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Evaluation and comparison of biofungicides and fungicides for the control of post harvest potato tuber diseases.

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Summary

Potatoes are susceptible to a variety of storage pathogens, including late blight (*Phytophthora infestans*), Fusarium dry rot (*Fusarium sambucinum*), Pythium leak (*Pythium ultimum*) and silver scurf (*Helminthosporium solani*). Current recommendations for potato storage diseases include sanitation and exclusion as the primary controls for these pathogens in storage facilities. Few fungicides are registered for direct application to tubers for control of these important pathogens and few compounds are available for potato tuber treatment in storage, including chlorine-based disinfectants such as, sodium hypochlorite, calcium hypochlorite and chlorine dioxide.

In recent years several new biofungicides based on the biocontrol bacteria *Bacillus subtilis* (Serenade) and *B. pumilis* (Sonata) have been registered or are awaiting EPA approval for use on potato, and have shown promise in the control of seed and soil borne diseases such as late blight, black scurf and pink rot. These products have been evaluated for the control of pathogens under post-harvest potato tuber storage conditions. Thus, studies were initiated to evaluate the efficacy of these biofungicides for the control of potato storage pathogens under post-harvest conditions. For a comparison, several commercial storage products Phostrol (sodium, potassium and ammonium phosphates), and Oxidate (hydrogen dioxide) and experimental treatments such as Quadris (azoxystrobin) and mixtures of azoxystrobin and fludioxinil (Maxim) at different rates ± thiabendazole (Mertect) and more recently difenoconazole were evaluated for their effectiveness under storage conditions. Preliminary results show that in general the conventional fungicides (azoxystrobin, fludioxinil and difenoconazole) provided the most effective disease control. The biofungicides provided moderate control. The objective of these trials was to continue the evaluation of fungicides and biofungicides against the most common storage disease encountered in Michigan potato production.

Materials and Methods

Experiments were carried out in November 2009 with potato cultivars "FL1879", and "Dark Red Norland". The tests were carried out at two storage temperatures used in the potato industry; 10°C (49°F), chip processing and 4°C (39°F), table stock and seed. The cultivars used in the 10°C test were cv. FL1879 a chip processing cultivar; cv. Dark Red Norland at 4°C a red skinned table-stock cultivar. Potatoes free from visible diseases [except cv. Dark Red Norland that was uniformly infected with *H. solani* (*Hs*, silver scurf, 20% of surface area affected) at the time of application] were selected for the trials from tubers harvested in October 2006. Tubers were prepared for inoculation with *Phytophthora infestans* (*Pi*), *P. erythroseptica* (*Pe*), *Pythium ultimum* (*Py*), and *Fusarium sambucinum* (*Fs*) by grazing with a single light stroke with a wire brush, sufficient to abrade the skin of the tubers to a depth of 0.01 mm. Solutions (1 x 10^3/ml) of sporangia/zoosporangia of *Pi* (late blight), oospores/sporangia of *Pe* (pink rot), oospores of *Py* (Pythium leak), and macroconidia of *Fs* (dry rot) were prepared from cultures of the pathogens previously isolated from potato tubers in Michigan. All pathogens were grown in Potato Dextrose Nutrient Broth for 20 days prior to preparation of inoculum solutions. Two non-treated controls, either inoculated with one of the pathogens or non-inoculated were included in the trial.

Inoculated and damaged/inoculated tubers, (50/replicate/treatment; total 200 tubers/treatment) were sprayed with 10 ml of pathogen suspension, for a final dosage of about 0.25 ml per tuber. Tubers were stored for 2-d after inoculation at 20°C before treatment. Fungicides were applied as liquid treatments in a water suspension with a single R&D XR11003VS spray nozzle at a rate of 1L/ton at 50 psi onto the tuber surfaces, with the entire tuber surface being coated. After inoculation, tubers were incubated in the dark in plastic boxes at 10°C or 4°C (depending on cultivar and disease combination) for 90 (oomycete pathogens and dry rot) to xxx days. The oomycete diseases were evaluated as the percent incidence of tubers with any signs or symptoms of the pathogen. Tubers with surface sporulation, discoloration of the skin or blackened/dead sprouts were considered infected. The remaining tubers were cut open and the number of tubers with symptoms or signs of the individual pathogens were counted to determine incidence of disease. Dry rot was and silver scurf disease severity was assessed using a disease severity index. Disease severity classes were determined as class 0 = 0%; 1 = 1 - 10%; 2 = 11 - 20%; 3 = 21 - 50; 4 > 51 – 100% internal area of tuber tissue with disease (dry rot) or surface area affected (silver scurf). The disease severity index was then calculated as the number in each class multiplied by the class number and summed. The sum was then multiplied by a constant to express severity on a 0 – 100 scale. Data were analyzed by two-way ANOVA using ARM software (Version 7, Gylling Data Management) and mean separation calculated using Fisher's protected least significant difference (LSD) test at P= 0.05.

