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Sensitivity to Hydrogen Peroxide *in vitro* of North American Isolates of *Phytophthora erythroseptica*, the Cause of Pink Rot of Potatoes

Khalil I. Al-Mughrabi
Potato Development Centre, Department of Agriculture, Fisheries and Aquaculture,
39 Barker Lane, Wicklow, New Brunswick, E7L 3S4, Canada

Abstract: *In vitro* laboratory studies were conducted to test the efficacy of hydrogen peroxide (OxiDate™) in inhibiting the growth of the pink rot of potato fungus, *Phytophthora erythroseptica*. In addition to the control, three concentrations (1:250, 1:100 and 1:50 of hydrogen peroxide to water) were tested. A total of 40 different *P. erythroseptica* isolates originated from New Brunswick and Prince Edward Island (Canada) and from Maine, Idaho, Minnesota, Oregon and Wisconsin (USA) were subjected to the various concentrations of hydrogen peroxide in a replicated experiment using a Completely Randomized Design. Growth of all isolates was completely inhibited when the 1:50 concentration was used. At the 1:100 concentration, growth of 94.2% of the isolates tested was completely inhibited. At the lowest concentration of 1:250, the growth of 41.4% of the isolates tested was completely inhibited. The results of this study suggest that hydrogen peroxide has the potential to be used for post harvest treatment of potatoes against *P. erythroseptica* that might be present on the surface of the tubers.

Key words: Hydrogen peroxide, *Phytophtora erythroseptica,* pink root, potato, *Solanum tuberosum,* storage

INTRODUCTION

Pink rot, caused by *Phytophthora erythroseptica* Pethyb. is a serious disease of potato (*Solanum tuberosum* L.)[1,2]. Poorly drained soils, combined with warm, wet climatic conditions at time of harvest promote disease incidence and severity[2,3]. Pink rot can often be found in the field before harvest. Infected tubers will appear dark and may be wet. When tubers are sliced open, infected tissues often turn pink or salmon colored after exposure to air for twenty to thirty minutes. Dark discoloration of the skin and lenticels of infected parts, purplish discoloration of the eyes and firm leathery texture of the rotted tissue are also symptoms of this disease[4-6]. All underground potato tissues, including roots, stolons, tubers and basal stems, can be infected by *P. erythroseptica*. Root and stem infection may cause wilting and death, while tuber infection leads to pink rot development in the field and in storage[7]. The pathogen can survive in the soil for several years by means of oospores[8]. The propagules of *P. erythroseptica* are endemic in most cultivated soils where potatoes are grown[7]. Superficially infected seed tubers in storage can produce infected daughter tubers and may act as a source of primary inoculum in uninfested soils[9]. Infection of tubers usually occurs via the growth of mycelium from diseased stolons. However, direct infection through buds or lenticels may also occur when adequate moisture and favorable temperatures are present[1]. Many of the commercially grown potato cultivars in Canada and the United States are considered to be susceptible to pink rot[10]. Metalaxyl-resistant strains of *P. erythroseptica* were found in Maine[11], New York[12] and Minnesota[13], USA., and therefore other alternative chemical control agents need to be evaluated for their efficacy against *P. erythroseptica*. Preventing potato spoilage during storage is of great economic concern to the industry[14]. Most of the registered treatments are disinfestants; they work as surface sterilants and are not curative. The EPA first approved hydrogen peroxide (OxiDate™) for use on potatoes in 1999. This product is considered environmentally-friendly, broad-spectrum disinfestant that provides immediate control of pathogens. It works by oxidising fungi and bacteria and has been used successfully during vegetable storage[15-17].

Hydrogen peroxide has been shown to effectively retard the progress of potato storage diseases[17-23]. Since the efficacy of hydrogen peroxide against the causal fungus of pink rot of potatoes has never been studied, this study was conducted to evaluate its efficacy against North American isolates of *P. erythroseptica*.

MATERIALS AND METHODS

Isolation of *Phytophthora erythroseptica*: Potato tubers with symptoms of pink rot were collected from potato

storage in New Brunswick and used as a source of pathogen for isolate 2004-100. For the isolation, culture establishment and maintenance of *P. erythroseptica*, a modified method of Peters *et al.*[24] was followed. Individual tubers were washed with water, surface sterilized with 10% commercial bleach (0.6% NaClO) for 30 sec, rinsed twice in sterile distilled water and then allowed to dry. A piece of skin was peeled 5 mm deep using a sterile scalpel in order to reveal inner pink tissues. Small tissue samples (~10x5x3 mm) were taken from the flesh and plated onto Potato Dextrose Agar (PDA) in Petri plates (90x15 mm, Fisher Scientific, Ontario, Canada). Petri plates were incubated at 22°C for 3-4 days. The isolate was identified as *P. erythroseptica* using the key of Stamps *et al.*[25] and descriptions from Erwin and Ribeiro[26].

