

A Review of Blackfoot, Petri, and Esca; Grapevine Fungal Diseases, their Treatments and the Impacts of Copper Based Fungicides

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August 14th 2021*

Abstract

Grapevines are a vital crop for the Canadian economy. Vineyards are a growth industry that bring tourists to Canada, and has the added benefit of being a profitable business that has significantly grown in popularity in British Columbia over the last 20 years. There are large gaps in knowledge on specific fungal diseases, and how to treat them. There are also issues with the knowledge from scientific studies not being effectively passed on to the people who can use it. This review seeks to gain insight from local vineyard managers to better understand both what their challenges with vineyard management are, and to look into the research behind fungal pathogens and pass that on to the vineyard managers who have helped with this review by filling out the survey. The fungal pathogens focused on in this review are actually fungal diseases that are made up of groups of harmful fungi those diseases are: Blackfoot, Petri, and Esca. The next step was looking into treatments for these diseases, with the specific intent to look into copper based fungicides. Lastly the impacts of copper based fungicides were looked into, to better understand their impact on the ecosystem and surrounding environment.

Introduction

The wine and grape industry in Canada is a significant economic driver as the industry generates \$6.2 billion in revenue annually, which increases to \$9 billion a year when taxes and wages are included (1). The industry in British Columbia (BC) generates almost a third of that total revenue, standing at \$2.8 billion annually. This is not revenue solely generated from selling grapes and wine, but also includes industry revenue that is a product of vineyards such as: specific tourism destinations, tours and, wedding venues (1, 2, 3). The vineyard industry employs roughly 12,000 people on average in every region in British Columbia (4). However, the vineyard industry in BC is extremely young. In BC the first vines planted are thought to have been by a priest in 1859 (5). At its oldest, the industry in BC is still under 200 years old, while the oldest vineyard in the world is the Staffhelter Hof vineyard in Germany, which is 1150 years old (6).

Fungal pathogens in vineyards represent a significant issue for the industry as they have huge negative impacts on the crops (7, 8, 9, 10, 11). This review focuses on three fungal diseases: Blackfoot, Petri, and Esca. These diseases excel at infecting grapevines and eventually killing them. Even though they have been around since the beginning of viticulture itself, there are no scientifically verified treatments that eliminate any of the diseases once the symptoms begin (7). These three diseases are not single fungal pathogens, but rather entire communities of fungi that work together to attack the host plant, in this case grapevines (10, 11). In addition, all of these diseases are active in BC, and have had massive negative impacts on grapevines both locally and globally (7, 8, 9, 12). Since the BC vineyard industry is relatively young, the impacts, although severe, are not well known, and have not made it to a level where the vineyard managers are well versed in how to combat the infections (7, 8, 9, 11, 12). Alternatively, it can be seen in older vineyard industries such as in Europe and Spain, how devastating these diseases are, with entire vineyards succumbing to them (12). The loss from these diseases equals to around 30-40% of all grapevines in Europe (12). In BC the Blackfoot disease showed symptoms in %75.5 (68/90) of the vineyards with young vine < 8 years old and, of those vineyards, %35.5

(32/90) had fungi that were identified as part of the black foot disease; 11% (21/191) vineyards had Esca disease, while 39.3% (75/191) vineyards had symptoms of Petri (10, 11).

Copper (Cu) based fungicides are the main treatment method for these diseases, along with a large number of other fungal pathogens (2, 3, 13, 14, 15, 16). Cu is the fourth most used ingredient in fungicides in BC (17). It has unique chemical properties, namely its' ability to bond with pretty much any element, that make working with Cu easy (14). Copper fungicides have long been prescribed as a treatment to fungal pathogens in agricultural soils, but Cu has not been thoroughly examined in terms of the impacts it has on the surrounding ecosystem (18).

This paper seeks to gain insight on current management practices when treating fungal pathogens as well as to learn whether or not these diseases are impacting individual vineyards. I surveyed local vineyards for their perception and treatment of the three target pathogens. I then reviewed scientific literature to synthesize recommended treatment methods for these pathogens, as well as the ecotoxicity impacts of Cu on the soil and surrounding environment.

