EXTENDED ABSTRACT

Field evaluation of bio-fungicides to control powdery mildew (*Erysiphe necator*) of wine grapes in California

Shijian Zhuang[1], Edgar Godoy Monterroso[2], Qun Sun[3], Yu-Chen Wang[4], Matthew Fidelibus[5], Philippe Rolshausen[6], Shunping Ding[2]
*Corresponding author: sding01@calpoly.edu

[1] University of California Cooperative Extension, Fresno Co.
[2] Wine and Viticulture Department, California Polytechnic State University-San Luis Obispo
[3] Department of Viticulture and Enology, California State University, Fresno
[4] University of California Cooperative Extension, Monterey Co.
[5] Department of Viticulture and Enology, University of California, Davis
[6] Department of Botany and Plant Sciences, University of California, Riverside

Keywords: powdery mildew, bio-fungicides, integrated pest management, fungicide rotation

ABSTRACT

Context and Purpose. Powdery mildew (*Erysiphe necator*) is one of the most significant fungal diseases affecting grapevines globally. Effective control typically requires an intensive spray program during the growing season, particularly before veraison, to mitigate impacts on yield and fruit quality. Integrated pest management (IPM), which combines timely fungicide applications with cultural practices, remains a cornerstone of disease control in vineyards. However, due to increasing regulatory restrictions and growing concerns over environmental sustainability and fungicide resistance, the California grape industry is shifting toward the use of biological fungicides (bio-fungicides) as alternatives to synthetic chemistries. This study aimed to evaluate the efficacy of bio-fungicides alone, synthetic fungicides alone, and rotational spray programs combining both, in managing powdery mildew under commercial vineyard conditions. Four field trials were conducted across two seasons (2023–2024) at Fresno site in the San Joaquin Valley and San Luis Obispo site in California's Central Coast, each with one white, Chardonnay, and one red, Carignan in Fresno and Pinot Noir in San Luis Obispo, *Vitis vinifera* cultivars selected.

Materials and Methods. A randomized complete block design with four replications was implemented at Fresno and San Luis Obispo vineyard sites. Thirty fungicide treatments, along with a no-spray control, were evaluated. Three bio-fungicides—*Bacillus subtilis* (Serenade®), *Streptomyces lydicus* (Actinovate®), and *Reynoutria sachalinensis* extract (Regalia®)—were selected. Sulfur-based products were applied pre-bloom, and experimental treatments began at bloom and continued until veraison. Sprays were scheduled weekly, biweekly, or based on the Thomas-Gubler Powdery Mildew Risk Index. Disease incidence and severity were assessed prior to veraison, and yield components, primary fruit chemistry, and secondary metabolites were measured at harvest.

Results. Spray rotation programs that integrated bio-fungicides with synthetic fungicides demonstrated comparable or superior control of powdery mildew compared to programs using synthetic or biological products alone.

INTRODUCTION

Powdery mildew is one of the most important fungal diseases for grape production worldwide (Gadoury et al., 2012). The obligatory parasite, *Erysiphe necator*, has the disease cycle starting from the sexual reproduction of chasmothecia as the overwinter structure where ascospores are released from and infect green tissues, establish mycelial colonies, and produce conidia under favorable environmental conditions. The asexual cycle can repeat every 5–9 days under optimal temperatures ranging from 21–30 °C, leading to rapid epidemic development (Carroll & Wilcox, 2003). *Vitis vinifera* cultivars are particularly susceptible, and environmental conditions such as temperature and high relative humidity (RH) strongly influence infection risk (Gadoury et al., 2012).

Vineyard cultural practices, such as pruning, shoot thinning, leaf removal, and hedging, can all reduce the disease risk by reducing RH and improving fungicide spray penetration (Austin & Wilcox, 2011). Canopy management practices that increase sunlight and UV exposure in the cluster zone, such as leaf removal, are also effective in suppressing mildew development (Gubler et al., 1999). Additionally, irrigation and nutrient strategies (e.g., deficit irrigation and balanced fertilization) help prevent excessive canopy growth and maintain light exposure (Smart & Robinson, 1991).

Timely fungicide application remains a critical control strategy. Preventative fungicides should be applied every 7–14 days depending on disease pressure, which is driven by temperature and humidity (Gubler et al., 1999). Sulfur-based fungicides have been widely used due to their efficacy and affordability. More recently, synthetic fungicides, including demethylation inhibitors (DMIs), strobilurins, and quinolines, have improved control efficacy, although resistance risk necessitates rotating modes of action (Brent & Hollomon, 2007).