In addition to isolates collected from New Brunswick (Canada), thirty eight other isolates of *P. erythroseptica* from Prince Edward Island (Canada), Maine (USA), Idaho (USA), Minnesota (USA), Oregon (USA) and Wisconsin (USA) were also obtained and used in this study (Table 1). *P. erythroseptica* isolates were courtesy of: (1) Dr. Rick D. Peters, Agriculture and Agri-Food Canada, Charlottetown, Prince Edward Island, Canada; (2) Dr. Gary A. Secor, North Dakota State University, Fargo, North Dakota, USA; (3) Dr. Jeff S. Miller, University of Idaho, Aberdeen, Idaho, USA and (4) Dr. David H. Lambert, University of Maine, Orono, Maine, USA.

Sensitivity of *Phytophthora erythroseptica* isolates to hydrogen peroxide: Forty *Phytophthora erythroseptica* isolates (Table 1) were characterized for sensitivity to hydrogen peroxide using an agar assay as described in Al-Mughrabi[23]. Hydrogen peroxide was diluted with sterile distilled water (SDW) to give a final concentration of 1:50, 1:100 and 1:250. A treatment containing SDW was used as an untreated check. Agar plugs (5 mm in diameter) taken from the margins of 4-day-old cultures were transferred into 3 sterile beakers, each containing one hydrogen peroxide dilution. The blugs were left to soak for 1 h and then transferred to Petri plates and allowed to grow for 3 days at 22°C, after which diameter of mycelial growth was measured using a traceable electronic digital caliper (VWR International, Ontario, Canada). Growth of isolates at each concentration was determined by measuring colony diameters in two perpendicular directions on each of three replicate plates for a total of six measurements per concentration per isolate. The relative growth inhibition for each concentration was calculated as follows: [100-(growth with hydrogen peroxide/ growth in control plate) x100]. The experiment was repeated and the results were pooled prior to statistical analyses.

Table 1: *Phytophthora erythroseptica* isolates identification, location, year collected and source

No.	ID	Location	Year	Source
1	PE9913-2DSZ1	Prince Edward Island	1999	R. Peters
2	2004-100	New Brunswick	2004	K. Al-Mughrabi
3	PE 403-2	Minnesota	2002	G. Secor
4	# 357-1	Oregon	2004	G. Secor
5	# 364-1	Maine	2004	G. Secor
6	# 364-2	New Brunswick	2004	G. Secor
7	# 364-4	Maine	2004	G. Secor
8	PE 403-1	Minnesota	2000	G. Secor
9	PE 614-1	Minnesota	2002	G. Secor
10	PE 618-3	Minnesota	2002	G. Secor
11	PE 621-4	Minnesota	2002	G. Secor
12	PE 627-3	Minnesota	2002	G. Secor
13	PE 632-4	Minnesota	2002	G. Secor
14	PR 152-1	Maine	2000	G. Secor
15	PR 193-2	Idaho	2000	G. Secor
16	PR 225-2	Idaho	2000	G. Secor
17	PR 230-2	Idaho	2000	G. Secor
18	PR 232-2	Idaho	2000	G. Secor
19	PR 291-5	Idaho	2000	G. Secor
20	PR 298-2	Wisconsin	2000	G. Secor
21	PR 400-1	Minnesota	2004	G. Secor
22	PR 506-1	Maine	2001	G. Secor
23	01-22g	Idaho	2001	J. Miller
24	01-1b	Idaho	2001	J. Miller
25	01-39e	Idaho	2001	J. Miller
26	02-105	Idaho	2002	J. Miller
27	02-28	Idaho	2002	J. Miller
28	01-39a	Idaho	2001	J. Miller
29	01-21	Idaho	2001	J. Miller
30	02-11	Idaho	2002	J. Miller
31	02-25	Idaho	2002	J. Miller
32	02-130	Idaho	2002	J. Miller
33	PR-R1	Maine	2004	D. Lambert
34	PR-R2	Maine	2004	D. Lambert
35	PR-R3	Maine	2004	D. Lambert
36	PR-R4	Maine	2004	D. Lambert
37	PR-R5	Maine	2004	D. Lambert
38	A-21S	Maine	2004	D. Lambert
39	PR-S1	Maine	2004	D. Lambert
40	PR-S2	Maine	2004	D. Lambert

Data were analyzed using CoStat Statistical Software (CoHort Software, Monterey, CA, USA) based on a completely randomized design. Mean separation was performed using Student-Newman-Keuls Test at $p = 0.05$.

