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Reducing cluster rots in Michigan wine grapes using combined pathogen and vinegar fly control

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ARTICLE INFO	ABSTRACT		
A R T I C L E I N F O Keywords: Sour rot Vitis Drosophila Botrytis cinerea Postharvest diseases	Cluster rots pose a significant threat to the wine grape industry, leading to substantial economic losses. This study aimed to determine the performance of multiple treatments targeting cluster rot pathogens and their insect vectors over three years in southwest Michigan. Grape clusters were rated for disease incidence and severity, insects were collected and identified, and pesticide residues were measured on harvested clusters. In 2020, disease incidence decreased significantly when BLAD was applied in combination with the insecticides spinetoram, imidacloprid with beta-cyfluthrin, or zeta-cypermethrin. Similarly, in 2021, clusters treated with cyprodinil plus fludioxonil mixed with insecticides cyclaniliprole, spinosad, zeta-cypermethrin, or imidacloprid plus beta-cyfluthrin significantly reduced disease incidence compared to fungicide alone. Notably, in 2022, treating clusters solely with an insecticide significantly lowered disease incidence compared to treating with only a fungicide. Disease incidence and severity were positively correlated with the number of <i>Drosophila</i> larvae or the adults emerging from clusters collected in the trial. The relationship between disease and insect incidence were significant in 2021 and 2022, highlighting the importance of controlling insect vectors to manage rots. Through chemical residue testing, we identified pesticide programs that resulted in lower pesticide residues on the grapes. Programs that incorporate organic insecticides or sterilants had statistically lower residues than programs with conventional products. Our findings highlight the effectiveness of incorporating insecticides into late-season IPM programs for reducing cluster rots in wine grapes as managing insect vectors mitigate the impact of cluster rots on grape production.		

1. Introduction

The term cluster rot in cultivated grapes (*Vitis* spp.) encompasses a number of diseases including Botrytis bunch rot (caused by *Botrytis cinerea*), ripe rot (caused by *Colletotrichum* spp.) and more recently sour rot. Sour rot is a late season polymicrobial disease complex consisting of yeasts, acetic acid bacteria, filamentous fungi, and insect vectors (Hall et al., 2018a) that affects grapes worldwide and costs the wine grape industry millions of dollars in lost revenue annually (Madden et al., 2017). Sour rot infections are characterized by oxidizing (browning) of the berry skin, an increased presence of insects, pulp oozing from the fruit, and a distinctive vinegar odor (Hall et al., 2018a). Disease severity is determined by the susceptibility and cluster architecture of the grapes, weather conditions, insect presence, and pathogen pressure. Conducive weather conditions include preharvest rains, warm temperatures, and high humidity, with the berries becoming increasingly susceptible after

veraison (Entling and Hoffmann, 2019). Tight clustered and thin-skinned varieties such as Pinot Noir, Vignoles, and Riesling are more susceptible to sour rot. Infected clusters are not harvested as they cannot be used for fresh product and using infected grapes for wine is associated with low quality wine, poor storage potential, and unacceptable levels of total and volatile acidity (Barata et al., 2011; Hall et al., 2018a).

Sour rot infection is initiated by wounds on the fruit caused by insects, birds, mechanical damage, cracks caused by powdery mildew or Botrytis fruit rot infection, or cracks formed during ripening (Pisani et al., 2015). Endophytic fungi, such as *Aspergillus carbonarius, Aspergillus niger, Cladosporium herbarum, Cladosporium cladosporioides, Rhizopus arrhizus,* and fermentative yeasts (*Saccharomyces spp., Candida spp., Hanesiaspora guilliermondii, Hanseniaspora uvarum, Metschnikowia spp., or Picha spp.*) will colonize and infect the wounded berries (Hall et al., 2018a; Hall and Wilcox, 2019; Pisani et al., 2015). The yeasts