Biological fungicides (bio-fungicides), composed of beneficial microbes, natural compounds, or plant extracts, offer environmentally friendly alternatives with lower resistance risks (Pertot et al., 2017). While their efficacy is generally lower than synthetics, their use is growing, especially in organic systems. The increasing regulatory pressure in California and market demand for sustainability have encouraged integrated use of synthetic and bio-fungicides through IPM (Integrated Pest Management) (Bailey et al., 2010).

MATERIAL AND METHODS

Vineyard Site

Two separate spray experiments were conducted in a commercial vineyard located in Fresno (36.674455, -119.915405) and San Luis Obispo (35.318768, -120.682448) and grapevines were planted on Pachappa fine sandy loam and Los Osos loam soil (www.nrcs.usda.gov). The vineyard in Fresno was planted in 2013 with Chardonnay and Carignan (*Vitis vinifera* L.) on Freedom (*solonis* × *Othello* × *Dogridge*) rootstock and the vineyard in San Luis Obispo was planted in 2018 with Chardonnay and Pinot Noir (*Vitis vinifera* L.) on 1103 Paulsen (*Berlandieri* × *Rupestris*) rootstock. The grapevine plant spacing was 1.5 m × 3.0 m (grapevine × row) with the rows-oriented East-West in Fresno and spacing was 1.5 m × 2.5 m (grapevine × row) with rows-oriented Southeast-Northwest in San Luis Obispo. The grapevines were quadrilateral cordon trained, with a 55 cm cross-arm, to 1.2 m height above vineyard floor with a pair of catch wires 30 cm above the cordons to form the "California Sprawl" in Fresno and grapevines were bilateral cordon trained, with four pairs of catch wire above the cordons to from the vertical shoot positioning canopy system. Shoot thinning and basal leaf removal were carried out during the growing season at San Luis Obispo site, and as a comparison, none of those practices were carried out at Fresno site. All cultural practices except shoot thinning and leaf removal were carried out according to University of California Cooperative Extension (Zhuang et al., 2019).

Experimental Design

The randomized complete block design was applied for both experimental sites in 2023 and 2024. Three adjacent grapevines comprised one experimental unit and one buffer vine was selected to separate experimental units. A total of 56 vines were chosen as an experimental block, replicated in four times. Thirty treatments plus no-spray control were randomly assigned in each experimental block. The treatment plan is illustrated in Table 1.

Weather Station

Two weather stations were installed at experimental vineyards: one at the Fresno site and one at the San Luis Obispo site. The weather stations (WatchDog 3240 wireless station – LTE-M modem, Spectrum Technologies, Inc. Aurora, IL) with temperature probe and leaf wetness sensor installed inside the grapevine canopy was established before the spray. The Thomas-Gubler index (index) was calculated based on the hourly temperature of 21-30 °C and the index was scaled from 0 to 100. Index < 30 was considered as low disease risk, 30-50 as moderate disease risk, and > 60 as high disease risk. The index was used by the end of each week to decide the spray interval for the following week.

Spray Program

Three bio-fungicides were applied in the experiment: *Bacillus subtilis* (Serenade® as the commercial product), *Streptomyces lydicus* (Acinovate® as the commercial product), and extract of *Reynoutria sachalinensis* (Regalia® as the commercial product). Three wettable or dust sulfur were applied prior to bloom and the treatment spray started at bloom. Four spray intervals were applied for each bio-fungicide: weekly, biweekly, based on index, and rotation with synthetic fungicide. Bio-fungicide spray interval based on index was scheduled weekly if the index > 30 and bi-weekly if the index < 30 and rotation program was scheduled using bio-fungicide if the index < 30 and using synthetic fungicide if the index > 30. Due to the high disease pressure based on the index in San Luis Obispo, for the rotational program in 2024, two bio-fungicide applications were intentionally scheduled after each synthetic fungicide application. Grower standard was consistent across two sites and two growing seasons starting with the bloom spray then followed by bi-weekly synthetic fungicide spray (Table 1). The final spray schedules in 2023 and 2024 were illustrated in Tables 2 and 3. Backpack sprayer (STHL SR 430, STIHL Incorporated, Virginia Beach, VA) and 100 gallons per acre equivalent were used to spray the canopy to achieve good coverage.