Results and Conclusions

In the late blight test at 10°C, late blight developed in several treatments and the inoculated check had 58.8% incidence (Table 1). No late blight developed in the non-inoculated check. Treatments with less than 58.8% incidence of late blight were significantly different from the inoculated check. Treatments with greater than 10.0% incidence of late blight were significantly different from the non-inoculated check.

In the pink rot test at 10°C, pink rot developed in several treatments and the inoculated check had 65.0% incidence (Table 2). No pink rot developed in the non-inoculated check. Treatments with less than 65.0% incidence of pink rot were significantly different from the inoculated check. Treatments with greater than 7.5% incidence of pink rot were significantly different from the non-inoculated check.

In the pythium leak test at 10°C, pythium developed in several treatments and the inoculated check had 60.0% incidence (Table 3). No pythium developed in the non-inoculated check. Treatments with less than 60.0% incidence of pythium were significantly different from the inoculated check. Treatments with greater than 6.3% incidence of pythium were significantly different from the non-inoculated check.

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In the Fusarium dry rot studies at 10°C Fusarium dry rot developed in several treatments and the inoculated check had 70% incidence and 31.9-severity index (Table 4). The non-inoculated check had significantly less dry rot incidence and severity than the inoculated check. Treatments with less than 56.3% incidence of dry rot were significantly different from the inoculated check. Treatments with greater than 13.8% dry rot incidence were significantly different from the non-inoculated check. Treatments with less than 24.1 dry rot severity index were significantly different from the inoculated check. Treatments with greater than 5.9 dry rot severity index were significantly different from the non-inoculated check.

In the silver scurf leak test at 4°C on Dark Red Norland, severe silver scurf developed in several treatments and the inoculated check had 93.1% incidence of silver scurf (percentage of tubers in class 0) and 55.6% severity index (Table 5). Although the percentage incidence and severity were relatively high in all treatments, they were all significantly lower than the untreated check.

In this study, the oomycetes were not all controlled equally by the same programs. For example, Ranman 400SC 0.25 fl oz gave excellent control of late blight and pythium leak but was moderately effective against pink rot. GWN-4700 alone and in mixture was effective against all three oomycetes and not significantly different from the standard program, Phostrol. Late blight was well controlled by many products including WE1042-1, which was possibly related to the inoculation technique in that the pathogen had not fully penetrated the lenticels by the time the fungicides/biofungicides were applied. This was also true of the Pythium study but the rate of growth and penetration of *Phytophthora erythroseptica* may have been at a rate great enough for the infection to occur and establish prior to the application of the products. The biological controls were generally les effective than the best chemical controls but still provide some useful reduction in the establishment of the oomycetes. For example, BUPots-4 in the Pythium trial and the late blight trial and BuPots-1 in the pink rot trial.

In the dry rot trial, the three-way mixtures of fludioxinil, azoxystrobin and difenoconazole produced excellent control and at all rates of the fungicides (especially at the highest mixture rate of difenoconazole). While treatment with fludioxinil and azoxystrobin together or difenoconazole alone provided effective but numerically less dry rot control than the three-way mixtures although in terms of severity there was no significant difference among treatments. The biological control products in some cases provided a moderate reduction in incidence and severity of dry rot.

In the silver scurf trial, azoxystrobin plus fludioxinil and all rates of difenoconazole in the three-way mixtures of fludioxinil, azoxystrobin and difenoconazole produced the best control but only suppressed the development of silver scurf. WE1042-1 75DS and the biological control products provided a moderate reduction in incidence and severity of silver scurf.

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Table 1. Incidence of tubers with potato late blight stored at 10°C for 90 days after treatment with fungicides/biofungicides.

