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Terroir

An Integrated Wine Science Publication

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WINE SCIENCE

HOW CAN SOMETHING THAT TASTES SO GOOD BE SO INTERESTING SCIENTIFICALLY?

Working in small groups, students in ISCI 3A12 examine the science behind wine making, from the art of viticulture to its eventual consumption by the public. Students perform literature-based reviews and original research in order to understand the wine industry and its complexity; including the environmental requirements and consequences of winemaking, the short- and long-term health effects of drinking wine, and what factors contribute to the quality, aroma, and taste of wine. This research was formatted to resemble a publicly accessible scientific article, and compiled to create each Vintage of *Terroir*.

This Publication is written by members of the ISCI 3A12 class of 2023

Cover Image: Jackson Triggs Vineyards. Melissa Cappelletto. 2023
Table of Contents Image: Barrels in the cellar of Rosewood Estates Winery. Melissa Cappelletto. 2023

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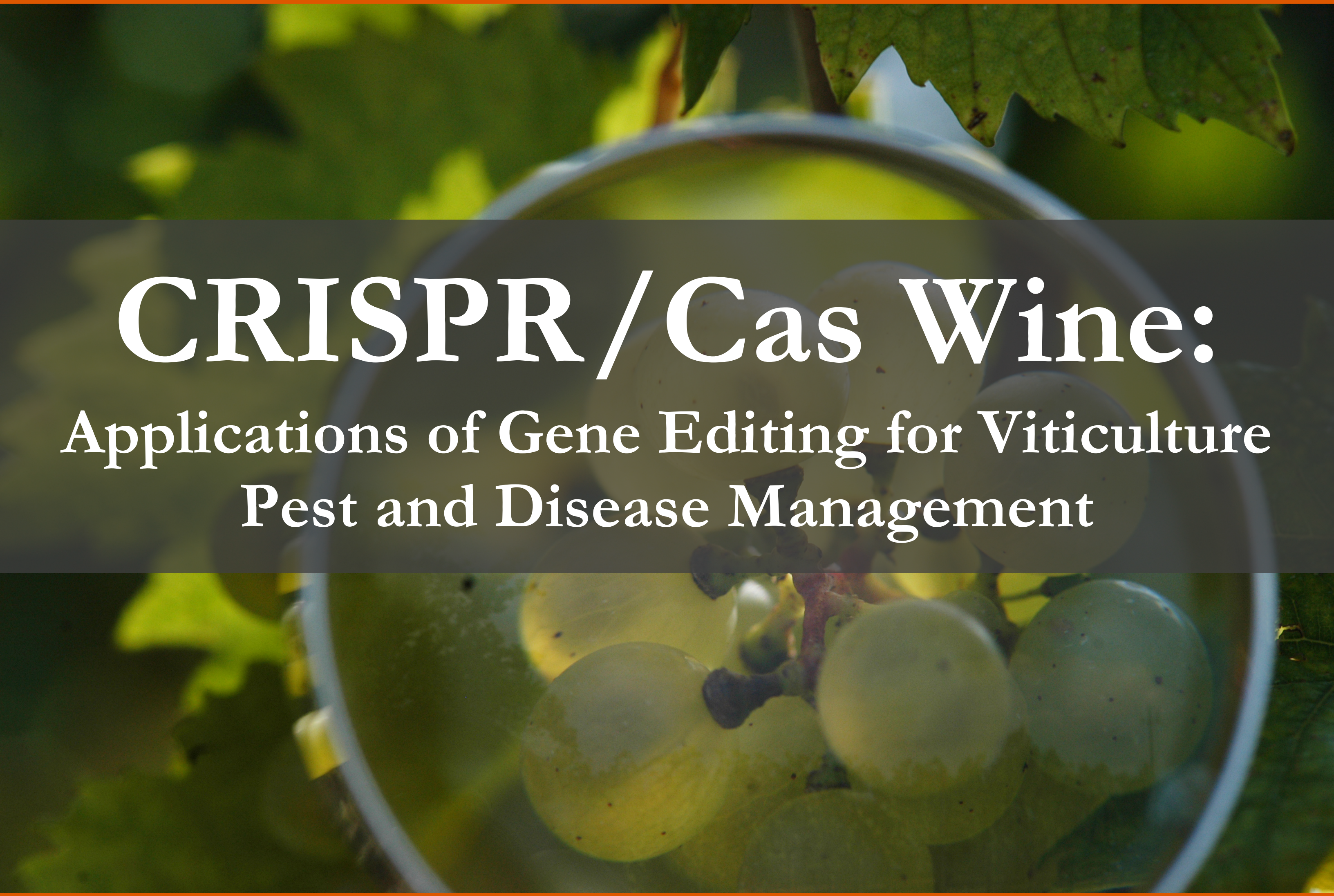
EDITORS: Melissa Cappelletto & Bronwen Elkins



CRISPR/Cas Wine:

Applications of Gene Editing for Viticulture Pest and Disease Management

Vikram Arora, Lauren Creasy, Abigail Hanna, and Adam Sutoski

Throughout the history of viticulture, diseases and pests have reduced the quality and production of wine. Diseases such as downy and powdery mildew can lead to necrotic leaves, whereas bunch rot infects fruits with fungal growth, causing undesirable odours and increased astringency. Harmful pests, notably phylloxera, have also devastated vineyards, leading to substantial financial losses. The wine industry had devised strategies to respond to these threats, including chemical treatments, grafting, and integrated pest management. However, these treatments are becoming less effective as emerging variants of diseases and pests arise due to chemical resistance and climate change. The seemingly endless battle against these threats emphasizes the need for innovative approaches to pest and disease control in order to sustain the wine industry.

CRISPR/Cas9 presents a promising future in the wine industry due to its capability of performing precise modifications to the genome of grapevines. By identifying relevant genes, scientists and winemakers can artificially enhance grapevine resistance without the use of chemicals or grafting. This method leads to reliable outcomes that ensure high crop yields and quality grapes for the winemaking process.

INTRODUCTION

The wine industry is no stranger to disease, pests, or disasters. From the powdery mildew, caused by Erysiphe necator, and *Daktulosphaira vitifoliae* (grape phylloxera) outbreaks of the nineteenth century to the recently detected invasive *Lycorma delicatula* (spotted lanternfly), pests and diseases continue to shape viticulture.

Phylloxera remains the most geographically distributed pest species, found in every viticultural region worldwide (1). Native to Eastern North America, phylloxera coevolved with *Vitis cinerea* (North American grapevines), developing resistance to the leaf and root attacks of phylloxera (2). After the pest appeared in France in 1863, a lack of resistance from *Vitis vinifera* (European grapevines) destroyed 40% of French grapevines over the next 27 years (1). Consequently, there was a loss of 15 billion French Francs in total income, equivalent to 75% of one year's economic output (3). In response, viticulture practices adopted a grafting technique, where European shoot systems are grafted onto the roots of American grapevines to create a pest-resistant hybrid. However, natural phylloxera resistance remains imperfect and susceptible to pest adaptation. For example, AxR#1 was a popular American rootstock hybrid that had shown resistance to the Napa phylloxera for decades (4). But in 1983, AxR#1 demonstrated susceptibility to a new genetic phylloxera strain that had adapted to feed and reproduce on the previously resistant rootstocks. When AxR#1 continued to fail throughout the 1980s and was ultimately replanted, the impact of the new phylloxera strain cost the Californian wine industry between USD 750 million to 1.25 billion (4).

Downy mildew, powdery mildew, and bunch rot also cause severe economic and yield losses in the wine industry. Unlike phylloxera, most varieties of grafted European grapevines possess no natural genetic resistance to the fungi that incite these diseases (5). As such, the extensive use of fungicides has been implemented worldwide, creating an increased risk of fungicide resistance. The naturally high genetic diversity of fungi makes mutations that lead to resistance common, further imposing difficulty in viticulture disease management.

Even more concerning than the influence of these historical pests and diseases is the danger of newly emerging threats due to climate change. As temperatures rise, species like *Lobesia botrana* (European grapevine moth) have begun to migrate northward, with other Mediterranean and tropical insects also expanding their distribution (6). Additionally, rising temperatures and longer growing seasons may increase the number of insect generations per year. Changing environments allow pests, including disease vectors, to adapt to lower temperatures in North America. As precipitation and humidity change, wetter and warmer soils increase the risk of fungus, mould, and mildew infestation. In the future, changes in global temperature, precipitation, and extreme weather events will continue to affect grapevine health, yield, disease incidence, novel pest emergence, and wine quality (6). Specifically, wineries in Niagara, Ontario may encounter severe impacts from climate change due to the microclimates of the region (6). Niagara vineyards are projected to experience significant temperature increases, alterations in precipitation throughout all seasons, and increased frequency of extreme weather events. The potential yield loss and reduced wine quality due to climate change will have significant economic impacts in Canada (6).

As climate conditions shift, so must the landscape of pest and disease resistance in viticulture. Current integrated pest management strategies, including pesticides, fungicides, and grafting, are imperfect and require innovation to compete in an adapting environment. One such innovation growing in popularity is CRISPR/Cas9 and gene editing to artificially enhance grapevine resistance, protecting against pests, diseases, and environmental stressors.

CRISPR:
Clustered Regularly Interspaced Short Palindromic Repeats
Cas9:
CRISPR-Associated Protein 9

CRISPR/Cas9

Gene editing is the modification of genetic material by inserting, replacing, or deleting a DNA sequence, resulting in the gain of genetic traits or inactivation of target genes (7). While numerous gene editing tools are available, the CRISPR/Cas9 system is most commonly used due to its high efficiency, low cost, and repeatability (7). The CRISPR/Cas system is a naturally occurring defence mechanism that protects prokaryotes from invading mobile genetic elements and viruses. CRISPR are DNA sequences found in prokaryotic genomes comprised of identical repeats and non-coding genes called spacers. These spacers are derived from protospacers, which are pieces of foreign DNA from invaders such as bacteriophages that are integrated into the pre-existing CRISPR sequence. When the foreign DNA enters the cell, CRISPR RNA (crRNA) recognizes and pairs with this DNA. The

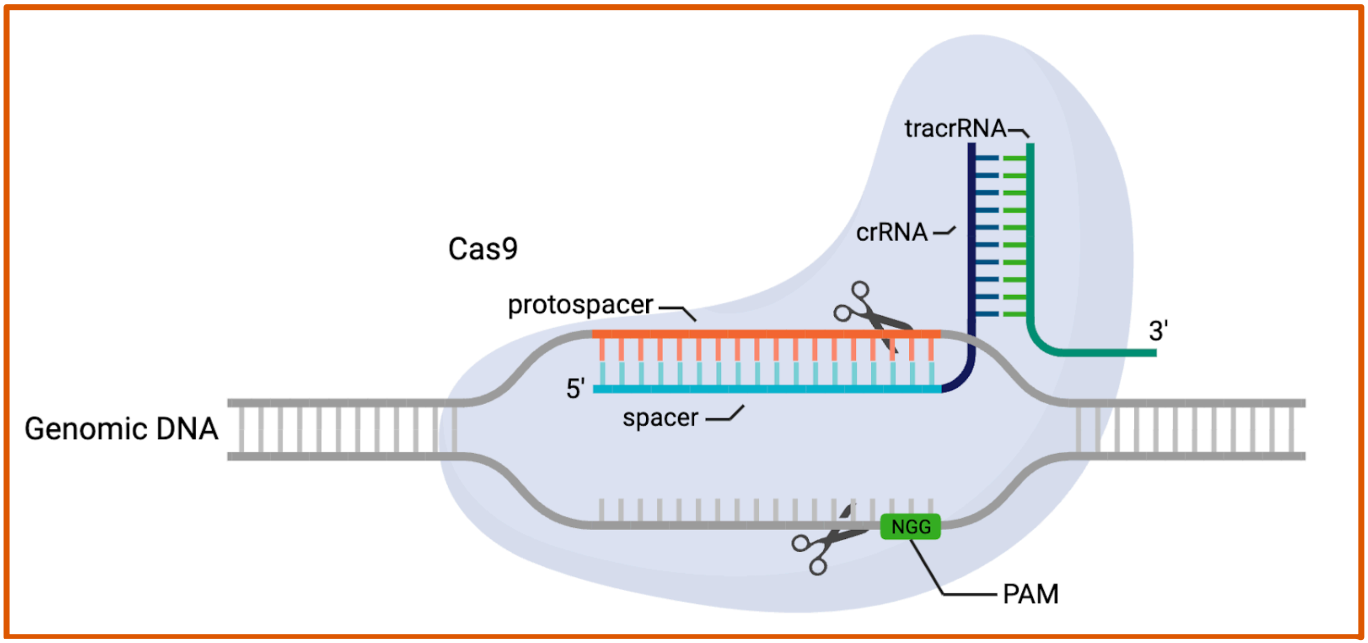


FIGURE 1: CRISPR/Cas9 DIAGRAM The protospacer-adjacent motif (PAM) sequence is typically comprised of the three-nucleotide sequence NGG, in which N can be any nucleotide followed by two guanines (G). The PAM sequence is recognized by the Cas9 nuclease downstream of the target site. Once recognized, an RNA-DNA pairing is made in the presence of a matching protospacer and spacer sequence on the CRISPR RNA (crRNA). The endonuclease can perform a double-stranded break once the crRNA recognizes the target sequence and transactivating crRNA (tracrRNA) is recognized by Cas9. If an NGG PAM sequence is not available near the target site, variants of the Cas9 nuclease can be engineered with alternative PAM requirements to diversify the number of target sites available (9).

pairing then guides a Cas protein to cut the foreign DNA, ultimately protecting the cell (7).

In gene editing applications, the endonuclease Cas9 works with a programmable single guide RNA (sgRNA). sgRNA is made from the fusion of a crRNA and a transactivating crRNA (tracrRNA). The crRNA is designed to be complementary to the target gene sequence, while the tracrRNA is a sequence recognized by Cas9 (8). Once sgRNA has directed Cas9 to the crRNA complementary target sequence in the foreign DNA, Cas9 recognizes the protospacer-adjacent motif (PAM) site, marking the target sequence (Figure 1) (9). Activation of Cas9 through its association with the sgRNA allows Cas9 to perform a double-stranded break (DSB) three base pairs upstream to the PAM site (8). The DSB is repaired by the cell through either non-homologous end joining or homology-directed repair. The error-prone nature of non-homologous end-joining repair can introduce mutations that act to knockout the desired gene. For a more precise knockout, specific regions of the gene sequence can be excised. Using CRISPR/Cas9, insertions or replacements of gene sequences are facilitated by adding a donor DNA template designed for the target DSB site (10).

Endonuclease:
A group of enzymes that break an internal phosphodiester bond within DNA.

Non-homologous End-Joining:
Pathway that repairs double strand breaks in DNA without the need for a homologous template.

Homology-Directed Repair:
DNA repair pathway that requires a homologous sequence to guide repair.

The selection of traits in the wine industry is not novel. For centuries, crossbreeding has been used to produce ideal characteristics of grapevines. Modern genetic modification techniques also include the insertion of plant DNA using vectors like bacteria and plasmids. Most recently, researchers have begun to utilize gene editing techniques, including CRISPR/Cas9, to generate grapevines with protection against environmental stressors and create wines with the best flavours and aromas (8). To date, CRISPR/Cas9 has been used to alter urea, ester, and glycerol production in wine yeasts to enhance taste, reveal genes associated with vulnerability to diseases, and even initiate targeted mutagenesis in winegrapes (8,11). While the many applications of gene editing in viticulture will contribute to the advancement and optimization of the industry, its application in grapevine resistance may be the most important.

DOWNY AND POWDERY MILDEW

Downy mildew (DM) is caused by *Plasmopara viticola* and is prevalent in growing areas with warm and wet weather conditions during the growth season of the vine. It can be identified by light green "oil spots" on the upper surface of leaves, which later turn yellow and become necrotic (Figure 2) (12). White growth of fungus consisting of sporangia and sporangiophores are seen on the lower surface of the leaf. When grapes and vines are infected, ultimately they turn dark brown or black and are shed. This decreases live foliage and grapes, reducing yield in future generations due to a depletion of the carbohydrate reserve of the vine (13). While there are various breeding approaches to create strains that are resistant to DM, the most effective and economically viable approach is chemical control (14). Specifically, copper-based fungicides are commonly employed, but they negatively affect soil fertility and microbiota, harming the ecosystem (14).

Sporangia:
Enclosure in which asexual spores are formed.
Sporangiophore:
Any structure that bears one or more sporangia.

Powdery mildew (PM) is incited by several types of fungi and is believed to have originated from North America. Symptoms of the disease include white powdery areas on the vine, leaves, inflorescence, and berries of *V. vinifera* (Figure 2) (12). When severely infected, leaves containing the fungus may curl and drop prematurely. Even when this superficial growth is cleaned from the grapes, discoloured areas remain. This can lead to premature fruit drop and rotten berries, decreasing the yield and quality of the wine (13). Currently, the most common management method for PM is the use of fungicides. These target different sites in the fungi to affect nucleic acid metabolism, cytoskeleton formation, respiration, and signal transduction (5). However, intensive spraying programs often lead to the emergence of PM strains resistant to various fungicides.

While these two diseases are typically treated with fungicides, CRISPR/Cas9 is being tested to develop disease-resistant crop varieties. Typically, this occurs via the introduction of dominant resistant genes into cultivars through classic breeding. However, this technology introduces recessive mutations in host susceptibility (S) genes to confer resistance.

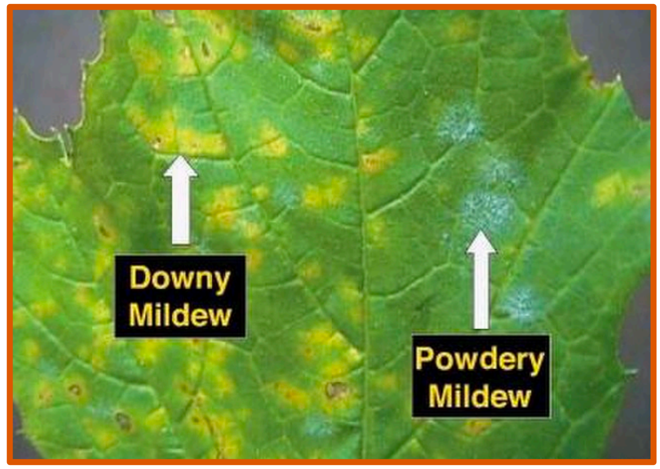


FIGURE 2: DOWNY AND POWDERY MILDEW
Downy mildew (left) causes light green to yellow angular spots on the upper surfaces of leaves. Powdery mildew (right) causes white to grey powdery spots and blotches on leaves, stems, and buds (12).

In many crops, the knockout of the *downy mildew resistant 6* (*DMR6*) S gene has proved successful in promoting DM resistance (15). In *V. vinifera*, the knockout of two highly similar *DMR6* genes (*VviDMR6*-1 and *VviDMR6*-2) can reduce susceptibility to DM. These genes typically encode for an enzyme that acts as a negative regulator of immunity. With *DMR6*-like oxygenase, it can act as a salicylic acid (SA) hydroxylase (16). When *DMR6* is knocked out using CRISPR/Cas9, endogenous SA is increased since SA hydroxylase is no longer present. Since SA regulates plant growth and enhances vigour under biotic and abiotic stresses, increased levels of SA from the knockout of *VviDMR6-1* and *VviDMR6-2* reduce susceptibility to DM compared to non-edited plants (15,17).

Oxygenase:
Enzyme that catalyzes oxidation reactions using molecular oxygen.
Hydroxylase:
Enzyme that catalyzes the addition of a hydroxyl group.

An S gene that can be targeted for PM resistance is the *mildew resistance locus O 3* (*MLO3*) gene. The mechanism by which MLO proteins act as PM susceptibility factors is unknown (18). However, *VvMLO3*-edited heterozygous *V. vinifera* shows enhanced resistance to PM through infected cell death, cell wall apposition (CWA), and hydrogen peroxide accumulation. CWA occurs when there are additional layers of cellulose and callose to the inner surface of the cell wall, which occurs during development and stress responses (19). The additional layers make it more difficult for PM to rupture the cell wall. In addition, increased hydrogen peroxide production and accumulation have been seen in mildew-infected lines, promoting PM resistance (20). Overall, targeted mutagenesis through CRISPR/Cas9 of *VvMLO3* can result in resistance to PM, allowing the potential development of disease-resistant cultivars (18).

Dimerization partner-E2F-like-1 (*VviDEL1*) also plays a role in disease resistance. Specifically, the knockout of *VviDEL1* shows over a 90% reduction in symptoms from PM infection (21). While the mechanism is still unknown, similar observations have been seen in *Arabidopsis* (22). In these plants, an increased accumulation of SA improved plant immunity and resistance to pathogens (21).

CRISPR/Cas9 also allows scientists to better understand the role of specific proteins and genes in plant disease resistance. Pathogenesis-related (PR) proteins comprise a large class of plant defence proteins (23). Transgenic plants expressing PR proteins show significantly higher resistance to various pathogens. PR4, produced by the *VvPR4b* gene, is a chitinase and chitin-binding protein that has deoxyribonuclease activity and can inhibit fungal mycelium growth (23). *VvPR4b* knockout mutations have been shown to increase susceptibility to DM due to the reduced accumulation of reactive oxygen species around stomata (23). Thus, it is predicted that if *VvPR4b* could be upregulated, increased resistance to DM could be conferred. However, the properties of this gene must be better understood before *VvPR4b* gene editing is applied on a large scale (23).

BUNCH ROT

Botrytis cinerea is a ubiquitous necrotrophic fungus responsible for diseases infecting over 1400 plant species (24). In viticulture, the persistence of this fungal pathogen results in worldwide yield losses of 20-50% of grapes in the post-harvest period (24). The disease associated with *B. cinerea* is commonly recognized as grey mould or bunch rot. Similar to the optimal environment for grapevine development, humidity over 90% and temperatures between 15-20 °C provide favourable conditions for bunch rot (25,26). During ripening, infected fruits form necrotic areas with fungal growth (Figure 3) (27). The enzymatic activities of *B. cinerea* lead to chemical alterations in the grape must, causing the production of undesirable odours, browning, increased astringency, and a coarse texture in the wine (28). Careful surveillance of *B. cinerea* is necessary because wineries often decrease the price of their product if the fungus infects more than 5% of the harvested crop (29).

FIGURE 3: BUNCH ROT INFECTED WINE GRAPES
The sporulating fungus is spreading across the grapes, which can result in lesions, leading to drying, premature drop, and ultimately, grapes suitable for winemaking (27).

B. cinerea induces grapevine cell death by producing phytotoxins and enzymes that degrade cell walls (25). As the fungus establishes its presence, it can also control the metabolism of the host to promote colonization. This parasitic activity of *B. cinerea* is further enhanced through the many virulence factors that increase plant susceptibility (30). Interference with these protective pathways allows *B. cinerea* to establish inside the host and develop biomass before the necrotrophic stage.

Did you know? *B. cinerea* is responsible for a unique natural sweet wine category called botrytized wine. Under optimal conditions, *B. cinerea* causes noble rot, a mild infection. Consecutive sunny days with morning fog and elevated humidity, followed by high temperatures in the afternoon, create ideal conditions for this phenomenon (31).

Botrytized wines are beloved for their varied residual sugar content and fruity aroma composition. During noble rot infection, *B. cinerea* increases the sweetness level of the wine while altering the flavour (31). When interacting with the grapes, *B. cinerea* punctures the skin of the berry, resulting in dehydration. This water loss concentrates the sugar content of the berries, ultimately decreasing the acidity of the grape.

Currently, chemical control is the standard approach to reduce the incidence and intensity of bunch rot (29). Fungicides are typically applied periodically throughout the growing season depending on factors such as the weather conditions and the susceptibility of the grape variety. However, chemical control of bunch rot is hindered by the emergence of many resistant strains and negative public perspectives on the safety of pesticides. To provide a novel solution, recently, the genomes of *V. vinifera* and *B. cinerea* have been sequenced, allowing for the editing of resistance and susceptibility genes to develop disease-resistant grape varieties (24).

Targeted mutagenesis through CRISPR/Cas9 in *V. vinifera* has produced promising results to increase resistance against *B. cinerea* (32). To induce resistance, *VvWRKY52* was selected as the target gene from the WRKY transcription factor due to previous evidence of its role in biotic stress responses (32). To assess if the targeted mutations influenced resistance against *B. cinerea*, leaves of non-mutant, monoallelic mutant, and biallelic mutant transgenic lines were compared to wild-type strains. The largest cell death was observed in the wild-type, while the mutated lines displayed an increased resistance (32). Of the transgenic lines, the biallelic mutant showed the highest resistance than the monoallelic mutant. Similar trends were observed when analyzing fungal structures, as the *B. cinerea* colonies growing on the wild-type plant were the largest, while the biallelic mutants had the smallest colonies (32).

Monoallelic Mutation:
An alteration that occurs on one of the alleles of a single gene.

Biallelic Mutation:
An alteration that occurs on both alleles of a single gene.

This study did not carry out the winemaking process with the edited lines of *V. vinifera* (32). However, it was noted that no differences in phenotype were observed across the various genotypes, suggesting that the gene knockout had no visible impact on the development of the plants. These results further suggest that CRISPR/Cas9 can increase the capacity of grapes to resist the dangers of bunch rot without compromising the integrity of the winemaking process.

PHYLLOXERA

Dactylosphaera vintifoliae, commonly known as the grape phylloxera, is the most notorious pest in the wine industry. Phylloxera are sap-sucking insects which feed on the major roots of *V. vinifera* and the leaves of *V. cinerea* (1). When attacking the roots, phylloxera produces nodosities and tuberosities, which allow other pathogens to feed on the roots (Figure 4) (33). These secondary infections strip the roots and gradually cut off food and water supply to the vines. Since there is limited chemical control available for phylloxera, grafting European vines onto North American roots is a commonly employed management strategy (1).

Nodosity:
A pouch-like gall formed on primary roots by swelling root cortexes.

Tuberosity:
A cancerous, scabby deformity.

Despite the dangers of phylloxera, gene-edited pest resistance in grapevines has been minimally researched, with efforts yielding mixed results. As a polygenic trait, phylloxera resistance is difficult to identify in the genome, leading researchers to use classical gene editing techniques to induce broad-spectrum resistance into grapevines (34). Resistance to phylloxera has been observed with the accumulation of dhurrin, a cyanogenic glucoside (35). The presence of dhurrin provides a strong defence mechanism against pests through its bitter flavour and ability to release cyanide, a respiratory inhibitor, when chewed. Thus, to induce phylloxera toxicity, researchers have upregulated the synthesis of dhurrin in *V. vinifera* roots (35).

FIGURE 4: PHYLLOXERA-INDUCED GALLS ON GRAPEVINE LEAF Hook-shaped galls, known as nodosities, are induced by phylloxera. During feeding, phylloxera secrete growth-inducing chemicals into the leaves, creating hollow swelling of plant tissue (33).

Grapevines were transformed with the genes *CYP79A1* and *CYP71E1*, which encode enzymes for the first step of the dhurrin biosynthesis, and *sbHMNGT*, which encodes for the enzyme of the last step of dhurrin biosynthesis, glucosyltransferase (36). Endogenous grapevine enzymes showed the ability to catalyze dhurrin degradation and release cyanide, suggesting grapevine root tissue has the capacity for cyanogenesis.

After *in vitro* co-culture of the cyanogenic line and phylloxera, nodosities, eggs, first instars, and total phylloxera were significantly higher than cultures with acyanogenic roots (36). However, cyanogenic plants had significantly fewer insects per root axis. This effect does not necessarily reflect an acquired resistance, rather limitations of the initial inoculation rate. Additionally, phylloxera may be able to avoid cyanogenesis when feeding, indicating that phylloxera susceptibility to cyanogenesis must be further examined (36). Since this technique has not been tested in the winemaking process, it is unknown how the taste and aroma of wine would be affected.

The progeny of high and low resistance grapevines can be examined using genetic framework maps to locate chromosomes with genes related to high resistance. This technique reveals the relative location of genetic markers on chromosomes. In various *Vitis* species, several loci have been identified as contributing to root resistance.

Quantitative Trait Loci: DNA region associated with a specific phenotype.

Quantitative trait loci (QTLs) associated with the maximum number of nodosities and larvae from phylloxera were identified in a *Vitis rotundifolia*/*V. vinifera* cross. This cross is typically difficult as the two species form a numerical hybrid since the parent plants have a different number of chromosomes. The identified QTLs are localized on chromosomes 3, 7, and 10 of the hybrid (34). Two QTLs within chromosome 7 account for 20% of the phenotypic variability in the maximum number of nodosities. Two other QTLs identified for the maximum number of larvae accounted for 87% of phenotypic variability. The *V. rotundifolia* chromosome carrying the resistance QTLs that control these traits have a low probability of pairing to its *V. vinifera* chromosome homolog. Mapping suggests the regions of interest fall on the lower arm of chromosome 7 in *V. rotundifolia* (34). Similarly, a Börner-Reisling hybrid possessed localized root resistance factors on chromosome 13 (37). If these regions of interest can be isolated and target genes can be identified, these regions can be modified, much like for bunch rot, DM, and PM. Since several loci are responsible for developing phylloxera resistance, isolating particular genes of interest is complex.

Although gene editing has shown promise to improve resistance against phylloxera, the lack of target genes prevents the application of CRISPR/Cas9. Once identified, CRISPR/Cas9 can introduce or knockout specific gene systems to induce phylloxera resistance in European grapevines. Additionally, by using CRISPR/Cas9, the traditional cross-breeding process can be optimized by directly inputting the resistance genes from North American varieties like *V. rotundifolia* into *V. vinifera.*

CONCLUSION

CRISPR/Cas9 has the potential to revolutionize the wine industry by allowing grapevines to withstand the highly volatile environmental conditions incited by climate change. While still in its infancy, existing advancements demonstrate promising results in engineered resistance to DM, PM, and bunch rot. As scientists begin to understand more about the genetic code of phylloxera and the genetic variation between *V. cinerea* and *V. vinifera*, there is the potential to develop resistance against the most lethal pest in viticulture. As CRISPR/Cas9 eliminates the need for cross-breeding, genetic engineering is becoming more time-efficient and less costly (34). Applications of CRISPR/Cas9 in the wine industry go beyond pest management. Other applications include the alteration of aroma, taste, and colour to better cater to the demands of consumers. CRISPR/Cas9 can also enhance the marketability of wines, as the industry can start altering traits without the need for hybrid varieties. Engineered natural pest resistance in grapevines allows for the sale of "more natural" wines to appeal to a new market that values pesticide- and additive-free products. Additionally, healthier plants will produce more predictable yields that require fewer chemical treatments. By preventing pest infestation, grapevines also gain an additional layer of protection against secondary diseases, which are often introduced to a system by invading pests. The stigma around CRISPR/Cas9 and genetic engineering presents a barrier to its use in viticulture. Many consumers still have suspicions around genetically modified organisms in agriculture, making the introduciton of CRISPR/Cas9 into the wine industry challenging. Transparency and clear communication of the technologies being used are vital steps towards breaking down these barreirs. Despite current obstacles, CRISPR-edited wines have the potential to achieve commercial success in the near future.

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Cover Image

Comugnero Silvana. *Controlo dell uva*.
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Less Water, More Wine:

Assessing Sustainable Water Use in Wine Making

Samiksha Babbar, Sophia Caranci, Maya Das, Keira Hum

As climate change becomes more prevalent, the need for sustainability and resource management is critical. In the wine industry, the use of water management strategies is inherent to the production of quality grape crops. Many grape-growing regions have drier microclimates, creating a need for water conservation in the vineyards. Factors such as climate, irrigation method, grapevine efficiency, and soil management are important to consider when assessing the overall environmental impacts of a winery and vineyard. This can be determined through total water footprint (WF) - a quantitative metric for water management strategies. This article presents several strategies that decrease the total WF of a region, including reduction methods in all stages of winemaking. From these findings, a novel case study exploring the viability of these strategies in two different climates were analysed: Niagara, Canada, and Sicily, Italy. Reducing WF is most economically feasible when one practice is focused on. The most effective water management strategies were determined for both the Niagara and Sicily regions, which can further be applied to create sustainable winery practices and reduce water scarcity in any winemaking region.

INTRODUCTION

The process of winemaking has remained an extremely selective and inherently complex process for centuries (1). This can be attributed to the unique characteristics of wine, which are easily impacted by a myriad of factors within every step of the vinification process. Issues can arise due to grapevine growth conditions, climate, and soil, which makes changing standard viticulture practices a challenge (2). However, amid concerning climate projections and a recent uprise in adaptable and sustainable agriculture, conforming to these practices is inevitable (1).

Sustainability in winemaking aims to reduce environmental impacts by identifying which common practices are not productive or efficient (2). One important to consider is water usage, which is a vital aspect of wine production. The estimated amount of required water varies by region, as well as the different processes employed by specific wineries. Although the wine industry was previously considered to be sustainable, rapid global expansion and clean water scarcity has shifted this perspective (3).