Disease Rating

Disease incidence and severity were rated three weeks, two weeks, and one week prior to veraison. Disease incidence was determined by counting the number of clusters that had any berries showing white mycelia, and the percentage of clusters with mildew was then calculated as the disease incidence. Disease severity was determined by sampling twenty random clusters with ten from one side of the canopy and another ten from the opposite side of the canopy at Fresno site and twenty-five random clusters at San Luis Obispo site with the similar sampling strategy, and disease severity was calculated as the percentage of berries with mildew on each sampled cluster.

Yield Components and Berry Chemistry

When the berry Brix reached approximately 21° for white and 24° for red cultivars, yield components (number of clusters, average cluster weight, average berry weight, and number of berries per cluster) were determined at harvest from each experimental grapevine using methods similar to those described by Zhuang et al. (2014). Berry Brix, pH, titratable acidity (TA, g/L of tartaric acid), anthocyanins and phenolics for red varieties were measured in the lab.

Statistical Analysis

All data were tested for normality using Shapiro-Wilk's test. When the normality test failed, data were log or square root transformed to pass the test. If the transformed data still failed the test, the test proceeded with the Kruskal-Wallis rank sum test, and differences among treatment means were separated by Dunn's test at $p < 0.05$. When the normality test succeeded, two-way ANOVA (spray × year) was run for disease incidence, severity, yield components, and harvest berry chemistry using the PROC MIXED procedure of SAS (v.9.4; SAS Institute, Inc., Cary, NC). Differences among treatment means were tested at $p < 0.05$ using Fisher's protected Least Squares Means under the Mixed Procedure. Results from Chardonnay in Fresno and San Luis Obispo were presented and discussed here.

RESULTS AND DISCUSSION

1. Year had a significant effect on disease pressure, with much more elevated disease pressure in 2023 than in 2024 (Figure 1 and 2).

2. For the Fresno trials, *B. subtilis,* when applied weekly showed the best disease control efficacy among three bio-fungicides and the same as the grower standard in 2023. The rotational program that integrated synthetic and bio-fungicides showed similar efficacy in comparison to the grower standard in 2023. All bio-fungicides showed the same disease control efficacy as the grower standard in 2024, which was a much lower disease pressure year (Table 4 and 5).

3. For the San Luis Obispo trials, *R. sachalinensis* applied at a higher frequency showed better disease control efficacy than the other bio-fungicides. The rotational program that integrated synthetic and bio-fungicides showed similar efficacy in comparison to the grower standard.

4. Despite different trellis and canopy management at Fresno and San Luis Obispo sites, the rotational program showed significantly better efficacy than bio-fungicides alone and the same as the grower standard.

5. Yield and berry chemistry were observed to be affected by disease severity, but not by the type of bio-fungicides or the frequency of bio-fungicides used in the programs.

CONCLUSION

Our study demonstrated that a rotational program integrated synthetic, and bio-fungicides achieved the same level of disease control as synthetic fungicides alone, across two vineyard sites with very different trellises and canopy management practices. The consistent results confirmed that a rotation strategy guided by the Thomas-Gubler index provided a sustainable and effective approach to managing powdery mildew while reducing the reliance on synthetic fungicide applications. As regulations tighten and market demand increases for environmentally friendly disease control solutions, our rotational program proved to be an effective tool for managing grapevine powdery mildew.

REFERENCES

Austin, C. N., & Wilcox, W. F. (2011). Effects of fruit-zone leaf removal, training systems, and irrigation on the development of grapevine powdery mildew. American Journal of Enology and Viticulture, 62(2), 193–198. https://doi.org/10.5344/ajev.2010.10067

Bailey, K. L., Boyetchko, S. M., & Längle, T. (2010). Social and economic drivers shaping the future of biological control: A Canadian perspective on the factors affecting the development and use of microbial biopesticides. Biological Control, 52(3), 221–229. https://doi.org/10.1016/j.biocontrol.2009.05.003

Brent, K. J., & Hollomon, D. W. (2007). Fungicide resistance in crop pathogens: How can it be managed? FRAC Monograph No. 1 (2nd ed.). Fungicide Resistance Action Committee.

Carroll, J. E., & Wilcox, W. F. (2003). Effects of humidity on the development of grapevine powdery mildew. Phytopathology, 93(9), 1137–1144. https://doi.org/10.1094/PHYTO.2003.93.9.1137

Gadoury, D. M., Cadle-Davidson, L., Wilcox, W. F., Dry, I. B., Seem, R. C., & Milgroom, M. G. (2012). Grapevine powdery mildew (Erysiphe necator): A fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Molecular Plant Pathology, 13(1), 1–16. https://doi.org/10.1111/j.1364-3703.2011.00728.x

Gubler, W. D., Rademacher, M. R., & Vasquez, S. J. (1999). Control of powdery mildew using the UC Davis Powdery Mildew Risk Assessment Index. University of California Agriculture and Natural Resources Publication.

