

A Final Report for a Research and Extension Project Submitted to:
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Efficacy of alternative fungicides for grape downy mildew disease management

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INTRODUCTION AND OBJECTIVES

As public concern over food safety continues to grow, there is always a need to provide more 'low impact' fungicide options for the juice grape industry to reduce synthetic inputs and continue maximizing the healthy profile of their product. Over the past several years, research has generated useful information regarding alternatives for powdery mildew control, but data on alternatives for control of other diseases is scarce and there are currently no low impact alternatives that can be recommended to control downy mildew on Concord or Niagara grapes.

Therefore, in 2018 we began a two-year trial in our experimental block of *Vitis* interspecific hybrid 'Chancellor' to examine and evaluate two alternative or 'reduced risk' crop protection materials for downy mildew control. Our goal was to determine the potential of these low impact materials for integration into juice grape disease control programs without endangering crop quality or yield.

The products we evaluated were LifeGard and Zonix. LifeGard is an OMRI listed, biological fungicide, based on a bacterium (*Bacillus mycoides*). LifeGard does not act directly on fungal pathogens but claims to control disease by priming/activating/heightening the natural defenses of grapevines against pathogens. LifeGard is already labeled for use on grapes and has a 4-hour reentry interval and a 0 day pre-harvest interval. LifeGard has provided good control of downy mildew in several New York efficacy trials in the Finger Lakes, but it has not been successfully tested in the Lake Erie region.

Zonix, another biofungicide is currently labeled for use on ornamentals and turf, but not yet on grapes. Zonix is a rhamnolipid biosurfactant that kills zoospores, the infective spores of the downy mildew pathogen. According to the marketer of this product, PropTera, 'When a zoospore comes in contact with a rhamnolipid, the zoospores' cell membrane explodes ending the pathogens life cycle. This physical mode of action will not contribute to or cause the development of resistant pathogen strains'. Initial results from replicated lab/greenhouse tests with Zonix on Chardonnay leaves in 2017, showed a high degree of efficacy against downy mildew, but we were not aware of any other data from tests on grape downy mildew.

EXPERIMENTAL PROCEDURES TO ACCOMPLISH OBJECTIVES

In 2018, a field trial was set up at the Lake Erie Regional Grape Research and Extension Center to examine the efficacy of these materials for control of downy mildew on Chancellor grapes under Lake Erie region climatic conditions. Chancellor grape was chosen (instead of Niagara) due its high susceptibility to this disease and greater likelihood of adequate disease levels coming off of two dry, 'downy mildew-less' seasons (2016 and 2017). This trial was conducted in a vineyard with mature vines trained to a single-curtain, high-wire cordon system. Treatments were applied to four-vine plots

in a randomized complete block design with four replications. All treatment applications started just before the 5-6 leaf stage (about 3 weeks prior to bloom), when downy mildew first becomes a threat. Applications were made with a Friend covered-boom plot sprayer at 125 psi and 50 gal/A. Our treatment objectives were: i) to compare the efficacy of solo programs of LifeGard and Zonix - applied every 10 days - to a program of a known standard (Manzate Prostick) applied every 14 days, for grape downy mildew control, and ii) to evaluate LifeGard and Zonix as rotational and tank mix partners with Manzate Prostick (can they be integrated with standard fungicides to maintain good downy mildew control and reduce the use of standard fungicides). Powdery mildew was controlled throughout the block in both years with regular cover sprays of Luna Experience (6 fl oz/A), Quintec (4 fl oz/A), Vivando (12 fl oz/A) and Torino (3.4 fl oz/A). Downy mildew incidence (percent clusters diseased) and severity (percent area diseased among all clusters) were determined on 7 August (2018) and 26 August (2019) from 50 randomly selected clusters per plot. Data were subjected to analysis of variance (ANOVA) using the general linear model function in Minitab 18 (2018) and Minitab 19 (2019).