Water Footprint (WF) is a novel method for mitigating the environmental impacts of large global industries, such as winemaking (3). Generally, WF serves as a measurement of the amount of freshwater that is consumed and degraded by human activities (4). Water can be "lost" from the system when used in certain industrial practices, which is what consumption refers to in terms of WF (5). Much like other agricultural processes, the usage of water in the growth and maintenance of grapes is a primary issue when looking at sustainability (1). The total WF of each industrial process has been presented for assessment through three components: blue, green, and grey (**Figure 1**) (5). **Blue WF** is associated with the consumption of water in agriculture and winemaking, such as the processes of irrigation and fertilisation. **Green WF** refers to the availability of soil moisture and total evapotranspiration by the plant. Finally, **grey WF** is associated with wastewater management and the volume of polluted water based on regional standards. These can be studied by either combining all three factors or individually assessing each method (3).

By combining these three components, an accurate assessment of water usage in a region can be determined and improvements can be made. It is therefore necessary to evaluate solutions considering all factors affecting total WF: climatic effects, irrigation, soil moisture, and plant water-use efficiency (WUE). These considerations will be applied to two specific regions that differ in environmental conditions: Niagara, Canada, and Sicily, Italy.

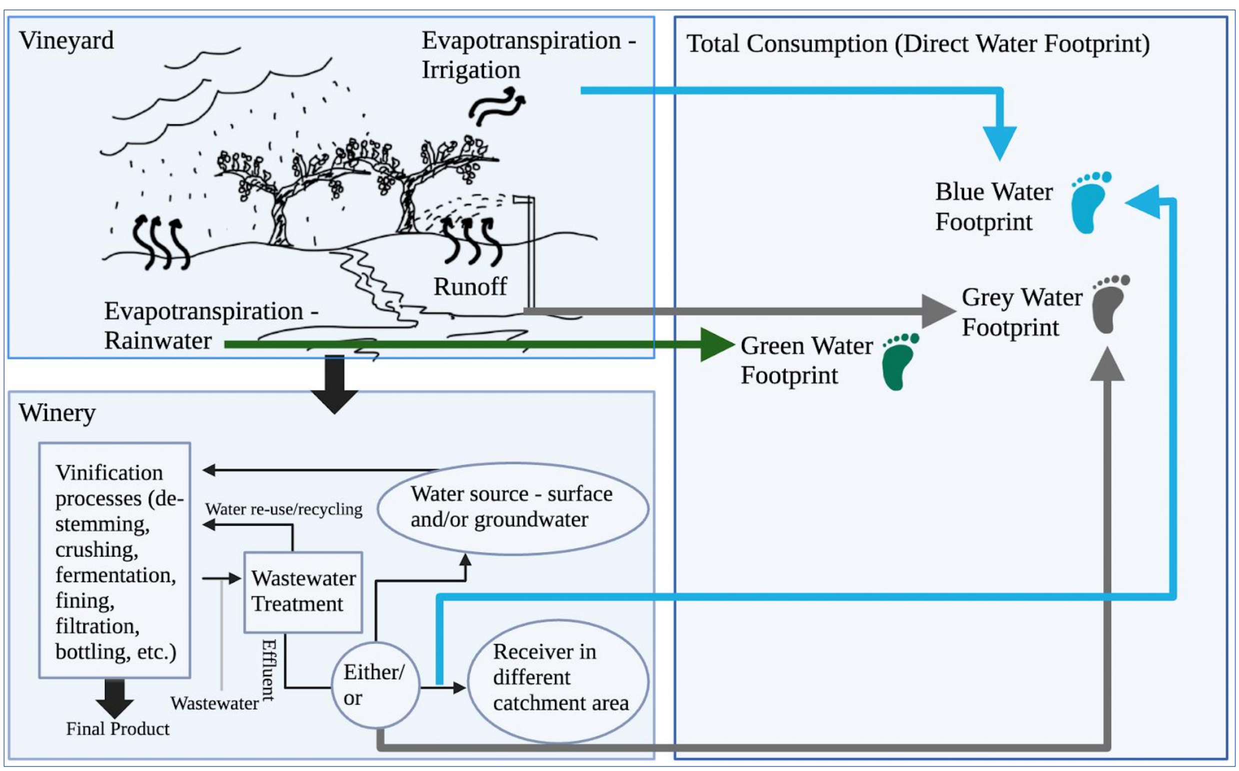

FIGURE 1: COMPONENTS OF WATER FOOTPRINT IN A WINERY. Summary of processes involved in generating the total water footprint of a region, including the production of wine and growth of grapevines. Total direct water consumption depends on the green, blue, and grey water footprints (Adapted from Johnson and Mehrvar (6)).

BLUE WATER FOOTPRINT

Standard methodology to calculate the blue WF includes the water consumption from the winery itself and the maintenance of its grapevines. Given these parameters, the vintner can determine how much water is used in the production of one bottle of wine, and gain an understanding of their water usage on a long-term basis (6). The total blue WF of an individual winery depends on three main factors: climatic conditions of the region, water used for crop management and irrigation, and the vinification process (3). However, climatic effects and irrigation tactics will be prioritised in this article as they heavily impact the blue WF associated with grapevine management processes (6).

CLIMATE CONDITIONS

In order to propose solutions for reducing blue WF, the microclimate conditions of a region must be considered, as it impacts the quality and quantity of wine produced. When analysing the microclimate of a region, various factors should be kept in mind (**Table 1**) (7). The overall microclimate and the correlation of various climate-altering components must be assessed in order to establish optimal sustainable vineyard practices. More specifically, to determine water-efficient methods, primary factors such as weather patterns and spatiotemporal variability must be studied further.

TABLE 1: CLIMATIC EFFECTS OF BLUE WATER FOOTPRINT. Climatic effects affecting the blue footprint of vineyards. Standard units of measurement are listed on the right side of the figure (Adapted from Lamastra et al. (3)).

Climatic Effect	Units
Daily Rainfall	mm
Daily Solar Radiation	MJ/m^2
Maximum/Maximum Daily Air Temperature	°C
Average Daily Wind Speed at 2m Height	m/s
Average Daily Relative Humidity	%

The temperature of a region greatly affects the growth stages of the grapevines in countries which experience continental climates consisting of four seasons. In 2019, Di Carlo, Aruffo and Brune (8) specifically investigated a vineyard that had maintained the same cultivation methods from 1820 to 2012 and found the grape harvest date (GHD) advanced as regional temperature increased. Vineyards must adapt to shorter and milder winters, as they affect the 50 to 400 chilling hours necessary for grape **endodormancy** (7,9). During these chilling hours, temperatures hover between 0 and 7.2 °C, causing buds to burst and allow for the vine to mature (7). The optimal temperature range for grape development depends on the wine produced, meaning this variable is specific to each microclimatic region. In warmer climates, the evaporative demand increases, heightening plant water loss and irrigation rates (10,11). If regional temperatures increase due to global warming effects, then agricultural methods to increase water availability in the vineyard must be redesigned. This may cause an imbalance in the vineyard's blue WF.

Endodormancy: A state where the plant conserves its resources, inhibits its growth, arrests cell division, and reduces metabolic and respiratory activity.

Precipitation and soil-water availability have substantial impacts on the quality and productivity of grapevines. It should be noted that soil-water availability is impacted by factors such as soil properties, spatial terrain patterns, meteorology, and irrigation practices (7). In the same study by Di Carlo, Aruffo and Brune (8), the effects of how climate change impacts precipitation and temperature were determined. These ultimately affect the GHD, premium wine quality, fertilisation usage, grape maturation, and irrigation patterns. Precipitation factors such as intensity, duration, and frequency impact the blue WF (8). Optimal vineyard conditions would consist of constant moderate rains, which aid in leaf area development and extend periods of canopy growth (7,8). However, in unfavourable arid environments, when precipitation patterns are unpredictable and less frequent, strategic irrigation and field management strategies must be employed (12).

IRRIGATION AND CROP MAINTENANCE

With an understanding of the climatic conditions of a region, one way to effectively manage water within a winery is through irrigation systems. Irrigation provides crops with a controlled supply of water to ensure they have optimal growing conditions (10). In wineries, irrigation can be manipulated, conditioning plant roots to become more water efficient over time, ultimately creating a more sustainable grape-growing process (13). Although there are many irrigation methods, a comparison between regulated deficit irrigation (RDI) and partial root-zone drying irrigation (PRD) will be focused on due to their prevalence in the industry (14).

When using RDI, the whole root-zone of the plant is irrigated for set periods of time (**Figure 2a**). This allows the vineyard to choose which parts of the growth cycle have an abundance of water, and which parts have a deficit (15). RDI applies the concept that a plant benefits from different amounts of water at different stages to produce the best quality of fruit (10). During **non-critical stages**, less water is provided, causing plants to develop drought resistant properties. This means they produce less vegetation, grow stronger root systems, and perform more efficient transpiration (16). In **critical stages**, more water is provided, allowing the plant to direct the majority of its resources towards fruit development. This will subsequently produce a higher-quality grape crop (13). RDI is more suited to variable climates, with more rainfall over the growing season. This irrigation system enables the vineyard to alter the water supply depending on the climate over the year (16).

Non-Critical Stages:
For grapes, this refers to the phases when the fruit is not actively developing. This includes the harvest, defoliating, dormancy, and budburst stages.
Critical Stages:
For grapes, these phases are centred around when the fruit themselves are developing. This includes the flowering, fruit setting, veraison, and ripening stages.

Alternatively, PRD is characterised by the separation of plants into a 'dry' and a 'wet' side. Half the root system is irrigated, and the other half is kept in an engineered drought (**Figure 2b**) (17). Typically, the two methods that can be used include maintaining designated sides or alternating irrigated sides in cycles of 10-20 days (10). PRD applies the concept of uneven growth into the root system, allowing the plant to benefit from both drought-resistant and fully watered characteristics (15). Half of the roots are forced to adapt to limited water conditions, eventually possessing drought-resistant properties. Correspondingly, the other half of the roots are supplied with enough water to ensure sufficient fruit growth (18). PRD is typically used in dry environments, with little precipitation over the growing season. The plants benefit from a constant supply of water on one side of the roots, and the health of the plants is maintained (18).

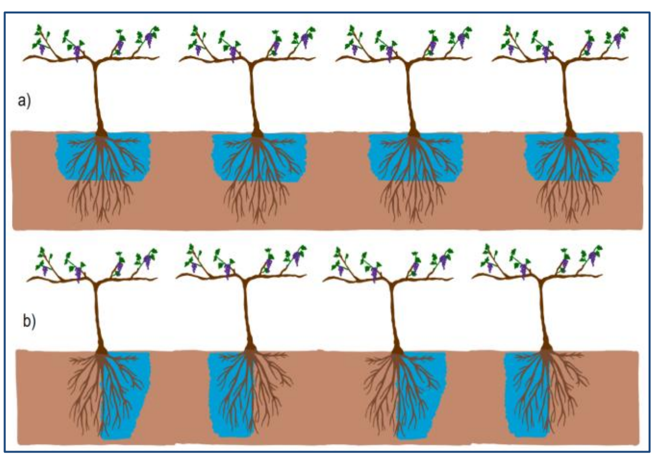

FIGURE 2: IRRIGATION METHODS. Diagram of different irrigation strategies, with the blue representing the areas where water is applied. a) shows regulated deficit irrigation, and b) shows partial root-zone drying irrigation.

Overall, irrigation presents many benefits for plants, including increased nutrients and decreased water use. The surplus and deficit watering cycle of both RDI and PRD creates drought-like stressors within the plant (13). When plants are exposed to these artificial conditions, the development of new shoots decreases, leaf-area growth is slowed, and transpiration is reduced (17). As a result, the excess nutrients from these processes are funnelled into growing stronger and thicker roots (16). This effectively improves the WUE of the plants for future seasons, allowing the vineyard to use less water over time (10).

GREEN WATER FOOTPRINT

When evaluating water-use in the vineyard, it is important to consider the grapevine itself, its ability to effectively retain water, and the availability of soil moisture. When precipitation occurs, the measurement of total water required and the amount lost from evaporation can be measured within the region to accurately assess green WF (3). It is therefore important to look at two aspects to increase WUE: grapevine physiology and soil-water availability through processes such as mulching and cover crops.

GRAPEVINE WATER-USE EFFICIENCY

Winemaking is an extremely selective process that requires distinct modifications to grapes and grapevines at all stages of plant growth. This results in the need to effectively select for specific varietals that can prevent excessive water use during agricultural practices (19). Understanding which grapevines perform most efficiently reduces the need for frequent maintenance, selects for plant endurance in specific climates, and further improves the quality of grapes during winemaking. Looking at plant WUE relies on two main physiological components: hormone-mediated plant response and signalling, and effective water conservation systems within the grapevine (20).

One of the main indicators of a plant's efficient response to drought is its ability to manage transpiration. Effective water usage relies on the xylem network in the plant, with upward regulation of water occurring from the soil to atmosphere (**Figure 3**) (21). Negative water potential causes water to be transpired, which relies on pressure. An important aspect to consider is the physiology of the stomata, which facilitate regulated water loss. During periods of drought stress, the stomata will close to conserve water within the plant, in which a safe range of water potential must be conserved for effective plant function (19). Varying genotypes exhibit different sensitivities regarding a physiological response to water deficits, resulting in varying times for which the stomata are closed (20).

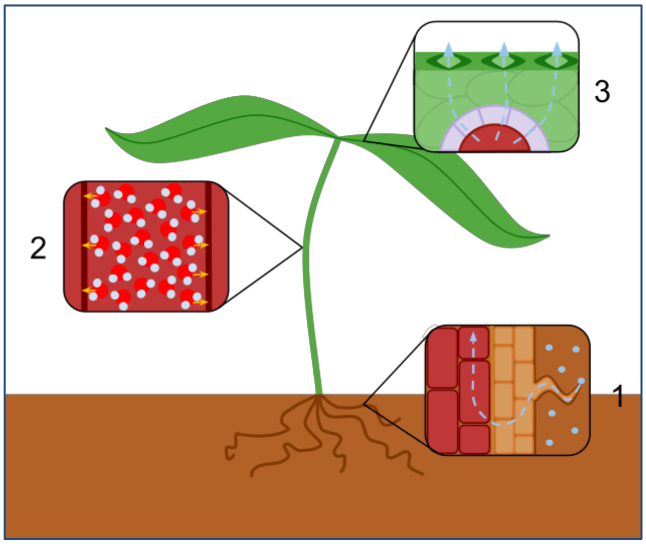

FIGURE 3: TRANSPIRATION IN PLANTS. Process of transpiration where 1) water passively enters through roots and is guided to the xylem, 2) cohesion and adhesion cause water to extend from roots to the top of the plant, and 3) the stomata open to allow water to escape (22).

The growth and adaptation of grapevines in response to varying climate conditions also rely on the hormone abscisic acid (ABA). ABA-mediated signalling maintains water balance and osmotic stress tolerance (23). Under deficit conditions, the concentration of ABA in xylem sap and leaves can help indicate the response to effectively conserve water within the grapevine. Among the *Vitis* genus, ABA concentrations and stomatal sensitivity to ABA can be measured to successfully select for highly responsive plants (19).

Modifying the response of grapevines is climate-specific, however, there can be general methods outlined to select for the most water-efficient grape cultivar. To categorise ABA within the stem xylem sap, the effects of limiting irrigation can be studied through gene expression in certain systems (24). There are also methods to measure the amount of evapotranspiration per millimetre per day to determine whether plants of a certain cultivar are performing well under water-deficient conditions. However, as outlined previously, drought-resistant strains highly depend on the region of interest, in which cultivars can be grown and selected (21). Identifying ideal varietals can help reduce the overall WF and irrigation needs of entire regions, as well as implementing sustainable farming practices (24).

GROWING CONDITIONS AND SOIL MOISTURE

Within wineries, promoting ideal growing conditions can beneficially impact grapevine quality while simultaneously increasing WUE. Through implementing various strategies during the growing process, soil-water evaporation and the amount of water required to grow grapes can be decreased (10). The sustainability of viticulture is extremely reliant on water, due to requirements of the vine typically exceeding average annual rainfall in arid environments (12). Projected warming and drying across the globe will pose a risk to viticulture practices through decreasing soil-water availability, inevitably decreasing grapevine yield, berry quality, and causing crop failure (25).

In order to reduce the amount of water supplied to crops, managing the water stored in soil after it rains is crucial (10). One tactic that has shown great success in increasing WUE is mulching. Mulching is a practice which involves distributing materials that cover the soil surface of crops with the intent to reduce moisture loss and minimise weed populations (26). This method has also been seen to decrease the amount of water runoff and soil erosion while increasing soil infiltration capacity and grapevine yield (27). Specifically, compost-mulch soils have been seen to increase permeability and water storage capacity, and decrease evaporation for grapevines compared to bare soil (28). However, it is important to consider that varying mulch materials are favoured based on specific conditions, and research must be completed to assess the optimal mulch for each vineyard. To ensure sufficient water is provided, the surrounding microclimate conditions must be considered (10). Mulching undoubtedly increases WUE by greatly minimising water loss and maximising its infiltration (12).

Did You Know?
Organic mulching made from natural sources, such as straw, crop residue, winery waste, and compost has the most beneficial impacts on vineyard crops (10). This is also great for sustainability!

Another common practice that has improved growing conditions within vineyards is the use of cover crops, which entails planting additional crops with the sole purpose of covering the soil. Cover crops have been established as a beneficial tactic due to their ability to minimise soil erosion and improve water-holding capacity (12). They have also been frequently used in tandem with mulching, successfully reducing the risk of water runoff. However, it is crucial to carefully select the correct species for specific regions, as certain cover crops may consume more water than the soil conditions can allow in dryer areas and risk grapevine health (10). By manipulating the growing conditions through various tactics, WUE can be substantially increased to reduce water usage and promote sustainability within wineries.

Mulching Versus Cover Crops:
As mulching is not competing for resources, it is less of a risk to vineyards, and is better in arid environments. Despite this, cover crops have considerable potential in more humid areas (27).

GREY WATER FOOTPRINT

Another important component of sustainable wineries is implementing effective systems to manage polluted water and runoff from agricultural processes (6). This can be further split into two different components: using freshwater to dilute pollutants and managing wastewater (5). Most solutions must consider policies on a legislative level, which means that they are dependent on the treatment strategies that are set as a standard in the specific region's agricultural sector (29). However, the intricacy of winemaking and freshwater usage in its processes brings in the need for a separate assessment (30). Two factors to consider are increasing treatment efficiency and reducing pollutants.

Treatment efficiency refers to choosing a suitable system, as well as consistently monitoring and maintaining its viability. For example, when focusing on winery wastewater, a 2009 study by Oliveira et al. (30) outlined a novel method using a bioreactor, measuring treated water related to vineyard irrigation, and identifying an efficient strategy that was able to effectively filter out pollutants. When this technology was further applied to testing treatment quality against other methods, it allowed for a 20% increase in efficiency and an overall reduction in grey WF (31). Similar systems, such as co-treatment, show a substantial decline in wastewater generated in vineyard and winery processes (6). Specificity in the region's treatment strategy and its efficiency in the wine industry is therefore a vital reduction strategy.

Individual wineries can also reduce their impact by using alternative strategies to pesticide and fertiliser use. As identified in grey WF assessment, the freshwater used to dilute these components can be a substantial amount within wine sectors (29). Identifying these key contributing pollutants and finding suitable alternatives is therefore necessary. From this, it is clear that a sustainable grey WF cannot readily be assessed in a generalised manner, but is rather highly context-dependent (3). This is because there are differing wastewater treatment systems and legislation regarding water runoff that must be considered to effectively measure the total impact of a winery in a sustainable way (6). Therefore, a focus on this WF component should be provided in a region-specific analysis.

CASE STUDY: APPLYING WATER FOOTPRINT IN DIFFERENT CLIMATES

As mentioned previously, water-use and calculation of the total WF is highly specific to individual regions. It is therefore necessary to apply these concepts to areas that have implemented or require the implementation of WF management strategies. The following case study will explain applying WF to two different regions: Niagara, Canada and Sicily, Italy. These regions have contrasting climate conditions and require focus on differing aspects of WF.

NIAGARA, CANADA

The Niagara region of Ontario, Canada is an ideal location for wineries, containing over 76% of Ontario's 7284 ha of grapes used in vinification. This region yields a temperate meso-climate that is ideal for grape growing. Additionally, it experiences approximately 500 millimetres of rainfall during the growing season of April to October (32). As a result, WUE is increased within grapevines, and blue and green WF are optimised (33). This means that any water management strategies should centre around grey WF. Managing grey WF includes implementing surface water pollution strategies, treatment

Did You Know?
The Niagara region is proximate to both Lake Erie and Lake Ontario, causing harsh winter conditions to be alleviated due to wind patterns (32). This is part of why the region is considered a moderate microclimate!

systems, and general regulatory requirements through regional legislation. A 2023 study conducted by Johnson and Mehrvar (6) determined municipal water treatment plants and their effects on management in the Niagara region, calculating a total grey WF of $1.47 \times 107\ m^3/yr$. This was based on how much treated wastewater was discharged into surface water, which is a large amount considering the proximity to freshwater lakes (33). Identified pollutants include total suspended solids, ammonia nitrogen, and phosphorus (6). Phosphorus is the most concerning due to its specific identification by Lake Ontario's wastewater legislations and detrimental effects on marine ecosystems (5).

The municipality of Niagara employs co-treatment strategies, which are shown to reduce winery wastewater by 99.3% (6). Co-treatment includes adding winery wastewater with other municipal solid waste, and treating them simultaneously (34). However, it is important to note that mitigation tactics on a winery level can also be a useful strategy to reduce grey WF. These include decreasing phosphorus and nitrogen fertilisation, subsequently reducing the volume of water required to dilute pollutants in wastewater (35).

While green and blue WFs are important in any context, the Niagara region's unique climate makes it less of a concern in managing total WUE. While standard irrigation strategies can be employed, the focus for this region should be on grey WF and continuing co-treatment strategies.

SICILY, ITALY

As one of the leading wine producers in the world, Italy is home to unique, well-developed wines that possess a complex taste profile (3). It has a Mediterranean climate, with dry conditions in the summer months and mild conditions with frequent precipitation in the winter months (36). Across Sicily, the temperature varies with elevation; the highest values are along the coast and flat-lands, and the lowest values are in higher elevation areas (37). Accordingly, increased precipitation occurs in areas of higher elevation (38).

Currently, the region is facing a heightened risk of desertification and increases in temperature, magnifying threats of heat waves and water stress on grapevines (39). It is therefore necessary to assess ways to sustainably manage wineries with respect to water-use, and apply more of a focus on green WF.

An analysis of wine regions in Sicily reported 81.4% of the total WF composition to be green, using between 704.5 and 915.9 litres of water per litre of wine (**Figure 4**) (3). This was calculated using the sum of all rainwater lost by evapotranspiration. Therefore, it is important to assess green WF as the primary focus of this region. With the risk of increased drought stress, plants and soil must be well adapted to water deficit conditions in order to maintain signature wine characteristics. One way to mitigate green WF is to look at ideal grapevine varieties, which heavily depend on the region's climate. A 2019 study conducted in a similar climate region, Turi, Italy, measured plant response for Italia and Autumn Royal grape cultivars (40). The varieties were grafted on two rootstocks, called 140Ru (*V. berlandieri* × *V. rupestris*), where it outperformed the other varieties in response to irrigation methods (40). Additionally, management of soil moisture during periods of limited rainfall can be obtained with a variety of factors, including the technique of organic mulching, which is suitable for Mediterranean climates. However, it is important to note that this should be done without tillage, since it causes high soil degradation rates (12).

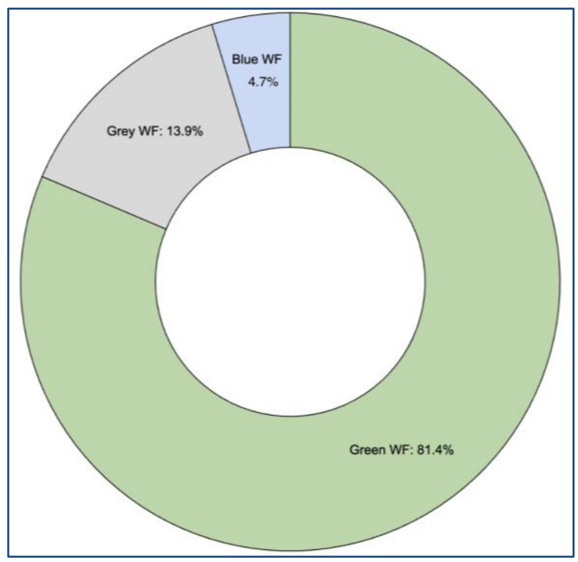

FIGURE 4: WATER FOOTPRINT COMPOSITION. Graph depicting the total average water footprint composition of six different vineyards in Sicily, Italy (Adapted from Lamastra et al. (3)).

Although some blue and grey WF strategies, such as irrigation, can be employed in Mediterranean regions such as Sicily, it is clear that managing green WF can have positive effects on overall WUE (3). It is recommended that this region focuses on mulching and grapevine cultivar selection based on the climate.

CONCLUSION

The assessed treatment strategies and regional case studies indicate that researching the WF of a vineyard relies on variables such as the microclimate of the region, irrigation practices, grapevine variety, and soil management strategies. With these aspects in mind, vineyards can manage their farming techniques, optimising water usage and grape quality. Through the analysis of Niagara and Sicily wineries, it is evident that depending on the region, one WF aspect must be prioritised to implement the most cost-effective and efficient strategies for sustainable water use. This means that a case-based approach should be applied when studying WF and implementing sustainable management strategies. WF is a novel method to accurately assess the water usage of a region, and is necessary for a sustainable future that mitigates the effects of climate change.

MORE TO EXPLORE

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Sonication:

Impacts on Red Wine Vinification

(1)

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The art of vinification paints a canvas of cultural richness, a symphony of age-old tradition and revolutionary science. However, red wine vinification is a highly sensitive process with a variable timeline. In the constant pursuit of enhanced efficiency and optimization while persevering quality, vintners seek to find new technologies to solve these issues. Ultrasound, a well-developed technology currently used in other areas of the food industry, has shown promise in revolutionizing the vinification process. Through chemical and physical interactions, ultrasound can accelerate the processes of maceration, fermentation, and ageing, all of which are time-dependent and strenuous aspects of traditional methods. While shortening the vinification timeframe, ultrasound can also enhance key quality characteristics of the wine, producing a product at par if not better than current red wines on the market. This suggests that implementing this technology will not sacrifice the wine drinking experience, but potentially enhance it. Implementing this technology will require a unique setup for each vintner and can introduce a degree of stability into an industry known for its volatility, ultimately transforming the way red wine is produced.

TRADITIONAL METHODS

The process of red wine vinification is deeply ingrained in history, with methods passed down through generations, shaping vinification into a cultural practice celebrated worldwide. While vinification can vary based on region, grape variety, and processing characteristics, all follow the same stages: maceration, fermentation, and ageing.

The maceration stage involves the crushing of harvested grapes (2). This process breaks down grape skins to facilitate juice release and increase skin contact during fermentation. The crushed grapes are added to vinification tanks where fermentation begins. The extracted grape juice is left to sit with residual grape skins to extract compounds, such as tannins, found primarily in the skins (3). This processing time ranges from 5-30 days, depending on desired compound concentrations (4). Fermentation begins during this rest phase in addition to the formation of a skin cap, resulting from the buoyancy of grape skins. Throughout the fermentation process, the grape skin cap is punched down to increase contact between the must and grape skins, in addition to increasing oxygen circulation, to elevate compound extraction (5). Following fermentation, wine is removed from the tanks for ageing. The ageing process varies in both time and methodology depending on the final desired wine characteristics. Most red wines are aged in oak barrels to undergo oxidative ageing before being bottled for reductive ageing (2).

Oxidation: The exposure of wine to oxygen.

Reduction: Limited oxygen exposure to wine.

While the three core stages of vinification remain consistent, regional terroir provides the final product's distinct flavour, texture, and identity (2). For instance, Bordeaux in Southwest France is renowned for its high-quality production of a variety of red wines, including Cabernet Sauvignon, Merlot, and Cabernet Franc (6). Bordeaux has strict regulations on grape varieties, vineyard practices, and vinification techniques rooted in its long history and culture of wine-production, reinforcing traditional methods. Traditional practices such as precise pruning, canopy management, and yield control are emphasized to optimize grape quality (7,8). Additionally, oak barrels are regulated, and vintners must adhere to prescribed ageing periods to achieve the desired balance and complexity in the wines. In contrast, vintners of the Niagara Peninsula in Canada are more open to experimentation and adaptation due to its shorter history as wine producers (6). The semi-continental climate and diverse soil types in the various regions of Niagara produce well-known red and hybrid varietals such as Pinot Noir, Baco Noir, and Cabernet Sauvignon. The greater flexibility in grape varietals and wine-making techniques allows for increased adaptability to changing consumer preferences. Emerging trends in Niagara may include a focus on sustainable and organic practices, a shift towards minimal intervention vinification, and advanced technology (9). While both regions celebrate the profound influence of terroir, they do so with different approaches, shaping the current landscape of the wine industry.

ULTRASOUND TECHNOLOGY

While ultrasound technology is well known in the healthcare field, its applications go beyond diagnostic or therapeutic purposes, expanding into various industries. This includes the food industry, where ultrasound plays a more significant role in food processing than consumers are aware of. Ultrasound is applied during various food processing procedures including extraction, fermentation, and enzymatic activation and deactivation (10). Additionally, ultrasound can aid in the compositional analysis of food products for quality assurance and assist in disinfection processes (10).

Ultrasonication: The process of applying ultrasonic waves to agitate particles in a liquid medium.

Ultrasound waves are defined as mechanical waves at frequencies above the human ear threshold, ranging from 20 kHz to greater than 10MHz (10,11). This broad range in frequencies can be subdivided into several categories, each responsible for varying effects. Low frequency ultrasound is characterised by frequencies ranging from 20-100 kHz (10). High frequency ultrasound described the range of frequencies from 100 kHz-1 MHz. Diagnostic ultrasound describes the range of frequencies from 1-10 MHz, however the food industry only uses low and high frequency subdivisions in food processing (5,11). Ultrasound waves can propagate through solids, liquids, and gases possessing elastic properties, generating two alternating cycles throughout their propagation:

Power: Amount of energy transferred or converted per unit time (W=Joules/s).

Intensity: The power transferred per unit area (W/m^2).

expansion and compression (Figure 1) (11). This alternating cycle is responsible for the phenomenon called cavitation, which can only occur in liquids (2). During the expansion phase, ultrasound waves cause local pressures to drop below liquid vapour pressure, promoting the expansion of dissolved gases (5,11). The gas bubbles continue to grow and oscillate until they eventually reach their critical size, defined by ultrasound frequency and intensity, and achieve resonance with applied waves (5). Subsequently, bubbles implode, drastically increasing local temperatures and pressure, and generating turbulence and disruptive forces in the fluid (12). These localized disruptions induce mechanical changes, such as grape cell wall damage, and chemical changes in the wine (5).

Cavitation: The expansion and implosion of gas bubbles in a substance because of the expansion and compression properties of ultrasound oxygen exposure to wine.

Must: Freshly pressed grape juice containing the skins, seeds, and stems.

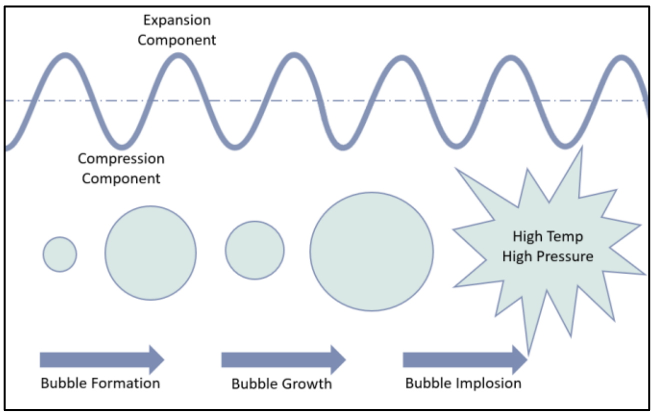

FIGURE 1: ULTRASOUND AND CAVITATION Visual depiction of ultrasound waves and the cavitation process caused by ultrasound. Ultrasound waves have compression and expansion cycles that cause bubble formation, bubble growth, and subsequently bubble implosion in liquid mediums. Bubble implosion produces notable localized increases in temperature and pressure of up to 5000°C and 500 bar. Inspired from (13).

Cavitation is predominant at low frequency ultrasounds, resulting in notable mechanical effects. However, cavitation is negligible at high frequency ultrasounds resulting in minimal mechanical and chemical effects (10). Conversely, low frequency ultrasounds may induce chemical and physical changes in the wine (10). These are often applied for three purposes: modifying compound extraction during maceration, hastening fermentation or ageing, and sterilization (2). While ultrasound frequencies modulate the degree of cavitation, the power or intensity of ultrasound waves also play a role in cavitation optimization and thus regulate the degree of chemical and mechanical changes taking place in the wine (10,11). However, each stage in vinification requires careful selection of the ultrasound intensity. Some processes, such as fermentation, require more gentle stimulation as compared to maceration, where grape cell destruction caused by high intensities is favourable for improved compound extraction (11).

ULTRASOUND TECHNOLOGY IN THE WINE INDUSTRY

Developed in collaboration with the University of Murcia and the University of Castilla-La Mancha, the Ultrawine Perseo system is the first machine that has obtained approval from the International Organization of Vine and Wine (OIV) for oenological practice (Figure 2) (14).