Survey methods

I have canvassed both the lower mainland and lower Vancouver Island regions for vineyards. I canvassed by networking with people in the industry as well as using the internet to find additional vineyards. I then contacted most of the vineyards in the area by calling and emailing them directly, and then asked to either have a short on the phone interview or ask that they fill out a survey. Both the interview and the survey had the same questions. The blank survey can be found, [here](#).

Survey results

Survey Name: The impact of heavy metal fungicides in the winery industry

Response Status: Partial & Completed

Filter: None

2/1/2021 3:22 PM EST

Are fungi affecting your crop yields? If so, approximately what percent of your crop is affected and what yield changes have you noticed?

Number of responses: 3

Response Ratio

Yes 2 66.66%

No 1 33.33 %

What specific fungi are causing damage to your crops?

2 Response(s)

Powdery mildew

Do you use fungicides to combat the problem? If yes, what is/are the products you use?

Number of responses: 3

Response Ratio

Yes 3 100.0 %

How frequently do you use the product(s)?

3 Response(s)

Weekly during high pressure periods

Usually three times per season

Every two weeks

How do you apply the fungicide?

3 Response(s)

Air blast sprayer

With a tractor-mounted hydraulically driven air blast sprayer (high speed fan directs atomized spray from nozzles onto canopy, ensuring coverage of all surfaces of the leaves)

Hand held sprayer

What time of year do you apply the fungicide?

2 Response(s)

Bud break to veraison

spring/early summer

March - August

What volume of the product do you typically use?

2 Response(s)

What ever is required. ~ 5lb./acre/week Sulfer (1)

330L every two weeks over 5 acres of land

In a typical year, what are your annual expenses of fungicides?

2 Response(s)

\$200/acre

\$150 a year (a bag costs \$300 and lasts 2 years)

What are the biggest challenges that your crops face?

1 = Least impact, 5 = Biggest impact

Number of responses: 3

Responses

Fungi 1 response score of 2

Fungi 3 responses: 2,2, and 2

Drought 2 responses: score of 2 and 3

Temp change 1 response: score 1

Pests 3 responses: 4, 5, and 5

Invasive species 2 responses: 3 and 3

Have the challenges that your crops face become worse in recent years? If so, please describe.

Number of responses: 2

Response Ratio

Yes 1 50.0 %

No 1 50.0 %

Have you used other control methods outside of fungicides? If so, can you describe which you use and why you have chosen that technique?

3 Response(s)

That is a complete essay. Suffice it to say that proper cultural practices mitigate many disease pressures. Canopy, water

and nutrient management all have a significant affect on disease and pest pressures.

Intensive canopy management to allow air circulation and exposure of the fruit to sunlight

Horsetail tea as a natural spray to use on harmful fungi

If you have anything else to add that you think would be relevant, please expand below.

3 Response(s)

I take Umbridge to your introduction where you state plainly that your goal is to reduce or eliminate the use of synthetic fungicides. Why? Where does this come from? It seems that you are assuming for some strange reason that they are inherently bad.

We practise natural viticulture: no herbicides or insecticides and only certified organic fungicides.

Birds that consume the grapes, or harm the grapevines looking for insects are a larger issue for me than fungi. Also when there is an excess of rain my crops suffer significantly. (19)

Research methods

I used Google scholar to source the entirety of the research papers. The parameters were using the key words: environment, fungi, vineyard, Canada, British Columbia, fungicide, impacts, ecological, grapes, disease, pathogen, Esca, Petri, Blackfoot, water, ecotoxicity, and management. Each of these words could be linked with “and” or “or”, as well as change to the plural or short form version of any particular word on the list. There are some additional single use phrases that I used for the introduction that were topics on the history of viticulture both locally and globally. To limit some issues with replication, only the search results from the first page were used. The specific goal was to look into fungal pathogens, and how they are currently treated, the environmental impacts of that treatment, and potential alternatives.