Pertot, I., et al. (2017). Integrated control of plant diseases using biological and other non-chemical strategies: Challenges and perspectives. Frontiers in Plant Science, 8, 1838. https://doi.org/10.3389/fpls.2017.01838

Smart, R. E., & Robinson, M. (1991). Sunlight into Wine: A handbook for winegrape canopy management. Winetitles.

Zhuang, S., Tozzini, L., Green, A., Acimovic, D., Howell, S., Castellarin, S., Sabbatini, P. (2014) Impact of Cluster Thinning and Basal Leaf Removal on Fruit Quality of Cabernet Franc (Vitis vinifera L.) Grapegrapevines Grown in Cool Climate Conditions. Hortscience 49:750-756.

Zhuang, S., Fidelibus, M., Kurtural, K., Lund, K., Torres, G., Stewart, D., and Summer, D. (2019) Sample Cost to Establish A Vineyard and Produce Winegrapes. University of California Agricultural and Natural Resources. 2019 Sample Costs to Establish a Vineyard and Produce Winegrapes – Fresno, Madera, Merced, and Stanislaus Counties – Cabernet Sauvignon Variety (ucdavis.edu)

TABLES AND FIGURES

Table 1. Fungicide spray program at Fresno and San Luis Obispo sites in 2023 and 2024.

Treatment ID													
1	2	3	4	5	6	7	8	9	10	11	12	13	14
R[a]	R	R	R	A[b]	A	A	A	S[c]	S	S	S	Grower standard	No-spray control
Weekly				Weekly				Weekly					
	Bi-w				Bi-w				Bi-w			Bi-w[e]	
		RI[d]				RI				RI			
			Rotation				Rotation				Rotation		

[a]*R. sachalinensis*
[b]*S. lydicus*
[c]*B. subtilis*
[d]Spray interval based on the Thomas-Gubler Powdery Mildew Risk Assessment Index
[e]Grower standard spray started at bloom and followed bi-weekly spray interval. Fungicides used in this program include Luna Experience, Quintec, Vivando, Elevate, Torino, Miravis Prime, and Gatton.

Table 2. Fungicide spray schedule at Fresno site in 2023 and 2024.

Year	2023									
Date	05/06	05/13	05/22	05/30	06/05	06/12	06/20	06/26	07/05	07/10
Weekly			x	x	x	x	x	x	x	x
Bi-weekly			x		x		x		x	
Based on Index			x		x	x	x	x	x	x
Rotation			x	x	x		x		x	
Grower Standard			x		x		x		x	
Year	2024									
Date	05/06	05/13	05/22	05/30	06/05	06/12	06/20	06/26	07/05	07/10
Weekly	x	x	x	x	x	x	x	x	x	
Bi-weekly	x		x		x		x		x	
Based on Index	x	x	x		x		x		x	
Rotation	x		x		x		x		x	
Grower Standard	x		x		x		x		x	

Table 3. Fungicide spray schedule at San Luis Obispo site in 2023 and 2024.

Year	2023											
Date	05/31	06/07	06/14	06/21	06/28	07/05	07/12	07/19	07/26	08/02	08/09	08/16
Weekly	x	x	x	x	x	x	x	x	x	x	x	x
Bi-weekly	x		x		x		x		x		x	
Based on Index	x					x	x	x	x	x	x	x
Rotation	x		x		x		x	x	x	x	x	x
Grower Standard	x		x		x		x		x		x	
Year	2024											
Date	05/22	05/29	06/05	06/12	06/19	06/26	07/03	07/10	07/17	07/24	07/31	08/07
Weekly	x	x	x	x	x	x	x	x	x	x	x	x
Bi-weekly	x		x		x		x		x		x	
Based on Index	x		x	x	x	x	x	x	x	x	x	x
Rotation	x		x	x	x		x	x	x		x	x
Grower Standard	x		x		x		x		x		x	

Table 4. Disease incidence and severity at Fresno site in 2023 and 2024.