The Ultrawine Perseo system has been granted international patents for its groundbreaking system in the thirty-nine member states of the European Patent Organization, USA and New Zealand. This system utilizes high-power, low-frequency ultrasound as an extraction technique (16). The primary objective of the machine is to facilitate the efficient transfer of phenolic compounds from grape skins to the liquid phase or must, with a particular emphasis on the anthocyanins responsible for wine colour. Moreover, the machine can serve as a continuous pre-treatment method for crushed red grapes before they are introduced into the vinification tank (14). Experiments conducted on numerous grape varietals using the machine demonstrated that a few minutes of ultrasound treatment at varying power levels could significantly enhance the extraction of the phenolic compounds (16). Concurrently,

FIGURE 2: ULTRAWINE PERSEO SYSTEM The Ultrawine Perseo system, which has potential application for advanced grape treatment during the fermentation process. The Perseo 6 model has a work output of 3000kg/h of product (Dimensions: 2400 x 1020 x 1700 mm) and weighs 425 kg (15).

it reduces traditional maceration times by up to 50% (17). This reduction in maceration time is a key factor in saving energy during the vinification process, with reported energy savings of up to 15% (16). Additionally, the final product yielded 35-40% more organoleptic attributes while achieving the same polyphenolic potential (16).

Each ultrasound module within the Ultrawine Perseo system consists of at least one sonoplate, a generator for supplying electrical energy, and a hexagonal pipe (Figure 3) (14). The hexagonal pipe functions as a protective and soundproofing enclosure for the sonoplates while supporting the continuous flow of grape must at varying rates between 1000-50000 L/hr. The system incorporates a cold exchange to limit overheating, thereby preventing the formation of unwanted compounds like the carcinogen, hydroxymethylfurfural, during extended treatments (14). Operating within the low frequency range of 15-35 kHz, with a preference for the 22-25 kHz range and an amplitude from 1-100 μm, the sonoplates ensure efficient cavitation and compound extraction (17).

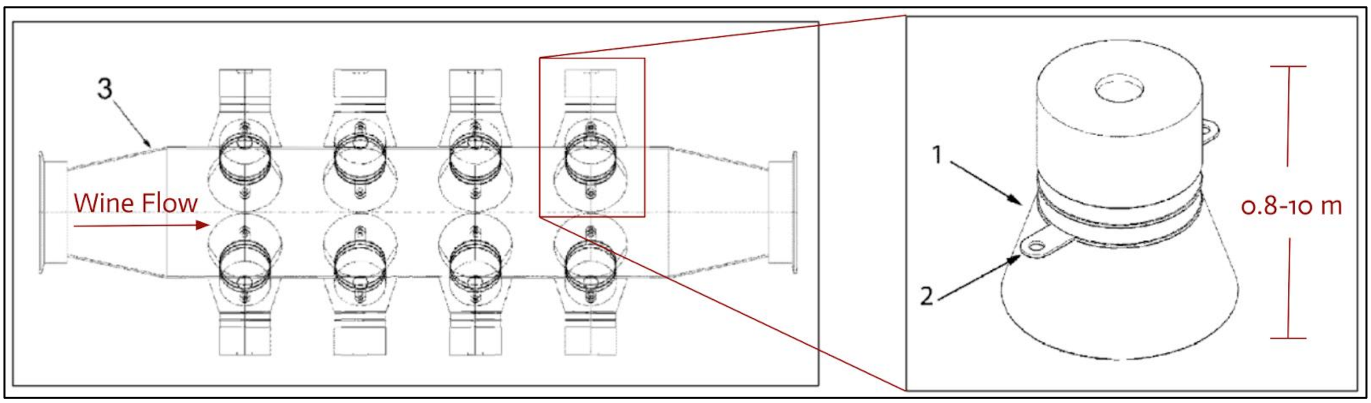

FIGURE 3: ULTRAWINE PERSEO DEVICE PATENT ILLUSTRATION where sonoplates (1) are connected to a hexagonal duct (3) for grape flow. The ultrasound system, created by connecting sonoplates via terminals (2), utilizes piezoceramic transducers, depicted in the inset. The hexagonal pipe, designed for grape must, has widened regions for insulation. Welded sonoplates form a protective enclosure for the ultrasound module (Adapted from 12).

The base model of the Ultrawine Perseo system, which is best suited for small wineries, costs approximately CAD 200,000. This makes it an attractive option for smaller wineries looking to integrate ultrasound technology without the extensive financial commitments required by larger industrial-grade systems which can cost millions. Conversely, larger wineries may necessitate higher-capacity industrial-grade ultrasound equipment to meet the demands of their extensive production lines. While the Ultrawine Perseo system offers scalability, accommodating the requirements of both small and large wineries, larger establishments might opt for advanced configurations with increased processing capabilities. In addition to the Ultrawine Perseo system, there are typical commercial ultrasonic machines used across various industries that are not specialized for vinification but can be adapted for use in the industry (17). Additional expenses may include professional installation services and training programs for personnel operating and maintaining the ultrasound technology. There are currently no companies or wine producers that openly utilize ultrasound technology in production. Current research efforts predominantly take place on a laboratory scale, yielding unrepresentative results, and emphasizing the need to bridge the gap between experimental findings and large-scale industrial applications (2). Clear requirements for ultrasound use in each vinification step should be established. This is due to the exact mechanisms by which ultrasound operates in different vinification steps are not fully understood, limiting commercial applications.

IMPLICATIONS OF ULTRASOUND ON MACERATION

Phenols: Compounds characterized by a hydroxyl (-OH) functional group attached to an aromatic ring.

Anthocyanins: A class of polar compounds that contribute to the red, purple, and blue colours in plants.

Flavonols: A class of polyphenolic compounds characterized by a ketone functional group.

Ultrasound assisted maceration has consistently shown to increase phenolic compounds, such as flavonols and anthocyanins, compared to manual maceration processes (12). The implosion of cavities within the liquid component causes immense physical damage to cell walls, membranes, and seed particles (10,12). Additionally, cavity implosion induces homolytic cleavage in water molecules, creating radicals. The increased presence of both phenols, a known antioxidant, and free radicals in the must solution allows for heightened antioxidant activity (2,12). Subsequently, ultrasound expedites the maceration process by extracting more chemical compounds via the mechanical breakup of grape skins. The increased surface area between the must and solid particles allows a greater dissolution of desired compounds into the must (2,12).

Homolytic cleavage: Breakage of a chemical bond, where each molecule involved keeps one of the electrons from the bond.

Antioxidants: A substance that protects cells against free radical damage.

However, if ultrasound intensity and duration are above an optimized threshold, an excessive degree of cavitation may occur. This can deplete the yeast culture in the solution and reduce future fermentation abilities in subsequent vinification stages (2). Thus, the vintner must accurately set the parameters of the ultrasound waves to match the type of vinifera, yeast concentration, and desired sensory profile (2). The cavitation process may also cause the solution to overheat due to the localized instantaneous release of energy in the form of heat and pressure (2). To accommodate for this, the solution should be constantly monitored to achieve the vintner's preferences for viscosity and density.

IMPLICATIONS OF ULTRASOUND ON FERMENTATION

One of the main fermentation pathways is alcoholic fermentation (2). In this process, *Saccharomyces cerevisiae* (*S. cerevisiae*) and other yeasts facilitate the breakdown of carbon-based sugars, producing alcohols, carbon dioxide (CO_2), and metabolites (5). Varying concentrations of these metabolites such as flavonols and anthocyanins alter the distinct profile of the wine and contribute to the overall drinking experience. Low intensity ultrasound can accelerate fermentation and extract compounds related to wine flavour and aromatic profiles (18). The ability of ultrasound to improve or degrade wine quality depends on species-specific enzyme tolerance. *S. cerevisiae* is the most common yeast used in red wine fermentation for its unique stress response pathways, allowing it to withstand extreme ethanol, osmotic pressure, and temperature conditions. The fermentation process indirectly assists in aromatic compound extraction by producing metabolites or dividing binders and their aromatic substituents. While its mechanism is poorly understood, the use of low frequency ultrasound promotes cell growth and anaerobic respiration (19). It is theorized that low intensity ultrasound reduces the dissolvability of CO_2, lowering the pH of the solution and maintaining an ideal environment for *S. cerevisiae* growth (20,21). Additionally, low intensity ultrasound is shown to promote *S. cerevisiae* cell growth by inducing changes in cell physiology. This includes increasing cell membrane permeation to enhance the uptake rate of enzyme substrates and metabolites, accelerating yeast fermentation (22). For instance, Dai et al. (23) demonstrated a substantial increase in the biomass growth of *S. cerevisiae* under optimal ultrasound conditions involving a frequency of 28 kHz, power of 140 W/L, and treatment time of 1 hour. The treatment heightened cell membrane permeability and activated the yeast, facilitating the migration of nutrients from yeast cells to the fermentation medium. This led to a 36-hour reduction in fermentation time compared to the control group (23).

The application of ultrasound during fermentation is also particularly valuable for its bactericidal effect, which eliminates or decreases the quantity of sulphur dioxide (SO_2) needed to sanitize the must (24,25). Cavitation creates sulphite or bisulphite ions, rendering it more reactive and effective in protecting the wine against spoilage. The sulphur-free radicals generated during cavitation also engage in oxidative reactions with key wine constituents, particularly carbonyl and phenolic compounds. This interaction significantly influences the wine's colour, creating a deeper and more vibrant red (24).

As with all technology, ultrasound applications have specific challenges that need to be addressed for optimal results. Excessively high intensity ultrasound and prolonged treatment times can be detrimental to the fermentation process (3). This is primarily due to excessive cavitation, which can kill the yeast cells (26).

IMPLICATIONS OF ULTRASOUND ON AGEING

During the wine ageing process, ultrasound technology can increase the rate of chemical reactions within the wine by promoting molecular interactions and decreasing activation energy (2,27). Ultrasound waves increase the affinity between polar molecules such as water and ethanol, and other functional

Esters: A class of acid-derived chemical compounds characterized by a -COOR group which reacts with water to produce alcohols.

groups such as esters. This leads to the formation of larger molecules that alter wine mouthfeel and balance the taste profile (2). Ultrasound waves have also been used in chemical reactions to reduce activation energy. When higher intensities are used, cavitation increases, releasing more heat into the system (2). Higher temperatures decrease the activation energy of various chemical reactions including esterification, condensation, and redox, resulting in an increased reaction rate and decreased ageing time. Reducing the length of the ageing process increases return on investment by allowing the producer to cut down production time, increase storage capacity, and produce a greater quantity at the same or higher quality.

QUALITY INDICATORS

While ultrasound may promote and accelerate all stages of the vinification process, it is imperative that the quality of wine is maintained. Wine quality is quantifiable using chemical indicators, including phenol and polysaccharide content, and colour (28). Colour gives a general visual appeal to wine, in both colour density and hue. Phenol content builds the aromatic and flavour profile of the wine., while polysaccharides contribute to wine viscosity and mouthfeel, balancing the aromatic profile.

It is generally believed that increases in polysaccharide concentrations correlate with improved mouthfeel and sensation. A study by Lapuente, et al. (29) tested the effects of varying ultrasound settings on polysaccharide concentrations during maceration. They determined that throughout the ultrasound application, polysaccharide release increased, surpassing levels reached by the control wine. This suggests that ultrasound can be used to decrease maceration time, while still maintaining or potentially increasing polysaccharide content.

The types of phenolic compounds found within one vintage are variable, providing different flavours, such as bitter, floral, and herbaceous notes. Natolino and Celotti (30), applied ultrasound during the ageing process which resulted in a similar total phenolic content to the control wine, while the proportion of complex polyphenols increased. Complex polyphenols are known to contribute to a smoother and less bitter aromatic profile. In the wine industry, complex polyphenol concentration positively correlates with wine quality, suggesting that increased polyphenol concentration may improve the perceived quality of ultrasonicated red wine (31). However, without promoting polymerization, excess polyphenols such as harsh tannins will remain unbound. This can increase astringency, depreciating the quality of the wine, thus justifying the need to optimize ultrasound frequency and intensity to minimize negative effects.

Colour is used as a visual indicator of flavour and extracts. To the consumer, increased colour is associated with increased wine quality and age (Figure 4). A study performed by Zhang and Wang (32) achieved the colour density and visual characteristic development associated with an aged red wine through ultrasound treatment on a young red wine. As a result, consumers may associate young ultrasonicated red wines with the colour characteristics of older wines, making it a more desirable product based on perceived quality and efficiency.

FIGURE 4: COLOUR EVOLUTION The colour evolution of a wine as it ages replicated through the application of ultrasound. Polymerization reactions overtime cause the browning of reds, and the paling of colour as molecules become too large to remain suspended (33).

FUTURE DIRECTIONS

While ultrasound technology produces a more desirable product, its implementation in the market may face rejection by consumers due to unfamiliarity, alterations in sensory characteristics, and potential stigma surrounding health and safety (34,35). It is also a concern that consumers will associate the usage of the technology with a reduction in authenticity of the luxury product (36). To minimize these challenges, marketers can capitalize on the limited label space on the wine bottle to inform consumers of the technology and assure them wine quality is upheld (Figure 5) (36). As a result, consumers may feel they are making a more informed purchase, thus associating positive emotions with ultrasound implementation in their luxury drink products (34). Transparency regarding ultrasound technology use builds trust between the brand and consumer, increasing positive associations and developing brand loyalty (34).

In recent years, consumers globally have also become more conscious of healthy eating and drinking. The potential increased health benefits associated with ultrasonicated wine may provide vintners a unique advertising opportunity (36). This is particularly advantageous in the red wine market, as it is commonly known as a healthier alternative compared to other alcoholic beverages and wine varieties for its antioxidant properties (36). As ultrasound extraction methodologies can be used to increase the antioxidant properties of wine through heightened phenol concentrations, the relative health benefits of the product are improved. Additionally, the evasion of SO_2 use in the fermentation process through ultrasound allows the product to be marketed as sulphite free, catering to consumers with sulphite sensitivities. (36). Given these properties, vintners may successfully market ultrasonicated red wines to health-conscious consumers as an alternative to traditional red wines.

FIGURE 5: LABEL MARKETING A bottle of red wine utilizing limited label space to emphasize buzz words such as 'healthier' and 'sulphite-free'. These words are used to catch the consumers attention, informing them of the benefits associated with the product (37).

Although ultrasonicated wine can offer numerous marketing opportunities for vintners, limited studies have been conducted on consumer acceptance, which is a key reason for its lacking presence in the industry and on shelves. It is expected that studies regarding consumer preference will emerge in innovative wine regions of the world including the Niagara Peninsula. However, extra virgin olive oil, a luxury product across Southern European countries, can be utilised as an analogue to investigate current consumer acceptance. A study by Roselli, et al. (38) found that consumers with a higher education status were more likely to buy ultrasonicated extra virgin olive oil. This may be attributed to enhanced comprehension of label information and rationalization of novel food processing techniques used in luxury food products (38). 51% of the surveyed participants proposed they would purchase ultrasonicated extra virgin olive oil. However, 33.8% of participants would only purchase the product at an equivalent price to that of the traditional product (38). Therefore, this analogy offers valuable perspectives on the specific markets where ultrasonicated wine might find traction.

Balancing the perceived benefits and addressing the concerns associated with ultrasound technology in wine production is crucial for both vintners and consumers. When considering how the ultrasound process reduces maceration, fermentation, and wine ageing time, the manufacturing process holds the potential to enhance the profitability and viability of vinification in non-traditional wine regions worldwide.

SUMMARY

The implementation of ultrasound in the wine industry offers vintners a unique opportunity to fuse tradition with new advancements. Using ultrasound decreases the overall time required for maceration, fermentation, and ageing. In evaluating the risks and rewards of ultrasound technology, vintners must carefully balance multiple parameters. These include adjusting frequency and intensity to meet their needs, while preserving the uniqueness associated with traditional vinification methods. Vintners should explore the technology's potential to improve efficiency and increase the quality of the vintage. This involves conducting further research to identify the precise application of ultrasound on an industrial scale. The future of ultrasound in the wine industry relies on market demand. Analogues in the food industry highlight a rising consumer embrace of ultrasonicated luxury products, presenting diverse advertising opportunities for ultrasonicated wine. However, with any novel product, the transition to retail availability will necessitate time and further research, setting the stage for an upcoming dynamic and pioneering era in vinification.

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Oppenwiner:
Using Radioisotope Dating to Detect Fraudulent Wine

Mehreen Chowdhury, Zachary Kazaz, Naira Woo

The production and distribution of counterfeit wine poses an immense threat to consumers, governments, and jeopardizes the global wine market at-large. Forging counterfeited bottles or reusing authentic bottles makes detection impossible without looking at its content. However, current authentication measures have proven ineffective. The answer to detecting wine fraud may lie in our nuclear past. Unnatural amounts of atmospheric Carbon-14 and Cesium-137 released by nuclear testing and their sequestration within viticulture offer a temporal framework that can be used to date and verify wine according to their vintage. As these atmospheric radionuclides diminish in concentration over time, we must also consider comprehensive future wine authentication methods.

We can use novel technologies to compensate for the shortcomings of radioisotope dating. Nonetheless, radioisotope dating remains the most promising remedy for wine fraud on the market to date.

BACKGROUND

THE FRAUDULENT WINE INDUSTRY

The global wine market was valued at nearly $410 billion USD in 2022 and is estimated to exceed $583 billion USD by 2032 (1). In the same year, fine wines comprised half of annual wine revenues and have continued to be the top-performing asset class in the luxury investment sector, growing by over 20% in the last 5 years (2). In particular, demand for fine wines of older vintages from notable wine-producing regions such as France, Italy and Spain have skyrocketed (3). The scarcity and desire for these fine wines result in exorbitant market prices, creating a variety of unregulated secondary markets such as wine merchants, private collectors, and auction houses (4).

Within this lucrative and high-demand industry, consumption and collection fraud in wine has become an ever-emerging threat. It offers a high profit and their seemingly impossible authentication presents low risk. Forging counterfeited bottles or reusing the glass, cork, label and capsule of authentic bottles makes detection nearly impossible without looking at its content (5). Moreover, it is notoriously difficult to estimate the scale of the wine fraud market as it is challenging to authenticate and often unreported, making its quantification impossible.

The fraudulent wine industry generates an estimated $9 billion annually, poaches tens of thousands of jobs while depriving governments of billions in tax revenues (6). Counterfeiting wines often involves replacing the contents of authentic bottles with mixtures of lesser quality or simply replacing the label of a cheaper bottle. This can increase the price of a bottle by over 20-fold, profiting tens-of-thousands of dollars per bottle. It is approximated that over 5% of wine sold on secondary markets are counterfeit (7). The extent of wine fraud varies geographically. For example, the EU estimates over 7% of wines in their market are inauthentic, whereas some regions that are heavily dependent on importing wines, such as China, report roughly 50% of wines being counterfeit (8).

Fake rare and fine wines are produced at limited scales in domestic kitchens to mass-production at commercial sites. Here, cheap wines are blended with additives to conceal their low quality. For example, elderberry among other colouring agents are added to achieve deeper colours, with spices such as cinnamon included to improve complexity, in addition to other sweeteners and chemicals (8). In extreme cases, fraudulent

FIGURE 1: WINE FRAUD WITH COUNTERFEIT LABEL
Side by side comparison of fake (left) and authentic (right) Rouget Vosne-Romanée (1).

wines have proven deadly. Sweetening and aromatic agents can contain hazardous materials such as diethylene glycol, lead(II) acetate and methanol, which can be fatal in small doses (9).

The wine industry is especially susceptible to fraud owing to the market's vast scale, lack of authentication and surveillance, and various supply channels (7). As the fake wine industry continues to expand, the ineffectiveness of current authentication measures have become increasingly clear. Chemical testing has proven unsuccessful as there is a lack of molecular markers with wine tastings indicating biased and inaccurate results (5). Recent findings have shown that the answer to wine fraud may lie in our radioactive past.

SIGNS OF OUR NUCLEAR PAST

Radionuclides related to atomic testing and historical events can be used to date wine as the amount of atmospheric radiocarbon and Cesium-137 (^{137}Cs) sequestered by a wine when compared to atmospheric concentrations of the corresponding year's vintage (5). Until the late 1940s, when above-ground nuclear testing began, the entirety of Carbon-14 (^{14}C) on Earth was generated via interactions between Nitrogen-14 and cosmic rays in the stratosphere. Between 1945 and 1963, atomic testing nearly doubled the atmospheric concentration of ^{14}C, which has decreased following the enforcement of the Nuclear Test Ban Treaty of 1963 (10). This decrease in radiocarbon is a result of the carbon cycle, where ^{14}C has been distributed between the atmosphere, biosphere, and oceans, as well as the Suess-effect.

Suess Effect: The combustion of fossil fuels releases a significant amount of C based CO_2 and reduces levels of ^{14}C that are naturally present in the atmosphere.

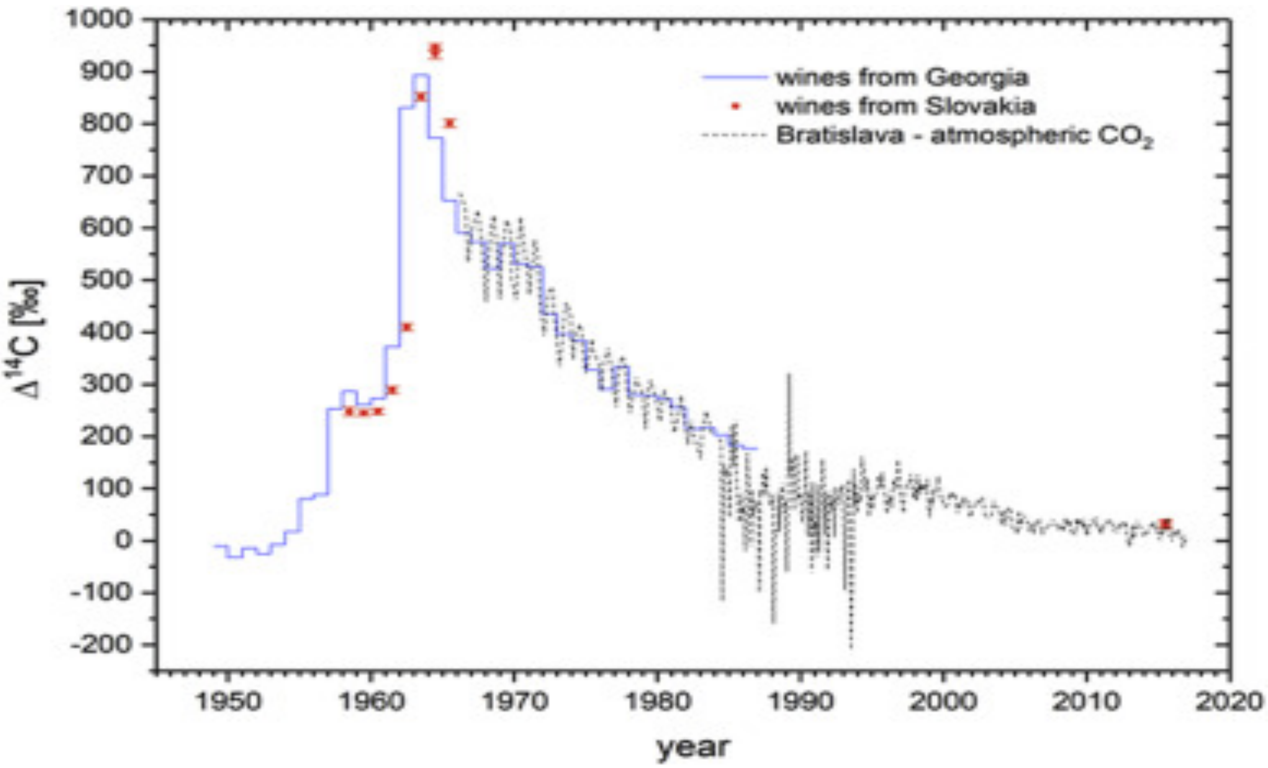

FIGURE 2: TRENDS IN ATMOSPHERIC ^{14}C OVER TIME Comparison of $\Delta^{14}C$ measurements in wines from Georgia and Slovakia. $\Delta^{14}C$ data in Slovakia was also measured from atmospheric CO_2 and is visibly impacted by the Suess effect from the 1980s to 1990s (11).

The release of unnatural amounts of radionuclides into the atmosphere through nuclear testing and catastrophes have created a 'bomb pulse' when measured over time. Observed changes in atmospheric ^{14}C offer a temporal framework at which biological materials produced after 1955 can be compared against with high precision (see Figure 2) (10). The amount of ^{14}C uptake and radiocarbon-containing $^{14}CO_2$ sequestered by grape vines, alongside their subsequent reformation and storage into metabolites and substrates in grape berries can correspond to atmospheric ^{14}C concentration of the year that sequestration occurred (10). Thus, radiocarbon dating can be used as an accurate measurement of time to determine the vintage of wines for authentication. Notably, ^{14}C can also be screened nondestructively. While radiocarbon dating remains effective at authenticating past vintages, ^{14}C levels will continue lowering to natural levels via dilution from fossil fuel emissions and dispersion via the carbon cycle. Consequently, ^{14}C measurement will require increasingly higher precision and should be supplemented with additional methods when authenticating future wine vintages.

Bomb Pulse: The sudden increase of ^{14}C in the Earth's atmosphere due to the hundreds of above ground nuclear bomb tests that started in 1945 and intensified after 1950 until 1963

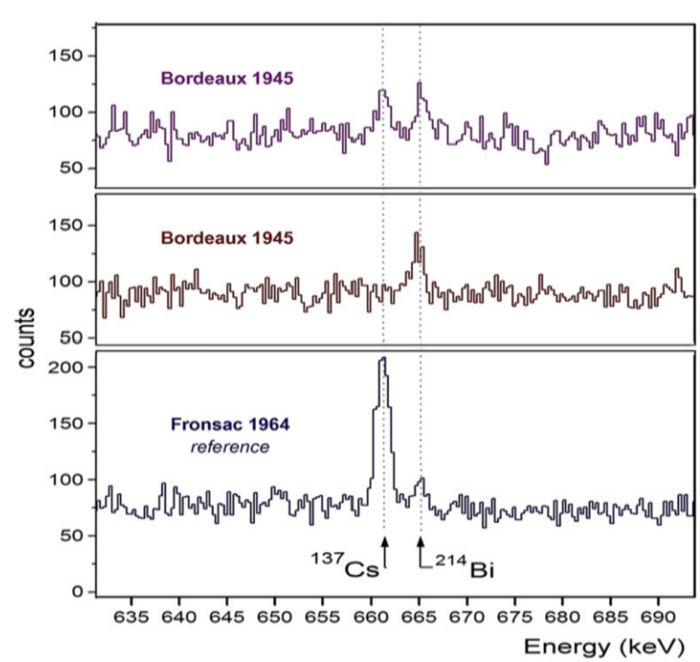

FIGURE 3: GAMMA SPECTROMETRY FOR NONDESTRUCTIVE WINE AUTHENTICATION
A partial gamma spectra of ^{137}Cs for three different wine magnums measured nondestructively. A verified Fronsac 1964 is used as a reference sample to two samples of 1945 Bordeaux. The lack of ^{137}Cs peak in the middle spectra indicates it is fraudulent (5). Note that ^{214}Bi is a daughter product of ^{226}Ra found in the glass of all bottles which is accounted for on the spectra.

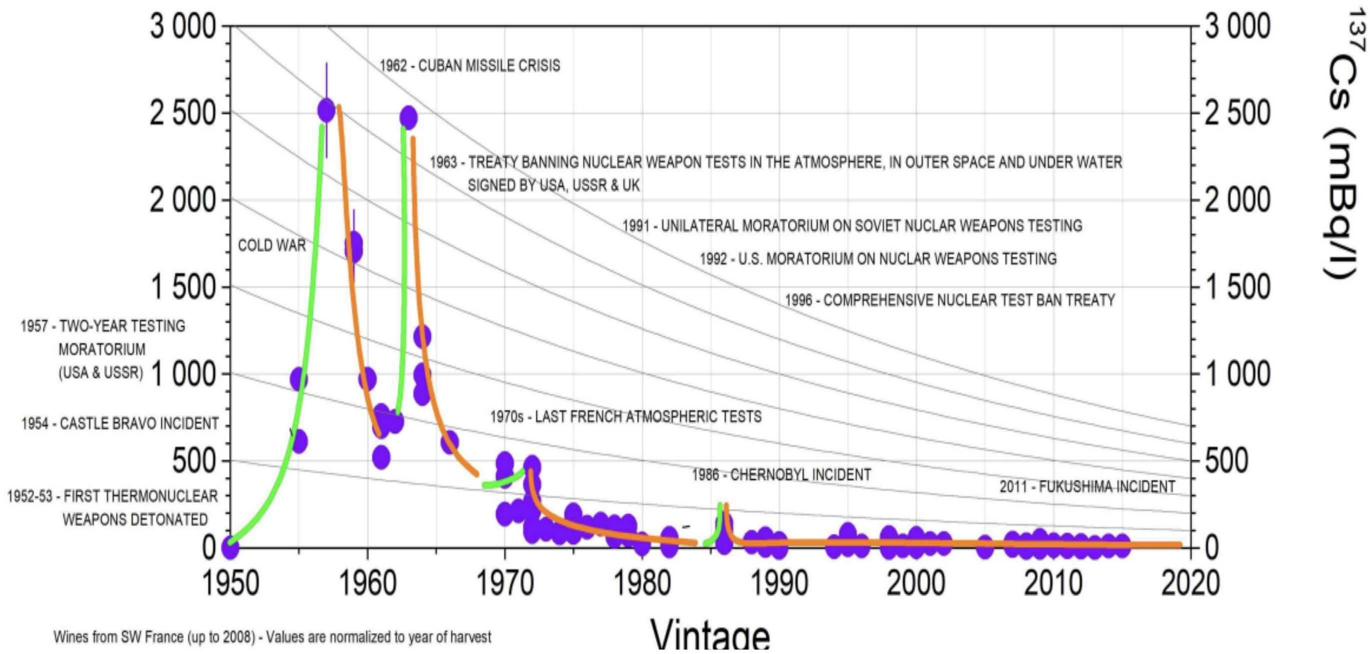

FIGURE 4: ^{137}Cs CONTAMINATION IN WINE FROM NUCLEAR TESTING ^{137}Cs content (mBq/l) of wines from 1950-2016 with ^{137}Cs activity as a function of wine vintage. Green and orange coloured lines are trendlines for corresponding patterns of growth and decay. Annotations made on the figure remark relevant above-ground nuclear testing, incidents, and notable historical events (4).

Similar to ^{14}C, the anthropogenic radionuclide ^{137}Cs can also be used for dating by measuring the gamma rays emitted (see Figure 3) (10). Owing to the penetrating power of γ-rays which can pass through glass and the precision of current detection equipment, nondestructive measurements can be made without disturbing the bottle or sample (5). Much like ^{14}C, ^{137}Cs is readily absorbed by vine leaves, translocation into berries and seeds, and soil-root uptake (11). Because ^{137}Cs is solely a product of nuclear fission and does not occur naturally, its detection can be used to identify the vintage of wines if it was grown when there was high atmospheric ^{137}Cs content. However, ^{137}Cs varies geographically and has a half-life of 30 years, resulting in its rapid depletion following nuclear events, namely, the bomb pulse of 1945-1963 (10). As a result, ^{137}Cs is most effective as a means of determining if a wine vintage was before or after this window of time (see Figure 4). For radiocarbon and ^{137}Cs dating to be used more commonly, methodologies can be simplified by creating a database containing a collection of pre-authenticated reference samples (10). Additionally, because ^{137}Cs and ^{14}C dating are most effective at authenticating past vintages and will eventually become ineffective, a future means of wine authentication must also be proposed.

^{14}C WINE DATING

^{14}C wine dating has many advantages such as its reliability and versatility of methods. The variation in historical atmospheric ^{14}C concentration allows wines from both the preatomic (pre-1945) and the atomic age (1945–present) to be dated (13). ^{14}C is more obvious in wines produced during the atomic bomb tests, which left behind large initial quantities of several radionuclides including ^{14}C and ^{137}Cs. This increase in ^{14}C is reflected in wines from the era, and even years later in 2016, atmospheric ^{14}C remains 15% above natural levels (12). Povinec et al. (13) dated both old and young Slovakian wines using observed relative ^{14}C levels following the bomb-produced ^{14}C in the atmosphere (Figure 5). The radiocarbon age was calculated using two methods. The first used the ^{14}C decay

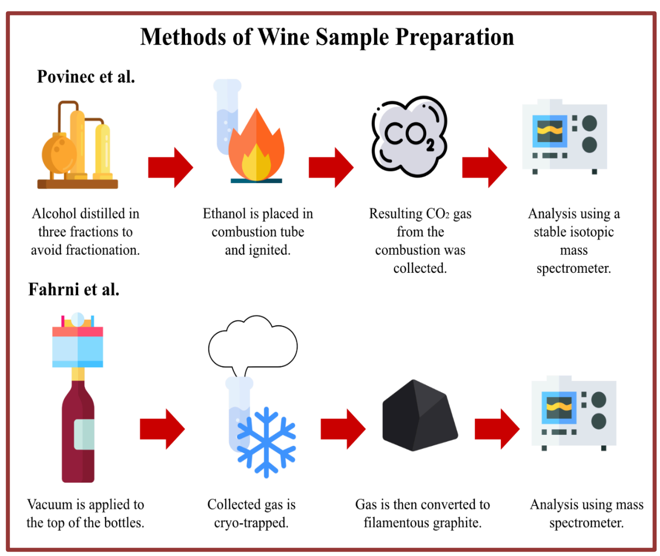

FIGURE 5: HOW TO SAMPLE WINE FOR DATING A flowchart depicting the sample preparation methods of Povinec et al, and Fahrni et al, respectively. Both methods involve the extraction of ethanol from wine and its subsequent conversion into a carbon product for analysis (13,14).

