Project Title: Efficacy of Natamycin for Control of Mucor Rot in Pear Fruit
Report Type: Final Project Report
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Budget 1
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Item	2024
Salaries	$ 22,162.00
Benefits	$ 1,695.00
Wages	
Benefits	
RCA Room Rental	$ 1,648.80
Shipping	$ 200.00
Supplies	$ 1,000.00
Travel	$ 1,500.00
Plot Fees	$ 1,750.00
Total	$ 29,955.80

Footnotes: Salary: 1.0 PTE Biological Science Technician (GS-5), (three 8-hr days per week) + 15 hours overtime.
Benefits: For Biological Science Technician (GS-5)
RCA room rental: per OSU-MCAREC fee book (cost per sq ft x time) (one room)
Shipping: Postage required to send fruit to Pace laboratory for residue testing.
Supplies: Harvest and storage supplies, pathogen culture and inoculation supplies.
Travel: To field sites and fruit transport (fuel).
Plot fees: 0.25-acre rental, OSU-MCAREC

Objectives

1. Compare the rates of different natamycin concentrations for prevention of post-packing Mucor rot on pears that have been in storage for six-months.
Determine the efficacy of natamycin for significant control of Mucor rot when applied as an aqueous dip post-storage in laboratory trials. This experiment will replicate and build upon previous baseline studies that have been conducted on citrus fruit but have not yet been tested on 'd'Anjou' pears (Kim et al. 2017, Saito et al. 2023).

2. Compare rates of different natamycin concentrations immediately post-harvest for preventative control of Mucor rot on pears.
Determine the amount of disease incidence incurred in 'd'Anjou' pears when the fungicide and inoculum are combined in an experimental dump tank in a lab setting. Results from this objective will provide insight into optimal concentrations for natamycin use on pear packing lines.

3. Evaluate efficacy of natamycin in combination with other postharvest chemicals for control of Mucor rot on pears at harvest.
Compare the efficacy of natamycin to control Mucor rot in pears at harvest with fruit that has been in long term storage. Demonstrate the best strategy for combinations of postharvest chemicals to be used with natamycin treatments. The data from this experiment will be beneficial to the industry as it will help to identify optimal chemical combinations for significant control of Mucor rot in pears.

Significant findings

- The postharvest timing of natamycin (BioSpectra 100SC) application and concentration of active ingredient influence treatment efficacy, with earlier application (shortly after harvest vs. five months postharvest) and higher concentration (highest label rate) generally leading to greater reduction of disease incidence.
- The 1000 ppm natamycin treatments resulted in numerically lower disease incidence in all experiments when compared to the 500 ppm natamycin treatments.
- Average Mucor rot lesion diameter was lower for 1000 ppm treatments (compared to 500 ppm treatments) when the near-harvest and at five-month postharvest trials were combined and averaged.
- In an experimental dump tank that contained the highest label rate of BioSpectra 100SC (1000 ppm natamycin) and *Mucor piriformis* spores, Mucor rot incidence was reduced by 66%.
- A low rate of propiconazole (Mentor 45WP, 0.3g/L) combined with the highest label rate of natamycin (BioSpectra 100SC, 1000 ppm) reduced Mucor rot by 22% in a preliminary trial.