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convert the fruit sugars into ethanol and acetic acid bacteria (Acetobacter spp., Gluconobacter spp.) oxidize the ethanol into acetic acid, which browns the berry skin and causes the distinctive vinegar smell (Hall et al., 2018a; Hall and Wilcox, 2019). The acetic acid and other volatiles produced by the rotting fruit attract insects, particularly vinegar flies including Drosophila melanogaster and D. suzukii, berry moths, and yellowjackets (Barata et al., 2012; Hall et al., 2018a; Ioriatti et al., 2017; Madden et al., 2017; Oliva et al., 1999). Not only do insects create fruit wounds that are prerequisite to the initial development of sour rot, but these insects also act as vectors by transporting yeasts and acetic acid bacteria and cause wounds on the fruit surface to provide entry points for additional infection. Drosophila spp. Play a critical role, as the developing larvae can decompose fruit and increase sour rot development (Entling and Hoffmann, 2019). Barata et al. (2012) found that sour rot was not observed when grape clusters were physically protected from insects using a mesh shield, even if the berries were artificially wounded. Hall et al. (2018a) also found that sour rot symptoms only developed when clusters were in the presence of Drosophila, indicating that the presence of vinegar flies plays a critical nonmicrobial role in the development of sour rots, catalyzing the ethanol to acetic acid conversion and through the loss of berry integrity due to egg laying behaviors. Even healthy fruit have the microorganisms required for the development of sour rot, but symptoms do not appear unless in the presence of Drosophila spp, which are critical in the wounding process to initiate disease (Hall et al., 2018a). With the invasion of grape production regions by D. suzukii, the risk from the insect component of the sour rot complex has increased, because this species can initiate infestation by laying eggs into intact berries (Baser et al., 2018). It may also facilitate infestation by Drosophila species that can only infest previously-damaged fruit (Ioriatti et al., 2015; Entling and Hoffmann, 2019).

Targeting insect pests that are active in vineyards after veraison appears to be a critical component of reducing the economic losses from cluster rots. Despite the scientific evidence of sour rot infections being mediated and spread through *Drosophila* activity, there has not been widespread adoption of insecticide applications to reduce rot vectors during the pre-harvest period. Controls applied near harvest must have low residues to fit regulatory requirements and some growers are aiming to minimize residues to meet consumer preferences. In New York and Michigan, there is evidence of vinegar fly populations that are resistant to commonly used insecticides such as zeta-cypermethrin, acetamiprid, and malathion (Hubhachen et al., 2022; Sun et al., 2019). This highlights the need to explore alternative chemical classes and new management options to control *Drosophila* flies in vineyards.

There is also a great need to explore alternative fungicide options for controlling late season rots. Wine production and fermentation can be negatively affected by fungicide residues. Pesticide residues on grapes can be transferred to the juice, which can hinder the metabolism of yeast strains used in fermentation and reduce efficiency. This can cause undesirable volatile compounds, alter wine color, and reduce the phenolic composition of the wine (Caboni and Cabras, 2010; Briz-Cid et al., 2018, 2019; Gava et al., 2021; Russo et al., 2019). Effects have also been reported on the increased production of glycerol and volatile acidity (Gava et al., 2021). For example, several studies have found that grapes treated with famoxadone, fenhexamid, fluquinconazole, metrafenone, boscalid, kresoxim-methyl, quinoxyfen, mepanipyrim, or trifloxystrobin will impact the color and alter the phenolic profile of wine (Briz-Cid et al., 2014, 2015, 2018; Mulero et al., 2015). Additionally, tetraconazole residues reduce phenolic content, including anthocyanins and flavan-3-ol monomers, of wine by more than 45% (Castro-Sobrino et al., 2019). Wine made from grapes with mepanipyrim and iprovalicarb residues resulted in an increase in volatile acidity, lactic acid, total phenolic index, but a reduction in total monomeric anthocyanins, with effects on the wine color (Briz-Cid et al., 2019). Sterilant fungicides based on acidic and peroxide compounds to prevent disease have potential to reduce residues on grapes at harvest. Sterilants containing

Table 1

Treatments used in the small plot efficacy trial in 2020, 2021 and 2022, along with the active ingredient and post-harvest interval (PHI). Treatments were applied once fruit reached 14 Brix following a standard program. The 2020 trial focused on conventional chemicals and sterilants (i.e. peroxyacetic acid) which has been the traditional management practices. In 2021 and 2022, an organic insecticide (spinosad) was added along with more combinations of products based on the 2020 data.