2018 trial:

In 2018, total rainfall for May, June, July, August, and September was 4.67, 3.74, 2.14, 4.02, and 5.01 in., respectively. Downy mildew disease development was greatly delayed in 2018 and was not observed on clusters until near the end of July when fruit were considered resistant to direct infection. Among the factors that contributed to this were: i) a relatively small overwintering inoculum pool from the previous two, relatively dry growing seasons, and ii) dry conditions during the ten days prior to bloom which provided little chance for pre-bloom infections of any kind. Wet conditions did occur from beginning of bloom until about ten days after end of bloom (3.65" rain in two and half weeks) but there was little overwintering inoculum and no disease in the vineyard for an epidemic to develop from. This wet period was again followed by extremely dry conditions for the next three and half weeks, until what was considered the end of fruit susceptibility. Nevertheless, all spray programs were continued to provide protection during this entire period (table 1).

Table 1: Downy mildew on Chancellor clusters on 7 August, 2018

Treatment and rate/A	Days after first application ^z						Incidence (%)	Severity (%) ^y	Control (%) ^x
Manzate Prostick 75DF 3 lb	0,	14,	28,	42,	56		4.0 b ^w	0.09 b ^w	99
Manzate Prostick 75DF 3 lb	0,	24,	48						
Zonix 38 fl oz		14,	38,	63		4.5 b	0.15 b	99	
Manzate Prostick 75DF 1.5 lbs + LifeGard 2.25 oz + Induce 0.125%	0,	14,	28,	42,	56	3.0 b	0.16 b	99	
Manzate Prostick 75DF 1.5 lbs + Zonix 19 fl oz	0,	14,	28,	42,	56	5.5 b	0.18 b	98	
Manzate Prostick 75DF 3 lb	0,	24,	48						
LifeGard 4.5 oz + Induce 0.125%		14,	38,	63		9.0 b	1.48 b	86	
LifeGard 4.5 oz + Induce 0.125%	0,10,	20,	29, 40,	50, 60		47.0 a	7.99 ab	26	
Zonix 38 fl oz	0,10,	20,	29, 40,	50, 60		61.5 a	11.73 a	0	
Untreated Control						63.5 a	10.73 a		

^zThe first fungicide application was on 24 May. 0 = 4-5" shoots; 10 = 10" shoots, 6 leaves; 14 = 12" shoots, 7 leaves; 20 = beginning bloom; 24 = late bloom; 28 = early post bloom; 29 = early post bloom; 38 = post fruit set; 40 = three weeks after trace bloom, 42, 48 = four weeks after trace bloom, 50, 56 = five weeks after trace bloom, 60, 63 = six weeks after trace bloom.

^ySeverity was rated using the Barratt-Horsfall scale (0-11) and was converted to % area infected (0-100 %) using Elanco conversion tables.

^xPercent control = control of disease severity on clusters relative to the untreated control.

^wMeans followed by the same letter within a column are not significantly different according to Fisher's LSD ($P \leq 0.05$).

When downy mildew was finally observed on clusters in late July, our cluster assessments determined that solo programs of LifeGard and Zonix failed to reduce the disease. However, rotational and tank mix programs of Manzate Prostick with either LifeGard or Zonix, provided nearly complete control in most cases, statistically equivalent to a typical conventional program of Manzate Prostick. There was no phytotoxicity associated with any of the treatments.

When we compare the levels of synthetic pesticide inputs for each program, the rotations and tank mixes reduced Manzate Prostick inputs by about 40 and 50%, respectively, over Manzate Prostick alone, without compromising disease control and crop quality. This is perhaps the most important result of this trial. However, the lack of disease pressure during most of the cluster susceptibility period, reduces the value of the trial and any conclusions we might draw from it.

2019 Trial:

In 2019, total rainfall for May, Jun, Jul, Aug, and Sep was 2.74, 4.58, 2.90, 4.04, and 2.04 in., respectively. Similar to 2018, downy mildew disease pressure was low and was not observed on clusters until after fruit were considered resistant or nearly resistant, to direct infection. Indeed, fruit losses to downy mildew in unsprayed vines was even lower than in the previous season. Most fruit symptoms were characterized as “leather berry” and likely occurred via infection through the berry stem. As in 2018, factors that contributed to this were: i) a relatively small overwintering inoculum pool from the previous three growing seasons, and ii) dry conditions from bloom through the first half of July. Over 2.5 inches of rain fell during the second half of July, inciting a modest level of disease in the trial block. All spray programs were continued to provide protection through the end of July.