Methods

The post-harvest fungicide BioSpectra 100SC (active ingredient natamycin) was evaluated for control of Mucor rot on 'd'Anjou' pears. Pears were wound inoculated with *Mucor piriformis* and then treated with the lowest label rate of BioSpectra 100SC (500 ppm natamycin) and the highest label rate of BioSpectra 100SC (1000 ppm natamycin). Untreated fruits were used as controls. The fruit were then placed on fruit trays, wrapped in a perforated plastic bag, and stored in cardboard boxes in regular air at -0.5°C ± 0.5°C (31°F). Lesion sizes were measured in two perpendicular directions using a digital caliper after three weeks. A total of 16 fruit samples (two experimental replicates of eight fruits per replicate) and two liquid samples per treatment were sent to AgroFresh/Pace in Wapato, WA for residue testing. These experiments were conducted four different times using fruit from two different growing seasons. For the first two experiments, fruit from the 2023 harvest that originated from two different growing locations was used to evaluate the efficacy of BioSpectra 100SC on 'd'Anjou' pears that had been stored in air storage for five months. For all other experiments, fruit from the 2024 harvest was used. Fruit from the 2024 harvest originated from two different growing locations (Hood River, OR, and Cashmere, WA). For objective two, a laboratory experiment was conducted to determine the amount of disease incidence incurred when 'd'Anjou' pears were dipped in an experimental dump tank that contained BioSpectra 100SC and *M. piriformis* sporangiospores. The high and low label rates were used for the BioSpectra 100SC concentrations in the dump dank water. Four holes were punctured on two opposite sides of the fruit at the equator region. The fruit was then dipped into the BioSpectra 100SC and *M. piriformis* spore solution for 10 seconds and allowed to dry at room temperature. The control dump tank contained only water along with *M. piriformis* sporangiospores. After fruit was dipped into the experimental dump tank they were then placed on fruit trays, wrapped in a perforated plastic bag, and stored at room temperature for 6-7 days. The percentage of infected fruit and the percentage of infected wounds per fruit were calculated after 6-7 days.

A final small-scale trial was conducted testing the efficacy of BioSpectra 100SC alone or in combination with the fungicide Mentor 45WP against Mucor rot. Fruit were wound inoculated with *M. piriformis* using the previously mentioned methods, then treated with the following treatments: BioSpectra 100SC (114oz/100 gal or 8.9g/L)), Mentor (4oz/100 gal or 0.3g/L), and BioSpectra 100SC + Mentor. A total of 18 'd'Anjou' pears were used per treatment. Lesion sizes were measured in two perpendicular directions using a digital caliper after the fruit was left in regular air cold storage for 7 days and then room temperature for 7 days.

Fruit quality evaluations were conducted on one replicate of 18 pears per treatment for trials in fall 2024 and experimental dump tank trials. Fruit quality was assessed using standard fruit quality measures which included fruit weight, index of absorption difference (I_{AD}; DA meter), firmness, soluble solids contents, and titratable acidity. Fruit was also inspected for any evidence of phytotoxicity such as marking, russeting, or other potential fruit finish issues.

Results and Discussion

Disease incidence

The individual trial with the lowest disease incidence was observed in the 'd'Anjou' pears grown near Cashmere, WA and treated at the near-harvest time point. There was 0% infection rate three weeks after Mucor rot inoculation and 1000 ppm natamycin treatment (Fig. 1A and Table 1). However, these fruit had a 9% infection rate when inspected again at the four-week time point (Fig. 1B), which is an indication that natamycin may reduce the rate of Mucor rot growth (fungistatic) but not eliminate (fungicidal) a portion of Mucor rot propagules. The 500 ppm natamycin treatment corroborated this outcome with a 30% infection rate after three weeks and an 83% infection rate at four weeks (Fig. 1A, 1B, and Table 1).

These results are consistent with findings from Saito et al. (2023) who found that Mucor rot incidence increased after three and four weeks in storage. Through our testing we found that trials could not continue beyond five weeks in air storage due to control fruit (fruit with no natamycin treatment) deteriorating. Additional research efforts will utilize controlled atmosphere storage or modified air packaging to conduct longer term experiments or evaluate repeated applications.

Results were similar for the two separate experiments conducted on fruit five-months postharvest. For these trials, the lowest label rate of BioSpectra 100SC (500 ppm natamycin) reduced Mucor rot incidence by 43-46% whereas the highest label rate (1000 ppm natamycin) reduced Mucor rot by 54-59% (Table 1). The natamycin fruit residue values were considerably lower for these two trials (five months postharvest, performed in spring 2024) compared to the other reported natamycin liquid and fruit residues (Table 1). This was a result of initial fungicide application methods that were later modified leading to higher natamycin fruit residue values. Considering this, these two trials represent minimal optimal natamycin residue amounts on aged fruit. As expected with deliberate inoculation, the control treatments for these experiments had a disease incidence of 98-100% (Table 1). This represents a severe situation that typically would represent higher incidence than would occur in a commercial setting. None-the-less, the 500 ppm and 1000 ppm natamycin dip treatments reduced Mucor rot by an average of 45-57% after three weeks.