Treatments, rate/hectare	Manufacturer	Years Tested	Active Ingredient	PHI (days)
Untreated		2020, 2021, 2022		
Oxidate 2.0 (1:100) + Mustang Maxx (292 ml)	Biosafe Systems (East Hartford, CA), FMC Corporation (Philadelphia, PA)	2020	Hydrogen peroxide, peroxyacetic acid + zeta- cypermethrin	1
Oxidate 2.0 (1:100) + Entrust (420g)	Biosafe Systems, Corteva Agriscience (Indianapolis, IN)	2021, 2022	Hydrogen peroxide, peroxyacetic acid + spinosad	7
JetAg (570 ml) + Mustang Maxx (292 ml)	Marrone Bio Innovations (Raleigh, NC), FMC Corporation	2020, 2021, 2022	Hydrogen peroxide, peroxyacetic acid + zeta- cypermethrin	1
Fracture (2.2L)	FMC Corporation	2020, 2021, 2022	BLAD	0
Fracture (2.2L) + Venom (210g)	FMC Corporation, Valent (San Ramon, CA)	2020	BLAD + dinotefuran	7
Fracture (2.2L) + Leverage 360 (234 ml)	FMC Corporation, Bayer CropScience (St. Louis, MO)	2020	BLAD + imidacloprid, beta-cyfluthrin	3
Fracture (2.2L) + Mustang Maxx (292 ml)	FMC Corporation	2020, 2021, 2022	BLAD + zeta- cypermethrin	1
Switch (981g)	Syngenta (Wilmington, DE)	2021, 2022	Cyprodinil, fludioxonil	7
Switch (981g) + Mustang Maxx (292 ml)	Syngenta., FMC Corporation	2020, 2021, 2022	Cyprodinil, fludioxonil + zeta- cypermethrin	7
Switch (981g) + Leverage 360 (234 ml)	Syngenta, Bayer CropScience	2021, 2022	Cyprodinil, fludioxonil + imidacloprid, beta-cyfluthrin	7
Switch (981g) + Entrust (420g)	Syngenta, Corteva Agriscience	2021, 2022	Cyprodinil, fludioxonil + spinosad	7
Switch (981g) + Verdepryn (600 ml)	Syngenta, Summit Agro (Durham, NC)	2021, 2022	Cyprodinil, fludioxonil + cyclaniliprole	7
Mustang Maxx (292 ml)	FMC Corporation	2022	Zeta- cypermethrin	1

peroxyacetic acid or BLAD can reduce sour rot and botrytis bunch rot (Hall et al., 2018b), with the potential to reduce the activity of *Drosophila* flies in fruit through which could have a synergistic effect on the sour rot complex (Van Timmeren et al., 2020).

The goal of this project was to evaluate the efficacy of multiple treatment combinations on cluster rots to improve late-season management of sour rot in wine grapes. The programs included various fungicides and/or insecticides, as well as some newly available biopesticides. We also sought to quantify the amount of pesticide residues on clusters at the time of harvest from each treatment.



Fig. 1. Sour rot on 'Vignoles' grapes near harvest in southwest Michigan (A). 'Vignoles' clusters sampled from Switch/Mustang Maxx treated blocks (left) and untreated blocks (right) in a small plot efficacy trial in southwest Michigan and incubated for *Drosophila* adults (B).

2. Materials and methods

2.1. Small plot trials

The trials were conducted in a Vitis interspecific hybrid cv. Vignoles commercial vineyard in Berrien Springs, Michigan in 2020, 2021, and 2022. Vines were spaced at seven x nine feet (2.1 \times 2.7 m) and were cordon trained on a two-wire trellis and were hand pruned. Each treatment (Table 1) was applied to a four-vine block, which was replicated four times in a randomized complete block design throughout the vineyard. The total size of the plot was approximately 0.25 ha. Treatments were applied using a RYOBI ONE+ 18V Cordless Battery 4-gallon (15 L) backpack sprayer (Ryobi, Fuchu, Japan) with a spray volume of 50 gpa (467.7 liters/ha). Once the shoots reached six inches (15.3 cm), all vines were treated with a rotation of mancozeb (Manzate Maxx 292 ml/ha, UPL, Cary, NC), azoxystrobin (Abound 1132 ml/ha, Syngenta, Wilmington, DE), pyraclostrobin, boscalid (Pristine 1611 g/ha, BASF, Florham Park, NJ), and cyprodinil (Vangard 700 g/ha, Syngenta). Starting at 12-14° Brix, the treatments of fungicides and insecticides were applied weekly as listed in Table 1. Seven days after the last treatment was applied, we measured sour rot and botrytis fruit rot infestation, by visually assessing 25 randomly selected clusters taken from the center vine in each plot (Fig. 1A). Incidence was defined as the percent of clusters exhibiting disease, and severity was defined as the percentage of diseased surface area on infected fruit.

