Thermo-Therapy and Use of Biofungicides and Fungicides for Management of Internal Discoloration of Horseradish Roots - 2006

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Summary

The results of this study and the results of other unpublished investigations showed that set-borne organisms of horseradish can be eradicated by treating the sets in hot-water [115ºF (46ºC) for 10 min]. Application of either the fungicide fludioxonil (Maxim 4FS or Maxim Potato WP) or a biofungicide [*Trichoderma virens* (SoilGard 12G or G-41) or *Bacillus subtilis* QST 713 (Serenade MAX)], onto sets (set treatment) prior to planting, can provide protection to growing roots for about 12 weeks (approximately 15 May to early August). Remaining time of the growing season, with favorable conditions (early August - end of September), is not long enough for *Verticillium* and *Fusarium* species to infect the roots and discolor them to unmarketable levels. Some roots might be infected, but severity of discoloration will be negligible. This is an effective integrated approach for management of the destructive disease complex of internal discoloration of horseradish roots. This strategy can be easily used in both traditional and organic horseradish production.

Introduction

Internal discoloration of horseradish root is a disease complex and is the most serious threat to horseradish production in the United States. At least three fungal pathogens, *Verticillium dahliae*, *V. longisporum*, and *Fusarium solani*, have been identified to be the causal agents of this disease. It is also possible that additional bacterial and/or fungal agents may be associated with internal discoloration of horseradish roots. At present, there is no method available to provide adequate control of this disease. This study was conducted to evaluate the effectiveness of hot-water treatment (thermo-therapy) of horseradish sets (planting stocks) and use of biofungicides and fungicides, applied as set treatments, for control of internal discoloration of horseradish roots.

Materials and Methods

A field trial was conducted in a commercial field near Collinsville, Illinois to determine effectiveness of hot-water treatment and application of biofungicides and fungicides for control of internal discoloration of horseradish roots. The field had a long history of internal discoloration of horseradish root. Three horseradish cultivars, 15-K, 1573, and 1722 were used in this study. The roots of all three cultivars were obtained from commercial horseradish growers in Collinsville, which were produced in 2005. No discoloration symptoms were detected by visual observation of the roots. Roots (0.4- to 0.6-inch in diameter) were selected, washed with tap water, and cut into 8-inch segments (sets). The sets were either heat-treated at 115°F (46°C) for 10 min or not treated. Then, two fungicides (Maxim 4FS, Maxim Potato WP) and three

biofungicides (Serenade MAX, SoilGard 12G, G-41) (Table 1) were applied separately onto the sets 4 days prior to planting. Untreated control sets were included with all treatments.

***Hot-water treatment* (*thermo-therapy*).** An electrical laboratory water bath, with 1,920 Sq inch (30,000 cm^3) capacity, was used for hot-water treatment of the sets. The bath was filled with water to approximately 80% of its capacity. The temperature of water bath was set to be 115°F (46°C). The bath was turned on and water temperature was stabilized at 115°F (46°C), which was monitored with a thermometer. Each time, 20 horseradish sets together were treated in a fabric mesh (similar to cheese cloth). After immersing the sets in the water bath, we waited several minutes to re-establish temperature of water at 115°F (46°C). The sets were kept in 115°F (46°C) for only 10 min. Then, the sets were dried on newspapers and treated with either biofungicides or fungicides or planted without further treatments.

Representatives of hot-water treated sets were cultured on acidified potato dextrose agar (A-PDA). Growing organisms in the plates were transferred onto PDA in Petri plates, grew for 1 to 2 weeks, and identified.

Set-treatment with fungicide Maxim 4FS. Two hundred milliliters of tap water was poured into a 2-gallon zip-lock plastic bag and 0.2 ml of the fungicide Maxim 4FS (fludioxonil) was added to water in the bag and mixed. The sets were placed in the bag and shaken for 5 min. Treated sets were drained and dried in an exhaust hood.

Set-treatment with fungicide Maxim Potato (WP). One hundred milliliter of tap water was poured into a 2-gallon zip-lock plastic bag and 5 g of the fungicide Maxim Potato WP (fludioxonil) was added to water in the bag and mixed thoroughly. Twenty horseradish sets were placed in the bag and shaken for 5 min. Treated sets were drained and dried in an exhaust hood.