Figure 1. **'**D'Anjou' pears grown near Cashmere, WA in the natamycin application rate trial performed shortly after harvest. The small spots in the center of the fruit are the site of wound inoculation with the Mucor rot organism, *M. piriformis.* **A.** Comparison of one replicate of fruit treated with natamycin after three weeks at 31˚F. Results illustrate reduction in incidence and lesion size in fruit treated with 500 or 1000 ppm natamycin; this trial yielded the best overall results at the three-week evaluation timepoint. **B.** One replicate from this trial that was also inspected after four weeks at 31˚F illustrating lesion growth, even in natamycin-treated fruit.

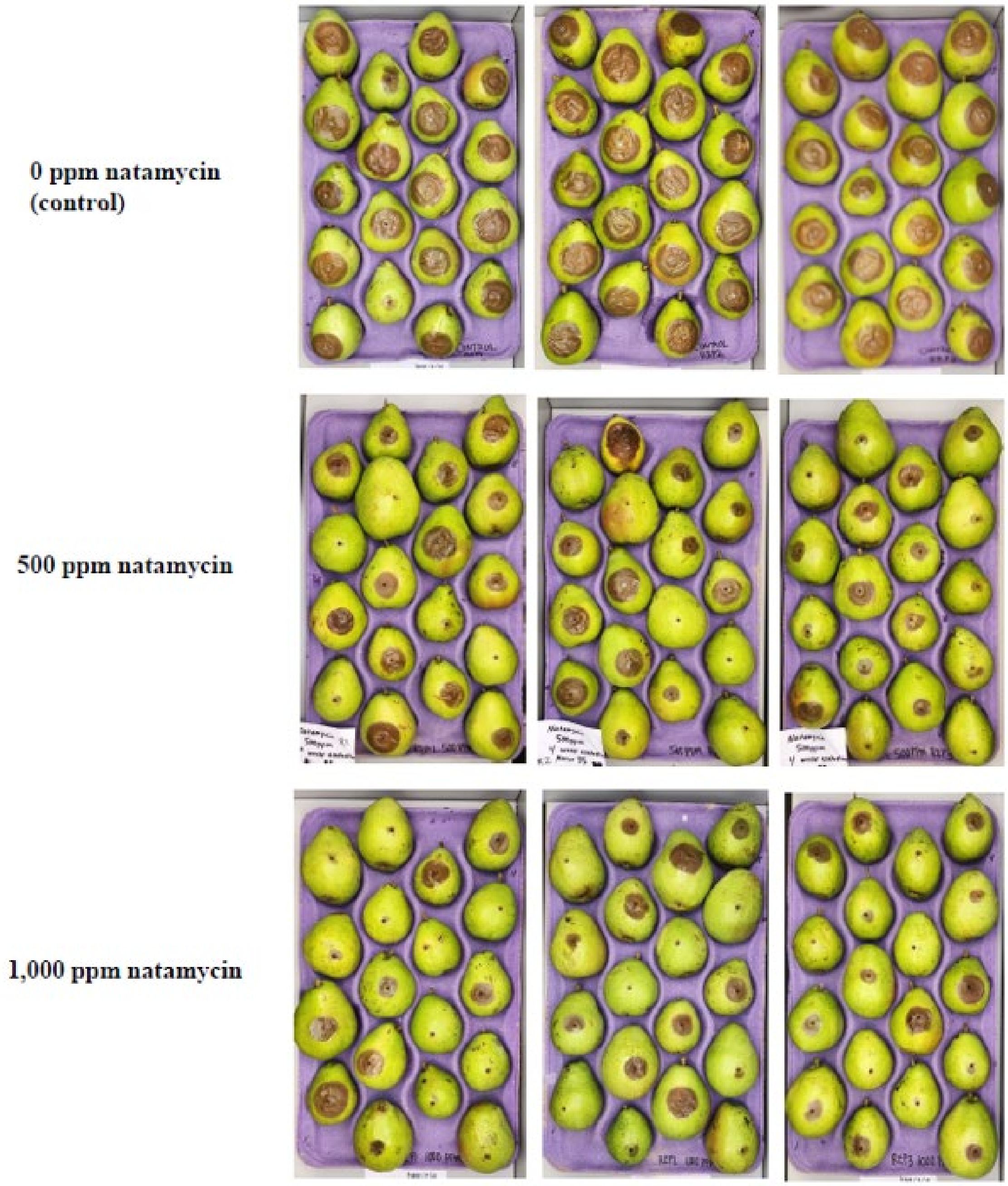

Figure 2. 'D'Anjou' pears from Hood River, OR in the natamycin rate application trial performed shortly after harvest; pictures were taken 3-weeks post-inoculation and subsequent treatment. The small spots in the center of the fruit are the site of wound inoculation with the Mucor rot organism, *M. piriformis.* Results illustrate reduction in disease incidence and lesion size in fruit treated with 500 or 1000 ppm natamycin.

Table 1. Natamycin liquid residue, fruit residue post-application, and infection rates of Mucor rot following *Mucor piriformis* inoculation and natamycin treatment for 'd'Anjou' pears from four separate experiments. Evaluations were conducted three weeks post-inoculation and natamycin treatment.

Location where fruit was grown	Time point (inoculation date)	Natamycin target rate (ppm)	Liquid residue (ppm)	Fruit residue (ppm)	Infection rate (%)[1]
Hood River, OR	At harvest (9/4/2024)	0	-	-	96 (n = 52/54) a
Hood River, OR	At harvest (9/4/2024)	500	391.74	2.99	70 (n = 38/54) b
Hood River, OR	At harvest (9/4/2024)	1000	781.57	5.52	54 (n = 29/54) c
Hood River OR	Five months (2/27/2024)	0	-	-	98 (n = 53/54) a
Hood River OR	Five months (2/27/2024)	500	-	0.30	54 (n = 29/54) c
Hood River OR	Five months (2/27/2024)	1000	-	0.42	46 (n = 25/54) c
Cashmere, WA	At harvest (9/17/2024)	0	-	-	100 (n = 54/54) a
Cashmere, WA	At harvest (9/17/2024)	500	457.61	0.75	30 (n = 16/54) cd
Cashmere, WA	At harvest (9/17/2024)	1000	927.80	1.29	0 (n = 0/54) d
Cashmere, WA	Five months (3/5/2024)	0	-	-	100 (n = 54/54) a
Cashmere, WA	Five months (3/5/2024)	500	-	0.31	57 (n = 31/54) c
Cashmere, WA	Five months (3/5/2024)	1000	-	0.77	41 (n = 22/54) c