Results

Blackfoot Disease

The Blackfoot Disease (BFD) is a fungal disease that impacts vegetation, although in this case the focus is specifically on grapevines. The first reported case of BFD was in 1961, although it has likely been active for significantly longer (7, 11). The fairly recent developments in the micro-science fields have allowed for increased precision when identifying specific aspects of various things, and in this case symptoms that were thought to be one disease turned out to be two. The two causal fungal agents to BFD were thought to be *Cylindrocarpon destructans* and *Cylindrocarpon obtusisporum*, however BFD turned out to be significantly more complex and there have been 18 harmful fungi associated with the disease (11). Those harmful fungi are: *Campylocarpon fasciculare*, *Campylocarpon pseudofasciculare*, *Cylindrocarpon pauciseptatum*, *Cylindrocladiella parva*, *Cylindrocladiella peruviana*, *Ilyonectria alcacerensis*, *Ilyonectria europaea*, *Ilyonectria estremocensis*, *I. liriiodendri*, *Ilyonectria lusitanica*, *I. macrodidyma*, *Ilyonectria novazelandica*, *Ilyonectria pseudodestructans*, *Ilyonectria robusta*, *Ilyonectria torresensis*, *Ilyonectria vitis*, and two as yet uncharacterized *Ilyonectria* species known as *Ilyonectria sp. 1* and *Ilyonectria sp. (Figure 1)* (11). Due to the hyper focused examination and identification of fungi being a relatively new practice, an issue arose when identifying the harmful fungi, which is that past records described some fungi and labelled them as a given thing, do not match up with the gene databases that are currently used to identify specific species (7, 11, 20). For instance, there have been issues deciphering the difference between *Cylindrocarpon obtusisporium* and *Cylindrocarpon macrodidymum*, as well as at least some

of the previously identified *Cylindrocarpon destructans* were actually *Cylindrocarpon liriodendra*. While these seem like small discrepancies, the reality is that there is a struggle even identifying specific species, let alone determining each specific species' role in the disease and then how to accurately deal with the harmful fungi (7, 20).

There are two infection sites where BFD infects the vine: nursery infections, and vineyard infections. The symptoms of a nursery infection are slightly different than those of a vineyard infection as they occur on the vine at different ages. Whether BFD occurs in the first two years of the vine's life, which is a nursery infection, or if the infection occurs between the ages of two and ten in the vineyard, the infection is fatal (7, 12). Nursery symptoms are shown as variations of stunted growth, which could be low volume of foliage produced, shorter trunks, thinner trunks, shorter internodes, uneven maturation specifically in regard to woody material, decreased root mass, and necrotic root lesions (7, 11). These symptoms are frequently coupled with discolouration of the vines and roots, along with vascular streaking. While vines infected in the vineyard have significant growth issues after winter, in comparison to uninfected vines **see image 2** (7, 11). In addition to reduced growth, infected vines have a dark brown discolouration that extends from ground level to 15cm in height on the trunk, and in some cases this discolouration extends up to 10cm underground on the root system (11, 20). The brown discolouration is the product of thick-walled tyloses, or brown gum. The gum plugs the phloem elements as well as the younger xylem (11). On a microscopic level there exists fungal hyphae, which only occurred in the discoloured areas of the vine (7, 11, 20). The blocking of the phloem cells actively prevents the movements of nutrients through the vine. The fungal hyphae exist within the ray cells which allows them to move through the phloem elements, and access stores of nutrients (7, 11). Simply put, the fungi are the perfect shape to slip into the space where nutrient uptake occurs within the vine, and instead of the nutrients going towards the vine's growth, they go to the fungi that have taken up residence inside the vine (7, 11).