2.2. Salt tests for Drosophila eggs, larvae, and pupae

On the same day as the samples described above, clusters were collected from each treatment block and one cluster was randomly selected for salt and one for filter testing (modified from Van Timmeren et al., 2017). The berries from each cluster were removed from the rachis. The total berries and the number of diseased berries were counted, and the cluster was weighed. Each cluster was placed in a plastic deli cup, covered in salt water (1 cup salt to 1 gallon water), and let sit at room temperature for 30 min. The fruit/liquid mixture was poured into a funnel with a 0.6 cm hardware cloth at the opening set over a 4 cup (0.9 L) Medelco reusable coffee filter (Medelco Inc., Bridgeport, CT). Fruit caught in the funnel was thoroughly rinsed. The coffee filters were examined under a dissection microscope for *Drosophila* eggs, first through third larval instars, and pupae.

2.3. Rearing cups for Drosophila adults

At the same time as the other samples were taken from the vines, a

cluster from each treatment block was randomly selected for rearing cups. Each cluster was weighed, and the total number of berries and the number of diseased berries were counted. The clusters were placed on top of a sponge inside a 946 ml deli cup, a sticky card was placed on top of the grape cluster, and the cup was covered with a mesh lid (Fig. 1B). Cups were held at room temperature (20 °C) for 10 days and then the number of adult flies and other insects on the sticky cards were counted using a dissection microscope.

2.4. Pesticide residue analysis

In 2022, a single cluster from each plot in the trial was sampled at harvest, frozen, and sent to Synergistic Pesticide Laboratories LLC (Portland, OR) on dry ice for pesticide residue analysis. Samples were homogenized via Robot Coupe (Vincennes, France) food processor and extracted using QuEChERS EN 15662 method extraction kit (Thermo Fisher Scientific, Waltham, MA) followed by cleanup with Dispersive Solid Phase Extraction (dSPE). All samples were promptly analyzed by LC/MS/MS using the TSQ Endura with Vanquish Binary Pump and Autosampler (Thermo Fisher Scientific, Waltham, MA) and GC/MS/MS using the TSQ 8000 Evo with Trace 1310 GC and Autosampler (Thermo Fisher Scientific, Waltham, MA) for the Multiresidue Screen consisting of 291 agrochemicals. Each analytical run was calibrated with curves prepared in matrix with Multiresidue Working Standard Mixes fitted to a linear curve consisting of 6 points, ranging from 10 to 500 ng/ml. Where analytical detection was confirmed by retention time and acceptable mass spectra ion ratios, residues were reportable below the calibration curve, down to 50% of the Reporting Limit, with qualification.

2.5. Weather data analysis

Weather data was collected from the Berrien Springs Enviroweather Automated Weather Station Network supported by Michigan State University. Precipitation, maximum temperature, minimum temperature, and maximum relative humidity values were recorded from August 15 through September 14 in 2020, 2021, and 2022 (Supplemental Fig. 1).

2.6. Statistical analysis

Data within each year were analyzed using a multifactor ANOVA, Fisher's least significant difference (LSD), and linear regression procedures with the Statgraphics Centurion XVLI program (Statgraphics Technologies, Inc., The Plains, VA).



Fig. 2. Cluster rot incidence and larval counts from small plot efficacy trials in 2020 (A), 2021 (B), and 2022 (C).

Table 2

Results of linear regression comparing cluster rot incidence and *Drosophila* larvae or adult counts. Bold cells indicate significance at the 95% confidence interval.