Treatments and rate of application per cwt of tubers	Late blight incidence (%)[a]	
Non-inoculated check…………………………………………	0.0	l[b]
Inoculated check………………………………………………	58.8	a
Ranman 400SC 0.025 fl oz…………………………………...	11.3	f-k
Ranman 400SC 0.05 fl oz…………………………………….	7.5	h-l
Ranman 400SC 0.125 fl oz…………………………………...	10.0	g-l
Ranman 400SC 0.25 fl oz…………………………………….	0.0	l
Phostrol 53.6SC 1.28 fl oz……………………………………	3.8	jkl
Oxidate 27SC 0.125 fl oz……………………………………..	6.3	i-l
GWN-4700 80WP 0.14 oz……………………………………	12.5	f-j
GWN-4700 80WP 0.14 oz + Phostrol 53.6SC 1.28 fl oz……….	15.0	e-i
GWN-4700 80WP 0.14 oz + Phostrol 53.6SC 1.28 fl oz[c]………	6.3	i-l
GWN-4700 80WP 0.14 oz + A8574 360FS[d] 0.03 fl oz…………	7.5	h-l
GWN-4700 80WP 0.14 oz + A9859 230SC[d] 0.03 fl oz…………	3.8	jkl
GWN-4700 80WP 0.14 oz + A8574 360FS 0.03 fl oz + A9859 230SC 0.03 fl oz………………………………………	1.3	kl
09 BuPots-1 100L 0.2 fl oz…………………………………...	18.8	d-g
09 BuPots-2 100WS 1.52 oz……………………………………	17.5	d-h
09 BuPots-3 100WS 1.52 oz……………………………………	23.8	de
09 BuPots-4 100LS 0.2 fl oz……………………………………	7.5	h-l
09 BuPots-5 100LS 0.3 fl oz……………………………………	13.8	e-j
WE1042-1 75DS 1 lb……………………………………………	3.8	jkl
ME02-7008A 100D 1 lb…………………………………………	26.3	cd
ME03-5709A 100D 1 lb…………………………………………	23.8	de
W103-5B 100D 1 lb……………………………………………..	36.3	bc
ND04-1A 100D 1 lb……………………………………………..	21.3	def
Vermiculite 100D 1 lb…………………………………………...	37.5	b
$LSD_{0.05}$	10.13	

[a] Late blight incidence (%); tubers with any symptoms and signs of *Phytophthora infestans*.
[b] Values followed by the same letter are not significantly different at $P = 0.05$ level according to Fisher's protected least significant difference (LSD) test.
[c] Applied in 4 pt H_2O/ton tubers.
[d] A8574D= difenoconazole; A9859= fludioxinil.

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Table 2. Incidence of tubers with pink rot stored at 10°C for 90 days after treatment with fungicides/biofungicides.

Treatments and rate of application per cwt of tubers	Pink Rot incidence (%)[a]	
Non-inoculated check……………………………………………	0.0	k
Inoculated check…………………………………………………	65.0	a
Ranman 400SC 0.025 fl oz……………………………………...	13.8	f-i
Ranman 400SC 0.05 fl oz………………………………………	18.8	efg
Ranman 400SC 0.125 fl oz……………………………………...	20.0	ef
Ranman 400SC 0.25 fl oz………………………………………	20.0	ef
Phostrol 53.6SC 1.28 fl oz………………………………………	10.0	hij
Oxidate 27SC 0.125 fl oz………………………………………..	3.8	jk
GWN-4700 80WP 0.14 oz………………………………………	8.8	ij
GWN-4700 80WP 0.14 oz + Phostrol 53.6SC 1.28 fl oz……….	7.5	ijk
GWN-4700 80WP 0.14 oz + Phostrol 53.6SC 1.28 fl oz[c]………	11.3	g-j
GWN-4700 80WP 0.14 oz + A8574 360FS[d] 0.03 fl oz…………	10.0	hij
GWN-4700 80WP 0.14 oz + A9859 230SC[d] 0.03 fl oz…………	8.8	ij
GWN-4700 80WP 0.14 oz + A8574 360FS 0.03 fl oz + A9859 230SC 0.03 fl oz…………………………………………	8.8	ij
09 BuPots-1 100L 0.2 fl oz……………………………………...	7.5	ijk
09 BuPots-2 100WS 1.52 oz……………………………………	10.0	hij
09 BuPots-3 100WS 1.52 oz……………………………………	21.3	def
09 BuPots-4 100LS 0.2 fl oz……………………………………	22.5	de
09 BuPots-5 100LS 0.3 fl oz……………………………………	17.5	e-h
WE1042-1 75DS 1 lb……………………………………………	21.3	def
ME02-7008A 100D 1 lb…………………………………………	8.8	ij
ME03-5709A 100D 1 lb…………………………………………	28.8	cd
W103-5B 100D 1 lb……………………………………………..	35.0	c
ND04-1A 100D 1 lb……………………………………………..	28.8	cd
Vermiculite 100D 1 lb…………………………………………...	33.8	c
Vermiculite 100D 1 lb	53.8	b
$LSD_{0.05}$	8.02	