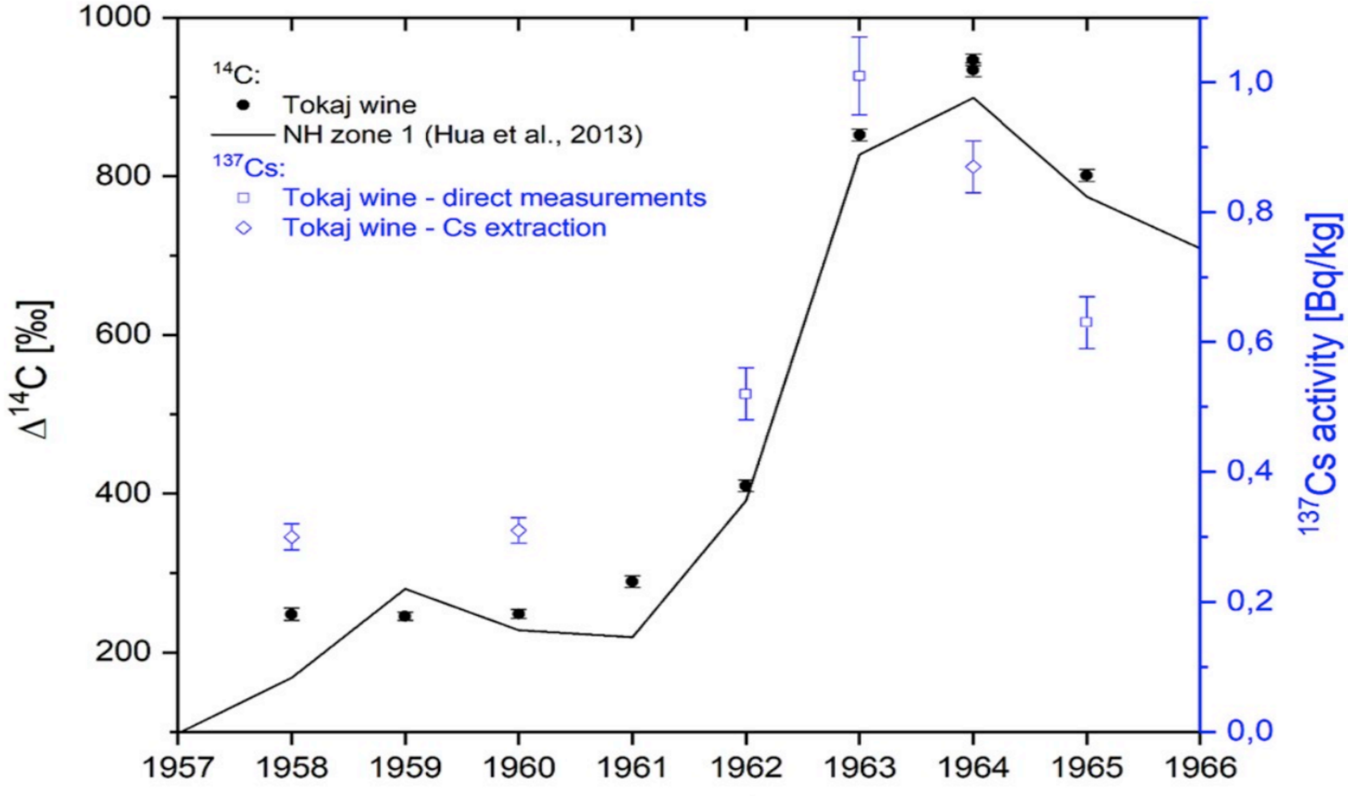

FIGURE 6: ^{14}C VS. ^{137}Cs The change in ^{14}C levels compared to ^{137}Cs activity for the Slovak wines. Also included is atmospheric regional $\Delta^{14}C$ data from Hua et al., 2013. This data shows a positive correlation between change in ^{14}C and ^{137}Cs levels in wines produced from 1957 to 1966 (13).

constant, while the second used the relative change in ^{14}C trend of the specific region the wine was from. Using these methods, they were able to successfully date wines with an uncertainty of about 5‰. The disadvantages to this method are that it requires the destruction of the wine, which is undesirable for collector's bottles. Other downsides are that any organic additives such as sugar added by the winemakers will incorporate additional carbon isotopes, which may add ^{14}C isotopes. Some of these flaws can be overcome by using different methods. Povinec et al. (13) note that use of accelerator mass spectrometry would yield higher accuracy and precision which would allow a total $\Delta^{14}C$ precision of about 2‰.

Fahrni et al. (14) provide an alternative, non-destructive ^{14}C dating method using the angels' share (Figure 5). They dated known-aged wines obtained from a private collection whose age ranged from 1934 to 2006 (14). Of the 32 wines tested, 72% were unambiguously identified as the correct vintage. Due to the presence of false-positives, this method is still under development to improve accuracy and account for other factors such as contaminants (14). Despite its flaws this method is highly attractive as it can date wines without uncorking the bottle, a necessary condition for collectors. Once improved, this process will be a highly valuable tool in preventing wine fraud.

Angel's Share: The ethanol vapours produced by wine that have diffused through the bottle's cork

A third method proposed by Asenstorfer et al. (10) uses bomb pulse ^{14}C generated during atomic bomb testing carried out from the 1950s to 1963 (10). Twenty wine vintages from 1958 to 1997 were distilled and combusted to form graphite. Accelerator mass spectrometry analysis of these samples managed to date the wines within 2 to 17 months of the date on the label, although major discrepancies were infrequent (10). In spite of its accuracy, this method only works over a short time period due to the Suess-effect, where ^{13}C fossil fuels dilute atmospheric ^{14}C. Furthermore, tannins derived from heartwood can contain ^{14}C which throws off the age calculation. Similarly, to Povinec. et al.[13] the sample preparation is destructive, which compromises wine integrity (10). Despite these drawbacks, this method is remarkably accurate when dating wines between 1950-1963, making it a valuable wine dating strategy.

^{137}Cs WINE DATING

^{137}Cs dating is another method that can accurately date wine. Unlike ^{14}C dating, it is not impacted by additives such as sugar and can be done non-destructively via gamma-spectrometry (13,15). ^{137}Cs activity was found to vary in wine temporally, which correlates to the anthropogenic ^{137}Cs released into the atmosphere as a result of atomic testing (Figure 3). Furthermore, due to nuclear incidents such as Chernobyl, atmospheric ^{137}Cs varies geographically, meaning that this dating method may be able to trace wine back to its geographical origin (15). While not as thoroughly used as ^{14}C dating, this method has been tested by Povinec. et al. (13) and Pravikoff et al. (16) who successfully used it to date a series of Californian wines at the PRISNA facility.

Despite its accuracy, there are several conditions that may affect ^{137}Cs wine dating.. Unlike ^{14}C, all ^{137}Cs in the atmosphere is a result of the atomic bomb explosion, which limits this method to only date wines from 1950 until the present (13). Secondly, dating wine without uncorking the bottle, comes at the cost of loss of sensitivity and an increase in measurement times (16). To increase sensitivity, the wine must be destroyed by being placed in a crystallizer under high temperature (100-500 ºC). The resulting ashes can be more accurately measured using gamma spectrometry. Furthermore, gamma spectrometry requires heavy instrumentation in order to be conducted. For example, Povinec et.al also used a high-Purity Germanium (HPGe) detector, which may be inaccessible to the general public (13). While its flaws must be recognized, ^{137}Cs dating is still a useful technique that can successfully date wines. As these dating methods continue to be developed and improved, some of these flaws may be overcome.

Both ^{14}C and ^{137}Cs dating have advantages and disadvantages. In terms of accurately dating wine. Anthropogenic ^{14}C dating becomes more ambiguous the further away the vintage is from the 1964 bomb pulse. Furthermore, large and irregular variations of ^{137}Cs distribution make it challenging to date wine to an exact year, which is why Povinec. et al. (13) suggest using a combination of these methods to circumvent these flaws (Figure 6). Given the variety of tested ^{14}C dating methods, different methods can be selected on a case-by-case basis according to the specific conditions required by the tester. These varied techniques provide both destructive and non-destructive methods which can be used to protect consumers from wine fraud.

WINE DATING USING OTHER ISOTOPES

Other isotopes may be used to date wine; however, they are not as well studied or accurate. ^{137}Cs activity is expected to eventually decrease to undetectable levels as more time passes since the bomb pulse. Thus, Lead-210 was investigated as a potential replacement as it is a gamma emitter that has been detected in wine (13). However, it is limited by its weak correlation between ^{210}Pb activity and vintage. The uncertainty using this method is high, with variations up to ± 50%, ultimately making it challenging to use in its current state (17). Natural cosmically produced tritium has also been used to date wines . Kaufman and Libby (18), found that the tritium contents of vintage wines matched the natural tritium abundances over the previous 18 years. However, this method's measurements are hampered by the spread out of nuclear power plants, rendering it not as effective in modern times. While wine dating techniques that use these radioisotopes are promising, they must undergo refinement to be effective, Currently, ^{14}C and ^{137}Cs dating remain as the most effective techniques used to date wine, however, these alternatives may see use in the future.

FURTHER APPLICATIONS

DETECTING FOSSIL FUEL EMISSIONS

A breadth of studies have investigated the use of radioisotopes, namely with ^{14}C and ^{137}Cs, in determining the vintage and origin of wines. In particular, ^{14}C datingcan elucidate spatial distributions of $^{14}CO_2$ over time, and relate this to fossil fuel emissions by observing ^{14}C levels in grape-derived wine ethanol (10). This is owing to the Suess effect, as seen previously in Figure 2 (19). Fossil fuel CO_2 emissions, especially in industrialized regions, overshadow the amount of CO_2 given off by the biosphere and coastal seas, making it difficult to differentiate between the two (20). However, since fossil fuel-derived CO_2 contains no traces of ^{14}C, when released it decreases the amount of ^{14}C that is naturally present in atmospheric $^{14}CO_2$.

Other sources of CO_2 such as respiration and decay of living organisms, do not contribute to the Suess effect because they recycle carbon in the carbon cycle, and do not introduce new carbon isotopes to the atmosphere, unlike fossil fuels (19). Since fossil fuel-derived CO_2 cannot be directly measured from the air, analyzing wine ethanol provides an innovative approach for comparing regional CO_2 measurements from air samples to residual ^{14}C levels in grapes from specific harvest years. Palstra et al. (20) analyzed 165 wines harvested between 1990 - 2004 and found clear spatial and temporal variations between atmospheric $^{14}CO_2$ concentrations and values of regional atmospheric fossil fuel-derived CO_2 in Europe. With improvements to this method by broadening its scope, the radiocarbon content in wines can help society better understand the environmental impacts that come from accumulating greenhouse gases from fossil fuel emissions (20).

BLOCKCHAIN

Despite the accuracy and precision of radioisotope dating, as mentioned previously, ^{14}C and ^{137}Cs dating are most applicable to wines produced before the 2000s due to data from the bomb pulse and thus may be relegated to rare and fine wines (13). As we look to the future of the wine industry, more cost-effective and scalable authentication methods, such as blockchain technology, are likely to emerge as commonplace. A blockchain is a shared database which has a decentralized system where information is shared across a network of computers. It is continually updated with records of transactions among individuals (entities), stakeholder information (i.e. farmers, engineers, IT experts, etc.), contracts, and other relevant supply chain information stemming from the beginning of the production process (Figure 7) (21).

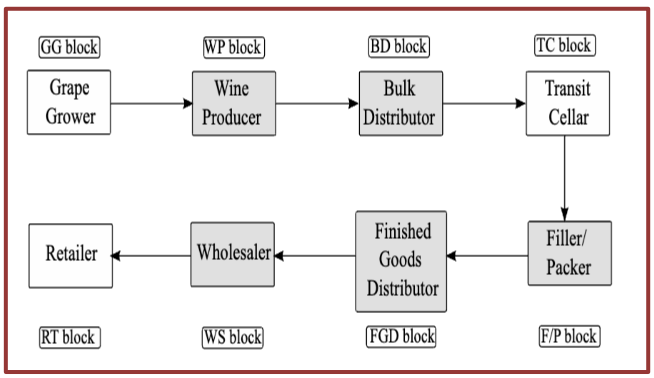

FIGURE 7: ENTITIES IN A BLOCKCHAIN BASED WINE SUPPLY CHAIN TRACEABILITIY SYSTEM Schematic showing the data flow between each entity in a proposed wine supply blockchain based traceability system. The gray blocks represent individuals that are part of a collective decision making process. The grape grower would generate the first block in this example (21).

Furthermore, companies can use data from the blockchain system to create item-specific information on each wine bottle for consumers to view. With a quick scan of the bottle using a smartphone, a unique traceability system is available for consumers to authenticate their wine thanks to blockchain (Figure 8) (22). The issue with traceability systems currently implemented in the wine industry (i.e. radio frequency identification, , Electronic Product Codes, etc.) include a lack of end-to-end encryption, which allows counterfeiters to gain unauthorized access to supplier information stored in a central database (23).

FIGURE 8: AUTHENTICATION BY SCANNING A wine bottle with a near field communication label can be scanned with a smartphone to allow a consumer to access information about its origin. Wine companies can program specific information to the label to cater to consumer preferences, using data from blockchain (22).

Blockchain differs from traditional database storage methods in many ways, most notably because it uses a software algorithm called cryptographic hash, which securely converts inputted information into an encrypted output to generate a block (Figure 9).

```
ID: GG001
Block# 2                                  Batch# 1
------------------------------------------------------------
Origin:          ABCD
Type of Vine:    XYZ
Treatments:      PQRST
........................................
........................................
Pruning Date:    D/M/YY
Delivery Date:   D/M/YY
Prev. Hash:      abcdef0000a0a0bbcdcdfddfa56781bb
New  Hash:       8bc9ef5080a0affbadadfedea56481bc
```

FIGURE 9: BLOCK Example of a block generated by a grape grower with relevant information pertaining to the grape batch being used to make wine. The batch number indicates the production batch supplied by the wine producer which is crucial since a wine producer may receive grapes from many different producers (21).

Several blocks "chain" together to create the blockchain. Blockchains are created on distributed ledgers that serve as database storage platforms, such as *Multichain*, that Biswas et al. (21) used for their proposed blockchain-based wine supply chain traceability system. Each entity tied to the blockchain system has access to the unique information stored in each block with a special encrypted key.

Other entities in the system follow similar procedures, so eventually all their blocks are verified and linked together and can then be traced back using a unique identification (ID), batch, or block number. Every individual bottle of wine has a unique ID, and once a consumer purchases a bottle that transaction information will be incorporated into the system. With this system, as mentioned previously, consumers can scan a QR code on their bottle and instantly receive information about the wine. The advanced security and technological intricacy of this system make counterfeiting extremely difficult as it is impossible for the same bottle to be sold twice by the retailer (21).

CONCLUSION

Whether intentionally misrepresenting the vintage, relabelling cheap wine as a higher quality brand, or using flavour additives to mimic the taste of sought after wines, the production and distribution of counterfeit wine is damaging to both consumers and the wine industry. Beyond the economic consequences of this industry, consuming the harmful and often unregulated additives present in fake wines can pose significant health risks. Nonetheless, the methods of using anthropogenic radionuclides, ^{14}C and ^{137}Cs, to detect the real vintage of a wine provides promising solutions to tackle wine fraud. Different methods of ^{14}C dating, including the use of the angel's share and mass spectrometry, have proven to be reliable in determining vintages spanning from preatomic to atomic ages. While ^{137}Cs dating has been used to accurately date wine, it is a anthropogenic nuclide which is a product of events such as above ground nuclear testing. In the wake of the Nuclear Test Ban Treaty of 1963, atmospheric ^{14}C and ^{137}Cs concentrations are to unlikely to peak again and will continue dissipating to levels demanding increasingly high precision as time progresses. As we look to future methods of wine authentication, methods such as blockchain traceability systems offer innovative solutions for forthcoming generations. Continuously improving available methods of wine dating as well as creating more refined authentication techniques has the potential to eventually eradicate the threat of wine fraud, and provide a safer wine tasting experience to all.

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Got a Blank Space, Baby?

Write Fenugreek's Name

Naya Davidson-Lindfors, Max Dressler, Jeremy Dykstra, Ed Perri

Planting vineyards as monocultures can have detrimental effects on soil health, which can affect the quality and quantity of grapes produced. The impacts on soil health relate to the physical properties of the soil, erosion rates, nutrient content, and bacterial and fungal community health. One way to mitigate these consequences is to plant an intercrop, a secondary crop, between the rows of grapevine *(Vitis vinifera)*. The roots of intercrops stabilize the soil, reduce erosion, and positively affect nutrient levels, such as nitrogen. Intercropping requires a clear management plan to effectively benefit the grapevine. Aromatic plants, particularly fenugreek (*Trigonella foenum-gracum*), are novel intercrop species which have biopesticide and bioherbicide effects, while improving soil health. Fenugreek has been found to increase growth and nutrient content in grapevine, likely due to its atmospheric nitrogen-fixing properties. After assessing the environmental requirements of the intercrop, Fenugreek has the potential to increase sustainable production of grapevine in the Niagara Region.

INTRODUCTION

Grapevine (*Vitis vinifera*) in vineyards is commonly grown as a monoculture, only one type of plant is grown in the field, simplifying the ecosystem, but potentially resulting in ecological issues (1). While this method may be temporarily beneficial to produce grapes in the wine industry, the impacts on the soil may be detrimental to the grapevine over time. This could have negative implications for other parts of the ecosystem of the vineyard and its surrounding areas (2).

Constant replanting can disrupt the soil microbial communities, which leads to damage to the grapevine itself (2). In a soil analysis, Liu et al. (2) found that the diversity and abundance of fungi and bacteria were significantly reduced due to twenty years of repeated grapevine replanting. This reduction in microorganism diversity leads to a decrease in soil quality, worsening the wine produced from the grapevine. Despite this research being conducted in the Liaoning province of China, the findings still provide insight into the potential negative implications of the continuous replanting of grapevine in vineyards (2). As the microbial characteristics of the soil represent an indicator of the soil's overall health, it is critical to improve poor soil conditions caused by monocultures (3). In tandem with this decline in microbial diversity, continuous replanting also leads to an increase of potentially harmful fungi (2). Relative to more diverse cropping systems, grapevine monocultures may also show a reduction in the overall species count, but there is an increase in grape herbivores that may need to be combated with pesticides. The increased pest population may create large detriments to the overall environment surrounding the vineyard (3). As such, the wine industry should attempt to transition away from monocultures due to potential negative impacts to environmental growing conditions over time.

The Niagara region of Ontario contains a large quantity of vineyards due to the soil and climatic characteristics in the area. This region is more likely to contain a mixture of soil types which is beneficial to the growth of grapevine (4). A study conducted by Ross and Cline (5) found that soils in the Niagara region contained clay content ranging from 13-43% and silt content ranging from 45-66%. Additionally, it was found that the pH ranged from 5.0-6.7 (5). The Ontario government recommends that grapevine is grown in soils with lower silt or clay content to ensure effective drainage. The Niagara region soils contain sandy loam, sandy clay loam, and loam, which are all advised for optimal grape production, making it a viable location for vineyard placement. Additionally, south-facing sloped land is recommended for grape production as it improves water drainage and receives an optimal amount of sunlight for the growth of the grapevine (5). These environmental factors make the Niagara region instrumental and highly conducive to wine production in Ontario.

Agricultural diversification through the implementation of intercropping is being introduced as a more positive alternative (6). Intercropping, the addition of another crop between rows of the primary crop, can provide agricultural benefits to the vineyard, such as improved soil retention and enhanced water infiltration, as well as decreased pest and weed influence. Specifically, aromatics such as fenugreek (*Trigonella foenum-gracum*) could be used to increase berry yield and the health of the soil. There are additional economic benefits to aromatics as the seeds can act as another product for vineyards to sell, creating a supplementary source of income. While the long-term impacts of intercropping still need further investigation, it remains a promising alternative to the problematic monoculture approach that is common in vineyards and should be considered by vineyard owners in the Niagara region (8).

Did you know?

Cover cropping is different than intercropping! Both cover crops and intercrops grow in the same spatial area in a field. Cover crops are not harvested, whereas intercrops are used as a secondary cash crop. Intercrops are a specific subset of cover crops since they have an alternative purpose beyond covering bare soil (7).

SELECTING AN INTERCROPPING METHOD

Intercropping is a valuable agricultural technique that requires accurate implementation and effective management. A detailed intercrop management plan (IMP) is crucial to maximize crop yield and promote long-term soil health. Ripoche et al. (6) indicates three primary variables to consider in an IMP: the type of intercrop plant used, the percentage of the soil surface area covered, and the intercropping period (8). Common intercrops in vineyards found in Canada include oats, ryegrass, and a perennial grass mix (containing ryegrass, red fescue, and bluegrass) (9). Fenugreek is the proposed intercrop choice because of its potential benefits to soil nutrients and structure and its ability to successfully grow in the Niagara region (10). The soil surface area coverage is dependent on the intercropping method, which varies with the physical placement of the primary and secondary crops. The proposed method employs strip-intercropping, in which two crops are grown simultaneously in strips close enough for ecological interaction yet separated to allow for independent cultivation (11). Replantation is performed every year for annuals and every three to five years for perennials (9). Fenugreek (Figure 1) is an annual aromatic, requiring a yearly plow-down and replanting at the beginning of the next season. To ensure that this novel intercropping method and its respective IMP are effective, multiple-criteria decision analyses will be conducted annually. This method evaluates criteria of interest, in this case, the change in grape yield, compared to factors measured in the field. These factors include sugar content, mass of grapes, and overall flavour (6). Fenugreek seeds will be harvested for increased economic opportunities on the same acreage. The annual planting of fenugreek using strip-intercropping will follow a strict IMP to ensure grape yields while preserving soil health.

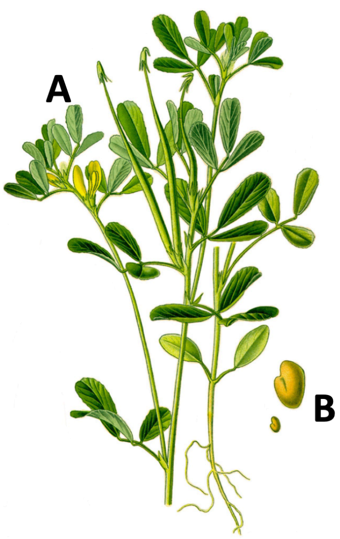

FIGURE 1: A FLOWERING FENUGREEK AND ITS SEEDS **a)** The fenugreek after flowering. Once the seeds have matured, the crop will be harvested. **b)** The fenugreek seeds that will be harvested through this novel approach

BENEFITS OF INTERCROPPING

MEDICINAL & AROMATIC PLANTS FOR INTERCROPPING

A novelty in the proposed intercropping method is the choice of secondary crop. This proposal suggests the implementation of medicinal and aromatic plants (MAPs), which are not yet considered viable for usage in vineyards (8). Notable agricultural advantages of MAP cultivation include improved soil health, biopesticide and bioherbicide activity, biocontrol of soil-borne phytopathogens, and adaptability to diverse ecological conditions (13). As such, the potential of using MAPs for intercropping is worthwhile despite the novelty of the research concerning these plants.

MAPs commonly produce compounds in their leaves that repel insects, and some MAPs exhibit allelopathic effects on common weed species through the production of volatile organic compounds (14,15). Natural products sourced from MAPs have potent insecticidal and herbicidal effects on major pests and weeds that plague crops (15,16). These natural pesticides from MAPs could serve crucial roles in integrated pest management strategies that reduce the use of conventional pesticides (15).

Further, intercropping of MAPs regulates soil-borne pathogenic fungi, nematodes, and viruses that typically result in a 15% loss of total crop production annually (17). MAPs can directly and indirectly affect phytopathogens due to the bioactivity of their secondary metabolites. These effects range from inducing systemic resistance responses, repelling viral vectors, or inhibiting phytopathogen growth through soil microbiome alteration (17). Furthermore, MAPs are resilient and grow in a wide range of climates and diverse conditions, including slopes of approximately a 45% incline (8). MAPs also grow on soil damaged by intensive management techniques such as copper-based fungicides, possibly allowing for MAPs to recover soil health and biodiversity through their inherent benefits (8).

Phytopathogen:
Refers to the soil-borne organisms that have serious negative effects on crops.

Fenugreek is an annual legume with valuable medicinal and pharmaceutical properties in its seeds and leaves (18). It was chosen due to its noted environmental and economic benefits in viticulture from a study conducted in Egypt by Belal et al. (18). However, due to the limited primary literature on this topic, no studies containing soil and climatic conditions analogous to the Niagara region have been published. Therefore, quantitative data from these experiments is extrapolated and examined for general trends. This study on intercropping grapevine with MAPs by Belal et al. (18) compared several characteristics shown in Table 1. These characteristics likely result from the atmospheric nitrogen-fixing ability of legumes in tandem with the reuptake of nutrients in the upper soil layer through intercrop roots (18). Some additional benefits of fenugreek include its pesticidal activity while planted, the bioactivity of its secondary metabolites, and the production of essential oils to create natural pesticides (17).

TABLE 1: EFFECT OF INTERCROPPING TREATMENTS ON VARIOUS CHARACTERISTICS This table compares data obtained from three experiments involving nitrogen (N) content measured as a % abundance in grapevine leaf petioles, grape yield, and mass of 100 berries. Intercropping with fenugreek shows a significant increase in these characteristics compared to the non-intercropped control. While intercropping with black cumin, another aromatic plant, does not increase any of the characteristics significantly. The significance of the results, indicated by the asterisks, is based on statistical analyses performed by Belal et al. (18). Adapted from Belal et al. (18).

	Characteristics		
Treatments	**N (%)**	**Yield (Kg/vine)**	**100 Berry Mass (g)**
Thompson Seedless Grapevine Alone (Control)	2.36	8.63	196
Thompson Seedless & Fenugreek	2.80*	9.35*	232*
Thompson Seedless & Black Cumin	2.42	8.35	200

Before fenugreek can be applied as an intercrop, its feasibility in Niagara must be assessed as it is native to the Mediterranean region (10). Fenugreek requires well-drained, loamy soils of pH 5.3-8.2, a planting temperature of a minimum of 10 °C, and an optimal growth temperature of 18-27 °C (19). Currently, fenugreek is grown in Saskatchewan as a specialty crop, which performs well when seeded between late April and the middle of May (10). Fenugreek should be seeded in early May which is late compared to other MAP intercrops, but the shorter maturity period of fenugreek seeds (~140 days) counteracts this drawback (19). The Niagara region does not have a homogenous climate due to factors such as lake distance, slope, and elevation (20). Regardless, there are general trends in the growing conditions of this region, such as a highly variable spring season and a warm summer season with mean temperatures in July ranging from 20.9-22.2 °C. A turbulent spring is concerning for fenugreek, supporting the suggestion for seeding in May, however, the summer temperatures in Niagara are optimal for fenugreek growth. Another consideration is soil type, Niagara is abundant in well-drained loams, which is preferable for fenugreek growth (20). Lastly, precipitation in July and August is low, while evapotranspiration is high, which is preferable for fenugreek due to its low water requirements (10,20). Overall, the MAP intercrop of choice is fenugreek because of its observed benefits and viability for the Niagara region.

SOIL STRUCTURE & ERODIBILITY

Soil erosion, particularly by water, is an important factor that can affect soil quality and subsequently grapevine growth (8,21). In the Niagara region, soil erosion is likely to become more prevalent due to climate change increasing the frequency of rainfall and drought conditions that promote erosion (22). Erosion can lead to reduced grape yield, smaller berries and clusters, lower acidity, and excessive sugar content, which may alter the quality of the wine produced (8). Soil erosion is dependent on soil aggregate stability, infiltration, and shear strength, which can be modified through the roots of plants (21). When the soil is bare, aggregates are more likely to be disrupted through the oxidation of soil organic carbon (SOC) and sediment transport. Intercrops can help to stabilize aggregates by increasing and maintaining SOC levels, while also physically trapping aggregates through the enmeshing effect of their roots (21,23). Mucilage, a root exudate, acts to stabilize soil particles since it expands to coat particles when wet and then pulls them closer together upon drying (Figure 2) (21).

Soil Aggregate: Refers to the clumping of small mineral particles of different sizes into a stable structure.

Shear Strength: Refers to the combination of both tensile and cohesive forces that provide resistance to soil movement.

Root Exudate: Refers to the chemical secretion of roots that allows for increased aggregate stability and facilitates bond formation between organic molecules and soil particles.

Intercrops can increase infiltration and decrease runoff that removes soil particles and nutrients. The roots can form macropores in the soil creating more space for water, increasing water absorption. They can also increase the permeability of the surface of the soil, as the presence of SOC reduces the formation of physical crusts that can prevent infiltration (23). Additional vegetation cover can also reduce the effects of heavy precipitation by shielding the soil surface from direct rainfall while allowing water infiltration. Runoff is prevented through roots increasing the shear strength of the soil. The tensile strength of roots holds soil particles in place through friction, increasing soil strength (21).

The physical structure of the roots including size, tap versus fibrous roots, and density, can greatly affect the intercrop's potential to reduce erosion (Figure 2). While tap roots increase the soil's saturated hydraulic conductivity, fibrous roots can limit infiltration if they are densely packed near the surface (21,24). Fibrous roots do, however, provide greater root cohesion as they typically have a greater surface area that is in contact with the soil. For tap roots, the diameter is particularly important as with a thicker diameter, there tends to be more erosion surrounding the root and it has weaker cohesive forces due to the limited surface area. Moreover, when determining the most suitable type of root for an intercrop, soil type must be considered; for example, less cohesive and drier soils, like sandy soils, require fibrous roots to maintain cohesiveness (24). Fenugreek has tap roots with large finger-like structures which means that it would be less suitable for preventing erosion in soils that have a higher sand content but can be useful for increasing water infiltration (24,25). This means that fenugreek would be most beneficial in the loamy soils within the Niagara region, rather than the sandy or sandy clay loam areas (5,24). To combat erosion in vineyards, the type of intercrop, where it is planted, irrigation practices, climate, and crop management techniques should all be considered (23).

Saturated Hydraulic Conductivity: Refers to characteristics of soil that allow for water transport when saturated. This parameter is useful in determining the quantity of solute transport and water infiltration.

SOIL NUTRIENTS & PHYSICAL PROPERTIES

Nitrogen plays a key role in plant growth and is often the limiting factor for agricultural systems. Atmospheric nitrogen must be converted into ammonia (NH_3) to become usable by plants through nitrogen fixation performed by free-living or symbiotic microorganisms (26). NH_3 can also be produced during nitrogen mineralization which requires organic nitrogen from the soil. Nitrification further breaks down NH_3 into nitrite via ammonia oxidation and subsequently produces nitrate (NO_3^-) through nitrite oxidation (26). When an intercrop is present, the nitrogen content in the soil is substantially lower than in a monoculture of grapevine (Figure 3) (27). A higher uptake of nitrogen was found directly under the grapevine rootstock for an intercropped system. This phenomenon is a result of fewer intercrop roots under the grapevine, thereby decreasing nitrogen competition due to the larger roots of the grapevine. This demonstrates the importance of physical separation between crops utilizing a bare soil strip underneath the grapevine. This bare soil is crucial to direct nitrogen towards the growth of the grapevine. Annual intercrops, including fenugreek, demonstrated less nitrogen competition than perennial intercrops. This is because there is a shorter growth and nitrogen uptake period of the roots in each growing system, where the intercrop requires less nitrogen than the grapevine.

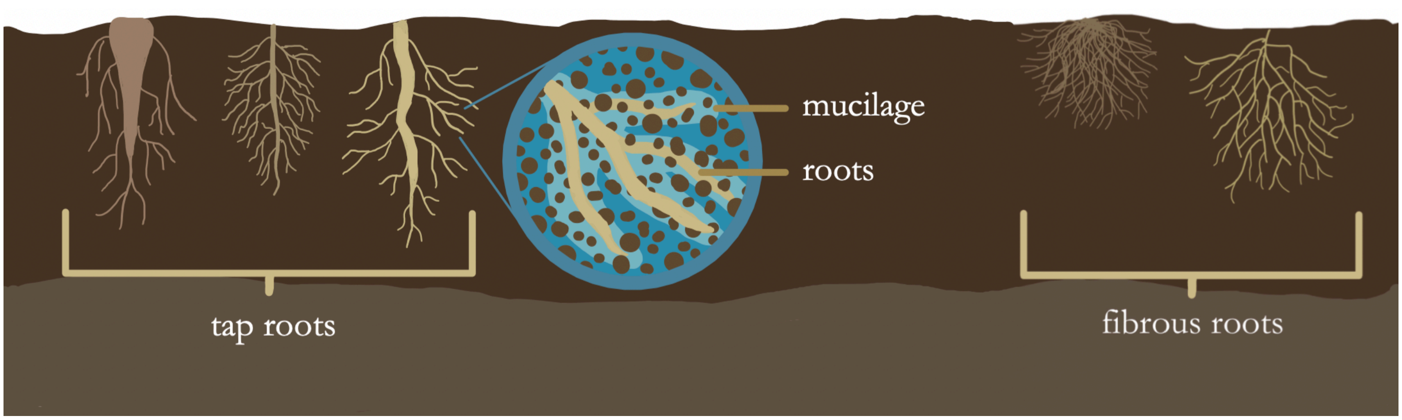

FIGURE 2: THE STRUCTURE OF TAP VERSUS FIBROUS ROOTS AND ROOT-SOIL INTERACTIONS The left side of the image shows a variety of taproot structures in which there is one main root with smaller offshoots. The fibrous roots (right) are thinner and have a more web-like structure. The blue insert (middle) shows how roots trap soil particles as they cover them in mucilage and physically envelop them.