Year	Variables	P value	R^2
2020	Incidence x Larvae	0.4586	8.1%
2020	Incidence x Adult	0.0598	41.8%
2021	Incidence x Larvae	0.0063	62.8%
2021	Incidence x Adult	0.9078	0.18%
2022	Incidence x Larvae	0.0217	46.1%
2022	Incidence x Adult	0.1146	25.3%

3. Results

3.1. Small plot trials

Cluster rot incidence was high in the untreated controls throughout all three years of the trial. Incidence ranged from 80 to 90%. The lowest disease incidence (80%) occurred in 2021 and the highest (90%) in 2020 (Fig. 2). The fewest number of *Drosophila* larvae and adults were recovered, regardless of treatment, in 2020, whereas 2021 had the most insects recovered from clusters (an average of 297 *Drosophila* larvae recovered from the untreated control).

In 2020, the cluster rot incidence of grape clusters treated with BLAD was 66% and the incidence was statistically lower when this treatment was applied with spinetoram, imidacloprid with beta-cyfluthrin, or zeta-cypermethrin (28%, 25%, and 16%, respectively). In 2021, grape clusters treated with the fungicides cyprodinil plus fludioxonil resulted in 67% cluster rot incidence, while cyprodinil plus fludioxonil applied with an insecticide such as cyclaniliprole, spinosad, zeta-cypermethrin, or imidacloprid plus beta-cyfluthrin all resulted in statistically significant lower disease incidence (49%, 18%, 9%, and 6% respectively). In 2022, grape clusters treated with only an insecticide had statistically significant lower disease incidence compared to those that received only a fungicide application; treating clusters with cyprodinil plus fludioxonil or BLAD resulted in 76% or 73% disease incidence.

The number of insects recovered from the salt tests and rearing cups was much lower in 2020 but higher in 2021 and 2022. There was a significant correlation between cluster rot incidence and recovered Drosophila larvae in 2021 and 2022 (Table 2).

Weather data was collected from August 15 through September 14, 2020, 2021, and 2022 from Michigan State University Enviro-weather. The year with the lowest precipitation was 2020 (3 cm, Supplemental Fig. 1). 2021 and 2022 had more precipitation at the end of the grape growing season (8.1 and 7.4 cm, respectively, Supplemental Fig. 1) and also had higher *Drosophila* infestation.

3.2. Chemical residues

291 pesticide residues were tested for, and 25 were detected in the harvested clusters (Fig. 3). Clusters treated with cyprodinil plus fludioxonil and imidacloprid plus beta-cyfluthrin had the highest ppm levels of pesticide residues detected, followed by BLAD applied with zeta-cypermethrin and cyprodinil plus fludioxinil applied with cyclaniliprole. The treatments with the lowest detectable residues were those treated with hydrogen peroxide plus peroxyacetic acid (JetAg) with Mustang Maxx, hydrogen peroxide plus peroxyacetic acid (Oxidate 2.0) with spinosad, and cyprodinil plus fludioxonil with spinosad.

4. Discussion

Our findings indicate that cluster rot incidence is significantly lower when fungicides are applied in combination with an insecticide. The most effective combinations included cyprodinil plus fludioxonil and BLAD fungicides when combined with an insecticide. We also found that zeta-cypermethrin was repeatedly included in the most effective treatments for rot and insect control across all years of the study, even when applied without a fungicide in 2022. Incidence of cluster rots was also significantly positively correlated with larval infestation by *Drosophila* in fruit in 2021 and 2022.

Although zeta-cypermethrin was one of the most effective treatments in our study, frequent use of the same insecticides to control *Drosophila* spp., and thus sour rot, has led to selection for insecticide resistance in many grape growing regions. Evidence of zeta-cypermethrin resistance developing in *Drosophila melanogaster* has been identified in New York vineyards in 2018, 2019, and 2020 (Mertz et al., 2021, 2022; Sun et al., 2019), a Missouri vineyard in 2020 (Mertz et al., 2021), and Michigan vineyards in 2020 and 2021 (Hubhachen et al., 2022). This highlights the need for alternative approaches to sour rot management to be



Fig. 3. Total chemical residues (ppm) of the applied treatment chemicals detected from each small plot efficacy trial treatment in 2022.

integrated into viticultural programs to limit the further development of resistance. Our study demonstrates that imidacloprid plus beta-cyfluthrin, spinosad, and cyclaniliprole also have potential for effective vinegar fly and cluster rot control when applied in combination with select fungicides, so there are other modes of action available to control vinegar flies.