			Year 2023		Year 2024	
ID	Fungicide	Interval	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
1	*R. sachalinensis*	Weekly	100	74 a*	9	2
2	*R. sachalinensis*	Biweekly	100	78 a	12	1
3	*R. sachalinensis*	RI Based	100	71 abc	13	2
4	*R. sachalinensis*	Rotate	100	53 bcd	9	2
5	*S. lydicus*	Weekly	100	71 ab	7	1
6	*S. lydicus*	Biweekly	100	67 abc	16	2
7	*S. lydicus*	RI Based	100	60 abcd	14	3
8	*S. lydicus*	Rotate	100	43 d	17	1
9	*B. subtilis*	Weekly	92	42 d	6	1
10	*B. subtilis*	Biweekly	94	52 bcd	10	2
11	*B. subtilis*	RI Based	98	42 d	10	2
12	*B. subtilis*	Rotate	99	48 cd	11	1
13	Grower standard	Biweekly	97	39 d	12	2
14	Control	No-spray	100	66 abc	19	3

*Different letters within columns represent significant differences according to the Kruskal-Wallis rank sum test and Dunn's test at $p < 0.05$.

Table 5. Disease incidence and severity in Chardonnay at San Luis Obispo site in 2023 and 2024

			Year 2023		Year 2024	
ID	Fungicide	Interval	Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
1	*R. sachalinensis*	Weekly	100.0 a*	23.5 gh	63 bc	11.25 e
2	*R. sachalinensis*	Biweekly	100.0 a	34.0 ef	57 bc	16.35 e
3	*R. sachalinensis*	RI Based	100.0 a	34.7 ef	44 cd	6.9 f
4	*R. sachalinensis*	Rotate	100.0 a	16.4 gh	8 e	0.45 g
5	*S. lydicus*	Weekly	100.0 a	55.0 d	81 ab	20.75 d
6	*S. lydicus*	Biweekly	100.0 a	65.1 bc	94 a	46.85 b
7	*S. lydicus*	RI Based	100.0 a	36.7 e	75 ab	22.5 d
8	*S. lydicus*	Rotate	100.0 a	13.5 h	14 e	1.65 g
9	*B. subtilis*	Weekly	100.0 a	37.3 e	78 ab	20.4 d
10	*B. subtilis*	Biweekly	100.0 a	68.1 b	78 ab	26.8 c
11	*B. subtilis*	RI Based	100.0 a	55.1 cd	77 ab	21.1 d
12	*B. subtilis*	Rotate	100.0 a	25.6 fg	19 de	2.05 g
13	Grower standard	Biweekly	93.0 b	13.8 h	4 e	0.95 g
14	Control	No-spray	100.0 a	91.9 a	99 a	74.85 a

*Different letters within columns represent significant differences according to Fisher's protected LSD test at $p < 0.05$.

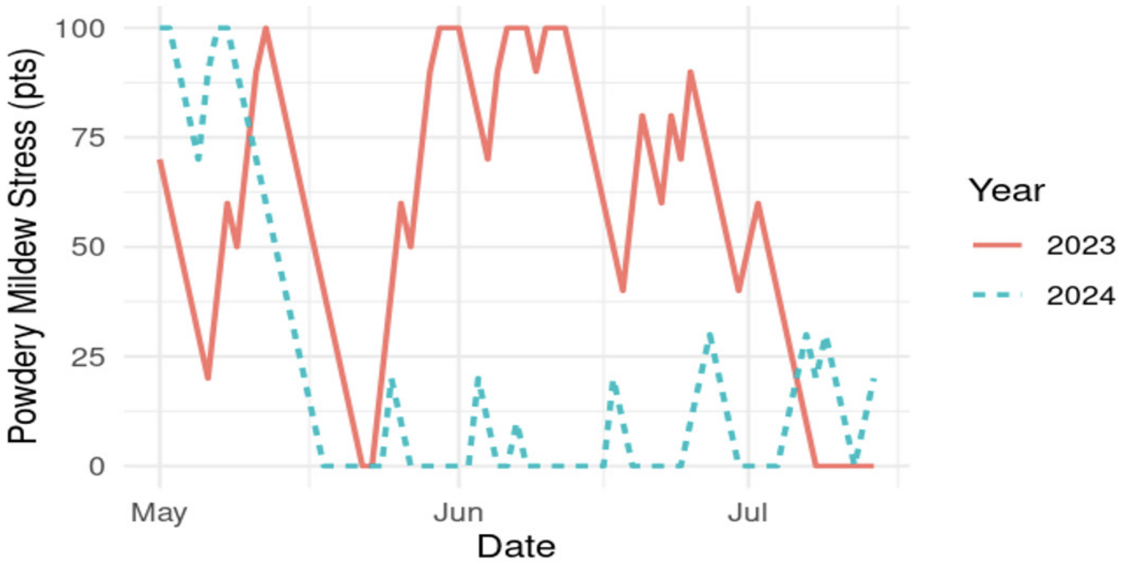

Figure 1. Thomas-Gubler index at the Fresno site in 2023 and 2024 from bloom to veraison.