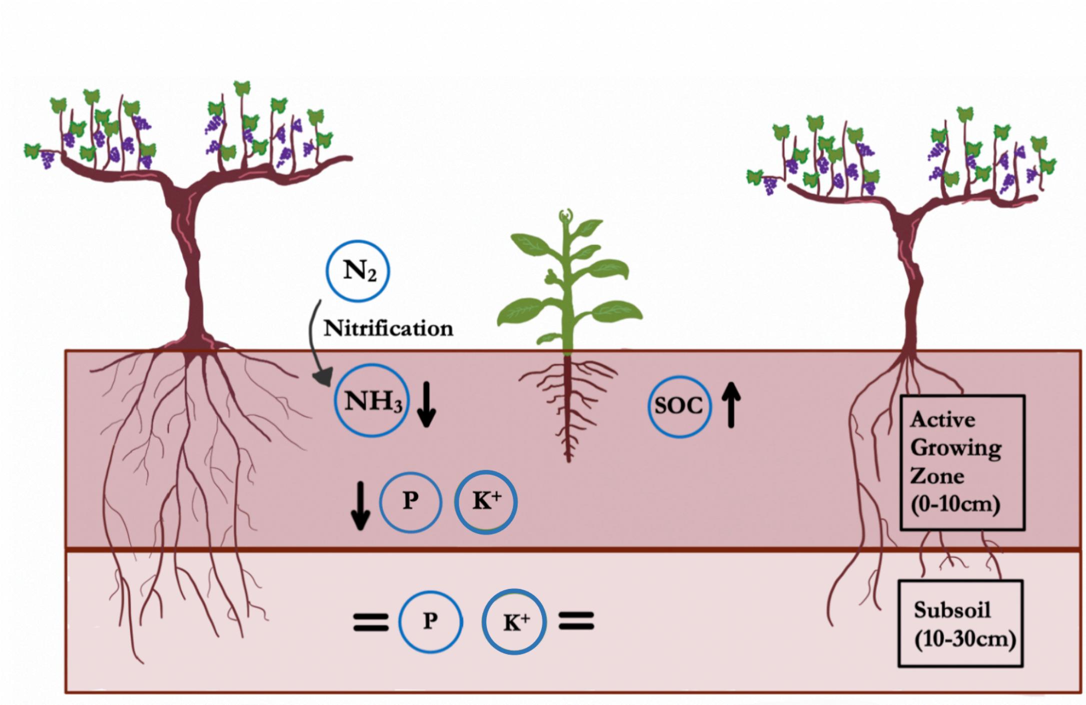

FIGURE 3: NUTRIENT CYCLING BETWEEN FENUGREEK AND GRAPEVINE ACROSS SOIL LAYERS Intercrops decrease nitrogen (N) in the active growing zone, but the grapevine roots penetrate deep enough to remain unaffected. Fenugreek can increase SOC, resulting in a higher effective cation exchange capacity, which allows for more efficient nutrient transfer to plants. Fenugreek has no effect on the phosphorus and potassium levels in the subsoil due to the shallow depths of its roots. The arrows indicate the direction of change in the amount of nutrients.

Without additional nitrogen fertilizer, grapevine growth was reduced for the growing season and the upcoming years (27). A study conducted by Dittrich et al. (8) found that the differences between nutrient uptake were highest near the soil surface. However, the majority of the grapevine roots are below the 0-10 cm layer where aromatics primarily grow, leaving them unaffected by the addition of an intercrop (8).

SOC tends to decrease with prolonged and continued agricultural use. Marks et al. (28) evaluated seven cover crops in a vineyard environment compared to bare soil and found a 22.7% increase on average in SOC at a depth of 0-30 cm within the soil. SOC levels change based on the chemical composition of the organic matter returned to the soil post-harvest. By allowing the fenugreek roots to remain in the soil over the fall and winter months, dead and decomposing material may increase SOC content in the topsoil over time. The amount and type of plant residue returned to the soil has been shown to prime the soil to different microorganisms (29). Organic matter, applied via fertilizer or as a direct effect of intercrop plant matter, has been shown to increase the effective cation exchange capacity (ECEC) of the soil (9). ECEC describes the capacity of soil to absorb cations, the most common of which are calcium (Ca^{2+}), potassium (K^+), and manganese (Mn^{2+}) (30). K^+ and phosphorus (P) levels were reduced in the topsoil layer by intercropping, but had little effect in the subsoil where grapes absorb the majority of their nutrients (8). Overall, intercropping can effect a variety of physical soil properties including SOC, K^+, P, and nitrogen levels.

THE SOIL MICROBIOME

Another benefit of intercropping is its effects on total soil microbial abundance, specifically regarding arbuscular mycorrhizal fungi (AMF). AMFs have been shown to improve grapevine health through the mycorrhizal network that they form in the soil (31). Some of the benefits of AMFs for grapevine include an increase in shoot and root biomass, nutrient absorption, and tolerance to abiotic and biotic stresses (31,32). AMF networks in the soil facilitate macronutrient uptake, increase the water absorption area, and compete with harmful soil-borne phytopathogens (31).

Arbuscular Mycorrhizal Fungi: Refers to soil microbes that colonize the roots of plants and establish a fungal network, allowing for increased nutrient and water uptake.

AMF networks are relevant to intercropping because their fungal links may have a substantial effect on nutrient transfer between the intercrop and grapevine (31). A study by Cheng et al. (31) found that fungal links can be formed between plants of different species and that intercropping increased fungal-mediated nutrient uptake through the overlap of root systems. Fenugreek is a nitrogen-fixing legume, therefore, AMF-mediated nitrogen transfer should be observed when intercropping, in addition to a mutualistic symbiosis between microbiota and fenugreek (10). Intercropping with fenugreek increased total microbial count and dehydrogenase and phosphatase enzyme activity (18). Notably, dehydrogenase and phosphatase activity indicates the measure of microbial redox and phosphorus transformation reactions, respectively. These reactions occur in the soil and are directly correlated with increased microbial count and nitrogen-fixation (33). This phenomenon is likely observed because fenugreek is symbiotically associated with common soil bacteria, increasing the size and nutrient-fixing capabilities of the mycorrhizal network (18,31).

CHALLENGES & CONSIDERATIONS

While intercropping can have many benefits, multiple factors must be assessed to implement this novel approach (Figure 4). A major challenge is the presence of competition between the intercrop and the grapevine. This can lead to reduced berry size, increased pests, and reduced vegetation growth, resulting in decreased grape yield (8). Staggering the harvest of fenugreek seeds and grapes will prevent nitrogen and water competition during the crucial final stages of berry enlargement from late September to October (34). Harvesting fenugreek in early September allows for sufficient time for fenugreek seeds to mature before the plants are plowed in the spring and replanted (35). By delaying the kill date of fenugreek, the loss of available nitrogen to the grapevine is reduced due to the cycling of nitrogen to the topsoil (36). Water competition can be mitigated through irrigation and is generally less evident when the soil has a higher water storage capacity. The time at which the intercrop is planted is also important as it may affect soil erosion. When the soil is freshly tilled in preparation for planting, it is left vulnerable to erosive forces (8). The timeline of intercropping must be considered to minimize the nitrogen and water competition while promoting long-term soil health.

Another thing to evaluate when intercropping is any secondary uses of the intercrop. Aromatics deserve further consideration as there is an increasing economic demand for products that contain them (8). Fenugreek is a spice and a medicinal plant that is marketed in curries, herbal blends, and tea. It has been found to lower blood sugar and cholesterol levels due to mucilaginous fibres in fenugreek seeds and leaves (19,20). The seeds can also be prepared into powders and extracts for medicinal purposes (10). Fenugreek has been used in the treatment of diabetes, infections, heart disease, and cancer. An additional benefit of fenugreek is it serves as feed for cattle, aiding in digestion and milk production (19). Despite the potential economic benefits associated with the use of fenugreek, it is important to consider extra costs that arise with the management of a secondary crop, as well as the potential for a decrease in grape yield (8). There is limited economic information on fenugreek in Canada as it is presently only grown in Saskatchewan. The Ministry of

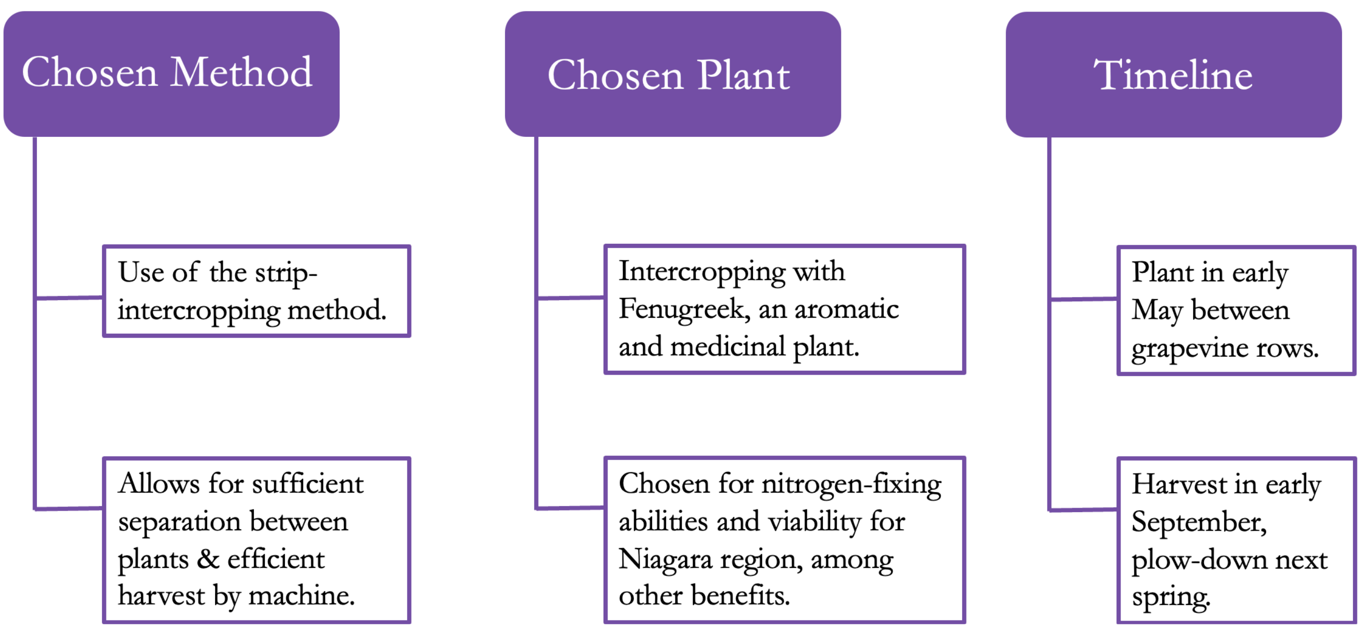

FIGURE 4: A SUMMARY OF THE PROPOSED METHOD FOR IMPLEMENTING FENUGREEK AS AN INTERCROP This figure outlines the chosen method, the chosen plant, and timeline of the proposition and the justification of this novel approach.

Agriculture of Saskatchewan created a guide for crop planning for farmers in 2023, in which they state that solely planting fenugreek in Saskatchewan would cost ~$332.47/acre, excluding labour or management costs. This accounts for costs such as seeds, fertilizers, pest management, and repairs and depreciation of buildings and machinery (37). The costs of growing fenugreek as an intercrop may vary from the estimate above due to factors such as existing infrastructure decreasing costs, as well as extra harvest machinery increasing costs (38). Overall, until there are further assessments of the viability and logistics of intercropping grapevine and fenugreek in the Niagara region, it is difficult to perform an accurate cost-benefit analysis.

CONCLUSION

The implications of intercropping aromatic plants in vineyards in the Niagara region have the potential to mitigate many of the negative impacts of monocultures. Fenugreek was determined to have the greatest potential as an intercrop due to its qualities as well as economic opportunities. Further research must be conducted into the viability of the growth of fenugreek as an intercrop within vineyards in the Niagara region, as it has tremendous potential.

MORE TO EXPLORE

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So Long SO_2:

Out With the Old and in With the New

Bronwen Elkins, Jessica Fisher, Cassidy West

White wines are beloved by consumers globally; however, there are emerging concerns over a common additive used to protect these wines as they age in the bottle. Oxidation and microbial growth both present risks to the quality of bottled wine, as they can result in the loss of fresh and fruity flavours and lead to spoilage. Due to its antioxidant and antimicrobial properties, sulfur dioxide is commonly added at the bottling stage to combat these problems. Unfortunately, sulfur dioxide poses health risks to consumers, particularly those who are sulfite-sensitive or have asthma, necessitating a reduction in the use of sulfur dioxide in wines.

Alternative antioxidant and antimicrobial agents that currently demonstrate promise include ascorbic acid, glutathione, and chitosan. They all impact the chemical profile, colouration, and sensory characteristics of wine. This article details the unique advantages and challenges of using each compound in wine preservation and evaluates the most promising alternatives to reduce or replace the use of sulfur dioxide. Based on this review, we believe that glutathione is a strong alternative to sulfur dioxide, but further research is required before its commercial implementation.

WHAT'S GOING ON IN YOUR WINE?

The winemaking process is very technical, and minor disruptions can affect the quality and value of the final product. Oxidation and microbial growth are two important factors that influence the colour and taste profile of white wines, requiring the implementation of antioxidants and antimicrobial agents before bottling (1,2). White wines are more susceptible to oxidation than red wines, placing them at the focus of this review (3).

Excessive oxidation can cause severe problems in white wine production, as it influences the development of colour, flavour, and aroma (3). During the bottling stage, chemical oxidation begins with the oxidation of phenolic compounds (Figure 1). Oxygen indirectly oxidizes polyphenols to produce ortho-quinones and hydrogen peroxide (H_2O_2) as by-products, through a reaction with iron and copper ions (1). Another reaction which occurs is the Fenton reaction, in which H_2O_2 reacts with iron ions to produce hydroxyl radicals (1). These reactive radical species can oxidize most organic molecules in wine, produce yellow or brown colours, and eliminate fruity aromas (3).

Phenolic Compounds:
Chemical compounds containing hydroxyl group(s) bonded to an aromatic hydrocarbon.

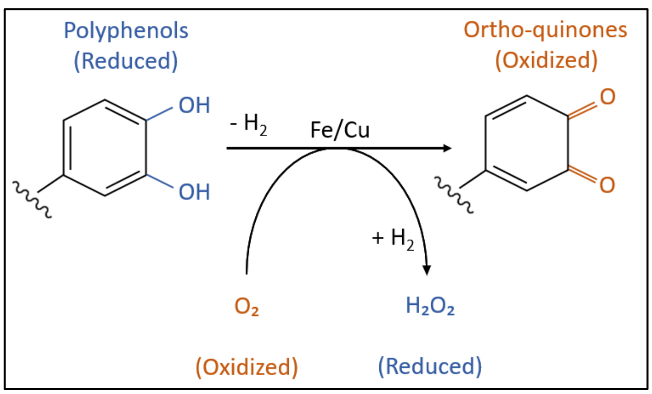

FIGURE 1: OXIDATION REACTIONS IN WINE involve polyphenols and ortho-quinones. In oxidation-reduction reactions, one compound becomes oxidized and loses two hydrogens, which are picked up by the oxidizing agent as it becomes reduced. The reduced forms of compounds are shown in blue and oxidized forms are shown in orange (Adapted from (6)).

Along with oxidation, microbial growth is a concern during the bottling stage. While there are many beneficial microorganisms in wine production, such as lactic acid bacteria and yeast, microbes may generate unwanted by-products (2,4). These compounds can spoil wine by producing unwanted volatiles. Acetic acid bacteria are commonly responsible for wine spoilage, producing compounds such as acetic acid, acetaldehyde, and ethyl acetate (2). Spoilage ruins large quantities of wine by adding bitterness, mousy taint, geranium notes, overt buttery characters, volatile acidity, and an oily or slimy texture to wine (2). Antimicrobial agents are added to minimize the effects of these unwanted compounds on wine.

(OUT WITH) THE OLD

The most frequently used additive in wine is sulfur dioxide (SO_2), due to its efficacy as an antimicrobial agent and antioxidant (5). It can control the oxidation of phenolic compounds and reduce growth of undesirable microorganisms during bottling and aging. SO_2 is found in three forms in wine: SO_2, HSO_3^-, and SO_3^{2-} (Equation 1). This additive is naturally found in low concentrations in wine (5).

Did you know?
Sulfur dioxide exists in 3 different forms that are in equilibrium, and the predominant form depends on the pH.

EQUATION 1: $SO_2 \rightleftharpoons HSO_3^- \rightleftharpoons SO_3^{2-}$

SO_2 is an effective antioxidant due to its ability to limit the production of radicals during oxidation in bottled wine. SO_2 reduces ortho-quinones back to their polyphenolic state, which preserves desired aromas. SO_2 also reacts with H_2O_2 directly, preventing the formation of hydroxyl radicals (Figure 2). Additionally, the enzymes which facilitate browning can be inactivated by SO_2 (5).

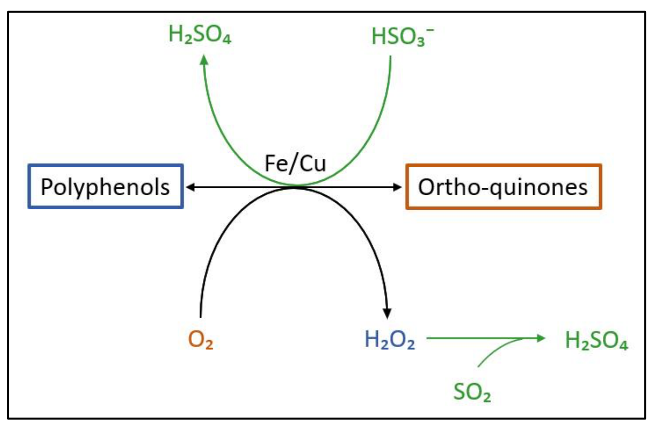

FIGURE 2: SULFUR DIOXIDE acts as an antioxidant in its molecular form (SO_2) by converting hydrogen peroxide (H_2O_2) to sulfuric acid (H_2SO_4). Hydrogen sulfite HSO_3^- reduces ortho-quinones back to polyphenols and is oxidized to H_2SO_4. Sulfur dioxide activity is shown in green (Adapted from (6)).

The antimicrobial efficacy of SO_2 is also important for its implementation in wine production. SO_2 inhibits the growth of lactic acid bacteria and yeasts, as well as acetic acid bacteria which is one of the main causes of wine spoilage (5). This use of SO_2 is limited by pH, as more acidic wine will have higher levels of molecular SO_2, which is the most effective antioxidant form. Low concentrations of free SO_2 may also be ineffective against some bacteria (5).

The main concern regarding the use of SO_2 in the wine industry is the effect of free sulfites in wine on individuals who have a sulfur allergy or are sulfite-sensitive (5). Additionally, when wines high in sulfites are consumed, individuals may experience symptoms including headaches, nausea, and stomach irritation, while asthmatic individuals may experience respiratory distress (7).

In Canada, under the Food and Drug Regulations, wines with more than 10 mg/L of added sulfites must display a sulfite warning label (Figure 3) (8). The International Organization of Vine and Wine (OIV) has set a maximum concentration of SO_2 in white wines at the time of sale to be 200-300 mg/L depending on the concentration of reducing agents (9).

FIGURE 3: SULFITE LABELS are displayed on wines with more than 10 mg/L of added sulfites to alert the consumer of the sulfur content.

(IN WITH) THE NEW

Though SO_2 acts as an effective antimicrobial and antioxidant, the health concerns surrounding it have prompted an investigation into alternative additives. When compared to the addition of SO_2 at bottling, a useful replacement for SO_2 would have no impact or improve the quality of the wine. To choose the most effective alternative, it is important to consider how the additive affects the chemical profile, colouration, and sensory characteristics of wine.

The presence of key compounds in the chemical composition, or chemical profile, of wine, can have profound effects on the quality of the wine. Lowering oxygen levels in white wines through the addition of reductive agents can prevent oxidative damage (3). When assessing some alternatives, their ability to preserve levels of free, active SO_2 in wine is critical, as they may not have adequate antioxidant or antimicrobial properties on their own (10). The ratio of polyphenols to ortho-quinones and other unique compounds that emerge during oxidation can affect the oxidative colouration and sensory attributes of the wine (1).

Oxidation initiates colour changes that reduce wine quality. The oxidation of phenols is a process catalyzed by copper and iron and results in the browning of wine (6). Browning occurs when oxygen is reduced to H_2O_2 and polyphenols are oxidized to ortho-quinones (6). Ortho-quinone products then undergo polymerization reactions that produce a brown or discoloured appearance to the wine. Polymerization reactions also occur between phenols and other compounds present in wine, such as acetaldehyde or glyoxylic acid. These reactions result in browning and other oxidative pigment formation, which are indicative of excessive oxygen levels and are viewed as a sign that the wine has gone stale (6,11). Darker white wines with a brown tint or yellow discolouration are perceived as lower in quality (11). Colour changes associated with oxidation are also correlated with loss of varietal aroma and negative impacts on other sensory characteristics (6).

> **Browning:** An oxidative colour change where white wine is darkened by a brown tint.

> **Varietal Aroma:** A distinctive fragrance that is specific to the type of grape used.

Wine taste and aroma highly influence consumer enjoyment. High quality wines are associated with a fruity and floral aroma and taste (6,12,13). Oxidation results in the loss of this fruity and floral aroma as important aromatic compounds such as esters and terpenes are lost (14).

ASCORBIC ACID (AA) is an antioxidant added to white wines at bottling to protect against oxidation (15). AA undergoes rapid oxidation to eliminate molecular oxygen and reacts with ortho-quinones to convert them back to polyphenols (6). When AA is oxidized by molecular oxygen to form dehydroascorbic acid, the molecular oxygen is reduced into an H_2O_2 by-product (Figure 4) (15).

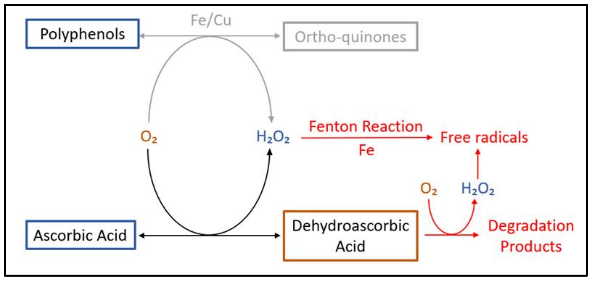

FIGURE 4: ASCORBIC ACID is a stronger reducing agent than polyphenols, and becomes oxidized into dehydroascorbic acid by molecular oxygen (O_2), producing hydrogen peroxide (H_2O_2). H_2O_2 participates in the Fenton reaction catalyzed by iron (Fe) ions to produce free radicals, while dehydroascorbic acid can be further oxidized into degradation products. Compounds in red are undesired products (Adapted from (6)).

To prevent the formation of H_2O_2 and subsequent hydroxyl radicals, SO_2 is often added in combination with AA (15). The reaction of SO_2 with H_2O_2 can prevent iron ions from reacting with H_2O_2 in the Fenton reaction (16). The oxidized form of AA is dehydroascorbic acid; this product may undergo subsequent oxidation or degradation reactions depending on the conditions (15). This leads to the production of compounds such as furfural, which is involved in the formation of spoilage pigments (17). AA also interacts with other oxidation products that are involved in the generation of xanthylium salts which produce yellow pigments (17,18). The OIV recommends using a maximum concentration of 250 mg/L AA during bottling (19). AA should be added at the bottling step to prevent exposure to oxygen which may cause greater damages to the wine than would occur without AA (19). The use of AA to prevent wine oxidation remains highly contested due to its various risks.

GLUTATHIONE is a powerful antioxidant that is naturally present in grapes and produced by yeasts during wine fermentation (20). The OIV has classified glutathione as a wine additive and has approved addition in its reduced form (GSH) to a maximum of 20 mg/L (21). Glutathione may serve as an alternative to SO_2 due to its ability to prevent oxidation while preserving desired aromas and preventing browning (22). Glutathione presents a particular advantage in preserving varietal character and colour (23). It eliminates free radicals and prevents oxygen from reacting with other compounds. GSH donates an electron from its thiol group to reactive oxygen species. Then, two of these reactive glutathione molecules form oxidized glutathione (GSSG) (20). The electrons released during this reaction are used in coupled reactions to protect other molecules from oxidation. Glutathione reduces ortho-quinones to phenolic compounds, preventing the ortho-quinones from undergoing reactions that produce oxidation and browning (Figure 5) (20). Mixed-culture fermentation has been explored to increase glutathione concentration above 20 mg/L and improve sensory characteristics. This effective method has been shown to increase the glutathione concentration up to 10 mg/L above the OIV limit (24).

Mixed-Culture Fermentation: To inoculate (add) two or more yeasts to grape must for fermentation.

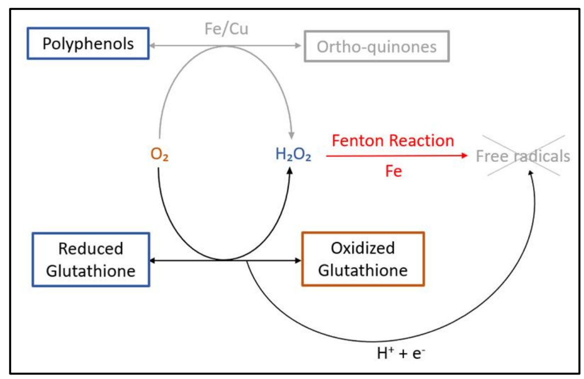

FIGURE 5: REDUCED GLUTATHIONE is a stronger reducing agent than polyphenols, and becomes oxidized by molecular oxygen (O_2), producing hydrogen peroxide (H_2O_2). As it becomes oxidized, glutathione releases a proton (H^+) and electron (e^-) which can neutralize free radicals produced through the Fenton reaction, or serve to reduce other oxidized compounds in wine (Adapted from (6,20)).

CHITOSAN is a natural polymer which is extracted from fungi and processed into films for use in wine (25,26). It has been classified as a processing aid by the OIV and can be used at a maximum concentration of 5000 mg/L (27). Chitosan is a viable and novel alternative to SO_2. It has antimicrobial properties allowing it to inhibit the growth of microorganisms, and function as an antioxidant (28).

As an antimicrobial agent, chitosan generally targets yeasts, lactic acid bacteria, and acetic acid bacteria (25). As an antioxidant, chitosan can directly scavenge radicals, or indirectly block radical formation through metal chelation (Figure 6). Through this mechanism, chitosan inhibits browning and prevents the loss of polyphenols (25).

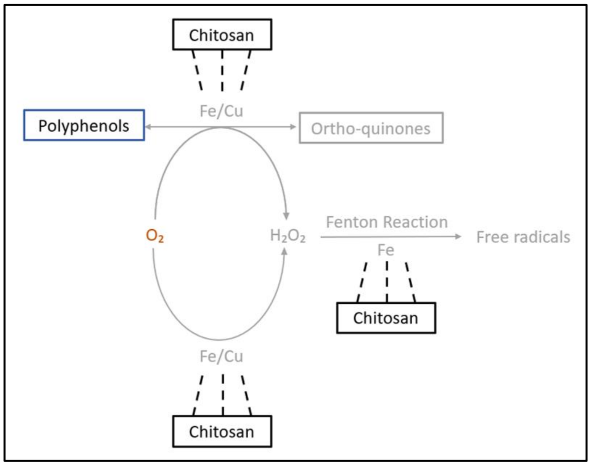

FIGURE 6: CHITOSAN is a chelating agent, which is a compound that forms coordinated bonds around metal ions, preventing them from participating in chemical reactions. Chitosan protects wines from damaging oxidation and radical-producing reactions by sequestering the metal ions needed to catalyze these processes (Adapted from (6,25)).

THE SEARCH FOR THE MOST EFFECTIVE ANTIOXIDANT

Preventing Chemical Oxidation: An Uphill Bottle?

A chemical analysis of wines supplemented with AA, glutathione, and chitosan reveals different effects of each compound on the composition of wine.

ASCORBIC ACID primarily affects the chemical profile of wines by altering the amount of oxygen in the bottle (15). When AA reacts with oxygen, higher SO_2 concentrations may be preserved, allowing for sustained antioxidant action (10,12,15,30). AA reacts rapidly to lower the oxygen concentration in the bottle, preventing oxidative damages (18,29). However, the products of AA oxidation are generally undesirable due to the emergence of oxidized colours and flavours (10,18). Greater levels of unwanted AA degradation products may be mitigated when SO_2 is supplemented in addition to AA (10). SO_2 is often required to be added with AA to scavenge H_2O_2 and other by-products, detracting from the efficacy of AA as a standalone antioxidant (16). However, the oxidation of AA is catalyzed by metal ions, and when metal ions are present they can act synergistically with AA to accelerate the loss of SO_2 (10). Thus, AA presents several benefits in maintaining desirable chemical profiles of wine, but there are risks and unknowns associated with its addition.

GLUTATHIONE is an additive that prevents the loss of desired aromatic chemical contributors during oxidation (31). Glutathione may lower the required amount of added SO_2 as it preserves higher levels of free SO_2 (32). Glutathione can be used in combination with other compounds such as caffeic acid or gallic acid to enhance its antioxidant effects (33). Glutathione also protects varietal characteristics; for example, it prevents the loss of 3-mercaptohexanol, a particularly important compound in Sauvignon Blanc wines (34).

CHITOSAN modifies the chemical profile of wine primarily through its activity as a chelating agent and a cation in acidic solutions (25). It can chelate heavy metals that act as catalysts in oxidation reactions. Chitosan is thought to inhibit Fenton reactions by chelating iron to prevent it from catalyzing reactions with H_2O_2 (35). It can also delay the formation of free radicals in bottled wine (35). Chitosan films are positively charged, facilitating electrostatic interactions which retain organic acids such as citrate and malate in their salt forms, lowering the organic acid levels detected in wines with chitosan films (36). However, the adsorption efficiency of chitosan may be reduced by competition for attraction between organic acids (25).

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AA and glutathione may both be added in combination with SO_2, permitting lower levels of SO_2 addition (12,32). Chitosan has performed well as a sole antioxidant, but needs further research to confirm its efficacy (36). Given the risks associated with AA and the lack of testing surrounding chitosan, glutathione added with SO_2 is currently the best candidate to improve the chemical composition of wine.

Judging a Wine by its Colour

Assessing the colour of wine is important in determining its quality, and the implementation of new alternatives may alter the traditional colour of white wine.

ASCORBIC ACID has the potential to both provoke and prevent browning. When combined with SO_2, AA decreases colour changes associated with oxidation, preventing browning. However, better results are seen when SO_2 is used as the sole antioxidant (12). When the use of AA is combined with a large headspace and greater oxygen intake, browning is accelerated,

Headspace: The space between the top of the wine and the bottom of the lid.

as degradation products such as furfural react with flavonols to produce the observed brown colour (12). A yellow pigmentation may also occur during AA degradation due to the production of xanthylium cations. This yellow pigmentation occurs in both the presence and absence of added SO_2 (6).

GLUTATHIONE itself is an antioxidant, but it also acts as a cofactor for antioxidant enzymes, such as glutathione peroxidase and glutathione reductase (14). Glutathione scavenges ortho-quinones, preventing the polymerization between these compounds and other wine components, such as catechin and caftaric acid. Preventing this reaction stops the production of brown or yellow products such as xanthylium salts (14). Glutathione also suppresses other colouration associated with oxidation by preventing the formation of pigment precursors which may generate xanthylium salts (14).

CHITOSAN adsorbs molecules such as the carboxymethine-linked catechin dimer intermediates and yellow xanthylium cations, due to its high affinity for oxidized phenolic compounds (25). This prevents browning and other oxidative colouration. The chelation of metal species also decreases oxidation and browning as these metals catalyze oxidation reactions. Chitosan effectively prevents oxidative colour change and browning on its own and does not need to be combined with SO_2 (25).

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The degradation of AA produces undesirable colour changes in wine, and these changes are exacerbated when the antioxidant is used in high oxygen conditions. Both glutathione and chitosan do not negatively impact the colour of the wine as these compounds partake in reactions to prevent the occurrence of browning and discolouration (14,25). Thus, glutathione and chitosan appear to be promising alternatives to reduce browning and oxidative colouration.

SO_2 Alternatives Put to the Taste

Effective SO_2 replacements should enhance desirable aromas or have a comparable effect on the taste and aroma of wine (Figure 7).

