surface. The treated potatoes were held in commercial storage for 238 days at about 7 °C and 95% relative humidity (RH). Four tubers were randomly taken for determination of surface residues at 1, 14, 28, 56, 84, 119, 147, 175, 203, and 238 days after treatment.

Sensitivity of Isolates of *H. solani* to Thiabendazole. A separate study was conducted in 1990 to determine the sensitivity of *H. solani* colonies isolated from infected potatoes. Potato samples of 21 cultivars consisting of 15–25 tubers per sample were taken at random from commercial storages in the Lower Fraser Valley and Pemberton areas of British Columbia, which are separated by 90 airline miles and many mountains. After washing, they were examined for visible symptoms of silver scurf.

A few tubers with lesions from each sample were selected. They were surface sterilized with a 0.5% solution of NaOCl, followed by washing three times with sterile distilled water. The potatoes were incubated as a whole at room temperature in the dark, or three pieces of the infected periderm from each tuber were incubated in a moist Petri plate kept at 15 °C. After 2–3 weeks, the conidia that appeared to be *Helminthosporium* spp. were picked singly with a sterile needle under a dissecting microscope and transferred to a specific antibiotic medium containing 2% malt extract agar amended with 625 mg/L of penicillin and 2700 mg/L of streptomycin. These plates were incubated in the dark at room temperature to obtain a contaminant-free culture of *H. solani*. After 3–4 weeks of growth, these isolates were examined and measured microscopically to confirm the presence of *H. solani*. Each isolate from a potato was assigned a number and maintained in our culture collection of *H. solani*.

Growth of the isolates collected was compared on six different media: 2% malt extract agar (MEA), kidney bean agar, potato dextrose agar (PDA), green bean agar, lima bean agar, and V-8 juice agar (Goth and Webb, 1983). MEA was selected as the growth medium used for all furthur testing with *H. solani*. The pure cultures were then transferred to the culture tubes containing MEA as stock cultures for testing the efficacy of thiabendazole.

Eight isolates were randomly selected from our culture collection for *in vitro* efficacy determination of thiabendazole. The fungicide was incorporated into MEA at concentrations of 5, 50, and 500 ppm. Three uniform plugs from an actively growing edge of a colony of each isolate were transferred to the growth medium plates containing the fungicide. Control plates received no fungicide amendments. The plates were then incubated in the dark at room temperature. After 4 weeks, the radial growth of each colony was measured and its increase in diameter calculated.

Removal of Surface Residues by Solvent Stripping. Ten tubers treated with thiabendazole were solvent stripped individually three times by sonication with methanol to remove surface residues. Each time, the treated tubers were placed separately in a 500-mL beaker containing 250 mL of methanol (HPLC grade) and the contents sonicated for 15 min. The stripping solution was then filtered through Whatman No. 1 filter paper. Aliquots of 10 mL filtrate were again filtered through a 0.2-μm cartridge filter, and the filtrate was appropriately diluted or concentrated for HPLC analysis.

After solvent stripping, four tubers were extracted separately by homogenization with ethyl acetate for tissue residues as decribed below.

Extraction of Tissue Residues. Sample tubers were macerated and thoroughly mixed in a food processor. Aliquots of 50 g of the potato pulp were mixed with 150 g of anhydrous Na_2SO_4, and the resultant mixtures were extracted twice with 150 and 100 mL of ethyl acetate (pesticide grade) by homogenizing for 1 min in a Polytron. The extracts were filtered through a glass fiber filter paper in a Büchner funnel. The filtrates were combined, concentrated to about 25 mL in a flash evaporator at 35 °C, and then quantitatively transferred to a 125-mL separatory funnel. The pooled filtrates were extracted three times with 25 mL of 0.1 N HCl. The acidic extracts were combined in another 250-mL separatory funnel. After the pH was adjusted to about 9 as indicated by litmus paper with about 12 mL of 1 M NaOH, the basic aqueous solutions were extracted twice with 25 mL of dichloromethane (pesticide grade). The combined extracts were then dried with anhydrous Na_2SO_4 and concentrated just to dryness in a flash evaporator at 35 °C. The residues were dissolved in an appropriate volume of methanol for HPLC analysis.