[a] Late blight incidence (%); tubers with any symptoms and signs of *Phytophthora infestans*.
[b] Values followed by the same letter are not significantly different at $P = 0.05$ level according to Fisher's protected least significant difference (LSD) test.
[c] Applied in 4 pt H_2O/ton tubers.
[d] A8574D= difenoconazole; A9859= fludioxinil.

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Table 3. Incidence of tubers with pythium leak stored at 10°C for 90 days after treatment with fungicides/biofungicides.

Treatments and rate of application per cwt of tubers	Pink Rot incidence (%)[a]	
Non-inoculated check…………………………………………	0.0	k
Inoculated check………………………………………………	60.0	a
Ranman 400SC 0.025 fl oz…………………………………...	8.8	g-j
Ranman 400SC 0.05 fl oz…………………………………….	13.8	e-h
Ranman 400SC 0.125 fl oz…………………………………...	8.8	g-j
Ranman 400SC 0.25 fl oz…………………………………….	6.3	ijk
Phostrol 53.6SC 1.28 fl oz……………………………………	3.8	jk
Oxidate 27SC 0.125 fl oz……………………………………..	5.0	ijk
GWN-4700 80WP 0.14 oz……………………………………	7.5	hij
GWN-4700 80WP 0.14 oz + Phostrol 53.6SC 1.28 fl oz……….	6.3	ijk
GWN-4700 80WP 0.14 oz + Phostrol 53.6SC 1.28 fl oz[c]………	3.8	jk
GWN-4700 80WP 0.14 oz + A8574 360FS[d] 0.03 fl oz…………	6.3	ijk
GWN-4700 80WP 0.14 oz + A9859 230SC[d] 0.03 fl oz…………	6.3	ijk
GWN-4700 80WP 0.14 oz + A8574 360FS 0.03 fl oz + A9859 230SC 0.03 fl oz………………………………………	6.3	ijk
09 BuPots-1 100L 0.2 fl oz…………………………………...	15.0	d-g
09 BuPots-2 100WS 1.52 oz…………………………………	13.8	e-h
09 BuPots-3 100WS 1.52 oz…………………………………	15.0	d-g
09 BuPots-4 100LS 0.2 fl oz…………………………………	11.3	f-i
09 BuPots-5 100LS 0.3 fl oz…………………………………	17.5	def
WE1042-1 75DS 1 lb…………………………………………	6.3	ijk
ME02-7008A 100D 1 lb………………………………………	18.8	de
ME03-5709A 100D 1 lb………………………………………	20.0	de
W103-5B 100D 1 lb…………………………………………..	27.5	c
ND04-1A 100D 1 lb…………………………………………..	21.3	cd
Vermiculite 100D 1 lb………………………………………...	35.0	b
$LSD_{0.05}$	7.25	

[a] Late blight incidence (%); tubers with any symptoms and signs of *Phytophthora infestans*.
[b] Values followed by the same letter are not significantly different at $P = 0.05$ level according to Fisher's protected least significant difference (LSD) test.
[c] Applied in 4 pt H_2O/ton tubers.
[d] A8574D= difenoconazole; A9859= fludioxinil.

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Table 4. Severity and incidence of tubers with Fusarium dry rot 90 days after treatment with fungicides/biofungicides at 10^{o}C.