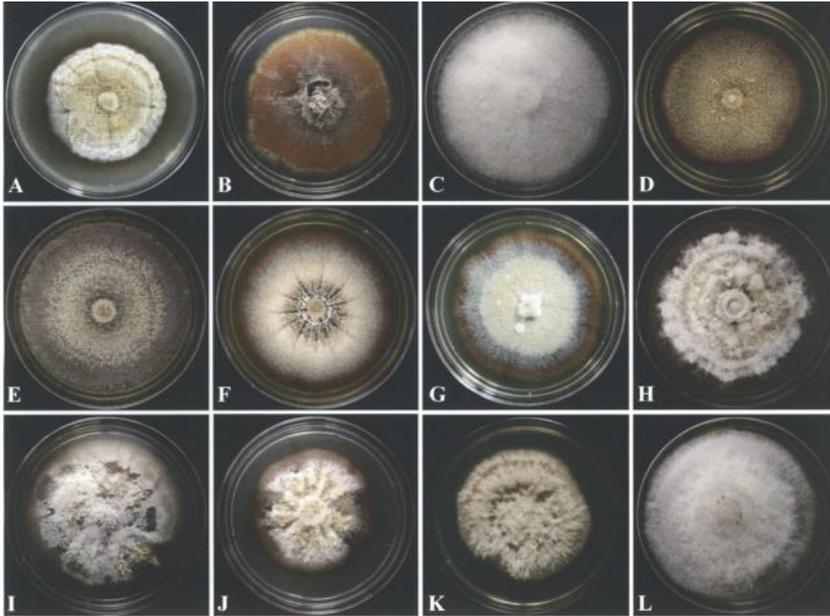


Image 1, shows some of the individual fungal species that are linked to BFD. A, *Cylindrocarpon destructans*. B, *C. pauciseptatum* ; C, *C. obtusisporum* ; D, *Ilyonectria alcacerensis* ; E, *J. liriodendri* ; F, *I. novozelandica* ; G, *I. macrodidyma* ; H, *J. torresensis* ; I, *Campylocarpon fasciculare* ; J, *Campyl. pseudofasciculare*; K, *Cylindrocladiella parva* ; L, *Cyl. Peruviana* (20)



Image 2, physical Blackfoot symptoms found in the trunk section of a grapevine (7)

Petri Disease

The Petri fungal disease only occurs in young grapevines 8 years and below (10, 21). Petri is thought to have existed since the beginning of viticulture, and has evolved alongside our agricultural growth through the centuries (10). The visible symptoms of Petri are indistinguishable from those of BFD in the field. However, Petri and BFD are thought to be two separate diseases as they have different fungal communities that make up each disease (10, 21). However, there seems to be an issue with listing the specific fungi that are associated with solely Petri as the full list is tangled with the fungi that are associated with the Esca disease. The fungi solely associated with Petri are: *Cadophora luteo-olivacea*,

Phaeoacremonium minimum, and *Phaeomoniella chlamydospora* (10, 12, 21). While the fungi associated with both Esca and Petri are: *Cadophora luteo-olivacea*, *Phaeomoniella chlamydospora*, *Phaeoacremonium canadense*, *Phaeoacremonium iraniamum*, *Phaeoacremonium roseum*, *Togninia fraxinopennsylvanica*, and *Togninia minima I* (10). The *Phaeomoniella* & *Chlamydospora* families are the main fungal types associated with Petri and Esca diseases. In addition to the above fungi, there have been 24 other *Phaeoacremonium* species and several *Cadophora* species; that have been associated with the esca and petri diseases. Not all of these fungi occur in both diseases, however it is unclear as to which fungi are specific to each disease (10).

Esca Disease

The Esca grapevine disease is also thought to have existed at the beginning of vineyard cultivation, and to have evolved alongside the industry . Esca is distinct from the Petri and Blackfoot diseases because, it specifically targets mature vines, 8 years and older (10). Esca infected vines can be identified by the observable symptom known as tiger stripes, which is a foliar symptom wherein the leaf veins turn light green and eventually rust coloured (10).

Two other symptoms are black measles, which is when the berries have grey to dark brown speckling; the other is a sudden wilting of the vine coupled with the grapes shrinking, this is known as vine apoplexy, which occurs in the summer (8, 10). The esca vascular symptoms are characterized by a white rot, which leads to a soft mass of wood in the center of the trunk, as well as black to brown spots in the xylem vessels (10). Tiger stripe is a slow burn symptom that is observable, whereas apoplexy produces a rapid death in the affected vines. The fungi live in the woody tissue of the vine, however the symptoms also occur in the foliage; which ranges from less productivity, to shriveling leaves and fruit, and to eventual death of the vine **see images 3 & 4**(8). *Basidiomycetous sp.*, *Fomitiporia mediterranea*, *Fomitiporia punctata*, *Phellinus igniarius*, and to a lesser extent *Stereum hirsutum*; are regarded as the causal organisms of the white rot symptom observed in the wood of trunks and/or cordons of esca-infected vines (10).



Image 3, Displaying tiger stripes which is a symptom of the Esca fungal disease.