Set-treatment with biofungicides. Three biofungicides, Serenade MAX (*Bacillus subtilis* QST 713), SoilGard 12G (*Trichoderma virens* GL-12), and G-41 (*Trichoderma virens* G-41) (Table 1) were used. The sets were dipped in tap water, and then placed in 2-gallon zip-lock plastic bag containing the biofungicide. The bag was gently shaken for 30 seconds. The sets were thoroughly covered with the biofungicide. Treated sets were dried in an exhaust hood.

Field trial. The field was plowed on 10 May, and the sets were planted on 12 May. The trial included three cultivars (15-K, 1573, 1722), two heat treatments (hot-water treated at 115°F for 10 min, not heat-treated), six treatments (Maxim 4FS, Maxim Potato WP, Serenade MAX, SoilGard 12G, G-41, control), five harvesting times (July, August, September, October, March), and four replications (10 sets each) for each treatment combination. Sets were planted 24-inch apart within the rows spaced 36-inch apart. Each plot consisted of one 20-foot row. A total of 10 sets were planted in each plot (10 plants per row). The plots were arranged in a split-plot design, cultivar being as the main plot, heat-treatment as sub-plots, treatment (fungicides and biofungicides) as sub-sub-plots, and harvesting times were randomly arranged in sub-sub-plots. Four replications of main-plots, sub-plots, and sub-sub-pots were arranged in a complete block design.

During the season, weeds were controlled by cultivation and hand weeding. The field was not irrigated. Precipitation and temperature in the fields were not recorded. Therefore, the data from the Belleville weather station, the nearest weather station to the experimental field, are presented. Precipitation was 5 days (0.54 in.) during 12-31 May, 6 days (2.66 in.) in June, 2 days (1.36 in.) in July, 6 days (0.96 in.) in August, 5 days (2.42 in.) in September, and 8 days (4.15 in.) during 1-29 October. Average monthly high and low temperatures (EF) were 78/55, 85/62, 89/66, 88/65, 77/52, and 65/41 during 12-31 May, June, July, August, September, and 1-29 October, respectively.

Number of plants in each plot was recorded and vigor of the plants in each plot was assessed on 14 July, 14 August, 15 September, and 28 October. Vigor of the plants was assessed using a scale of 1 to 4, 1 = the lowest vigor and 4 = the highest vigor of the plants. Plants were harvested on 14 July, 14 August, 15 September, and 29 October 2006. The remaining plots will be harvested in March 2007. Harvested roots in July, August, and September were assessed for incidence and severity of the internal discoloration in the field. Each root was sectioned at 1/3 (upper section) and 2/3 (lower section) of the length from the top and severity of discoloration was assessed at the cross sections. Harvested roots on 29 October were collected and transported to Champaign, IL. The roots were washed, weighed, and evaluated for internal discoloration. All the harvested roots from each plot were evaluated for the incidence (percentage of roots discolored) and severity (percentage of root area affected) of the main roots as described above. Also, two lateral roots from each of the main roots were sectioned in the middle and incidence and severity of discoloration were assessed at the cross section. Five roots with discoloration symptoms (symptomatic roots) and roots with no internal discoloration to un-aided eyes were cultured on acidified potato dextrose agar for determining presence of the pathogens in the roots. The remaining plots in the field will be harvested in March 2007 and evaluated for internal discoloration of roots according to the procedures described above.

Results and Discussion

Fusarium, *Verticillium*, and other fungi and bacteria were detected in very few hot-water treated sets cultured on PDA (data not presented). *Fusarium*, *Verticillium*, and other fungi and bacteria grew out from a higher number of untreated sets with hot-water. The results of the field trial showed very low incidence of the internal discoloration in the roots grown from untreated sets in July. There was higher incidence and severity of the disease in October (Tables 1, 2, 3). This indicated that the infection of plants was caused by soil-borne inoculum rather than the set-borne inoculum.

Internal discoloration of horseradish roots developed overtime (Table 2). In all three cultivars, incidence of the discoloration in October was significantly higher than those in July, August, and September. Also, incidence and severity of the disease increased steadily from July to October. This is in agreement with the previous reports that expression of internal discoloration in horseradish roots takes place approximately 12 weeks from the time of contacting pathogen with the root.

In all three cultivars, root discoloration originated in the vascular area, and then expanded inwards and outwards. This pattern of symptom development is expected as *Verticillium* and *Fusarium* species invade horseradish plants through vascular system. Percentage of disease incidence and severity of internal discoloration of roots grown from treated sets with either fungicides (Maxim 4FS, Maxim Potato WP) or biopesticides (Serenade MAX, SoilGard 12 G, G-41) were lower than those of roots grown from untreated sets (Tables 2, 3). The fungicides and biofungicides provided approximately a three month(from 14 May to 14 August) protection to the roots. During remaining time (middle of August to 29 October), infection of roots took place, but severity of discoloration was very low and almost all roots grown from treated sets were acceptable for processing.