Once again, LifeGard, applied on a 10-day schedule, failed to control both incidence and severity of fruit disease. Zonix alone, controlled incidence but like LifeGard, failed to control severity. All treatments containing Manzate Prostick provided significant control of both incidence and severity and were significantly more effective than Zonix and LifeGard. There was no phytotoxicity associated with any of the treatments.

Table 2: Downy mildew on Chancellor clusters on 26 August, 2019

Treatment and rate/A	Days after first application ^z						Incidence (%)	Severity (%) ^y	Control (%) ^x
Manzate Pro-Stick 75DF 3 lb	0,	14,	29,	43,	56		5.5 c ^w	0.18 b ^w	86
Manzate Pro-Stick 75DF 3 lb	0,		25,		49				
Zonix 38 fl oz		14,		39,		63	5.5 c	0.17 b	87
Manzate Pro-Stick 75DF 1.5 lbs + LifeGard WP 2.25 oz + Induce 0.125%	0,	14,	29,	43,	56		7.5 c	0.19 b	85
Manzate Pro-Stick 75DF 1.5 lbs + Zonix 19 fl oz	0,	14,	29,	43,	56		8.5 c	0.25 b	81
Manzate Pro-Stick 75DF 3 lb	0,		25,		49				
LifeGard WP 4.5 oz + Induce 0.125%		14,		39,		63	6.5 c	0.25 b	81
LifeGard WP 4.5 oz + Induce 0.125%	0,11,	21,	31,	41,	50,	60	27.0 ab	1.76 a	0
Zonix 38 fl oz	0,11,	21,	31,	41,	50,	60	23.5 b	1.09 a	16
Untreated Control							36.0 a	1.30 a	

^zThe first fungicide application was on 27 May. 0 = 4 leaves/shoot; 11 = 12” shoots, 7 leaves; 14 = 8 leaves; 21 = 9 leaves; 25 = 11 leaves; 29 = trace bloom; 31 = mid bloom; 39 = early post bloom; 41 = two weeks after trace bloom, 43, 49 = three weeks after trace bloom, 50, 56 = four weeks after trace bloom, 60, 63 = five weeks after trace bloom.

^ySeverity was rated using the Barratt-Horsfall scale (0-11) and was converted to % area infected (0-100 %) using Elanco conversion tables.

^xPercent control = control of disease severity on clusters relative to the untreated control.

^wMeans followed by the same letter within a column are not significantly different according to

Fisher's LSD ($P \leq 0.05$).

Field Inoculations

In addition to small plot, whole vine field trial evaluations (spray and wait for disease to develop naturally), we planned to conduct inoculations of field grown clusters to more directly assess the activity and rain-fastness of materials under test. Unfortunately, this failed in 2018 as inoculum did not become available until it was too late to make field inoculations. In fact, the dry spring/early summer conditions coupled with low overwintering inoculum (from two previous dry years), left even untreated Chancellor grapevines free of downy mildew during the entire period when clusters were susceptible to direct downy mildew infection.

In year two, we successfully collected an isolate of the downy mildew pathogen and generated lab cultures for direct inoculation of clusters, which would enable us to more directly assess the activity of materials under test. A separate group of 10 clusters of the unsprayed check, Manzate Pro-Stick, Zonix, and LifeGard solo treatments were inoculated with the downy mildew pathogen. Clusters were subsequently evaluated for disease development over the subsequent 4 weeks after inoculation. Inoculation was with 5×10^4 spores per ml (first two inoculations) and 1×10^5 spores per ml (last two inoculations) harvested from leaf cultures maintained in the laboratory, and applied with a small, hand-held preva sprayer. With all inoculations, inoculum was tested and viability confirmed. After inoculation, clusters were enclosed within Ziploc bags for 8 hours (first 3 inoculations) or overnight (16 hours/last inoculation) at field temperatures. Bagged clusters were covered with white sheets to prevent exposure to direct sun which would otherwise 'cook' clusters inside. After the infection period, the bags were removed and the clusters observed for symptom development over the next few weeks.