1. Chi-square statistical tests. Numbers in columns followed by different letters are statistically different.

Although not statistically different, on average for all combined treatments, the fruit treated with natamycin (BioSpectra 100SC) shortly after harvest had a lower disease incidence (58%) when compared to the fruit that had been treated five-months postharvest (66%). The 1000 ppm natamycin treatments resulted in lower disease incidence in all cases when compared with the 500 ppm treatments. Based on these results, applying the highest label rate of natamycin as soon as possible after harvest may be beneficial for preventative control of Mucor rot, although the duration of control is unknown, as experiments terminated at 3 weeks post-inoculation. However, postharvest Mucor rot incidence varies year-to-year, with risk factors unclear and an area of research need. Additional research incorporating principals of microbial ecology may lend insight into microbially-based *M. piriformis* suppression; for example Silvestri et al. (2020).

Lesion diameters

The width of the rotted area of the fruit surrounding the Mucor-inoculated wound (mean lesion diameters) were generally smaller shortly after harvest, relative to fruit wounded and inoculated five-months after harvest (Fig. 3). The present study did not result in a statistical separation in lesion sizes in response to 500 ppm vs. 1000 ppm treatments with the exception of the Cashmere fruit near-harvest trial, although trends suggest that 1000 ppm treatments reduced lesion diameters relative to 500 ppm treatments; results were also variable given site and storage (Fig. 3 and Fig. 4). The control, 500 ppm, and 1000 ppm treatments had mean lesion diameters of 26 mm (1 inch), 12 mm (0.5 inch), and 10 mm (0.4 inch) for the combined near-harvest testing. The combined five-month post-harvest experiments resulted in average lesion sizes of 29 mm (1.1 inch), 19 mm (0.7 inch), and 19 mm (0.7 inch) for the control, 500 ppm, and 1000 ppm treatments respectively (Fig. 4). In summary, trends from these trials suggest that the highest label rate of natamycin (BioSpectra 100SC) reduced the rate of Mucor rot growth but there was not statistically significant differences in lesion sizes between the 500 and 1000 ppm label rates in most scenarios.