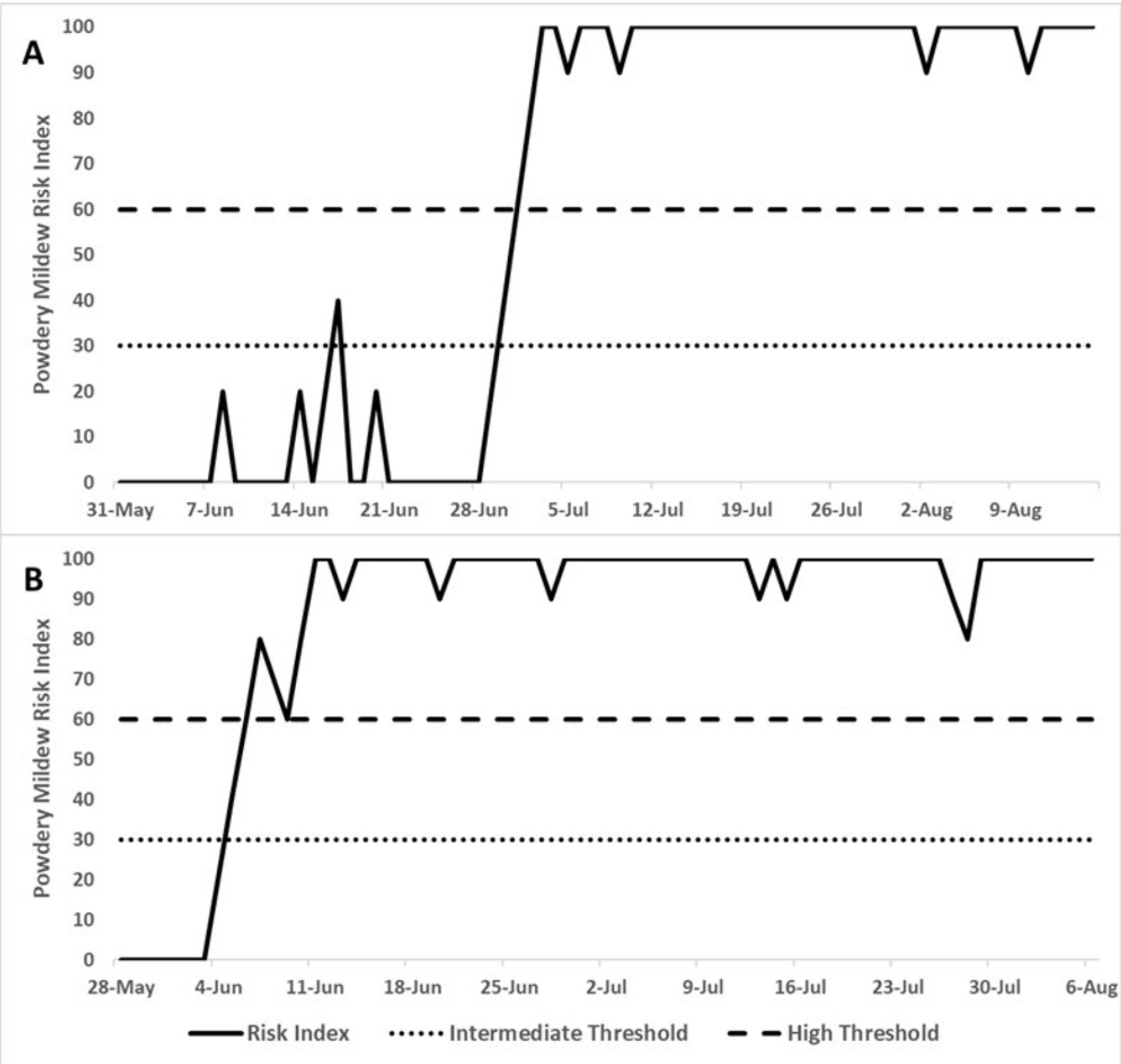

Figure 2. Thomas-Gubler index at the San Luis Obispo site in 2023 (A) and 2024 (B) from bloom to veraison.