Analysis of Thiabendazole by High-Pressure Liquid Chromatography (HPLC). A Varian Model 5000 high-pressure liquid chromatograph equipped with a Hewlett-Packard Model 1040A high-speed spectrometric detector was used to detect and quantify thiabendazole. The operating parameters were as follows: column, Supelcosil LC-8 (25.0 cm × 4.6 mm i.d., 5 μm); mobile solvent system, 65% methanol and 35% 0.0025 M phosphate buffer of pH 7.0, isocratic at 1.0 mL/min; UV detector wavelength, 300 ± 2 nm. Aliquots of 20 μL of thiabendazole standards or purified potato tissue extracts were injected into the HPLC for measurement. Under the chromatographic conditions described, the absolute retention time of thiabendazole was 4.24 min. Quantification was based on external standards. Detector response was calibrated for each analysis, and the calculation was based on average peak areas of these external standards, which were injected before and after the samples.

Evaluation of Method for Determination of Tissue Residues. Potato tubers that had never been treated with pesticides were processed and analyzed by the described method. Quadruplicates of 50-g aliquots of macerated tubers were fortified with thiabendazole at 5.0 and 0.1 ppm by adding 0.5 mL of an appropriate stock solution in acetone. After equilibration for about 0.5 h, they were analyzed by the method described to determine the recovery.

RESULTS AND DISCUSSION

Efficiency of Method for Tissue Residues. There was no detectable chromatographic response that interfered with the analysis of thiabendazole present in unfortified macerated tubers. The mean recoveries of thiabendazole and standard deviations (n = 4) were 97.9 ± 3.1% from fortification with 0.1 ppm and 85.8 ± 3.1% from fortification with 5.0 ppm. The higher recovery from 0.1 ppm fortification than from 5.0 ppm fortification was attributed to the higher background resulting from the injection of a larger sample equivalent for the 0.1 ppm fortification. The recoveries by the described method were highly reproducible, as indicated by the small standard deviations. On the basis of the sensitivity of detector response and the effectiveness of sample cleanup, the detection limit of the method was at least 0.025 ppm (fresh weight).

Efficiency of Method for Surface Residues. The amounts of thiabendazole present in the first methanol stripping and in the tissue extract in ethyl acetate accounted for >95% and <5% of the total applied,

Table I. Surface Residues of Thiabendazole and Occurrence of Silver Scurf on Potatoes Taken from Four Storages in British Columbia and Alberta

potato sample[a]	venue (dates)	days after treatment[b]	storage conditions		residues,[c] ppm (n)	evidence of silver scurf
			T, °C	RH, %		
1	BC (9/88–6/89)	255	7	95	20.9 ± 7.5 (4)	extensive
2	AB (9/89–6/90)	265	10	95	28.6 ± 5.4 (10)	little
3	AB (9/89–6/90)	265	10	95	24.0 ± 3.2 (10)	little
4	AB (9/89–6/90)	265	10	95	2.65 ± 1.01 (10)	some
5	AB (9/89–6/90)	250	3	90	1.02 ± 1.46 (10)	none
6	AB (9/89–6/90)	260	10	85–90	1.97 ± 1.37 (10)	extensive

[a] Sample 1 was taken from a storage in British Columbia. Samples 2–6 were taken from three different storages in Alberta of which samples 2–4 were from the same storage. [b] Thiabendazole was applied to all samples at the recommended rate of 40 g of ai/metric ton by power sprayers, except samples 2 and 3 for which a hand sprayer was used. [c] Mean ± SD.

respectively. These results clearly indicated that a single solvent stripping with sonication effectively removed surface residues from potatoes.