(10)

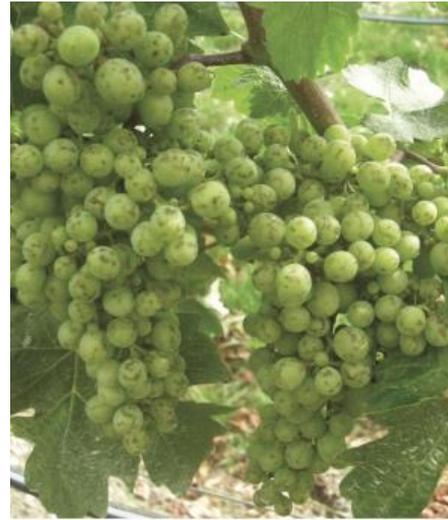


Image 4, shows black measles a symptom of Esca.

(10)

Treatment

At this current point in time there are no curative methods for the Esca, Petri, or Blackfoot diseases (7, 12). Due to this, along with a lack of available information on the topic, the treatments outlined will not be designated to one disease or another. There are two methods to managing fungal diseases: one is to prevent the disease from ever occurring, and the second is to eliminate a given disease after it has infected the host (20, 22). There are multiple ways through which a grapevine can be infected. Infection occurs from pruning wounds, grafting wounds, and soil borne pathogens (12, 20, 22). These infections can occur either in the nursery or in the vineyard (9, 10, 11). Currently, in the British Columbian database on treatments for fungal diseases in vineyards there is little to no mention of the Esca, Petri, or Blackfoot diseases; however, there are recommended fungicides for vineyards, **see tables 1 & 2 for details** (15, 16).

The preventative methods for managing fungal diseases are still in the early stages of being proven as effective. There are quite a few methods that have been tested (20, 22). Preventative methods focus on preventing infections, which occur in three currently known ways: nursery infections, grafting infections, and pruning infections. The first method is the hot water treatment (HWT) method, in this method water is heated to 50C and a nursery grapevine is uprooted, and then the roots are submerged for at least 30 minutes in the hot water (20). After this treatment there were no harmful fungi detected. The control vine had a 4.1% incidence of harmful fungi, while the normal rootstocks had an incidence of harmful fungi in 16.8% of vines (20).

Using chemical treatments to inhibit the growth of groups of harmful fungi has had some success, both in South Africa (23), as well as Portugal (20). In Portugal it was found that, the fungicides benomyl, prochloraz and the combining of carbendazim with flusilazole and, separately cyprodinil with fludioxonil inhibited mycelial growth of some *Cylindrocarpons* (20). While the use of benomyl, tebuconazole, and the mixtures of carbendazim with flusilazole and cyprodinil with fludioxonil significantly improved plant growth and decreased disease incidence compared with non-treated vines. In South Africa, studies were done on vines prior to grafting or planting, wherein the vine was subjected to a chemical bath (20, 23). The roots of the vines were tested 8 months after the bath and the chemical baths that had positive results were those that had benomyl didecyl-dimethylammonium chloride and captan (20, 23). There was another study which used captan, carbendazim, copper oxychloride, didecyl-dimethylammonium chloride, hydroxyquinoline sulfate, and prochloraz; to try and prevent fungal infections on the roots, there was some success. Captan and didecyl-dimethylammonium chloride were still the best performers of the bunch (20).

There has been some effort to discover an effective biological control agent, however the only slightly viable one found so far during a study in South Africa was done through using a fungal paste of *Trichoderma*. This paste is applied to grafting and pruning wounds which makes it more difficult for other fungi to effectively infect the vine (20). *Trichoderma* also has some growth stimulating effects. This has also been used to stimulate root growth, with the theory that the faster growth gives the vine added resistance against infections (20, 22). There is some belief that different species of grapevine have higher levels of resistance to fungal infections, however in a study done in California in a greenhouse, all the varieties of grapevine showed some level of susceptibility to fungal infection. It is unclear whether that level of susceptibility differs between species (20).