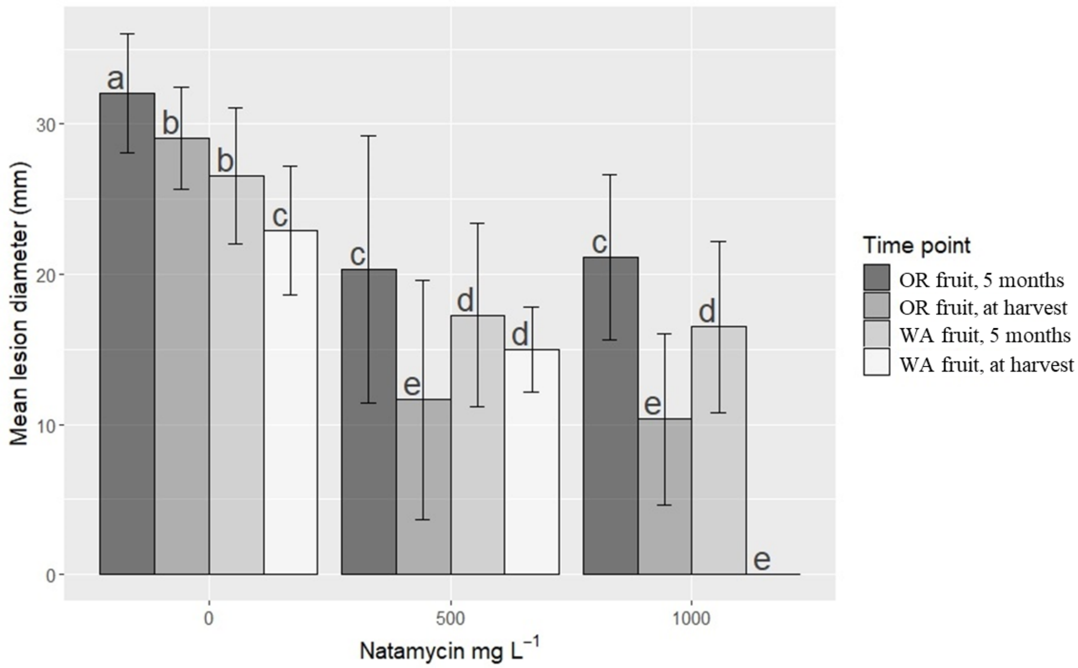

Figure 3. Mean lesion diameter of infected fruit inoculated with *M. piriformis* after three weeks in regular air storage at 31˚F. Values followed by the same letter are not statistically different according to Duncan's multiple range test ($p < 0.05$). 1 mg L^{-1} = 1 ppm.