Surface Residues and Occurrence of Disease. Results of our surveys on surface residues of thiabendazole and occurrence of silver scurf in six potato samples taken randomly from the four commercial storages in British Columbia and Alberta in 1989 and 1990 are given in Table I. On the basis of the recommended rate of 40 g of thiabendazole/ton, the theoretical concentration of surface residues should be 40 ppm (fresh weight). After 250–265 days (8–9 months) of storage, the actual concentrations varied greatly between samples, ranging from 1.02 to 28.6 ppm (fresh weight). In samples with relatively high levels of surface residues (samples 1–3), the variability was relatively small within each sample, as indicated by the small standard deviation. By comparison, the variability was much larger in samples with relatively low levels of surface residues (samples 4–6) (Table I). Since the differences in temperature and relative humidity among the four storages were relatively small, the great variation in surface residues among the six samples could not be attributed to their difference in degradation due to the environmental conditions of the storages (Table I). Rather, the difference in spray coverage by the methods of spray application among samples may be the primary cause of such variation. Samples 2–4 were taken from the same storage. A power sprayer was used for sample 4, whereas samples 2 and 3 were thoroughly sprayed by hand. The surface residues were about 10 times higher on samples 2 and 3 than on sample 4. It is evident that to ensure coverage of 40 g of thiabendazole/ton of potatoes, proper calibration of the individual sprayer is an absolute essential. Otherwise, under- or overspray will result.

The surface residues on samples 1–3 ranged from 20.9 to 28.8 ppm 255–265 days after thiabendazole treatment (Table I). They exceeded the tolerance of 4 ppm in raw unpeeled potatoes currently established in Canada. However, washing under running tap water appeared to be highly effective in removing soil as well as surface residues. The unwashed tubers of sample 1 contained 20.9 ± 7.7 ppm (n = 4), whereas the washed tubers of sample 1A contained 0.78 ± 0.22 ppm, which was well below the Canadian tolerance.

There was no direct correlation between the occurrence of silver scurf and the concentrations of thiabendazole surface residues on potatoes. Even after nearly 9 months, sample 1 contained 20.9 ppm of surface residues, which was about 50% of the theoretical concentration immediately after application. Even so, the treated potatoes in the storage where sample 1 was taken showed extensive occurrence of silver scurf. By comparison, the surface residues varied greatly on samples 2–4, depending on how the fungicide spray was applied. The potatoes in the storage where these three samples were taken showed only little or some evidence of the disease. Although sample 5 contained only 1.02 ppm of surface residues, potatoes in the storage where this sample was taken showed no evidence of the disease. These data demonstrated that insufficient surface residues alone do not explain failure to control silver scurf with thiabendazole. Rather, they suggest the possible existence of tolerant or even resistant strains of the pathogen.

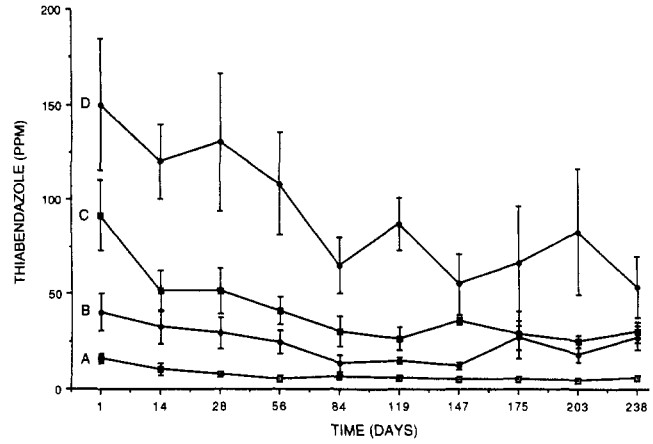

Figure 1. Concentrations of surface residues on potatoes stored at 7 °C and 95% RH after treatment with thiabendazole at (A) 20, (B) 40, (C) 80, and (D) 160 g of ai/ton.

It is established that the minimum conditions for lesion inception of silver scurf are 2.8 °C and 90% RH, and continuing infection can occur gradually during storage in conditions of high temperature and high RH (Boyd, 1972). Our survey data appeared to confirm the importance of environmental conditions in the development of silver scurf (Table I). Regardless of levels of surface residues, all of the samples (samples 1–4 and 6) that were stored at 7–10 °C and 85–95% RH had various levels of infection, ranging from little to extensive. Although sample 5 contained low surface residues, there was no evidence of disease on the potatoes. The temperature (3 °C) and RH (90%) of the storage where sample 5 was stored may have prevented the development of lesions or the spread of infections. More research is needed to understand better the interaction between the persistence of surface residues and storage conditions and how this interaction may affect the efficacy of the protectant.