Widespread fungicide resistance in Botrytis cinerea of grapes has also been detected due to repeated use of fungicides with the same mode of action. B. cinerea populations resistant to multiple active ingredients has been widely reported (Alzohairy et al., 2021; Fernandez-Ortuno et al., 2015) which has reduced the number of effective fungicides for gray mold, rendering it very difficult to control. In Michigan specifically, B. cinerea strains collected from vineyards were found to have multiple fungicide resistance to seven out of eight of the major chemical classes commonly used for control including thiabendazole (Mertect, Syngenta), boscalid (Endura, BASF), pyraclostrobin (Pristine, BASF), fenhexamid (Elevate 50WDG, UPL), iprodione (Rovral, FMC), fluopyram (Luna Sensation, Bayer), and cyrpodinil (Vangard, Syngenta) (Alzohairy et al., 2021). Better chemical resistance management through rotational programs, effective alternative products, and new chemistries are needed for the control of gray mold. Our study demonstrates that fludioxonil is an effective conventional product and BLAD is an effective organic treatment alternative for gray mold management.

There is evidence for endophytic yeasts being an important component in driving cluster rot development (Hall et al., 2018a). While fruit is primarily a carbohydrate source, yeasts are an important protein, vitamin, and nutrient source for many Drosophila spp. (Barata et al., 2012; Hardin et al., 2015) and are essential for larval development (Lewis and Hamby, 2019). Larvae reared in an environment without yeasts as a food source do not pupate (Lewis and Hamby, 2019). This could help explain why fungicides are effective at reducing cluster rot incidence. By applying fungicides and reducing the yeast populations, it also limits the amount of protein available for insect feeding and thus decreases the insect pressure. Barata et al. (2012) found that grape clusters have a plant defensive response that can heal the fruit skin when it is damaged by biotic or abiotic factors, but that this defensive healing was only observed in the absence of Drosophila. In that same study, if vinegar flies were present on the damaged clusters, they were able to transport the yeasts and acetic acid bacteria on the berry surface to neighboring clusters faster than the defensive plant response could heal the wounds, therefore increasing damage from associated rots. Furthermore, oviposition by Drosophila suzukii has been found to exponentially increase the concentration of acetic acid bacteria, and the oviposition and subsequent larval development also increases the risk of spoilage bacteria vectored by D. suzukii adults (Ioriatti et al., 2018). These findings indicate a positive feedback loop where spoilage bacteria attract Drosophila spp., which may then contribute to additional spread of harmful microbes. In this study, we found that these relationships were evident in commercial vineyards where Drosophila larvae infestation in fruit was significantly positively correlated with higher incidence of cluster rot diseases. Interestingly, we did not find as strong of a relationship between adult flies emerging from fruit and disease incidence. The precise mechanisms involved in the role that Drosophila adult oviposition and larval development play in cluster rots remains a topic for further research as it is possible that D. suzukii are facilitating grape infestation by D. melanogaster, similar to reports from other regions (Entling and Hoffmann, 2019; Ioriatti et al., 2017). Full sour rot symptom development has previously been shown to require the presence of Drosophila spp. (Hall et al., 2018a), thus it is not surprising that our study found treatments including insecticides that target vinegar flies to be more successful at controlling sour rot than treatments that only included a fungicide. This is consistent with other studies showing that insecticide treatments are more effective than antimicrobial pesticides for controlling sour rot (Hall et al., 2018b).

Cluster rots are complicated disease complexes involving interactions with multiple pathogens and vectoring pests. The pathogen that dominates infection on the fruit depends on many factors including cluster architecture, cultivar susceptibility, and varying environmental conditions such as moisture, humidity, and temperature (Crandall et al., 2022). Understanding the factors that influence insect vector pressure and the development of late season cluster rots are key to improving best management practices. Based on the results from this study, our current management recommendation for cluster rots is to apply cyprodinil and fludioxonil (Switch, Syngenta) to control the fungal and microbial pathogens, and imidacloprid and beta-cyfluthrin (Leverage 360, Bayer Crop Science) or other options active on vinegar flies, starting at 12-14° Brix. Not only are these products effective at reducing cluster rot incidence, but they also provide different modes of action to reduce the development of resistance.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Timothy Miles reports financial support was provided by Michigan Craft Beverage Council.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2023.106528.

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K.A. Neugebauer et al.

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