Relatively dry condition of soil during June, July, and August in the experimental field was unfavorable for development of the internal discoloration of horseradish roots. Therefore, the discoloration of roots occurred mainly during late August and September, which resulted in low disease severity. Otherwise, incidence and severity of the discoloration could have been much higher in the roots grown from untreated sets.

All of the treated and untreated sets gave rise to plants that grew well during the season. The results indicated that there was no adverse effect of the thermo-therapy, the fungicides, or biofungicides used in this study on either set germination or plant growth.

Among the three cultivars tested in this study, cultivar 15-K appeared to be less susceptible to the internal discoloration than cultivars 1573 and 1722. Further investigation, however, is needed to determine if development of the internal discoloration in the roots of cultivar 15-K is slower than other horseradish cultivars.

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Table 1. Fungicides and biofungicides tested for control of internal discoloration of horseradish roots in 2004.

Material used			Manufacturer	Rate (product)	Treatment
Trade name	**Agent**	**Active ingredient**			
Maxim 4FS	Fungicide	Fludioxonil	Syngenta, Inc.	1 ml/L	Soaking*
Maxim Potato WP	Fungicide	Fludioxonil	Syngenta, Inc.	50 g/L	Slurry*
Serenade MAX	Bacterium	*Bacillus subtilis* QST 713	AgraQuest, Inc	Set-cover	Slurry**
SoilGard 12G	Fungus	*Trichoderma virens* GL-12	Certis USAL.L.C.	Set-cover	Slurry**
G-41	Fungus	*Trichoderma virens* G-41	Biowork, Inc.	Set-cover	Slurry**
Untreated control		—	—	—	—

* Fungicide was added to water in a plastic bag, the sets placed in the bag, and shaken for 5 min.
** The sets were dipped in water and shaken with the agent in a plastic bag.

Table 2. Development of internal discoloration of horseradish roots during the growing season in Collinsville in 2006.

		Internal root discoloration (%)			
		Heat treated		Non-heat treated	
Cultivar	Month*	Incidence	Severity	Incidence	Severity
15-K	July	0.0 b**	0.00 b	2.5 b	0.51 a
	August	2.1 b	0.12 b	5.0 b	0.61 a
	September	2.5 b	0.22 ab	5.0 b	0.85 a
	October	7.5 a	0.46 a	10.8 a	0.94 a
	LSD (P=0.05)	***3.0***	***0.25***	***4.2***	***0.76***
1573	July	3.3 c	0.55 b	4.2 c	0.52 b
	August	10.0 b	1.55 b	4.2 c	0.76 b
	September	14.6 b	3.08 a	16.3 b	1.81 a
	October	41.2 a	3.23 a	32.1 a	2.21 a
	LSD (P=0.05)	***6.3***	***1.16***	***5.9***	***0.80***
1722	July	2.9 c	0.11 c	5.0 c	0.49 b
	August	13.8 b	1.38 b	5.4 c	0.81 b
	September	14.2 b	2.37 a	17.1 b	2.73 a
	October	34.6 a	3.00 a	35.8 a	3.21 a
	LSD (P=0.05)	***6.3***	***0.95***	***6.2***	***0.91***

* Sets were planted on 14 May. Plants were harvested on 14 July, 14 August, 15 September, and 29 October and evaluated for incidence and severity of the internal discoloration.
** Each value represents the mean of four plots each with 10 plants.

Table 3. Incidence and severity of the internal discoloration of horseradish root during the growing season in Collinsville in 2006.