The results of the field inoculations:

Inoculations were made on July 1, 3, 8, and 17, and the dryness of the post bloom period limited our ability to assess rainfastness. The first inoculation was performed under hot, dry conditions, from morning to mid-day and yielded very low levels of infection. We suspect that the majority of inoculum dried up too quickly on clusters, despite being enclosed in plastic Ziploc bags immediately after inoculation. Wash-off by rain could not have been a factor since almost no rain fell (0.01") between the last fungicide application and this first inoculation. However, the subsequent three inoculations were more successful and were pooled as separate replications to an overall mean that could be statistically analyzed.

Among the inoculations, Manzate ProStick provided almost complete control of downy mildew from inoculations made about a week after the last application, regardless of whether 0.3" or 0.8" of rain fell. However, an inoculation made about two weeks after the last application (at the very end of a typical 14 day spray interval) yielded only about 68% control of the disease with Manzate, despite only being challenged by 0.3" of rain. The reason for the lower control could relate to the rapid expansion of berries over that time, leaving breaches in the film of fungicide residue that the pathogen could exploit. For the Zonix and LifeGard treatments, control was more consistent with Zonix, ranging from 54-89% control (average of 78%, which was significant). On the other hand, LifeGard provided the least consistent, and lowest level of control; 0-82% control across the three inoculations (average of 51% control, which was not significant). The best control for LifeGard came from inoculations 1 and 6 days after an application, whereas no control was achieved after an inoculation made just hours after an application (and 10 days from the previous application). This could relate to the claim that LifeGard does not act directly on fungal pathogens but claims to control disease by priming/activating/heightening the natural defenses of grapevines against pathogens; in other words, at

least a day or two is required for an application to establish some level of natural defense against the downy mildew pathogen, but perhaps 10 days is too long to continue to expect protection. Overall, the average of the incidence ratings did not render a significant difference between the Zonix and LifeGard treatments and the check. On the other hand, Manzate did significantly reduce the average incidence of downy mildew (data not shown). The average severity rating showed that Manzate and Zonix provided for significant reductions in downy mildew, but LifeGard did not (Table 3).

Table 3: Downy mildew on Chancellor clusters from inoculations performed during the early post bloom period.

Treatment and rate/A	Dates of application						Average
	July 3		July 8		July 17		
	Incidence	Severity ^z	Incidence	Severity ^z	Incidence	Severity ^z	Severity ^y
*Manzate Pro-Stick 75DF 3 lb	0.0	0.00	70.0	4.91	25.0	0.94	1.95 b ^x
Zonix 38 fl oz	50.0	2.81	100.0	7.02	90.0	2.81	4.21 b
LifeGard WP 4.5 oz + Induce 0.125%	100.0	6.55	100.0	17.32	90.0	4.68	9.52 ab
Untreated Control	100.0	16.78	100.0	15.21	100.0	26.44	19.48 a

^zSeverity was rated using the Barratt-Horsfall scale (0-11) and was converted to % area infected (0-100 %) using Elanco conversion tables.

^yOverall severity from pooled ratings, showing significant separations between treatments.

^xMeans followed by the same letter within a column are not significantly different according to Fisher's LSD ($P \leq 0.05$).

Outreach: The results of these trials have and will be presented and discussed with processors at grower processor field rep meetings, and with growers at 'coffee pot' meetings and other extension based, grower meetings in the Lake Erie area in 2019 and 2020.

Impact Statement: Manzate Prostick inputs were reduced by about 40 and 50% by rotations and tank mixes, respectively (with either Zonix or Lifeguard), over Manzate Prostick alone, without compromising disease control and crop quality in either year. However, the lack of disease pressure during most of the cluster susceptibility period in both years, reduces the value of the conclusions we might draw from it.

Publications: The results of the 2018 trial were published in Plant Disease Management Reports, an online publication through the American Phytopathological Society.