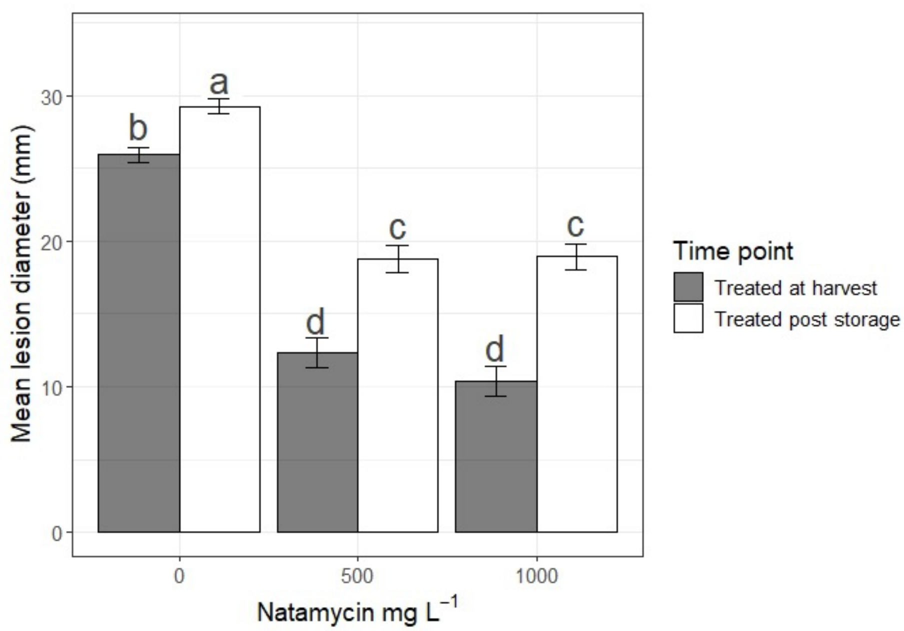

Figure 4. Mean lesion diameter among four combined experiments. Lesions were measured three weeks after inoculations. 'D'Anjou' pears were stored in air storage at 31˚F. Values followed by the same letter are not statistically different according to Duncan's multiple range test ($p < 0.05$). 1 mg L^{-1} = 1 ppm.

Experimental dump tank

The experimental dump tank trials were designed to simulate a pear packing line dump tank where natamycin was added to the water. Chlorine was not used within these trials as natamycin is chemically incompatible with chlorine (Adaskaveg et al. 2019). Further research is needed to determine the cost efficacy of 1000 ppm BioSpectra 100SC in dump tank water at pear packing houses; line spray applications or gas/vapor applications may be more economically feasible.

In agreement with the previous wound inoculation trials, the treatments with the lowest percent of infected fruit (17%) and the lowest percent of wounds infected (6%) occurred in the Cashmere, WA fruit that had been treated with 1000 ppm natamycin (Table 2). Aside from the controls, the fruit with the highest percent of infected fruit (65%) and the highest percent of wounds infected (31%) was observed in the fruit grown in Hood River, OR that was treated with 500 ppm natamycin (Table 2).

When the two dump tank trials were combined, the 500 ppm and 1000ppm natamycin treatments reduced the average percent of fruit infected by 53% and 72% respectively. Experimental dump tanks treated with 500 ppm and 1000 ppm natamycin decreased the average percentage of infected wounds by 82% and 86%, respectively.

Table 2. Average natamycin residues in experimental dump tank solutions, percent of fruit infected (any one or more of four wounds infected), and percent of wounds infected. Fruit were dipped in experimental dump tank for 10 seconds. Lesions were determined after an incubation for 6 days (Hood River, OR fruit) and 7 days (Cashmere, WA fruit).

Location where fruit was grown	Natamycin target rate (ppm)	Mean natamycin liquid residue (ppm)	Mean natamycin fruit residue (ppm)	Percent of fruit infected (%)[1]	Percent of individual wounds infected (%)[1]
Hood River, OR	0	-	-	100 (n=54/54) a	97 (n = 209/216) a
	500	434.93	0.48	65 (n = 35/54) ab	31 (n = 66/216) b
	1000	925.85	1.27	39 (n = 21/54) b	17 (n = 37/216) bc
Cashmere, WA	0	-	-	96 (n = 52/54) a	83 (n = 179/216) a
	500	547.43	0.75	30 (n = 16/54) b	11 (n = 23/216) c
	1000	967.09	1.60	17 (n = 9/54) c	6 (n = 12/216) c

1. Chi-square goodness-of-fit statistical tests. Numbers in columns followed by different letters are statistically different.

Natamycin combined with other postharvest chemicals

A small-scale trial was conducted testing natamycin in combination with the post-harvest chemical propiconazole (Mentor 45WP). More thorough trials testing propiconazole and other postharvest products with larger sample sizes and more detailed results will be presented at the research review (February 20, 2025) but are not available in this report due to the submission deadline. Testing from the original proposal was going to involve testing natamycin with postharvest wax however these plans were changed after learning that in some packinghouses post-harvest waxes are used on red 'd'Anjou' pears but are not commonly used on green 'd'Anjou' pears (industry communication). Furthermore, while the lab results have demonstrated a certain amount of initial Mucor rot suppression via natamycin (BioSpectra 100SC), high Mucor rot disease variability year-to-year, as well as the potentially limited term fungistatic nature of natamycin may necessitate additional disease control strategies. As a result, we propose to continue testing BioSpectra 100SC in combination with additional products such as BioSave, Mentor, and Aureo Shield. We will then move on to testing these products in combination with postharvest waxes to demonstrate the best strategy for combinations of post-harvest chemicals to be used for controlling Mucor rot.