Cultivar	Month[x]	Treatment	Internal root discoloration (%)			
			Heat treated		Non-heat treated	
			Incidence	Severity	Incidence	Severity
15-K	July	Treatments combined[y]	2.50[z]	0.26	3.00	0.74
		Control	0.00	0.00	0.00	0.00
	August	Treatments combined	0.00	0.00	5.50	0.92
		Control	0.00	0.00	2.50	0.50
	September	Treatments combined	3.00	0.15	5.50	0.32
		Control	0.00	0.00	2.50	0.12
	October	Treatments combined	9.00	0.55	4.00	0.62
		Control	10.00	0.50	45.00	2.50
1573	July	Treatments combined	3.50	0.61	4.00	0.62
		Control	2.50	0.25	5.00	1.58
	August	Treatments combined	9.50	1.61	5.00	0.61
		Control	12.50	1.25	5.00	1.13
	September	Treatments combined	11.00	1.78	15.50	1.75
		Control	32.50	1.62	32.00	2.12
	October	Treatments combined	39.50	2.75	29.00	2.65
		Control	50.00	5.32	47.50	3.25
1722	July	Treatments combined	3.50	0.14	5.50	0.52
		Control	2.50	0.32	7.50	0.35
	August	Treatments combined	17.00	1.47	6.50	0.62
		Control	10.00	0.67	10.00	1.75
	September	Treatments combined	16.50	2.32	17.50	2.60
		Control	15.00	2.62	15.00	3.38
	October	Treatments combined	32.50	2.67	39.50	3.15
		Control	45.00	4.62	30.00	3.50

[x] Sets were planted on 14 May. Plants were harvested on 14 July, 14 August, 15 September, and 29 October and evaluated for incidence and severity of the internal discoloration.

[y] Treatments included set-treatments with (i) Maxim 4FS, (ii) Maxim Potato WP, (iii) Serenade MAX, (iv) SoilGard 12G, and (v) G-41.

[z] Each value represents the mean of four plots each with 10 plants.

Table 4. Effects of set-treatment with hot-water, fungicides, and biofungicides on the incidence and severity of internal discoloration of horseradish roots and yield in 2006[v].

Cultivar	Heat treatment	Treatment	Internal root discoloration (%)						Plants per plot		Root weight (lb/plant)
			Main root				Lateral root				
			Upper[w]		Lower[w]						
			Incidence	Severity	Incidence	Severity	Incidence	Severity	Number	Vigor[x]	
15-K	**Heat treated[y]**	Maxim 4FS	7.5 b[z]	0.37 bc	5.0 b	0.25 cd	2.5 ab	0.12 b	9.50 ac	4.00 a	1.33 b
		Maxim Potato WP	7.5 b	0.87 bc	5.0 b	0.75 a-d	0.0 b	0.00 b	9.75 ab	4.00 a	1.10 cd
		Serenade MAX	10.0 b	0.50 bc	5.0 b	0.25 cd	0.0 b	0.00 b	7.75 e	4.00 a	1.15 c
		SoilGard 12G	0.0 b	0.00 c	0.0 b	0.00 d	0.0 b	0.00 b	10.00 a	4.00 a	1.54 a
		G-41	10.0 b	0.50 bc	10.0 b	0.50 b-d	7.5 a	0.5 ab	9.00 cd	4.00 a	1.50 a
		Control	10.0 b	0.50 bc	10.0 b	0.50 b-d	0.0 b	0.00 b	9.50 a-c	4.00 a	1.54 a
	Non-heat treated	Maxim 4FS	0.0 b	0.00 c	0.0 b	0.00 d	0.0 b	0.00 b	9.75 ab	4.00 a	1.37 b
		Maxim Potato WP	0.0 b	0.00 c	0.0 b	0.00 d	0.0 b	0.00 b	8.75 d	4.00 a	1.44 ab
		Serenade MAX	0.0 b	0.00 c	0.0 b	0.00 d	0.0 b	0.00 b	10.00 a	4.00 a	1.32 b
		SoilGard 12G	10.0 b	1.63 ab	7.5 b	1.25 a-c	5.0 ab	1.13 a	9.00 cd	4.00 a	1.43 ab
		G-41	10.0 b	1.50 ab	10.0 b	1.50 ab	2.5 ab	0.12 b	6.75 f	3.75 b	1.18 c
		Control	45.0 a	2.50 a	35.0 a	1.75 a	0.0 b	0.00 b	9.25 b-d	4.00 a	0.98 d
		LSD$_{(P=0.05)}$	***11.8***	***1.25***	***10.8***	***1.19***	***5.2***	***0.69***	***0.62***	***0.06***	***0.11***

[v] Plants were harvested on 29 October.

[w] Upper= upper section of root, sectioned at 1/3 of the root from the top; Lower=lower section of root, sectioned at 2/3 of the root from the top.

[x] Vigor of plants was assessed using a scale of 1 to 4, 1 = lowest plant vigor and 4 = highest plant vigor.

[y] Sets were treated with hot water at 115°F (46°C) for 10 min.

[z] Values within each column with a letter in common are not significantly different from each other according to Fisher's protected LSD (*P*=0.05).