ASCORBIC ACID improves the sensory characteristics of white wines if a delicate balance is achieved between lowering the production of oxidized flavours and limiting the emergence of reductive off-odours (6). AA protects desired aromas by preserving polyphenols which are typically lost during oxidation (16). This increases the fruity and fresh characteristics of AA supplemented wines (12). The addition of AA can also increase the perception of citrus, peach, and green apple aromas, and produce a higher quality wine with a less oxidized flavour (12,30). The perceived intensity of oxidized apple, honey, and sherry aromas may be increased through the addition of AA (12). However, some studies find none or few significant differences in aroma with AA addition, highlighting the need for further research (29,30). Findings are mixed: Riesling without AA was perceived as fresher on the palate, while a preference by tasters for Sauvignon blanc with AA emerged in another study (29,30). The preference for AA wines was stronger when there was higher oxygen content in the bottle (29).

FIGURE 7: THE TASTE AND AROMA OF WINE is dependent on a variety of factors ranging from grape varietal to terroir to additives.

GLUTATHIONE can impact the aroma and taste of wine, and its effect is particularly important as it preserves varietal aroma compounds (20). The aroma of wine is dependent on the concentration of glutathione as it controls the loss of particular compounds (20). When added in a concentration of 20 mg/L, glutathione prevents the loss of important fruity aromatic compounds (isoamyl acetate, ethyl hexanoate, and linalool) associated with quality wine, and this concentration more effectively preserves these compounds than 50 mg/L SO_2 (14). Glutathione prevents the development of flavours associated with aging, improving aromatic characteristics of the wine, and also preserves fruity aromas of young wines (20).

CHITOSAN can also impact the taste and aroma of wine through its application in the winemaking process. Chitosan can significantly decrease thiol oxidation, maintaining the aromas associated with thiols which are directly influenced by the grape varietal (37). In addition to these compounds, terpenols, which also vary based on the grape varietal used, greatly affect the aroma of wine (38). Chitosan can positively impact the aromatic volatile profile of wine, keeping the levels of these compounds above their perception threshold allowing consumers to note the increase in floral and fruity taste (35,36).

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Due to contrasting results between studies, the efficacy of AA to preserve wine taste and aroma may be limited. Alternatively, glutathione and chitosan improve the taste and aroma of wine, suggesting they could be viable SO_2 replacements.

SUMMARY

The wine-making process is highly experimental, which allows for the development of new wine preservation strategies, including the use of SO_2 alternatives (5). The health risks of sulfur compounds are one of the main motivations for their replacement and are well studied. However, the presented alternatives have lesser known risks. The large-scale feasibility of implementing SO_2 alternatives into the wine-making process is unknown, but these alternatives provide an opportunity to experiment with new and intriguing flavours that may be of interest to winemakers for marketing initiatives.

Through a critical analysis of the effects on chemical profile, oxidative colouration and browning, and sensory characteristics of wines supplemented with SO_2 alternatives, we have evaluated the efficacy of each alternative. AA demonstrates a lack of efficacy as it reduces the quality of wine, lowering its viability as a replacement for SO_2. Glutathione effectively protects important compounds and enhances desirable characteristics in wine, suggesting it is a promising option to replace or reduce SO_2 usage. Chitosan compounds are an emerging SO_2 alternative with some positive benefits, but they have not been as extensively studied as the other alternatives. Currently, the most promising sulfur dioxide alternative is glutathione, as there has been some research into its effects on human health, though chitosan may be a more viable substitute depending on its health impacts. The limitations in research on SO_2 alternatives highlight the need for further comparative analyses of their usage alone or in combination with SO_2. These prominent and innovative replacements present both challenges and opportunities for the advancement of wine preservation.

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Oak Barrels:

The Standard for Wine Ageing

Giancarlo Farruggia, Andrew Graham, Benjamin Swan, Kieron Walters

Barrel ageing of wine is a very important stage in the winemaking process. The ageing of wine affects its taste, aromas, texture, and composition. The most commonly used vessels for this process are American oak (*Quercus alba*) or French oak (*Quercus robur or Quercus petraea*) barrels, though other containers are also used. There are many different factors that affect the results of ageing. The size and shape of the barrel affect taste, with a larger surface area-to-volume ratio having a greater effect on flavour. The influence of toasting varies with temperature and area of the barrel, and can affect wine taste, pH, and colour. The material chosen for the vessel affects wine ageing, primarily through porosity, as greater permeability to oxygen increases the amount of oxidation in the barrel. Ageing can also be done artificially through various methods like simulating the micro-oxygenation environment of an oak barrel to attain similar effects. In the end, with the precise sciences coming together to produce a premium product, the price that individuals are willing to pay for such wine is underwhelming, due to the nature of the inexperience of novice wine tasters.

INTRODUCTION

Ageing is a critical stage of wine production. The process of fermentation is quite complex and occurs independently of the vessel in which the wine is created. However, additional nuanced processes occur afterwards while ageing, such as oxidation, esterification, and polymerization, affecting the taste, aromas, and texture of the wine (1). These vary significantly across wines, and a large factor in how these processes develop is the vessel containing the ageing wine. The most common of these are wood barrels, with the overwhelming majority being crafted with either American oak (*Q. alba*) or French oak (*Q. robur or Q. petraea*) (2). Many different methods have developed over the millennia to age wine with unique characteristics, many of which are being used with modern technology by winemakers in an effort to produce novel wine experiences (3). The difference in processes and results between these methods allow winemakers to better understand their products and adjust parameters to achieve desired results.

BRIEF HISTORY

Wine production in various forms has been around for many millennia, with a drastic evolution of the storage and ageing of wine throughout its history. Some of the earliest recorded instances of wine production indicate that wine was stored in containers made from either clay or animal skin thousands of years ago. Clay was a great discovery for wine ageing as its porosity allowed for more oxygen ingress than other sediments. Animal skin displayed this same property, but being composed of organic matter resulted in animal skin storage contraptions not lasting for a significant amount of time (4). A more recent historical wine storing and ageing vessel was the amphora pot, which was used from approximately 1500 B.C. to 500 A.D. and was the standard at that time for wine storage. Amphora pots were the standard due to their longevity as they degraded at a slow rate since they were ultimately composed of ceramic materials. Furthermore, amphora pots were designed into structurally stable shapes, leading to less breaks during the transportation of wine overseas. In addition, the porosity of ceramic materials allowed for the wine within the amphora pots to receive many benefits from oxygen intake (5). The first recorded instances of wine being aged and stored in barrels occurred over 1500 years ago in the Byzantine Empire. During this time period, storing wine in wooden barrels was not common (4). Over time, the benefits of barreling wine were recognized, and it became more popular. The first acknowledged benefit of barrel-aged wine was the release of wood odorants into the wine, altering its flavour. As time progressed, further benefits from barreling wine were discovered, leading to the now widespread use of wooden barrels to age wine (4).

Oxygen Ingress: The entrance of oxygen through a substance.

BARREL SIZE AND SHAPE

The wine manufacturing industry often ages wine in barrels of numerous sizes to yield different flavours at various intensities (6). The influence that a barrel has on wine flavour depends on the ratio between barrel surface area and wine volume. Barrels with a greater surface area-to-volume ratio tend to impart more intense flavours into wine (6). As a result, wine barrels of smaller volumes typically have the largest influence on wine taste, yielding a woodier flavour (6).

Regarding the shape of barrels, a cylindrical structure is ideal. In general, comparing the volume and surface area formulas of various shapes shows that more spherical shapes require less material to manufacture than rectangular containers with the same volume. As a result, the cylindrical shape maximizes the volume of wine that can be stored. However, cylindrical barrels are used over fully spherical containers because cylindrical containers are more structurally stable for storing fluids. Overall, barrel shape only has economic and stability benefits, and has not been proven to affect the development of wine flavour (7).

OAK BARRELS AS THE STANDARD FOR BARREL AGEING

Malolactic Fermentation: The process that converts malic acid into lactic acid.

In the wine industry, the default method for ageing wine is in oak barrels. A key reason for oak barrel superiority is that oak barrels provide a suitable environment for metabolic reactions to occur. Malolactic fermentation is one specific metabolic reaction which is very beneficial for wine development (8). Promoting malolactic fermentation improves biological stability and enhances the aroma and flavour of wine (9,10). In general, wood consists of many microscopic pores, making it superior to stone and metallic containers which are generally less porous. Oak wood pores are small enough to keep all liquid in while allowing for the transfer of gasses, most importantly oxygen, allowing a greater amount of oxygen ingress to occur. In oak wood, oxygen ingress is slow and controlled (11). Oxygen ingress is necessary for wine development as it allows for proper maturity of a multitude of organoleptic properties such as colour, smell, taste, and texture (12). Another key reason oak barrels are the standard for barrel ageing is that they are very effective containers for infusing beneficial flavour compounds into wine. The high porosity of oak wood allows wine to easily absorb into the wood, whereas in other woods, absorption of the wine would take longer and occur at a smaller and less efficient scale. In addition to easier absorption, the extraction wine extraction from oak barrels is also smoother, compared to with other wood types. Furthermore, from the ideal absorptivity properties of oak wood, wine absorbs more nutrients from oak wood than from other types of wood (13).

French oak is the most common type used to age wine. Barrels made from French oak are slightly tighter-grained than other types of oak wood such as American oak, which is another commonly used oak type. Tighter-grained wood increases the complexity of wood and spice aromatics in wine, which consumers generally find pleasant (2). Pleasant nutty flavours and aromas have also been discovered to be dominant in wines aged with French oak. The multitude of unique flavours and aromas presented in French oak-aged wine has resulted in greater positive gustative and odour impressions compared to other common wine ageing woods like American oak, Spanish oak, and chestnut wood (Figure 1) (14). Furthermore, tighter- grained woods are shown to have a greater oxygen ingress rate than wider-grained woods (15). Greater oxygen ingress leads to more refined wines. As a result, the tighter grains composing French oak allow it to be preferred for wine ageing due to enhanced flavours and increased oxygen ingress, benefiting the wine.

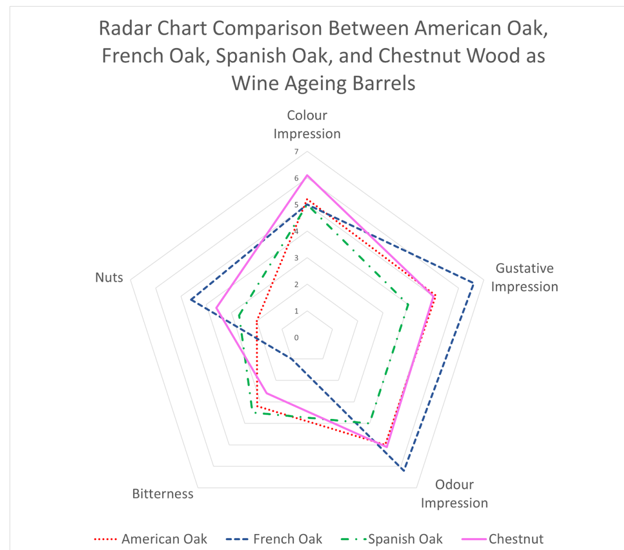

FIGURE 1: OAK BARREL SPECIES COMPARISON Radar chart comparison of American Oak, French Oak, Spanish Oak, and chestnut wood in wine ageing evaluated on colour impression, gustative impression, odour impression, bitterness, and nutty flavour. The further from the center a point is, the stronger the impression is for the respective category. Overall, French oak is shown to be the dominant wood for wine ageing as it excels in 60% of the categories over the other types of wood types. The studies were performed on experienced and trained wine tasters, and the data used to create this figure is from García-Moreno et al. (2021).

EFFECTS OF BARREL AGE & SEASONING ON QUALITY OF WINE

Wine barrel seasoning is the process of intentionally exposing the raw oak to natural weathering before the wine is barreled for a specific period of time to yield a higher quality wine (16). In general, seasoning can affect the oxygen transfer between the wood used for the barrel and the atmosphere, as well as the infusion of specific phenolic compounds from the

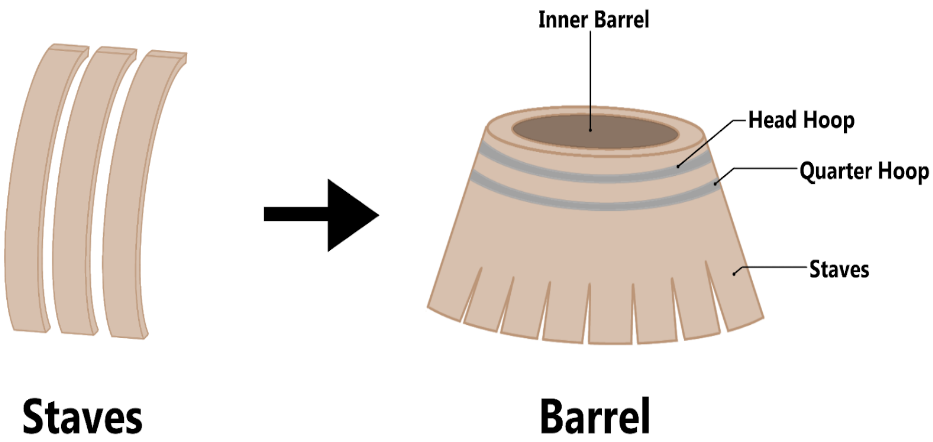

FIGURE 2: BARREL ANATOMY DURING TOASTING The anatomy of the barrel during the toasting process. Wooden staves are assembled and secured to metal bands known as hoops. The barrel is mounted to at least the head hoop, and more hoops could be added for stability. The inner barrel is then exposed to a temperature controlled flame, toasted to a specific degree based on the cooper's preference (20).

wood to the wine (16,17). Oxygen transfer is essential in wine, allowing for the yeast present to ferment the grapes into the desired alcoholic beverage. By weathering the wood prior to barreling, the porosity of the wood is increased, allowing yeast to flourish in a more oxygen-rich environment (16). In addition, it has been shown that seasoning will have a direct effect on anthocyanin content within the wine. Studies have been conducted comparing the natural and artificial seasoning of *Q. pyrenaica,* commonly known as Spanish oak (17). Natural seasoning consisted of weathering the oak wood for two years outside. Artificial seasoning consisted of weathering the oak for four months, then washing the wood with unchlorinated water and drying it in a 50°C oven every 10 days for a total of seven cycles (17). The wood was then stored at room temperature, unexposed to any weather. It was found that artificial seasoning yielded a greater amount of anthocyanins, as well as incorporating a greater depth of tannins within the wine compared to the natural seasoning method. This increases the bitterness and astringency of artificially seasoned wine when compared to naturally seasoned wine. Overall, it is recognized that two years of natural weathering is the maximum limit in terms of economic efficiency. This is due to a general stabilization trend during the third year, where compounds stay relatively unchanged in the final wine (17,18).

Tannin:
A water-soluble flavour compound found in the wood of grapevines.

Due to porosity increases caused by seasoning, overall oxygenation of wine barrels must be monitored, as barrel yeast contamination can ruin a batch (19). This is due to rapid yeast growth from presence of oxygen, leading to the sugar present in wine being consumed too fast, shortening shelf-life and producing unpleasant or undesired flavours (19). As wine sits within an oak barrel, the oak fibers on the inside become saturated with the wine. Therefore, barrels must keep a strict limit of wood saturation to prevent leakage or excessive evaporation of wine (15). Due to the increased oxygenation of the seasoning process, as well as the constant natural weathering of the barrel itself over time, it is suggested that barrels not be used for over eight years (16,19). The wood itself will have used a significant portion of its phenolic compounds after eight years from the time of harvest, leading to inconsistent wine quality (16).

Anthocyanin:
A water-soluble flavour compound found in grapes.

EFFECTS OF BARREL TOASTING ON QUALITY OF WINE

Barrel toasting is another method vintners may use to introduce a desired taste and aroma on wine, increasing the quality of a wine. The toasting process consists of exposing the barrel to extreme heat for relatively short periods of time (16). The anatomy of the toasting process is shown in Figure 2 (20). The staves are first mounted to at least the head hoop before being exposed to an open fire with a controlled temperature. Finally, it is secured with more hoops, and the head is placed on. It is important to note that typically, only the staves are toasted (16). This is because the lids, commonly referred to as "heads", must be fitted for their specific barrel (16). Heat levels and time intervals vary depending on factors such as quality of the oak wood, species of oak, vintner preference and customer demand. Common toasting temperatures vary between 160°C and 210°C, and exposure times from 10 to 25 minutes for 500L barrels (17,21,22). The consensus among studies proves that increasing the heat and time of toasting on a barrel decreases the bitterness and astringency of the finished wine (17,19,21). Specifically, gallic acid and tannin content are stripped from the burning process, and further oxygenation, leading to a stronger wine with less acidic properties and a deeper colour (16,17). Protocatechuic acid and syringic acid, which contribute to antioxidative properties and gastric acid secretion, are both increased by toasting (15). These acids can contribute both positively and negatively to the wine experience, though they contribute to improved blood circulation and gastrointestinal reflux (15). Vanillins are also expressed further in heavy toasting, increasing sweetness and vanilla flavour (21). Toasting additionally allows for more smoky aromatics to come through via the release of oak lactones (16). These lactones are commonly seen in other alcoholic beverages such as brandy or whisky, leading to a similar oaky or smoke-like aroma (16,17).

CHEMICAL PROCESSES OF AGEING

Several chemical reactions take place during ageing. Barrel ageing typically occurs in a small volume and high relative surface area container. Since the barrel is usually highly permeable to carbon dioxide (CO_2), barrel ageing improves the quality of wine by allowing the release of CO_2 produced during fermentation (23). With prolonged time and temperature change, the tartrate salts in the wine begin to separate and precipitate (24). This affects the pH balance and structure of the wine. The yeast eventually begins to undergo autolysis, releasing proteins and amino acids which contribute to wine texture and taste (23). When barrel ageing is used, some volatile compounds in the wine are absorbed by the barrel, improving wine stability and flavour (25). The barrel also introduces new aromas and flavours through hydrophobic interactions with the wine (26). Furthermore, the barrel supplies the wine with several compounds not produced simply by the fermentation of grapes, such as oak lactone, and can provide several aromas including smokey, toasty, coffee-like, oily, or fruity scents (23).

Autolysis: The automatic destruction of a cell through its own enzymes.

The oak barrel is a breathable vessel which creates a micro-oxidation environment, allowing for processes like oxidation, esterification, and polymerization to occur (Figure 3) (1). **Oxidation** occurs by adding oxygen gas to an alcohol, primarily ethanol in the case of wine, and converting it to another compound, such as an aldehyde, which can be further oxidized to an acid or ester product (1). This produces various changes to the wine's flavour,

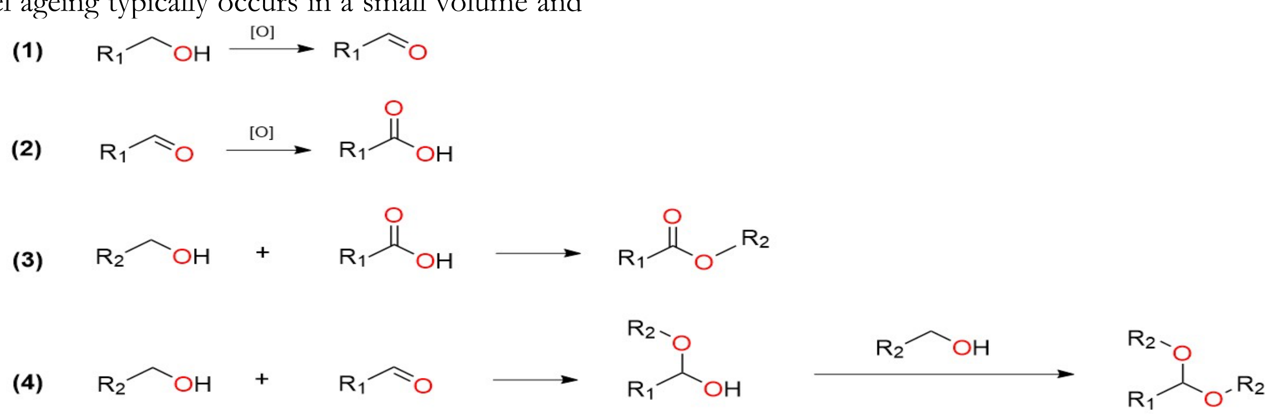

FIGURE 3: WINE AGEING CHEMICAL REACTION OVERVIEW (Reaction 1) An alcohol being oxidized to an aldehyde. (Reaction 2) An aldehyde being further oxidized to an acid. (Reaction 3) An alcohol and aldehyde being combined to form an ester. (Reaction 4) An alcohol and an aldehyde polymerizing (27).

scent, and composition. **Esterification** specifically occurs when an alcohol and an acid are combined (1). Esters are largely responsible for a wide variety of flavours and scents found in wine. **Polymerization** occurs when various compounds like polysaccharides, proteins, alcohols, aldehydes, and polyphenols link together with each other (1). This greatly affects the colour profile of wine, causing white wines to get darker and red wines to get lighter.

AGEING USING AMPHORA POTS

Many wineries worldwide are experimenting with alternative methods of ageing wine, and these can be used to fully understand why oak barrels are seen as a standard in the industry. One of the main alternative methods is amphora pots, made of different materials. Many amphora pots are made using an extremely porous form of pottery, leading to high oxygen transmission rates (28). However, these pots are so porous that they can lose liquid over time (3). Another common ceramic material for amphora pots is claystone, which varies in density based on the temperature at which it was created. Certain claystone amphora pots with low permeability results in all pores within the material being occupied by gas. This allows it to maintain constant oxidation rates, an impossible feature in oak barrel ageing (3).

To decrease the transmission of oxygen into the pot while sealing the liquid inside, many winemakers coat their pots with beeswax. Baiano et al. (2015) observed the effect of amphora pots on the chemical composition of wine. This study found that wines aged in waxed amphora pots had a lower alcohol content and less free sulfite, but raw amphora pots showed a significant decrease in flavonoids. All amphora pots showed lower acidity and smaller concentrations of aromatics, which is likely due to relative differences in compound stability and activation energies of various redox reactions (29).

A comparison of amphora pots and oak barrels can be difficult considering the wide range of factors involved with both techniques. Like all matters related to wine, there is a high level of subjectivity related to the benefits of each technique.

ARTIFICIAL AGEING

Due to various limitations of traditional ageing such as the lengthy time requirement and the cost of oak barrels, artificial ageing methods have been considered as an alternative. This is done by simulating the ageing process by replicating the environment of a traditional oak barrel, including the micro-oxygenation climate or porous nature of the barrel (23). One common alternative to barrel ageing is the use of oak wood chips submerged in the wine rather than having a container made of the wood. This provides all the same benefits as an oak barrel, but over a shorter time span, as the chips cover a greater surface area and remain more volatile than the oak in a traditional barrel (25). The wine is also able to penetrate chips more easily than a barrel, allowing for the transfer of more oak-related aromatic compounds. Creating a micro-oxygenation environment is another method used, as it allows for the oxidation reactions of a normal barrel to occur (23). Ageing on lees is another common strategy, which entails wine aged on particles leftover from yeast autolysis. These can release oak-related aromatic compounds and various macromolecules like polysaccharides, amino acids, and lipids, removing the need for an oak barrel. Changing the temperature of the wine to accelerate chemical reactions is also a viable option (23). While these options are useful for reducing the time and costs associated with winemaking, consumers may place importance on buying and consuming wine aged authentically in a barrel.

EFFECTS OF BARREL AGEING ON THE PRICE OF WINE

The many costs associated with barrel ageing lead to increased costs of barrel aged wines (30). A study by Combris, Lecocq and Visser (1997) on barrel-aged wine prices showed that price is mostly determined by variables such as the vintage, winemaker, and ranking by experts (31). These are also characteristics which all consumers can understand, regardless of expertise. A group of tasters with varying levels of wine expertise were given samples of Bordeaux wines and asked to each give a grade between 0 and 20, not knowing any of the factors which affect the price of wine. The tasters valued qualities related to the aromas, harmony of components, and finish. These sensory variables, along with concentrations of various compounds like tannins, were not found to affect the price of wine (31). Naturally, consumers are more willing to pay for wines with flavours they enjoy. A different study of consumer willingness specifically to pay for Chardonnay wines aged in different oak barrels found that consumer enjoyment of wine decreased with wines aged with more oak treatment. However, the participants in the study were novice wine tasters who may not be able to taste the differences that oak barrel ageing provides (30).

CONCLUSION

Overall, it is clear why the process of barrel ageing wine has been prominent in the industry for many centuries. First, the versatility of the barrel cannot be ignored. Often crafted from American or French oak, the barrels' abilities, such as catalyzing metabolic reactions such as malolactic fermentation cannot be ignored. These reactions are directly responsible for enhancing stability, aroma and flavour of wine. Furthermore, the porous nature of the barrel creates a unique micro-oxidation environment, allowing for fermentation without the significant risk of spoilage. This micro-oxidation environment enables processes like oxidation, esterification, and polymerization, which, in turn, contribute to the complexity and depth of the final wine. Additionally, the ability to season and toast the barrel allows winemakers to manipulate a dynamic range of phenolic compounds and anthocyanins, resulting in wines with precisely tailored notes in taste and aroma. Toasting and seasoning also directly affect acidity and astringency, emphasizing the impact of barrel ageing on the sensory profile of wine. Despite potential competition from amphora pots, oak barrels are deep-rooted within wine culture and will remain relevant. Nonetheless, it is essential to recognize that despite the improvements in wine quality achieved through barrel ageing, this effect may not always be apparent to the average consumer, as demonstrated by studies revealing the challenge novice wine tasters face in distinguishing differences in barrel-aged wines and non-barrel-aged wines.

MORE TO EXPLORE

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Beyond the Barrel: Redefining Tradition with Micro-Oxygenation

Dinithi Fernando, Fatemeh Ilkhani, Jodi Overwijk, Flora Sun

Traditional wine ageing occurs in oak barrels, and while prolonged contact with wood introduces desirable characteristics into wines, this method has numerous drawbacks. Between high manufacturing costs and unpredictable oxygenation of wine due to variations in wood grain, the limitations of barrel ageing highlight the importance of alternative ageing techniques in the wine industry. One of the most promising alternative techniques, specifically in the ageing of red wines, is permeable membrane diffusion micro-oxygenation (PMD-MOX). This technique introduces oxygen into wine as a controlled stream of fine bubbles via a semipermeable ceramic diffuser. The control afforded to winemakers who implement PMD-MOX results in wines with improved colour stability and mouthfeel, which is reflected in the sensory preference of PMD-MOX-treated wines by consumers. While advantageous, it is imperative that winemakers are precise and cautious with the introduction of oxygen into wine, as excessive oxidation can result in the overgrowth of aerobic microorganisms and wine spoilage. When used correctly, PMD-MOX has substantiated itself as a viable alternative to barrel ageing, and should continue to garner international recognition and implementation.

INTRODUCTION

Throughout history, winemaking has been deeply intertwined with many social cultures and traditions. Often synonymous with celebration and union, wine is enjoyed internationally, offering diverse flavours and experiences. Due to its endless potential, industry professionals continuously strive to improve the winemaking process in attempts to create products that adapt to consumer demand and optimise production. In pursuit of this, the process of oxygenating wine has advanced outside of traditional barrel ageing techniques. The ingenuity of micro-oxygenation (MOX) first began in the 1990s when Patrick Ducournau, a French winemaker, and his family of winemakers, the Laplace Family, attempted to create a novel technique for wine ageing (1,2). MOX is the controlled, periodic introduction of oxygen as a fine stream of bubbles into wine via a diffuser or membrane in stainless steel tanks (2). Ducournau's experiment aimed to replicate barrel conditions for wines aged in stainless steel. Since the development of MOX, its processes have remained relatively unchanged for the past 15 years. Furthermore, MOX has become marketed as a more cost-effective and expedited technique for wine-ageing. MOX is more viable for ageing red rather than white wines, as red wines are kept in contact with their skins and seeds, increasing the amount of phenolic compounds for MOX to interact with. While this technique has been implemented in some wineries, this paper aims to assess the viability of MOX as a more prominent alternative ageing technique for red wines (1,3–6).

In modern winemaking, there are two well-known approaches for conducting MOX (Figure 1). The first technique, bubble plume diffusion, was originally patented by Ducournau and the Laplace Family. The process involves the periodic introduction of oxygen as a stream of fine bubbles creating a semi-continuous dose rate. As the name suggests, oxygen bubbles are one of the most critical aspects of this technique. Among

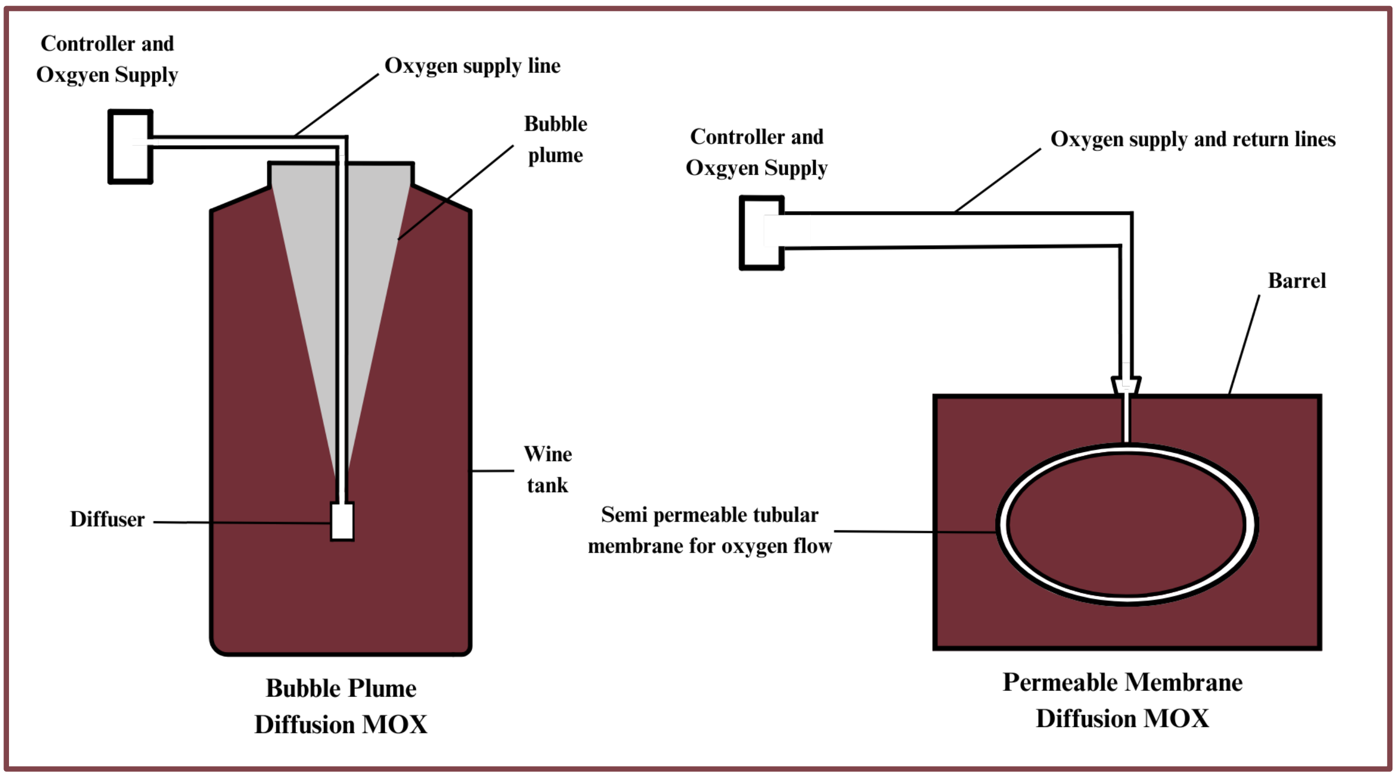

FIGURE 1: THE TWO TECHNIQUES OF MOX. On the left is the bubble plume diffusion method, as patented by Ducournau and the Laplace Family. This method relies on the formation of oxygen bubbles, creating a semi-continuous stream of oxygenation. Note that this method suspends the diffuser such that it is a few centimeters from the bottom of the tank. Accurate dosage of oxygenation is then reliant on bubble size as this determines surface area, bubble-rise velocity, and the mass transfer coefficient (7). This method is also susceptible to forces that act upon the rising oxygen bubble, such as gravity, buoyancy, and surface tension, creating a vulnerable process. On the right is the permeable membrane diffusion technique, which is not reliant on bubble formation or the physical rising of oxygen bubbles and utilizes a semi-permeable membrane to provide a continuous stream of oxygen. Instead, this method follows a process of gas absorption onto the inner tube, diffusion, and then desorption, ultimately leading to the release of oxygen into the wine. Adapted from Schmidtke et al. (7).

other factors, the bubble-rise velocity and size play important roles in determining its success (7).

In contrast, the PMD-MOX approach involves the use of a porous diffuser, allowing for a more controlled introduction of oxygen. This technique also avoids the impact of water pressure and prevents the formation and rise of bubbles by using a pressure-controlled tube, allowing for oxygen to consistently pass through.

TRADITIONAL OAK BARREL AGEING

Wine maturation is a crucial part of the winemaking process in order to enhance the quality of wine and allow for its preservation. This process is categorised into two main ageing phases: the oxidative phase, which traditionally takes place within an oak barrel, and the reductive phase, which occurs once the wine is bottled (8). Examining the oxidative phase, oak barrels (Figure 2) have made an exceptional vessel for this process as chemicals from the wooden barrels are released into the wine, impacting its physical and sensory properties (9).