Fruit quality

Within the fruit quality evaluations there were no statistically significant differences between the fruit weight and I_{AD} in response to treatment, and while there were effects on firmness and titratable acidity, result were not consistent throughout the experiments (Table 3, 4, 5, 6). Fruit quality near-harvest for fruit from Hood River, OR (Table 3) suggests a slight reduction in firmness in response to natamycin dip application, but this result is not consistent (Tables 4, 5, and 6). Similarly, titratable acidity was slightly lower for natamycin treated fruit near-harvest for the Cashmere, WA location (Table 4), but this result is also inconsistent (Tables 3, 5, and 6).

Fruit quality may have contributed to the statistically significant differences between the trial with the least amount of Mucor infection (Cashmere, WA at harvest) and the trial with the most amount of Mucor rot infection (Hood River, OR, at harvest) (Table 1). The fruit from Cashmere was smaller and firmer at harvest with an average weight of 206 grams and an average firmness of 13.3 lbs. at harvest. The fruit from Hood River, OR had an average weight of 217 grams with an average firmness of 14.4 lbf (Table 3, 4). Spotts (1985) reported that immature ‘d’Anjou’ pears are generally more resistant to decay, but the present results (two sites) do not conform to this observation, as the firmness of 13.3 lbf (less firm) would suggest that the fruit from Cashmere, WA were more mature at harvest. Additionally, 13% of the fruit from the Hood River, OR at harvest trial was reported to have had cork spot which likely also contributed to the advanced decay. There was no phytotoxicity observed within any of the fruit quality evaluations with less than 1% scald or scuff reported.

Table 3. Fruit quality for at harvest ‘d’Anjou’ pears treated with natamycin compared with untreated control. Fruit was grown at Hood River, OR and harvested on 9/4/2024. Natamycin treatments and day 0 fruit quality evaluations were conducted on 9/4/2024. Fruit was stored in cold storage at -0.5°C ± 0.5°C (31°F). Follow-up fruit quality evaluation was conducted on 9/25/2024.

Treatment	Days post-treatment	Weight (g)	I_{AD}[1]	Firmness (lbf)	Soluble solids content	Titratable acidity (%)
Control	0	223.8 ns[2]	1.9 ns	15.3 ac[3]	11.9 ns	0.36 a
	21	212.0 ns	1.8 ns	14.1 b	11.9 ns	0.37 a
Natamycin (500 ppm)	0	214.2 ns	1.9 ns	14.8 ab	11.9 ns	0.38 a
	21	209.9 ns	1.8 ns	13.9 c	11.9 ns	0.35 a
Natamycin (1000 ppm)	0	213.3 ns	1.9 ns	14.7 abc	11.9 ns	0.24 b
	21	214.5 ns	1.9 ns	13.8 c	11.9 ns	0.36 a

1. Higher values indicates more green.
2. ns, not significant.
3. Where the overall model was significant (p <0.05), Tukey’s *post hoc* was performed. Means in a column followed by different letters indicates a significant difference between these means.

Table 4. Fruit quality for at harvest ‘d’Anjou’ pears treated with natamycin compared with untreated control. Fruit was grown near Cashmere, WA and was harvested on 9/14/2024. Fruit was stored in cold storage at -0.5°C ± 1.5°C (31°F). Natamycin treatments and fruit quality evaluations were completed on 9/17/2024.

Treatment	Weight (g)	I_{AD}	Firmness (lbf)	Soluble solids content	Titratable acidity (%)
Control	207.4 ns[1]	1.7 ns	13.6 ns	12.8 ns	0.37 a[2]
Natamycin (500 ppm)	208.7 ns	1.7 ns	13.4 ns	13.1 ns	0.25 c
Natamycin (1000 ppm)	201.2 ns	1.7 ns	12.9 ns	12.8 ns	0.30 b

1. ns, not significant.
2. Where the overall model was significant (p <0.05), Tukey's *post hoc* was performed. Means in a column followed by different letters indicates a significant difference between these means.