Treatments and rate of application per cwt of tubers	Dry rot Incidence (%)		Dry rot Severity index[a]	
Non-inoculated check…………………………………………	2.5	f[b]	0.6	f
Inoculated check………………………………………………	70.0	a	30.6	ab
A12705 250SC[c] 0.03 fl oz + A9859 230SC[c] 0.03 fl oz……….	22.5	d	5.9	f
A8574D 360FS[c] 0.015 fl oz…………………………………...	20.0	de	5.0	f
A12705P 250SC 0.03 fl oz + A8574D 360FS 0.0075 fl oz + A9859 230SC 0.03 fl oz………………………………………	13.8	def	4.4	f
A12705P 250SC 0.03 fl oz + A8574D 360FS 0.015 fl oz + A9859 230SC 0.03 fl oz………………………………………	7.5	ef	2.2	f
A12705P 250SC 0.03 fl oz + A8574D 360FS 0.0226 fl oz + A9859 230SC 0.03 fl oz………………………………………	8.8	def	2.8	f
A12705P 250SC 0.03 fl oz + A8574D 360FS 0.03 fl oz + A9859 230SC 0.03 fl oz………………………………………	3.8	f	0.9	f
09 BuPots-1 100L 0.2 fl oz……………………………………	62.5	abc	29.1	abc
09 BuPots-2 100WS 1.52 oz…………………………………..	61.3	abc	26.9	a-e
09 BuPots-3 100WS 1.52 oz…………………………………..	53.8	c	22.2	cde
09 BuPots-4 100LS 0.2 fl oz…………………………………..	52.5	c	20.9	cde
09 BuPots-5 100LS 0.3 fl oz…………………………………..	68.8	ab	31.9	a
WE1042-1 75DS 1 lb………………………………………….	55.0	bc	22.5	b-e
ME02-7008A 100D 1 lb………………………………………	51.3	c	20.3	de
ME03-5709A 100D 1 lb………………………………………	51.3	c	20.3	de
W103-5B 100D 1 lb…………………………………………..	56.3	abc	24.1	a-e
ND04-1A 100D 1 lb…………………………………………..	50.0	c	19.7	e
Vermiculite 100D 1 lb………………………………………...	68.8	ab	28.1	a-d
$LSD_{0.05}$	14.33		8.33	

[a] Severity classes were determined as class 0 = 0%; 1 = 1 - 10%; 2 = 11 - 20%; 3 = 21 - 50%; 4 > 51 – 100% internal area of tuber tissue with disease and incidence is percentage of tubers in class 1 - 4.

[b] Values followed by the same letter are not significantly different at *P* = 0.05 level according to Fisher's protected least significant difference (LSD) test.

[c] A12705= azoxystrobin; A8574D= difenoconazole; A9859= fludioxinil.

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Table 5. Severity and incidence of tubers with silver scurf 101 days after treatment with fungicides/biofungicides at 4°C.

Treatments and rate of application per cwt of tubers	Silver scurf incidence (%)		Silver scurf Severity index[a] (%)	
Non-inoculated check…………………………………………	93.1	a[b]	55.6	a
A12705 250SC[c] 0.03 fl oz + A9859 230SC[c] 0.03 fl oz………	85.6	b	43.2	de
A8574D 360FS[c] 0.015 fl oz…………………………………..	69.7	gh	32.2	h
A12705P 250SC 0.03 fl oz + A8574D 360FS 0.0075 fl oz + A9859 230SC 0.03 fl oz………………………………………	74.1	fg	32.3	gh
A12705P 250SC 0.03 fl oz + A8574D 360FS 0.015 fl oz + A9859 230SC 0.03 fl oz………………………………………	84.7	bcd	36.6	fg
A12705P 250SC 0.03 fl oz + A8574D 360FS 0.0226 fl oz + A9859 230SC 0.03 fl oz………………………………………	80.9	cde	36.9	f
A12705P 250SC 0.03 fl oz + A8574D 360FS 0.03 fl oz + A9859 230SC 0.03 fl oz………………………………………	66.3	h	33.0	fgh
09 BuPots-1 100L 0.2 fl oz……………………………………	86.9	b	46.3	b-e
09 BuPots-2 100WS 1.52 oz…………………………………..	85.0	bc	49.0	b
09 BuPots-3 100WS 1.52 oz…………………………………..	78.1	ef	44.5	cde
09 BuPots-4 100LS 0.2 fl oz…………………………………..	85.9	b	47.7	bc
09 BuPots-5 100LS 0.3 fl oz…………………………………..	80.3	de	42.4	e
WE1042-1 75DS 1 lb…………………………………………	84.1	bcd	47.5	bcd
$LSD_{0.05}$				

[a] Severity classes were determined as class 0 = 0%; 1 = 1 - 10%; 2 = 11 - 20%; 3 = 21 - 50; 4 > 51 – 100% internal area of tuber tissue with disease and incidence is percentage of tubers in class 1 - 4.
[b] Values followed by the same letter are not significantly different at $P = 0.05$ level according to Fisher's protected least significant difference (LSD) test.
[c] A12705= azoxystrobin; A8574D= difenoconazole; A9859= fludioxinil.