RESULTS AND DISCUSSION

Results of the analysis of variance (ANOVA) showed that there were significant difference between hydrogen peroxide treatments in their effect on inhibiting the growth of *P. erythroseptica* (Table 2). Significant differences were also observed among isolates in their response to the 1:100 and 1:250 concentrations (Table 3).

Results of mean separation, using Student-Newman-Keuls Test (Table 4), showed that the 1:50 and 1:100 hydrogen peroxide treatments were not significantly different from each other in their effect on percent inhibition of *P. erythroseptica*. The growth of all isolates was inhibited at 1:50 dilution.

Table 2: ANOVA table for hydrogen peroxide treatments used in laboratory studies against *Phytophthora erythroseptica*

Source	DF	MS	F-value	P-value
Blocks	39	2.2	0.0001	***
Treatment	2	238.0	<0.0001	***
Model	41	13.7	<0.0001	***
Error	318			
Total	359			

Table 3: Mean Squares and significant differences of percent inhibition of 40 isolates of *Phytophthora erythroseptica* at various hydrogen peroxide concentrations tested *in vitro*

	Mean squares		
	Concentration		
Source	DF	1:50	1:100	1:250
Isolates	39	0[1]	2.186**[2]	2.663***
Error	80	0		
Total	119			

[1]The growth of all 40 isolates was completely inhibited at 1:50 hydrogen peroxide to water dilution,
[2]** and ***=Significant at 5% level using Bartlett's Test

Table 4: Percent inhibition of *Phytophthora erythroseptica* averaged for 40 isolates using various concentrations of hydrogen peroxide

Hydrogen peroxide [2]	Percent inhibition[1]		
	Min.	Max.	Average[3]
1:50	100.0	100	100.0[a]
1:100	46.3	100	94.2[a]
1:250	4.3	100	41.4[b]

[1]Percent inhibition was calculated relative to the untreated control which received sterile distilled water only; [2]Diluted solutions were prepared with sterile distilled water; [3]Average values of 40 isolates per concentration, each replicated three times (Table 1 for details). Average values followed by the same letter are not significantly different from each other at p = 0.05

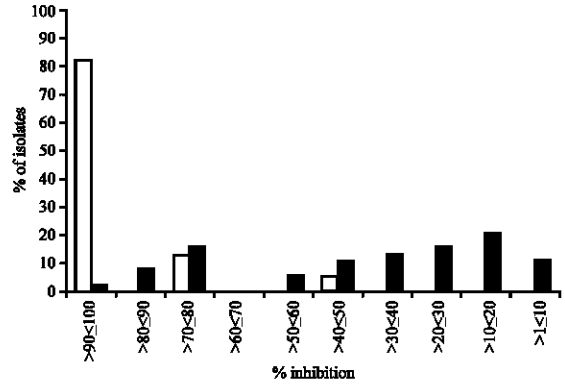

Fig. 1: Isolates of *Phytophthora erythroseptica* classed into 10 groups based on percent inhibition *in vitro* with various concentrations of hydrogen peroxide (□=1:100; ■=1:250). All isolates were completely inhibited at 1:50 concentration

Since all isolates were completely inhibited at 1:50 concentration, this concentration is not shown in the Fig. 1. At the 1:100 concentration, the growth of 82.5% of the isolates was completely inhibited. At 1:250 concentration, more than 50% growth inhibition was observed in 32.5% of the isolates. The lowest % inhibition observed at this concentration was 4.3% (Fig. 1).

Based on these results, hydrogen peroxide was effective in inhibiting *P. erythroseptica in-vitro*. Both of the 1:100 and 1:50 concentrations gave excellent inhibitory effect. Application of hydrogen peroxide should be done immediately after harvest and before storage. The initial application at the conveyer belt might be useful to prevent the spores present on the surface of the tubers from surviving and penetrating through wounds or natural orifices. In recent years, the recovery of mefenoxam-resistant strains of *P. erythroseptica* in the USA and Canada[11-13] has created concern that successful control of pink rot may be in jeopardy.

Post harvest application of hydrogen peroxide might help preventing post harvest pink rot infections and provide an alternative to mefenoxam. However, more research (greenhouse, growth chamber, etc.) is recommended to test the effect of hydrogen peroxide (OxiDate™) on potato tubers.

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