Table 5. Fruit quality evaluation for Hood River, OR fruit used in the experimental dump tank trial. The fruit was commercially harvested on 9/8/2024 and kept in cold storage at -0.5°C ± 1.5°C (31°F). Natamycin treatments occurred on 10/22/2024. Fruit was left at room temperature for one week with a follow up fruit quality evaluation conducted on 10/29/2024.

Treatment	Days post-treatment	Weight (g)	I_{AD}	Firmness (lbf)	Soluble solids content	Titratable acidity (%)
Control	0	220.3 ns[1]	1.8 ns	13.5 a[2]	6.9 a	0.30 a
	7	228.0 ns	1.6 ns	8.1 b	7.1 b	0.27 ab
Natamycin (500 ppm)	7	205.3 ns	1.6 ns	8.2 b	8.2 b	0.25 b
Natamycin (1000 ppm)	7	225.5 ns	1.7 ns	10.3 b	10.3 b	0.29 a

1. ns, not significant.
2. Where the overall model was significant (p <0.05), Tukey's *post hoc* was performed. Means in a column followed by different letters indicates a significant difference between these means.

Table 6. Fruit quality evaluation for 'd'Anjou' pears grown near Cashmere, WA that were used in the experimental dump tank trials. Fruit was harvested on 9/14/2024 and was stored in cold storage at -0.5°C ± 1.5°C (31°F). Experimental dump tank trials were conducted on 11/5/2024. Fruit was left at room temperature with a fruit quality evaluation being conducted on 11/13/2024.

Treatment	Weight (g)	I_{AD}	Firmness (lbf)	Soluble solids content	Titratable acidity (%)
Control	168.1 ns[1]	1.5 ns	3.1 a[2]	2.9 a	0.25 a
Natamycin (500 ppm)	165.7 ns	1.5 ns	5.6 b	5.7 b	0.27 b
Natamycin (1000 ppm)	180.5 ns	1.6 ns	4.9 b	4.9 b	0.26 ab

1. ns, not significant.
2. Where the overall model was significant (p <0.05), Tukey's *post hoc* was performed. Means in a column followed by different letters indicates a significant difference between these means.

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Executive summary

Title: Efficacy of Natamycin for Control of Mucor Rot in Pear Fruit

Keywords: 'd'Anjou' pears, BioSpectra 100SC, Mentor, natamycin, Mucor rot

Abstract:

Mucor rot is caused by the pathogen *Mucor piriformis*, which can be responsible for severe post-harvest decay in pears. For this study, the efficacy of BioSpectra 100SC (active ingredient natamycin) was tested in a laboratory setting. Testing was conducted at harvest and at five-months post-harvest. The most effective treatment was found to be the highest label rate of BioSpectra 100SC (1000 ppm natamycin) applied at harvest which reduced Mucor rot infection by 73%. Applying BioSpectra 100SC as soon as possible after harvest was more effective than applying later in the storage season. The 1000 ppm natamycin treatments resulted in lower numerical disease incidence and smaller average lesion diameters when compared to the lowest label rate of BioSpectra 100SC (500 ppm natamycin) when the at harvest and at five-month postharvest trials were combined and averaged. Additional laboratory trials were also conducted to determine the amount of disease incidence incurred in 'd'Anjou' pears when BioSpectra 100SC and *Mucor piriformis* inoculum are combined in an experimental dump tank. On average, the dump tanks with 500ppm and 1000ppm natamycin treatments reduced the average percent of fruit infected by 53% and 72% respectively. The fungicide Mentor 45WP combined with the highest label rate of BioSpectra 100SC reduced Mucor rot by 22% in a small preliminary trial. Further testing of BioSpectra 100SC in combination with other post-harvest chemicals is proposed for 2025-2026. This research has improved our understanding of the efficacy of natamycin for controlling Mucor rot on 'd'Anjou' pears in a laboratory setting. BioSpectra 100SC is currently the only commercially available postharvest fungicide that we are aware of that has been shown to help reduce Mucor rot incidence. Aside from the Mucor rot control, BioSpectra 100SC can be used as a rotational fungicide to help reduce fungicide resistance as according to the label BioSpectra 100SC also helps to control blue and grey mold.