FIGURE 2: TRADITIONAL BARREL AGEING. Oak barrels filled with wine, stored in a cellar during the oxidative ageing phase (12).

To begin the process, grapes are destemmed and pressed. The must is then filled into barrels and aged for a particular amount of time as determined by winemakers, typically ranging from months to years. The porosity of oak barrels enables the slow introduction of oxygen into the wine, initiating the polymerization of pigmented polymers and tannins over the ageing process. Ultimately, this contributes to the colour stability and texture of the wine (10). In addition to the transfer of oxygen through the barrel, aromatic compounds from the wood are transferred into the wine, altering various characteristics (9). One such desirable compound includes the oak lactones that are released into the wine during this process. Oak lactones play a major role in the sensory characteristics of a wine, contributing to a coconut and woody aroma. The cellulose, acids, and sugars in the oak also contribute to the wine quality (8). Through the prolonged contact between wine and oak, these compounds ultimately result in a wine that is more complex, which is often sought out by winemakers (11). Once the wine is deemed finished, it undergoes filtration and is bottled for the second ageing phase.

The use of oak wood for these barrels, while traditional, can result in negative effects for the wine industry. Oak wood makes an attractive candidate for the ageing of wine due to the compounds released during maturation, its permeability to oxygen, as well as its resilience and flexibility (9). Unfortunately, there are additional factors to oak wood that must be considered. While oak is relatively impermeable to liquids, some wine is absorbed into the wood through a process called wood impregnation, causing a headspace of gas in the barrel leading to evaporation and decreasing wine yield (9). Traditionally, wineries use either French or American oak. Each piece of wood differs in levels of density, which impacts its permeability and results in unpredictable changes to the wine. French oak tends to be more desirable due to the higher levels of volatile compounds released into the wine during maturation, contributing to the wine's organoleptic properties (13). While desirable, French oak is also associated with higher manufacturing costs when compared to American oak, given its irregular grain structure and higher porosity which makes it more susceptible to wood splintering (14). Due to the costly manufacturing process and the substantial quantity needed, barrels are becoming unsuitable for modern production and its increasing demand (10). Some ageing alternatives have been explored to mitigate the high costs and lengthy ageing times associated with barrels, including the use of toasted oak chips or dust in varying accelerated ageing methods (15). One of the more notable candidates to mitigate the issues with barrel ageing is MOX, where the wine is oxygenated and allowed to mature within steel tanks, eliminating the need for wooden barrels.

MECHANISM OF PMD-MOX

The process of MOX can be an alternative or complementary method for accelerating the process of barrel ageing for wines, by introducing controlled, continuous rates of oxygen (2). Although the timeline of this process is variable, the typical duration of MOX treatment spans from one to six months, relative to the three to thirty months seen in traditional barrel ageing (16,17). The MOX mechanism is meant to ensure there is a complete mass transfer of oxygen from the gaseous state to the dissolved state (7). A primary benefit of MOX is to improve the colour, aroma and texture of wine. For red wines, this takes the form of improving notes of fruit and giving the wine a fuller body by replicating the ingress of oxygen that occurs during traditional barrel ageing.

Specifically looking at PMD-MOX, one of the key devices to this approach is the porous diffuser. Outside of the wine-making industry, this apparatus is commonly used in aeration systems and primarily functions to allow for the dispersal of fine gas bubbles into a liquid (18,19). For this process to occur, the material of the porous diffuser plays a critical role in controlling oxygen flux. Generally, oxygen flux into wine is controlled by the gas pressure and membrane surface area. Typically, polydimethylsiloxane, a synthetic ceramic composite, is used for the apparatus (20). The hydrophilic properties of this coating prevent the formation of undesirable bubbles in the wine (7). The tubular shape of the diffuser also aids in controlling oxygen flux by modifying gas pressure. Note that the length of the tube can vary, impacting the surface area of the diffuser.

As mentioned, one of the most critical aspects of MOX remains to be the controlled dose rate of oxygen that is infused into the wine. Excessive oxygen can lead to a myriad of negative attributes and is associated with diminished sensory qualities, growth of aerobic microorganisms, spoilage potential, and reduced shelf life (7). To prevent the accumulation of oxygen in the wine, MOX requires the rate of introduction to be weak and equal to, or less than, the oxygen uptake rate of the wine (21). This meticulous control allows wine phenols to absorb oxygen without acquiring negative traits such as high astringency and tannin dryness (2). Typically, oxygen doses can range from 2 – 90mg of O_2/L of wine per month (22). However, it is important to note that the duration of MOX is variable and depends on the desired wine. Wine volume, temperature, and composition can all affect the calculation of the required dose of oxygenation. Most winemakers that use MOX will choose to monitor the increasing flux of the reaction intermediate, acetaldehyde, as an indicator of the degree of oxygenation throughout the procedure.

CHEMICAL AND SENSORIAL EFFECTS OF MOX

When considering the traditional winemaking process, oxygen plays a key role on a molecular level, as it is involved in oxidation, condensation, and polymerization reactions of phenolic compounds (2). The most notable phenolic compounds influencing the organoleptic properties of wine are also those which serve as the main substrates for oxygen consumption. These are anthocyanins and flavonoids, which are primarily responsible for the colour and astringent properties of wines, respectively. The presence and manner in which these compounds are present in wines (i.e., the type, concentration, polymer length, and distribution of each type) are the factors which alter the colour, taste, and mouthfeel of wines (2,23). With MOX, the consideration given to the role of oxygen in the winemaking process is heightened, and as long as necessary precautions are taken to avoid excessive oxidation of wine, MOX can lead to a variety of benefits at a molecular level (24).

Lost in a sea of jargon? Here are some helpful terms:

Micro-oxygenation (MOX): the controlled, periodic introduction of oxygen as a fine stream of bubbles into wine via a diffuser or membrane in stainless steel tanks.

Anthocyanins: water soluble phenolic pigments found in food, which are responsible for the red hues seen in wine.

Pyroanthocyanins: anthocyanin derivatives formed during fermentation. These compounds stabilize wine colour as they are resistant to the colour drop that is typically associated with the addition of sulphur dioxide.

Proanthocyanidins: also known as condensed tannins, these polyphenolic compounds occur naturally in the skins and seeds of grapes. These compounds are responsible for the astringent sensations in wine.

Sulphur dioxide: a common preservative added to wine, which mitigates wine spoilage through its antimicrobial properties.

The specific effects of MOX on all wines cannot be generalised, as the concentration of phenolic compounds in grapes and initial wines determines the varying dosages of MOX treatments (25). Despite this variability, there are certain beneficial trends which are exhibited when MOX is used to age red wines. These benefits are specific to red wines since they have high initial phenolic concentrations due to the prolonged contact with grape skins. One such trend is that MOX enhances the colour stability of red wines by oxidising ethanol into acetaldehyde in a controlled manner. The resulting increase in acetaldehyde along with anthocyanins, is involved in the formation of pyroanthocyanins (Figure 3) (2,3,26).

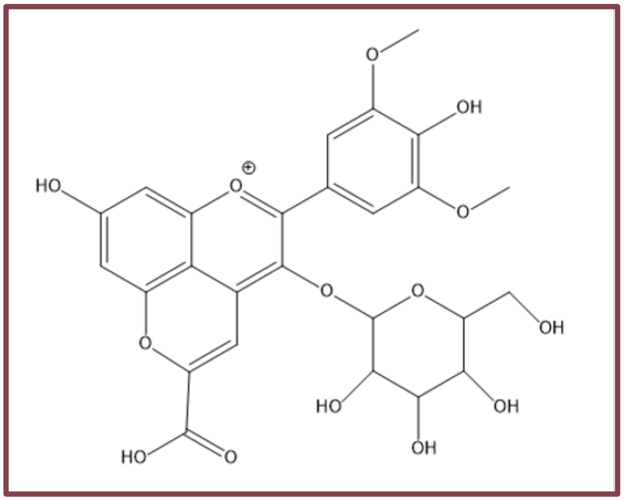

FIGURE 3: VITISIN A. The structure of Vitisin A, which is an example of one of the main pyroanthocyanins involved in the colour stability of red wines (27).

With the addition of sulphur dioxide throughout the winemaking process, a common hindrance to the colour stability of red wines is the phenomenon recognized as "sulphite bleaching". This occurs when sulphur dioxide binds with anthocyanins to form a colourless complex (28). This phenomenon is particularly prevalent after malolactic fermentation, where sulphur dioxide is added and a colour drop is typically noted (26,28). The formation of pyroanthocyanins, which is enhanced by MOX treatment, helps to stabilize the wine's colour. This is because these compounds do not form colourless complexes, unlike anthocyanins, making them resistant to sulphite bleaching (2,3,26).

Another beneficial trend seen in red wines aged via MOX is a decrease in the perception of astringent sensations (1,2,6). In the traditional ageing process of wine, young red wines typically have high degrees of astringency due to the presence of condensed tannins, or proanthocyanidins (i.e., a type of flavonoid). These are present in the skin and seeds of grapes, which leech into red wine through prolonged contact (Figure 4) (23). Over time, the condensed tannins are polymerized and cross-linked with anthocyanins, to form larger flavonoid compounds that have less astringent properties; partially leading to the association of aged red wines being of a higher quality due to their improved mouthfeel (2). MOX is able to promote

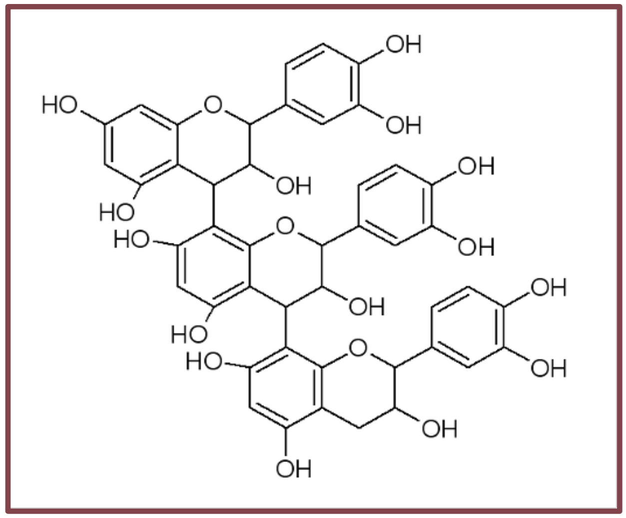

FIGURE 4: PROANTHOCYANIDIN C1. The structure of proanthocyanidin C1, which is one of the condensed tannins found in grape skins that is mainly responsible for the astringency of young red wines (29).

the formation of polymeric phenols because the oxidation of ethanol into acetaldehyde allows for the polymerization of tannins by forming ethyl-bridges between tannin molecules (2,23,24). The decrease in astringency due to the formation of larger flavonoids in a shorter time span yields a softer mouthfeel for MOX-treated wines, relative to traditionally aged wines when compared over the same timespan (1,2,6).

LIMITATIONS OF MOX

While MOX has been explored as an attractive alternative to traditional ageing methods, there are several limitations which must be considered. Winemakers are especially cognizant of the potential for microbial spoilage throughout winemaking, a possibility made more likely when oxygen levels are increased in the MOX process (4). Higher oxygen content in wine provides the ideal environment for the proliferation of spoilage microorganisms, namely acetic acid bacteria (AAB) and *Brettanomyces*. AAB are part of the natural microbial flora of grapes and wine, but may lead to the spoilage of wine as they form acetic acid through the oxidative metabolism of ethanol (7). The chemical process involves the transformation of ethanol to acetaldehyde through the enzyme alcohol dehydrogenase and the subsequent conversion of acetaldehyde to acetic acid by the enzyme acetaldehyde dehydrogenase (30). High acetic acid content in wine can lead to increased volatile acidity, negatively affecting the sensory profiles of the wine. *Brettanomyces* are yeasts that lead to unsavoury characteristics in wine through the production of volatile phenols from the organic acids present in grape musts (Figure 5). These volatile

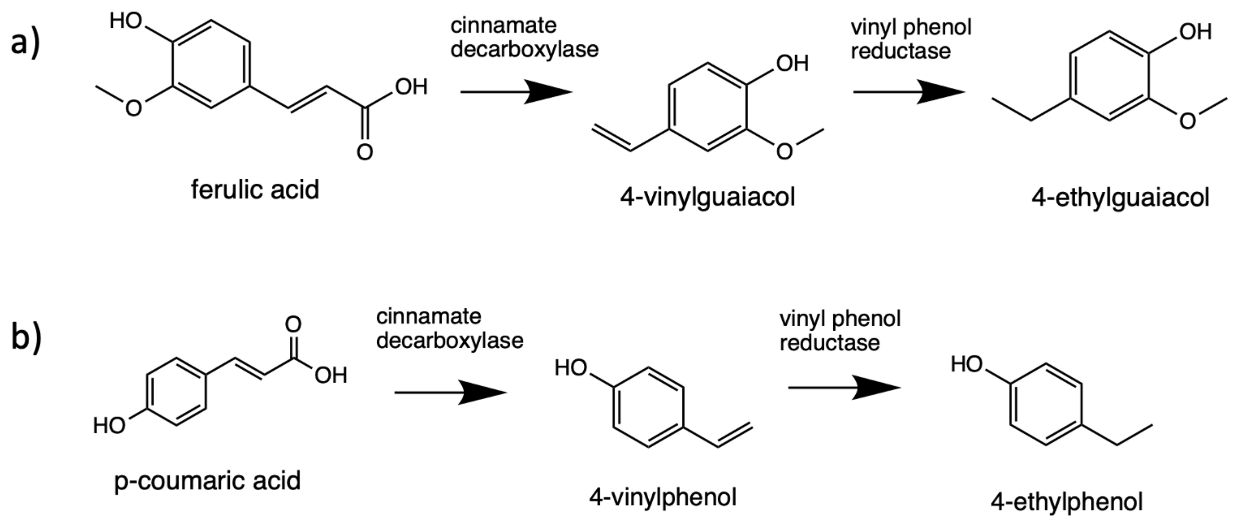

FIGURE 5: ENZYMATIC REACTIONS PRODUCED BY *BRETTANOMYCES*. The conversion of organic acids, found in wine musts, into volatile phenols, using cinnamate decarboxylase and vinyl phenol reductase, as per the reaction schemes. A) The conversion of ferulic acid into 4-vinylguaiacol, which is reduced to 4-ethylguaiacol. B) The conversion of p-coumaric acid into 4-vinylphenol, which is reduced into 4-ethylphenol (Created by authors using ChemDraw, (30,31)).

phenols impart flavours reminiscent of farmyards, horse sweat, and medicine, adding undesirable characteristics to wine flavour profiles (30).

Since both spoilage microorganisms are aerobic bacteria, the introduction of oxygen into wine can support their survival (4). During the MOX process, increases in dissolved oxygen concentrations and accumulation of gaseous oxygen in tank headspaces can support microbial growth, amplifying the risk of wine spoilage. Du Toit et al. (4) found that rates of oxygenation between 1.5 – 3.0 mg/L/month, over a 20-week period, led to changes in AAB and *Brettanomyces* levels in red wine. Throughout the trial, AAB populations in control wines decreased, while in MOX-treated wines, levels of AAB and *Brettanomyces* increased, demonstrating the caution required when using this technique. However, current MOX practices have tailored oxygenation levels to prevent this outcome. A rate of 3.0 mg/L/month, as determined by Du Toit et al. (4), serves as a maximum oxygenation rate, with typical ranges including 0.5 – 3.0 mg/L/month, 0.5 – 2.0 mg/L/month, and 0.5 – 1.0 mg/L/month, depending on the ageing timelines required by each wine (32). Additionally, some techniques to limit microbial growth include closely monitoring concentrations of free sulphur dioxide and dissolved oxygen every two weeks to ensure critical levels of oxygen are not reached. Decreases in sulphur dioxide levels point to high oxygen concentrations, as free sulphur dioxide interacts with oxygen in the headspace of the tank and depletes its free form (32).

Though the use of oxygen in the MOX process poses risks for microbial spoilage, it must be noted that the oxygen addition rates are considerably low, especially after malolactic fermentation. Solutions to mitigate risk include closely monitoring acetaldehyde formation during MOX and the addition of sulphur dioxide. Carlton et al. (5) determined a sensory threshold for acetaldehyde concentration to be 40 – 100 mg/L, but the large range points to greater variability, illustrating the need for a more reliable and consistent approach. The use of solid-phase microextraction coupled to on fibre derivatization, in conjunction with gas chromatography, and flame ionisation detection (SPME-GC/FID) serves to address this issue, providing a more accurate, though more costly, measurement of acetaldehyde formation during MOX (7). The use of this method may negate the cost-effective benefits MOX proposes. Previously, a study of the SPME-GC/FID technique as an analytical measure following MOX in merlot

Microorganisms in your wine!

***Brettanomyces*:** a yeast commonly associated with red wine spoilage that produces volatile phenols, leading to a loss of fruity character in wine.

Acetic acid bacteria (AAB): aerobic microorganisms that cause acidification of wine through the production of acetic acid.

wine found that acetaldehyde concentration increased by 1 mg/L/day once the concentration of anthocyanins and flavanols had been depleted to rate-limiting concentrations. This provided a marker to which flavanol consumption during MOX can be compared against and monitored (5).

To limit the growth of *Brettanomyces*, Beech et al. (33) found additions of 0.5 – 0.8 mg/L of molecular sulphur dioxide to be effective. Free sulphur dioxide can also bind to acetaldehyde and limit its conversion to acetic acid by AAB. Additionally, the use of the compound is associated with negative effects on organoleptic qualities and consumer health, resulting in efforts made in the wine industry to limit its use (34). The dangers of excessive oxygenation due to poorly monitored MOX and the limitations of the proposed solutions must be considered before adopting this alternate ageing method.

ECONOMIC AND CONSUMER IMPLICATIONS

Since its development, the use of MOX in the wine industry and its consumer reception has been debated due to its deviation from traditional winemaking. The adoption of MOX techniques began in France, before spreading to Italy, South Africa, Chile, and ultimately to the United States (35). MOX is now widely employed around the world, though not as a primary alternative to traditional ageing methods. Winemakers were motivated to adopt the new technique as it provides control in the unpredictable, albeit conventional, winemaking process. This control is afforded through more efficient manipulation of tannins, mouthfeel, colour stability, aroma integration, decreased sulphide, and increased longevity potential. MOX is also appreciated for its accessibility, in checking for microbial issues and flavour development throughout the process. In the early years of MOX's introduction to the wine industry, winemakers were attracted to reduced labour and cost. With the use of large stainless steel tanks as required in MOX, the expensive upkeep of oak barrels and the labour-intensive wine-racking processes is no longer necessary. While the initial implementation of MOX is costly, its marginal upkeep and increased longevity relative to oak barrels demonstrates its positive return on investment (36). Wineries can also increase profit margins as MOX accelerates the ageing process and limits wine loss due to evaporation from wood barrels, boosting production efficiency (37).

There is concern that this technique may not be well-received, with consumers opting for traditionally aged wine in lieu of MOX-treated wine (Figure 6) (10). A blind study on consumer perceptions of MOX wine (36), tested consumer sensory preferences for wine aged for 6 days using a dosage of 25 mL O_2/L/month and 50 mL O_2/L/month compared to a control wine. The results found that consumers preferred the wine aged with 25 mL O_2/L/month due to its olfactory complexity, roundness, and general complexity compared to the more highly-oxygenated and control wines (36). The results of the study show the potential for consumer approval of the sensory qualities MOX imparts on the wine, and further studies should seek to corroborate such findings and explore whether marketing wine as MOX-treated would deter consumers.

CONCLUSION

MOX has presented itself as an innovative and effective

FIGURE 6: CONSUMER RED WINE PREFERENCES. Results point to consumers' preference for micro-oxygenated wine, supporting its viability as a more established alternative ageing method (36,38).

method of wine ageing, especially as the wine industry strives to rectify the manufacturing issues which are seen in traditional barrel ageing. Contrary to the passive oxygen exchange seen in barrels, MOX techniques, such as the use of PMD-MOX allows for a stream of fine bubbles to be dissolved within wine, initiating desired oxidation, condensation and polymerization reactions that alter its characteristics. Since MOX techniques allow for the increased control of oxygen, MOX-treated wines are associated with enhanced colour stability and a decrease in perceived astringency when compared to traditionally aged red wines. Additionally, when looking at consumer perception directly, MOX-treated wines were favoured over those that were conventionally aged in a blind study, demonstrating the viability of this technique as an advantageous replacement to the latter.

While MOX is an attractive candidate for alternative wine ageing, its limitations cannot be ignored. The increased quantity and rate at which oxygen is introduced, while beneficial to an extent, can support microbial survival, leading to wine spoilage. Although there are analytical techniques in place to monitor the growth of microbial organisms, these methods are often associated with higher costs, potentially negating the cost-benefit of adopting MOX techniques. The integration of MOX ageing techniques into the wine industry does, however, have the potential to be more financially beneficial than traditional methods as it allows winemakers more control over their products. Further research should be dedicated to refining the ideal rates at which oxygen is introduced among various wines, not only to differentiate characteristics of wine at different rates, but to also mitigate risks associated with microbial spoilage. Overall, MOX appears to be a viable alternative to the barrel ageing of wine, and further research should be focused on optimising this technique in an effort to increase its presence in the wine industry.

MORE TO EXPLORE

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COVER IMAGE

star5112. Wine fermentation tanks at Cakebread Cellars in Rutherford, California. Available from: https://commons.wikimedia.org/wiki/File:Cakebread_Cellars_-_Johnn_05.jpg [Accessed 2023 Nov 17].

Spoiling the Fun:

Preventing *Brettanomyces*-Caused Spoilage in the Winemaking Process

Ella Gendler, Erica Li & Jonah Walker

In the coming years, climate change will have a sweeping effect on the Niagara grape growing region, and a considerable reduction in grape yield is forecasted. To maintain profitability, processes behind wine spoilage must be studied to facilitate their prevention. *Brettanomyces*, a genus of yeast, is among the most infamous causes of wine spoilage worldwide. An infestation of *Brettanomyces* imparts flavours described as "sweaty sock" and "barnyard" onto wine. These malodours originate from *Brettanomyces'* metabolites, mainly 4-ethylphenol and 4-ethylguaiacol. The key difficulty of dealing with *Brettanomyces* is its ability to grow on a large variety of carbon sources, particularly oak barrels and the ethanol inherently found in wine. Given this, detecting *Brettanomyces* requires rigorous testing. Agar plating and PCR are the most common methods, but they are not always accurate and cost-prohibitive, respectively. Removing *Brettanomyces* from wine is difficult but not impossible. Typically, small amounts of sulphur dioxide (SO_2) are added to the wine barrel, which prevents growth of *Brettanomyces* altogether. However, SO_2 can alter taste, maybe pose a risk to the health of the consumer, and can promote microbial resistance within the genus, reducing the treatment's efficacy. We recommend non-invasive physical fungicidal interventions such as pulsed electric fields and high hydrostatic pressure, but their high cost will require the economic collaboration of multiple vineyards.

INTRODUCTION

The Ontario wine industry produces an enormous 100.9 million bottles of wine per year (1). The unique terroir of the Niagara grape-growing region is a considerable part of what makes grape yields so large, and thus wineries so profitable. Unfortunately, it is currently under threat by climate change, bringing changes to temperature, precipitation, wind patterns, and frequency of extreme weather in the coming years. These changes will not only reduce crop yields, but will also increase the coverage of pests and disease, increase the rate of soil erosion, cause winter injury to vines, and compromise grape quality (2,3). Solutions to these problems are expensive, and their economic strain on wineries will be passed to the consumer. It is now paramount that vineyards have the capability to maximise the amount of wine produced from their grape yield. This highlights the need for research into wine spoilage.

One of the most infamous causes of wine spoilage are yeasts of the genus *Brettanomyces* (Figure 1). *Brettanomyces* has a significant history in the beverage industry. The genus was initially described by brewer Niels Hjelte Claussen in 1904 (4). The name "*Brettanomyces*" translates to "British fungus," reflecting its initial discovery in the British Isles (5). Claussen's isolate was derived from beer, where he and many other brewers considered it to be a desirable and necessary component of fermentation. This led to *Brettanomyces* becoming the first patented microorganism in history (6).

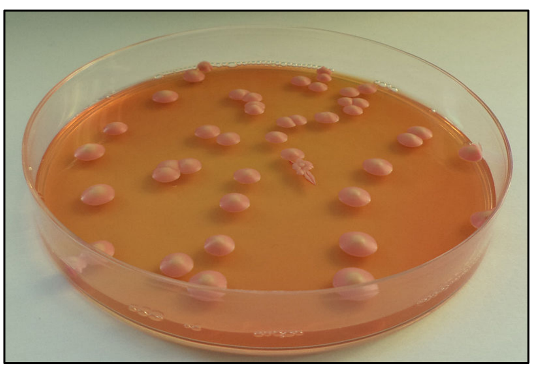

FIGURE 1: *BRETTANOMYCES* CULTURES ON AN AGAR PLATE. Each dot on the plate is one colony forming unit (CFU), , which represents a single viable microorganism capable of growing into a colony under the conditions (9).

Conversely, *Brettanomyces* is considered one of the yeasts "most feared by the winemaker," historically causing panic in the wine industry (7). It was first described in wine in 1950, but its role in spoilage was not discovered until more recently, when winemakers started noticing distinct changes in flavour profiles (6). In 1990, its presence was systematically associated with the development of two unique metabolites, 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG), two volatile phenols found to be responsible for creating the off-odours characteristic of *Brettanomyces* (8).

A complete understanding of *Brettanomyces*, including its inner workings as an organism, how it proliferates in the winemaking process, as well as current-day control strategies is important to understand to prevent wine spoilage. This article will discuss how the presence of *Brettanomyces* yeast in different stages of wine production affect its potential to cause spoilage, and the strategies that can be implemented at each stage to mitigate spoilage risk.

BRETTANOMYCES AND WINE

Brettanomyces is known to spoil red wine by imparting undesirable smells and flavours, commonly described as "sweaty sock," "barnyard," and "wet goat." However, at low concentrations, *Brettanomyces* can impart pleasant odours onto wine described as "clove," "smoke," and "spice" (9). The preference threshold, or maximum desirable concentration, for 4-EP is reported from 230–650 µg/L, and for 4-EG from 33 to 135 µg/L (10). These low sensory perception thresholds for 4-EP and 4-EG demonstrate the delicate equilibrium between contributing unpleasant and agreeable aromas to wine. Most winemakers view *Brettanomyces* as a risk to the winery, as its growth is difficult to control and can easily spread throughout the facility (11).

Winemakers who wish to prevent *Brettanomyces* growth will add small amounts of sulphur dioxide (SO_2) as a fungicide to their wine during the fermentation process, else they are branded as 'natural wines.' *Brettanomyces* is such a difficult and costly problem for wineries to solve because it can occupy many different niches in a winery besides within wine, including wooden barrels, valves used to transfer grape must, and even on the grape berry surface (11).

BRETTANOMYCES AS AN ORGANISM

A member of the fungi kingdom, *Brettanomyces* is an anamorphic yeast genus in the family Saccharomycetaceae (13). The classification of *Brettanomyces* has sparked considerable debate since Claussen's initial description. He noted that, unlike *Saccharomyces*, the primary genus involved in fermentation, *Brettanomyces* did not produce spores (4). However, a few decades later, a sexual form of the fungus was observed, marked by the formation of ascospores; this was named the *Dekkera* genus (14). Yeasts classified under the genus *Brettanomyces* do not form spores and are known as anamorphs, in contrast to those belonging to the *Dekkera* which are referred to as teleomorphs. Within modern mycological taxonomic guidelines, it is unlikely the two are genetically distinct, based on results from molecular DNA detection techniques such as polymerase chain reaction (PCR) testing (15). The two genera names are often used as synonyms, though *Brettanomyces* is more commonly used in the food industry. This article considers the two names to be synonymous (6).

Over the years, many different species of *Brettanomyces* have been suggested and many reclassifications have occurred (13). The species *Brettanomyces bruxellensis* is of primary concern for winemakers as it has been isolated from several fermented products, such as wine.

Brettanomyces is a well-adapted specialist, possessing the ability to enter a viable but non-culturable (VBNC) state. The removal of the external stressors may allow the cell to resuscitate thus exiting the VBNC state and becoming culturable again (16). A *Brettanomyces* cell may enter this state from stresses such as the presence of sulfites, which are often used in winemaking.

Viable but non-culturable (VBNC) state: The VBNC state is an adaptive response against adverse external stresses and leads to a reduction in the cell's size but still allows it to produce some metabolites.

To support the description of *Brettanomyces* as a well-adapted specialist, Conterno et al (17) conducted a study which genetically characterised 47 *Brettanomyces* strains and performed physiological characterization on 35 of these isolates. The strains studied were isolated from diverse geographic regions, from different vintage years and made with different varietals. They concluded that isolates were able to grow on a variety of nitrogen sources, specifically, both arginine and proline served all isolates as a source. This is noteworthy as the two amino acids can be the most abundant in grape juice, and may be left behind in finished wines if no other nitrogen source is available (17). Similarly, they concluded that isolates were able to grow on a variety of carbon sources due to the impressive diversity of carbon utilisation patterns. Most notably they found approximately 25% of isolates were able to grow on ethanol as a sole carbon source. This is of concern to winemakers who do not attempt to control the growth of *Brettanomyces* in finished dry wines since it would be possible for those strains to grow on the ethanol in the bottle without residual sugar traces. The study also found that all strains resisted at least 10% ethanol, grew at low pH levels of 2.5 and were tolerant to a variety of temperatures making barrels an optimal environment for their growth (16).

It is known that *Brettanomyces* produces a wide range of metabolites, most significant of which are acetic acid and ethyl phenols, notably 4-EP and 4-EG (18,19). Although many compounds are denoted as being related to the "Brett defect" in wine, 4-EP and 4-EG have been specifically linked to the presence and activity of *Brettanomyces* (19).

Brettanomyces induces wine spoilage by converting cinnamic acids to ethyl derivatives (Figure 2). Cinnamic acids are aromatic compounds naturally occurring in grapes and are thus present in the wines produced. Due to their aromatic nature, cinnamic acids contribute to the wine's flavour. Therefore, when reacting with *Brettanomyces*, the wine not only gains compounds inducing undesired flavours but also loses some of the compounds responsible for its original flavour and aroma (20).

Brettanomyces' ability to adapt so well to its environment has allowed it to grow in wine barrels as well as the grapes themselves. Since it can grow and succeed in many environments, *Brettanomyces* may induce wine spoilage during the winemaking process.

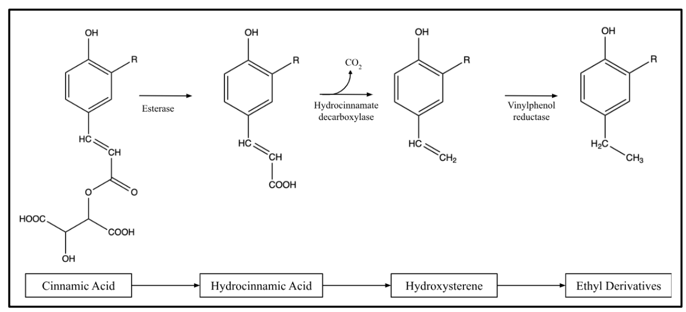

FIGURE 2: FORMATION OF ETHYL DERIVATIVES FROM CINNAMIC ACID BY *BRETTANOMYCES*. The cinnamic acids first go through an esterification reaction to create a hydroxycinnamic acid, followed by decarboxylation to hydroxystyrenes which are finally converted to ethyl derivatives through reduction (22).

BRETTANOMYCES DURING WINEMAKING

In winemaking, the primary growth period of *Brettanomyces* is during the ageing process (Figure 3). Grape skin is a natural reservoir of the yeast, as it makes its way to the vineyard through the air (7). Oro et al.(11) found that in the winemaking process, the strains from a vineyard and its respective winery were identical, suggesting that the vineyard is the contamination source. During the crushing process, the yeast can be transferred into the must, which begins its journey into the winemaking process (7).

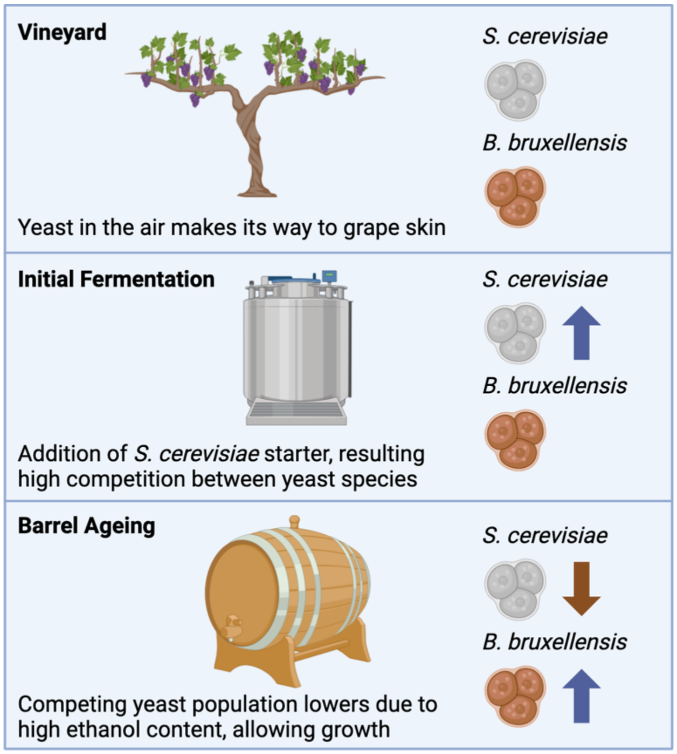

FIGURE 3: THE PROGRESSION OF BRETTANOMYCES YEAST DURING THE WINEMAKING PROCESS. It begins with the yeast on grape skins in the vineyard, which is then transferred through the air during the initial fermentation. The presence of *Saccharomyces cerevisiae* through the addition of a starter culture increases competition among yeast species. During barrel ageing, the population of competing yeast decreases due to the high ethanol content, allowing *Brettanomyces bruxellensis* to grow (7,22)

Brettanomyces growth is minimal during the initial fermentation due to competition with *Saccharomyces* yeasts (7). Its high resilience in specialised environments compared to *Saccharomyces* allows for it to survive in a low-resource state, termed *viable but nonculturable* (VBNC) (21). *Brettanomyces* remains largely inactive until the wine is transferred to oak barrels for ageing (21). The porosity of oak, combined with the ethanol, sugar, and nitrogen in wine, makes barrels the preferred ecological niche for *Brettanomyces* (21).