Persistence of Thiabendazole Surface Residues. The mean concentrations and standard deviations of thiabendazole on potatoes (n = 4) treated with the fungicide at 20, 40, 80, and 160 g of ai/ton are given in Figure 1. The actual mean concentrations of 16.1, 40.3, 91.7, and 150 ppm 1 day after application were in close agreement with the theoretical values. However, the concentrations on individual potatoes varied as indicated by the standard deviations, probably due to differences in size (157 ± 56 g, n = 200). The surface area per unit weight varied among

Table II. Effect of Thiabendazole on Growth of *H. solani* Isolates in Growth Medium Incubated at Room Temperature for 4 Weeks

	increase in colony diameter, mm			
isolate	0 ppm	5 ppm	50 ppm	500 ppm
8	25.8	24.3	22.1	16.2
12	27.0	27.3	23.5	19.8
15	29.0	28.6	27.3	23.8
39	19.0	18.2	0.0	0.0
61	29.2	27.5	24.9	17.7
65	29.3	24.6	0.0	0.0
187	23.6	22.3	4.6	0.0
294	20.3	20.8	18.7	7.8

potatoes of different sizes. Small potatoes with larger surface area per unit weight would have contained higher concentrations of surface residues. Because the treated potatoes were stored at about 7 °C, the degradation of residues was expected to be slow. In fact, the concentrations decreased by about 56.7–67.4% in 84 days and persisted at that level thereafter (Figure 1). These results showed that the surface residues of thiabendazole persisted for at least 238 days when stored at about 7 °C and 95% RH.

The concentration of surface residues was 26.7 ± 6.1 ppm in potatoes treated at the recommended rate with thiabendazole and stored at about 7 °C for 238 days (Figure 1). In our surveys conducted in 1989 and 1990, the mean concentrations of surface residues were 20.9 ± 7.5, 28.6 ± 5.4, and 24.0 ± 3.2 ppm, respectively, in potato samples 1, 2, and 3 taken from commercial storages in British Columbia and Alberta 255–265 days after treatment (Table I). These survey data were in agreement with those generated from our persistence study under controlled conditions. It appears that the exceedingly low levels of surface residues on samples 4–6 were the result of poor application (Table I).

Efficacy of Thiabendazole against Randomly Selected Isolates of *H. solani*. All isolates of *H. solani* grown on the six media tested produced a characteristic pigmentation and pattern of growth. Green bean agar and V-8 juice agar were observed to be good media for producing the spores (conidia). PDA and MEA were equally good for the mycelial growth. Therefore, MEA was chosen as the growth medium for the pathogen.

The growth in culture of eight isolates of *H. solani* isolated from locally collected potatoes infected with silver scurf was inhibited to varying degrees by thiabendazole (Table II). The infected potatoes were taken from storages with long histories of thiabendazole use and reported failures of control in recent years. Three isolates, no. 39, 65, and 187, were sensitive to thiabendazole at 50 ppm since little or no growth of the colonies occurred after 4 weeks of incubation. The other five isolates tolerated 50 ppm of the fungicide, and their growth rates were comparable with that of the control. When exposed to 500 ppm, one isolate, no. 294, was strongly inhibited and the increase in colony diameter in the other four isolates was only somewhat less than that of the controls (Table II). Our results indicate that these isolates were a mixed population of sensitive and tolerant or resistant strains of *H. solani*. Isolates of *H. solani* obtained from potato tubers that were resistant to thiabendazole had been reported previously by Hide *et al.* (1988). They classified the isolates inhibited by 5 ppm as sensitive, those that grew on 100 ppm as resistant, and those inhibited by 5–100 ppm as intermediate.

A mixed population of the pathogen is probably the major cause of failures in controlling silver scurf with thiabendazole in commercial storage in recent years. In our surveys of 1989 and 1990 (Table I) there was evidence of severe symptoms of silver scurf occurring in potatoes in spite of the presence of high levels of thiabendazole surface residues. Other factors such as date of harvest may be important also in affecting the severity of the disease (Merida *et al.*, 1992).

On the basis of the results of our surveys and studies under controlled conditions, it is evident that successfully combatting the disease requires proper management of the storage, maintaining temperature and RH unfavorable to *H. solani*, and efficient application of effective fungicide. Because of the existence of strains of the pathogen tolerant or resistant to thiabendazole, it is necessary to develop alternative fungicides that are more effective.

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