BRETTANOMYCES CONTROL

DETECTION OF *BRETTANOMYCES*

The adoption of novel control *Brettanomyces* technologies requires widespread implementation of detection techniques in order to be economically feasible. Currently, in the wine industry, detection is still largely reliant on human sensory evaluation for 4-EP and 4-EG aromas (22). Common direct and indirect detection methods have limitations, often being either imprecise, costly, or overly complex (22).

Plating is the most common method of direct detection due to its low cost (Figure 4). This involves culturing the sample in a nutrient source combined with antibiotic (e.g., cycloheximide) and bactericidal agents (e.g., ethanol) (7,22,23). Morphological characteristics and colony quantity are used to evaluate the presence and concentration of the yeast. Though plating is cost effective, it is only useful for viable cells, as Brettanomyces can exist in the VBNC state, undetectable through plating (9). Additionally, due to the sheer diversity of native non-Saccharomyces yeasts in winemaking, identification through morphological and physiological characteristics can be inaccurate (22).

FIGURE 4: BACTERIAL PLATING. Bacterial plating showing isolated colonies grown on an agar plate, each derived from a single bacterial cell or cluster (23).

The most common molecular detection is PCR, which uses the amplification of RNA and ribosomal DNA fragments to identify *Brettanomyces* (22). Its cost is often prohibitive, but compared to plating, PCR is precise, fast, and can detect VBNC *Brettanomyces* cells (22). Reverse transcriptase PCR (RT-PCR) utilises an enzyme to convert RNA into single-stranded DNA. This method can differentiate between viable and non-viable cells because it can detect RNA. Specifically, mRNA is typically unstable and rapidly breaks down following cell death. If mRNA is detected in significantly lower amounts relative to an actively growing sample, this can indicate that the *Brettanomyces* sample does not contain viable cells. RT-PCR is a commonly used molecular detection technique that can determine viability if *Brettanomyces* is detected.

Microscopy and mass spectrometry are used in research, but rarely by wineries. Often, these laboratory techniques require specialised training and are cost-prohibitive. Although, new biosensors equipped with microelectrodes can identify Brettanomyces at concentrations as low as 2 log cfu/mL by monitoring its metabolic activities and growth directly on the sensor's surface (22). Biosensors present the possibility of in-situ detection of *Brettanomyces* in wine and oak barrels, potentially revolutionizing quality control in the wine industry (22).

Currently, the most common intervention for Brettanomyces control is sulfur dioxide (SO_2) (24). SO_2 is an antifungal, antioxidant additive that is used throughout the winemaking process (25). There are small differences regarding the recommended doses of total SO2 use; the International Organization of the Wine and Vine recommends the use of 150 mg/L for red wines, the European Union limit is 160 mg/L for red wines, and Australia permits 350 mg/L for all wines (26).

CURRENT CONTROL STRATEGIES

The use of SO_2 dates back to the end of the 18th century. Currently it is widely used in the food and drink production industry, especially those involving low pH foods notably fruit juices and fermented drinks such as wine (27). SO_2 is used to control unwanted microorganisms and polyphenol oxidase activity during winemaking. It is added to machine harvested grapes, and in the process of red winemaking it is also added to the wine itself following malolactic fermentation (28,29). This process allows for the control of both oxidative processes and undesirable fermentations (27).

Despite its widespread use, SO_2 comes with limitations. From an oenological point of view, the use of SO_2 in inaccurate concentrations may cause more harm than good. As an example, the excessive use of SO_2 can cause organoleptic alterations in the final product such as aromatic neutralisation and production of characteristic aromatic defects. However, an insufficient concentration does not ensure the adequate stability of the wine against excessive oxidation or microbial development which could compromise its quality (27). Additionally, SO_2 can cause adverse effects in some individuals including allergic reactions, headaches, asthma and abdominal pain (26). As a result, there are strict regulations that govern the use of this preservative in the wine industry and thus research for new Brettanomyces control strategies continues.

EMERGING CONTROL STRATEGIES

For all winemakers, achieving the desired flavour in the wine is the highest priority, so any *Brettanomyces* control solution that alters flavour is unable to be adopted universally. In addition to their flavour-altering effects, the overuse of sulphites is initiating an antimicrobial resistance (24).

One less invasive method to inactivate *Brettanomyces* without impacting wine quality is pulsed electric field (PEF) technology. In this process, intense electric fields are applied directly to the wine, leading to a rapid increase in membrane permeability called electropermeabilization, which subsequently causes cell death (30). PEF works by altering the resting transmembrane voltage in *Brettanomyces* cells. All cells maintain a potential difference across their membrane by selectively pumping in and out oppositely charged ions. It is an extremely important and delicate balance which leads to a resting potential difference in a cell that ranges from -40 to -70 mV. Exposure to a sufficiently charged external electric field will upset this balance and lead to electropermeabilization. In microbial cells, this will trigger a homeostatic imbalance that will cause cell death (31). In the PEF machine, the liquid will be passed through several chambers where it will be directly exposed to a pair of electrodes emitting an electric field. According to Puertolas et al.(32), the optimal PEF treatment uses an electric field strength of 29 kV/cm with energy 186 kJ/kg.

Another common physical method of removing Brettanomyces from wine is high hydrostatic pressure (HHP) technology. HHP subjects food to immense pressure which destroys microorganisms and denatures proteins. For wine, the pressure range is typically between 100 and 500 MPa (24). HHP predominantly affects noncovalent bonds, harming the tertiary structure of essential proteins in a *Brettanomyces* cell. Food components that are necessary for the nutritional and sensory characteristics of the wine are low molecular-weight covalent structures, and are mostly unaffected (33). Thermodynamic principles dictate that an increase in pressure will lead to a proportional increase in the temperature, which would affect wine flavour. A good proxy for the effect of HHP on wine temperature is water, which will heat approximately 3°C for every increase of 100 MPa. Despite this, one of the main advantages of HHP is the elimination of heat damage, as HHP machines use a heat jacket or an internal heat exchanger to maintain a uniform temperature. Morata et al.(34) demonstrated that wines could be completely decontaminated at a pressure of 100 MPa over 24 hours at 25°C. Importantly, this did not affect the global aroma and colour parameters of the wine, but did reduce concentrations of the anthocyanin pigment affecting wine colour.

Both of these methods are well-established in the food production industry, though HHP typically outperforms PEF in effectiveness.

It achieves reductions greater than 5 log cfu/mL, in contrast to PEF which achieves reductions around 3 log cfu/mL (24).

BRETTANOMYCES IN NIAGARA

The Niagara wine industry, though significant within Canada, is relatively modest on the global stage, ranked 29th in worldwide wine production (35). Dominated by small wineries, with Niagara's constituting 84% of those in Ontario (Figure 5), the region grapples with *Brettanomyces*, which is particularly problematic for its economically vital Pinot Noir grapes (36). These grapes, grown in tight bunches, are especially susceptible to mould and yeast growth when combined with Niagara's cool and wet climate, which is further propagated in the winery.

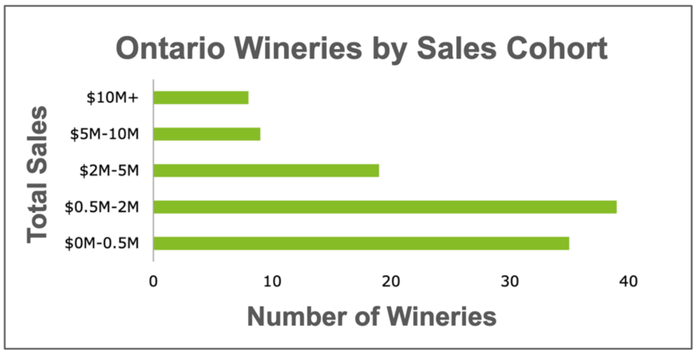

FIGURE 5: ONTARIO WINERIES BY TOTAL ANNUAL SALES IN CANADIAN DOLLARS. Most have annual sales under five million dollars (38).

The few larger, industrial wineries in Niagara, which are expected to maintain consistent standards, may be able to afford advanced treatments. The costs of implementing HHP and PEF treatments can range from $40,000 to $1,100,000 CAD and $60,000 to $700,000 CAD, respectively (24). These figures don't account for additional equipment or operational costs. HHP, recognized by Health Canada as a non-novel food processing method since 2016, and PEF, explored as a potential technology for parasite control in foods, are established methods in the food and beverage industry (39). With the higher energy consumption of HHP relative to PEF, wineries must weigh potential environmental impacts against the benefits of reduced spoilage. Reducing *Brettanomyces* contamination levels will extend shelf life, prevent spoilage, and ensure a consistent product, offering long-term advantages.

For the majority of smaller wineries, these costs will be prohibitive. Instead of focusing on treating *Brettanomyces*, they might benefit from focusing on enhancing preventive sanitation practices. High Powered Ultrasound has emerged as the most effective barrel sanitation technique. Its costs, ranging from $4000 to $10,000 CAD, are also considerably more manageable than HHP or PEF (24).

Effective treatment hinges on timely detection of *Brettanomyces*. Traditional detection methods fall short; their high costs and the need for specialised expertise make them less accessible (22). There is a pressing need for a detection platform that is both sensitive and economically viable. Emerging solutions, such as biosensor-based methods, could potentially bridge this gap (22).

The tightly knit Niagara wine community, exemplified by the Niagara Pest Monitoring Club, and collaborative efforts of groups such as Ontario Craft Wineries, Tourism Partnership Niagara, and Wine Growers Ontario, (38) reflects a collaborative spirit that could be harnessed to address the challenge of *Brettanomyces*. Joint ventures between large and small wineries, leasing equipment, and community-wide education initiatives could be the way forward.

CONCLUSION

Brettanomyces is a persistent yeast that is able to occupy many ecological niches and thrives in the presence of alcohol. Its metabolites, 4-EP and 4-EG, can create malodours easily detectable to the average wine consumer and can easily ruin an entire years' worth of wine. The main sources of contamination for *Brettanomyces* are grape skin, oak barrels, and the air. Ideally, *Brettanomyces* can be detected early in the winemaking process via plating or PCR, but it is difficult to swab entire wineries due to *Brettanomyces'* ability to occupy various niches. Inevitably, *Brettanomyces* has to be removed from wine before reaching the shelves. Niagara winemakers could continue using SO_2 to decontaminate their wine, but they risk compromising their signature wine flavour. Larger vineyards may be able to afford effective non-invasive decontamination technologies like HHP and PEF, but they remain out of reach for smaller wineries. To secure the future and maintain the quality of Niagara wines, further research into physical methods of decontamination and detection must be done.

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Pesky Pests:

Climate Change and Pest Interactions in Ontario Vineyards

Abdullah Islam, Raiyan Rubab, Sayna Salehzadeh, Shreya Sharma

When examining the effects of climate change on viticulture, changes to grape physiology, and cultivar adaptation should be considered, along with the implications of grapevine pest interactions. We assess the spotted lanternfly and potato leafhopper in Canada, exploring their distribution, development, and environmental and economic implications. While the potato leafhopper feeds on over 200 agricultural host plants, the spotted lanternfly is a potential future invader, spreading rapidly across the United States of America (1). Predictive niche models and experimental studies on high-temperature tolerance suggest further range expansion on a global scale, potentially including Ontario. Spotted lanternfly introduction to the United States of America will be used as a reference point to extrapolate potential outcomes of establishment in Ontario. Pest management strategies and preventative measures will further be explored while emphasising the importance of reducing invasion phenomena. Climate change is an enduring concern, and effective vineyard protection from infestation requires both prevention and mitigation measures. Pests are ecologically destructive and economically costly; thus, governments must continue to research this issue to prevent further pest proliferation and downstream harm.

INTRODUCTION

Accounting for one percent of the global wine production, viticulture is growing exponentially in Canada (2). This encourages international businesses to contribute to the Canadian economy through increased capital investments in the industry (2). Dating back to the 1860s, the history of wine production in Canada is short but successful (3). The wine industry is now regarded as the 'highest value-added agricultural product,' with contributions of over 11.5 billion CAD to the economy in 2022 (4). However, the success of this industry may begin to dwindle due to climate change, which has previously caused major economic losses to vineyards across the country, especially in Ontario.

Since the peak of the Second Industrial Revolution, the average global temperature has increased by at least 1.1 °C (5). Rising temperatures may have effects on environmental, economic, and ecological factors impacting vineyards across Ontario. This includes changes in seasonal precipitation patterns and an increased frequency of extreme weather events, which raises concerns for Niagara vineyards, globally ranked 27th for wine production quantities (6). By 2080, it is expected that Niagara will experience increased precipitation in the winter, spring, and fall seasons, but a decreased amount in the summers. Vineyard conditions may be exacerbated by predicted increases in average annual temperature, increasing by 11.4 °C in 2050, and 13.2 °C in 2080 (6). These issues will lead to grapevine water stress, novel pests, and grape yield loss, resulting in an overall reduction in both the production and quality of wine (7). Viticulture plays an important role in the Canadian economy and will be jeopardised by this issue. In 2014, the Niagara region reported losses to the Merlot, Sauvignon, and Syrah varieties due to changing weather patterns (8). This resulted in a decrease in the overall production of wine and the available varieties.

Along with greenhouse gas emissions, and changing precipitation patterns, temperature is one of the leading causes of changing pest population dynamics (9). Climate change could lead to an expansion of a species' geographic range, increased winter survival, and increased generations (9). Affecting the pre-existing protection programs against species and organisms in Ontario, and generating unforeseen species interactions, pests are known to cause serious damage to the grapevine. This has been previously observed with many pests, as severe infestations may produce crops that are inviable for winemaking (10). Though the degree of damage and infestation likelihood is variable depending on the pest, there are common characteristics that may be observed. These include disfigured leaves and shoots, poor vigour, and reduced berry size (10). Pests of interest include the potato leafhopper and the spotted lanternfly due to their potential impacts on wine production in Ontario vineyards.

EMERGING SPOTTED LANTERNFLIES

Native to Southeast Asia, *Lycorma delicatula* or the spotted lanternfly (SLF) is one of the most destructive invasive species in North America (11,12). SLF nymphs and adults consume plant phloem from the trunk or shoot tissue, diminishing photosynthesis capabilities and vine vigour. Individual vines can host over 400 adult SLFs, with a seasonal average of 7.5 SLFs per vine due to their high reproductive potential. Ineffective pest control measures can result in reduced vine growth, or even complete plant loss (13). Since the species' initial detection in 2014 in Pennsylvania, impacted vineyards have experienced a disconcerting 45% to 100% reduction in yield (11). In Canada, SLFs are already recognized as a potential threat to the grape industry and were added to the regulated pest list in 2018 to prevent introduction from previously invaded regions (14).

Phloem: The delivery mechanism of a plant, transporting nutrients and sugars produced during photosynthesis from the leaves to other parts of the plant.

Current literature on global SLF distribution is based on predictions made by niche modelling software, such as CLIMEX (12) or MaxEnt (Figure 1) (15). These models use broad-scale climatic data to estimate SLF development and mortality in both invaded and uninvaded regions. Such models indicate potential expansion into Europe, and Australia as well as increased regional distribution in China and South Korea (12,15). This demonstrates the profound establishment capabilities of the SLF, which are further compounded by potential climatic changes, as regions grow warmer.

Niche models: Tools used in ecology and biology to predict locations or niches where a particular species is likely to thrive based on environmental factors.

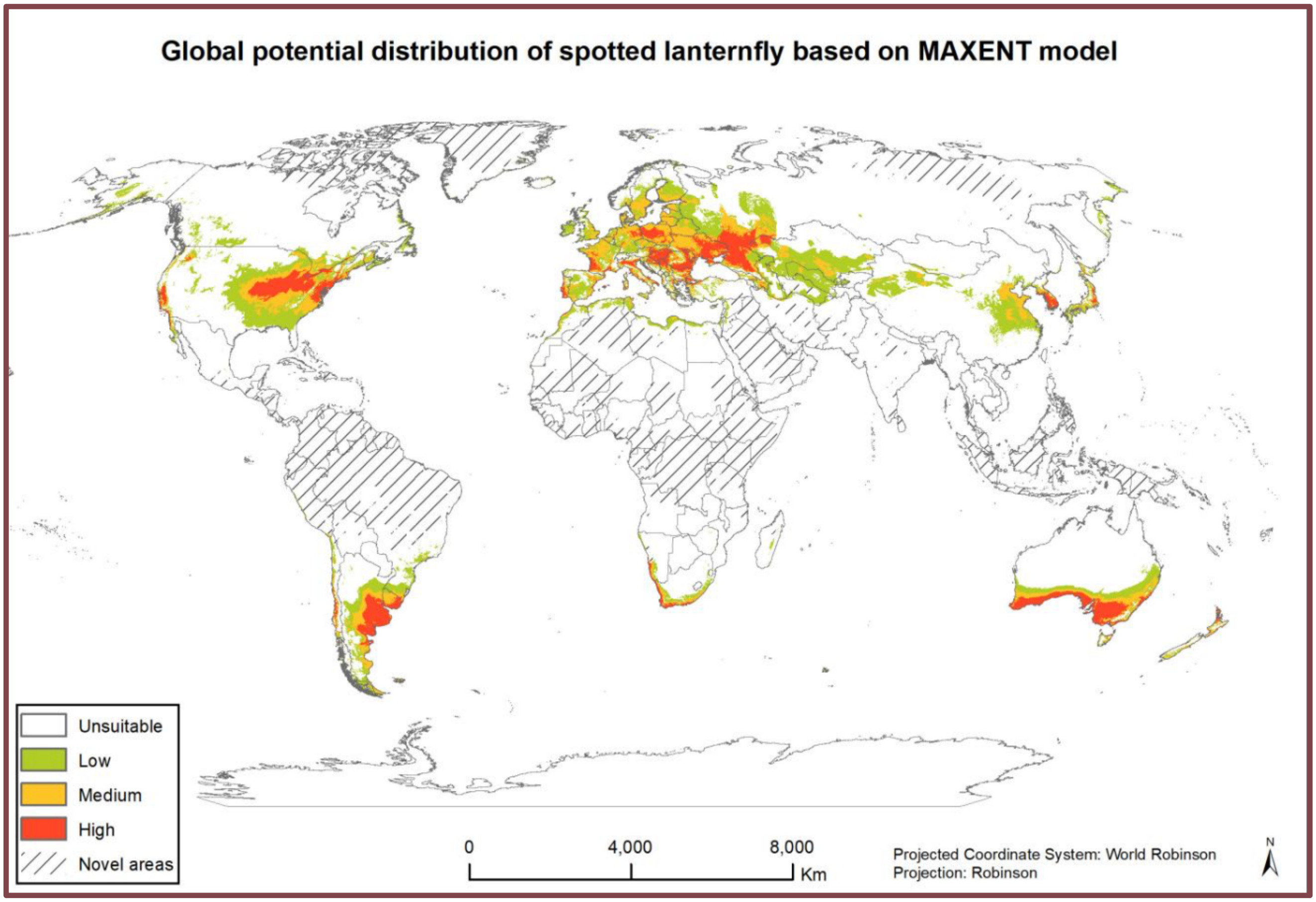

FIGURE 1: THE GLOBAL DISTRIBUTION OF *LYCORMA DELICATULA*, through the MaxEnt model. The red, yellow, and green hues highlight high, medium, and low suitability zones, respectively. Blank spaces denote unsuitable habitats, while hash marks point out environmentally unique areas between training and projection datasets (novel areas) (18).

However, vineyards are known to be sensitive to minor weather changes and have their own respective microclimates. The models fail to consider microclimatic variability in a given environment which is central to understanding the invasion of SLF specific to vineyards (16). Thus, the gap in the literature regarding this consideration is increasingly salient in the case of Ontario vineyards, given the current absence of SLF in Canada. Consequently, the effect of climate change on SLF voltinism and distribution in Ontario vineyards can only be understood from the perspective of existing studies on SLF life-cycle and environmental preferences.

Important environmental variables for predicting SLF distribution include the temperature ranges and thresholds for development, humidity, and soil moisture (15,16). While the recognized thermal value for each developmental stage varies between studies, in general, developmental delay and higher mortality were noticed at higher temperatures (17,18). At warmer temperatures, egg development time shortens, though it comes with a trade-off, namely a reduced hatching rate (19). In moderate temperatures of 15 – 30 °C, both developmental and survival rates are maximised (18). Colder temperatures ranging from -7 – 8.14 °C also prove suitable for the survival of overwintering eggs (15,19). As such, the SLF may be able to adapt to lower-temperature climates, allowing it to endure warmer Ontario winters resulting from global warming (12,15). It should be noted that these studies were conducted in laboratory settings and field applications of their results may not be an accurate reflection of natural conditions. However, this signifies that the increasingly warmer climate in Canada caused by global warming may aid SLFs to establish in Ontario vineyards in the coming years.

The Prairie Climate Centre predicts that the seasonal temperatures in Ontario will rise significantly by 2080 due to climate change. Average winter and summer temperatures are projected to rise to 2.3 °C and 25.2 °C, respectively. Fall and spring temperatures are predicted to increase by 3.5 °C approximately (6). The increased seasonal temperatures fall within a suitable range for SLF development and establishment, suggesting increased vulnerability of Ontario vineyards to SLF invasion. Climate change influence on precipitation, wind patterns, and pest interactions in Ontario may further alter SLF suitability in Ontario. Current studies on these parameters and their influence on SLF are insufficient to make more nuanced predictions (16).

The forest composition surrounding vineyards can also increase susceptibility to SLF invasion. The species feeds on over 73 host species, with a particular preference for *Ailanthus altissima* or tree-of-heaven, which supports both adult and nymph SLFs (20,21). Grapevines are similarly capable of sustaining adult and nymph SLF feeding and egg laying (22). Therefore, there is broad support for developing SLF in Canada, allowing for long-term proliferation. However, SLF populations cannot be maintained on a single diet of grapevines, although grape plants can improve the fecundity of SLF in the presence of *A. ailanthus* (20,23). A combination of hosts that support multiple stages of SLF in vineyards is therefore more likely to provide a preferable habitat. The wide establishment of tree-of-heaven in Eastern Canada renders Ontario vineyards highly vulnerable to SLF invasion (Figure 2) (22).

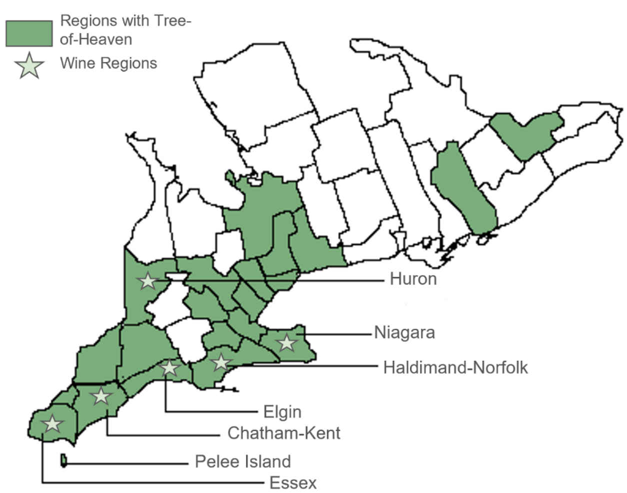

FIGURE 2: THE DISTRIBUTION OF THE TREE-OF-HEAVEN IN ONTARIO WINE REGIONS, as indicated by regions shaded in green with a star symbol (27–29).

Understanding the global distribution and environmental inclinations of SLF is crucial when addressing the potential threats posed by this invasive species. The projected future seasonal temperatures in Ontario align with the SLF's temperature range, which highlights the serious risk of invasion. Consideration of host species and temperature fluctuations in vineyards ensures high agricultural yield, while minimising economic risks from potential SLF invasion.

EXPANDING RANGE OF LEAFHOPPERS

Native to Canada, the *Empoasca fabae,* or potato leafhopper (PLH), is a prominent leafhopper species in Eastern Canada (27). It belongs to the family *Cicadellidae*, which are insects that use their piercing stylets to feed on phloem and xylem sap, along with mesophyll tissue of host plants (1). PLH are polyphagous pests, with their main host being the potato, while grapes are a secondary host species (27). The PLH is also a dynamic feeder, demonstrating both sheath- and cell rupture-feeding behaviours (28). As a sheath feeder, it feeds from a single phloem cell using its stylets that are sheathed with hardened saliva. As cell rupture feeders, the PLH uses its stylets to tear cells and consumes the phloem inside. A thin layer of saliva secretion prevents the plant from reacting to the wound (28). This may result in 'hopper burn', a plant response known to reduce grape yield, characterised by leaf discoloration, leaf curling, tip withering, and reduced vine growth (1). Hopper burn may affect grape quality, as it leaves faeces, honeydew, and sooty mould fungus on the leaves and berries of grapevines. This could account for the PLH's low economic threshold and the need for a standardised threshold in Ontario (29–31).

Polyphagous feeder: A pest or an organism with a diverse diet that can feed on a variety of different plants or substances.

Moreover, the temperature and climate have an immense impact on the distribution and growth of PLH. Every spring, PLH migrates from the Northern United States of America (USA) to Canada when the weather is warm, beginning in mid-June. Since the species cannot withstand cooler climates, it migrates back to the Northern USA as soon as the temperature drops. (27). In particular, DeLong (32) reported that elevation, humidity, and rainfall are responsible for the PLH's eastern Canadian distribution (32). Eastern regions below 950 metres in elevation with an average humidity of 40-60%, are of high economic importance, implying that PLH will proliferate in this area, requiring rapid control efforts. For PLH to cause economic devastation, moderate humidity and precipitation are required, along with warmer temperatures (32). As temperatures are expected to rise due to global warming, it is predicted that PLH populations will grow in the coming decades. This is because winters will become more temperate, allowing for PLH to remain in Canada for longer periods of time.

The fitness and maturation rate of PLH is impacted by temperature as well. When temperatures are above 10 °C, female PLH tend to lay four eggs on average per day. Over the course of their 35-day life cycle, each female can lay up to 124 eggs (32). In warmer weather, eggs may hatch in 7–10 days, while in colder weather, it may take up to 23 days. Therefore, PLH eggs mature in a temperature mediated process. Prior to feeding on the host, nymphs emerge later in the day during cooler periods. They complete their development from egg to adult in around 15 days at temperatures between 15-20 °C (32). Their development time decreases as temperatures rise. In colder weather, nymphs may seek shelter in soil crevices with slightly warmer temperatures. In Ontario, there are typically three to four generations of PLH during the summer (32). As temperatures rise, the overall lifespan of the PLH and the time it takes for eggs and nymphs to develop may shorten (32). As PLH develops from eggs to adults, their cold hardiness rises - nymphs tend to drop into soil cracks, where temperatures are warmer than surroundings when spring temperatures drop below 5 °C (32). Meaning, along with increased populations, adults may develop increased resistance to the cold.

In summary, the fitness and distribution of PLH will increase with the rise of temperature resulting from climate change. After spending their winters in the Southern USA, the PLH migrate to the Northern USA and Eastern Canada in the spring. This is because humidity, elevation, and temperature impact their distribution. In particular, they cannot tolerate cold climates (1,32). It is expected that the species will demonstrate increased range expansion and longer establishment periods in Canada in the coming decades. Furthermore, the PLH maturation is also affected by temperature, as with warmer temperatures the species demonstrates shorter development times, thus increasing their population in Canada.

IMPLICATIONS FOR ONTARIO VINEYARDS

Examination of the SLF and PLH makes it evident that the wine industry is at a rising risk for pest invasion, crop reduction, and overall economic and environmental loss. As a generalist feeder, the SLF may prey on over 100 species of plants, demonstrating the potential for widespread agricultural decimation. This is already seen in the USA, where the SLF has been a fierce invader, further exacerbated by human-mediated transport (33). The widespread invasion of SLF in Pennsylvanian vineyards has cost the state alone over $324 million USD annually, indicating severe economic and agricultural harm (34).

Previously, the harsh winter temperatures in Canada had been a major deterrent for many pests, but this invasion barrier may now be circumvented as a result of global warming (35). Ontario may now be a preferable environment for new invaders while providing a more rapid species establishment opportunity. Naturally, this will present long-term ecological concerns, including increased propagule pressure. This pressure presents downstream effects, including influences on species interactions and habitats in a pest's range of establishment. SLF has previously demonstrated the ability to interact with other invasive species in Pennsylvanian ecosystems, such as the previously mentioned tree-of-heaven plant (36). As this species is the preferred host for this pest, this interaction presents a serious concern, with the increased proliferation of the SLF in the presence of the species (24). This combined predatory establishment may further yield severe harm to native species, due to their inability to naturally defend themselves against alien invaders.

Propagule pressure: Quantity and frequency of introduction of potential invasive species into a new or non-native environment.

Unfortunately, it is difficult to extrapolate the exact extent of harm to vineyard ecosystems due to the novel phenomenon of SLF invasion in Canadian ecosystems. However, from the examination of the scenarios above, it is increasingly apparent that the species will have devastating impacts on Canadian vineyards if given the opportunity to expand its range northwards. The government must invest in pest prevention, as a more economically feasible and environmentally effective solution. As the SLF has yet to establish spread in Canada, species monitoring and predictive modelling efforts would mitigate invasion. From the investigation into the USA integrated pest management (IPM) efforts targeting the SLF, tools such as quarantining of key invader hosts such as the tree-of-heaven may be implemented (36).

Conversely, the PLH is a known pest in Ontario vineyards and presents similar responses to climate change as the SLF. With increasing temperatures, it is expected for PLHs to experience longer growing seasons (37). This would encourage an increased presence and proliferation of the species, particularly in the Niagara and North Erie regions (38,39). With rising temperatures within the Niagara regions, there has been an increased frequency of PLH infestations within the past 15 years, causing greater damage to crops including grapes (32). This is corroborated by major economic costs resulting from the pest, costing over $250 million in losses to Ontario agriculture annually (32). This pattern only emphasises the increasingly negative implications associated with climate change and pest proliferation.

Despite the future implications as a result of climate change, traditional insecticide usage remains worthwhile. Insecticides including acetamiprid, carbaryl, and phosmet, work to effectively minimise leafhopper damage to grapes upon their arrival in Canada (40). They further present little risk to human health, with minimal bioaccumulation potential. However, these insecticides must be integrated with predictive methods and scouting, to ensure administration is in line with shifting temporal pest patterns resulting from climate change. Monitoring methods in Ontario include the usage of sweep net sampling and yellow sticky cards (32). These methods remain ideal in diminishing rising infestations before widespread proliferation while mitigating excessive pesticide usage. Particularly, as the SLF is not yet present, emphasising the prevention and monitoring of the species is paramount.

A rapid response along with a thorough examination of climate change impacts must be used to act immediately. By examining the PLH, it is evident that climate change serves to exacerbate current invasive species and provide attractive conditions for novel invaders including the SLF in the near future. Preventive measures and predictive methods must be emphasised since, whereas invasion is preventable, climate change is an inevitable trend. Therefore, along with using IPM techniques, the Ontario government must also ensure widespread awareness of this rising issue.

CONCLUSION

The Ontario wine industry is at a critical point where navigating the intersection of climate change and invasive pests must inform future directions. The wine industry plays a vital role in the economy, thus addressing the changing pest-plant interactions in Ontario is imperative. The shift in climatic patterns poses a direct threat to vineyards, resulting in reduced overall grape yield and quality. The economic repercussions are severe, with potential losses amounting to billions of dollars.

The presence of pests, such as the SLF and PLH, serves to exacerbate climate change-related concerns. While SLF is not yet established in Canada, its presence and resultant economic downturn in the proximal USA demonstrate a potentially comparable outcome for Ontario vineyards. The threat extends beyond economic losses, with a lack of knowledge surrounding implications for nearby ecosystems, raising concerns about the long-term ecological balance. On the other hand, the PLH, a known adversary in Ontario vineyards, is predicted to have longer growing seasons and increased infestations. The economic toll already surpasses 250 million CAD annually, underscoring the urgency for adaptive measures for current and future invaders.

To secure the future of the Ontario wine industry, a twofold strategy is essential. Firstly, proactive measures must be implemented to address climate change impacts—embracing sustainable practices, water management, and vineyard resilience. Secondly, further research and investment in advanced monitoring systems, predictive modelling, and collaborative efforts are required to minimise pest invasion caused by climate change.

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