There is a British Columbia specific issue with vineyards, which is that there was a shift from apple orchards to vineyards. There is significant evidence that *Cylindrocarpon spp.* and *Ilyonectria spp.*, are associated in some way with orchards and that these fungi remain in the soil even after the orchard has been removed and then converted into a vineyard (11). There are multiple other vegetation groups that coexist or even promote the growth of fungi that is harmful for vineyards (11). In this case it is important to treat the soil prior to planting. There was a study done in New Zealand that used various cover crops as a potential biofumigation control agent and it found that mustard (*Brassica juncea*) had a significantly positive impact on the reduction of harmful fungi in the soil (20). There have also been some

less scientifically rigorous treatment attempts that have had some success. However, it should be noted that effectiveness of each treatment was based on field observations (22). The two most effective of the treatments were injections of hydrogen peroxide into the trunk of the grapevine, and separately the use of copper nails by hammering them into the trunk. The hydrogen peroxide was injected into the trunk by first drilling a small hole where the fungi were most active and then 3-4ml of hydrogen peroxide was injected into the new cavity (22). Growers reported good results with no fungi being visible after the injections. In the case of the treatment using copper nails; the copper nails were placed in the trunk of infected vines to slow the encroaching rot symptoms. The growers mentioned that the Cu enhanced area could be seen around where the nails were placed, and that the rot did not pass the areas of Cu accumulation (22).

Kumulus DF or Cosavet DF (sulphur 80%)		Cantus WDG (boscalid 70%)
PureSpray Green Spray Oil 13E (mineral oil)		Luna Tranquility (fluopyram + pyrimethanil)
		Merivon (Pyraclostrobin + Fluxapyroxad)
SuffOil-X		Pristine WG (boscalid + pyraclostrobin)
Vegol Crop Oil (canola oil)		Miravis Prime (Pydiflumetofen + Fludioxonil)
Vivando (metrafenone 300g/L)		Flint (trifloxystrobin 50%)
Property 300 SC (pyriofenone)	Actinovate SP (<i>Streptomyces lydicus</i>)	Sovran WG (kresoxim-methyl 50%)
Priwen 500EC (spiromamine)	Buran (garlic powder)	Diplomat 55C (polyoxin D zinc salt)
Nova (myclobutanil 40%)	OxiDate ⚡ (hydrogen peroxide + peroxyacetic acid)	Milstop or Sirocco (potassium bicarbonate)
Mettle (tetraconazole)	Fracture (BLAD polypeptide)	Serifel (<i>Bacillus amyloliquefaciens</i>)
Fullback (flutriafol)	Lime Sulphur (sulphide sulphur 30%)	Serenade Opti (<i>Bacillus subtilis</i>)
Cevya (mefentrifluconazole)	Cueva (copper octanoate)	Double Nickel (<i>Bacillus amyloliquefaciens</i>)
Inspire Super (difenoconazole + cyprodinil)	Parasol WG ⚡ (copper hydroxide)	Timorex Gold (tea tree oil)
Aprovia (benzovindiflupyr)		Regalia Maxx (<i>Reynoutria sachalinensis</i>)
Aprovia Top (benzovindiflupyr + difenoconazole)		
Sercadis fungicide (fluxapyroxad)		

Table 1, recommended fungicides for use on grapevines in British Columbia. (21)

COPPER 53W	Copper Spray	Coppercide
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Table 2, other recommended fungicides for use on grapevines in British Columbia. (22)

Copper impact on soils

Cu is a highly variable element. While there exists a natural bioavailability in soils, it has unique properties that make it easy to work with and to synthetically create fungicides (14). The unique aspect of Cu is that it can bond to a variety of other elements. While this property makes Cu easy to manipulate, it also creates a complicated issue when introduced into any environment outside of a lab where there is almost complete control of the chemical interactions. When Cu is added to the vineyard system, regardless of the application method, it will eventually find its way into the soil (13, 18, 24, 25). Once Cu makes contact with the soil, its speciation changes into chemical fractions as it bonds with the soil organic materials (SOM). This decreases the mobility of the Cu. The SOM effects the Cu mobility in two different ways. If the soil has a higher acidity level the mobility decreases (24, 25). The level of mobility decreases in this order: Mn-(hydr)oxides > SOM > Fe-(hydr)oxides > clay minerals, SOM > Fe-, and Al-oxyhydroxides > clays and SOM > silicate clays > Fe-(hydr) oxides > ferrihydrite (25). The decrease in mobility also refers to the decrease in the ability for living things to utilize the Cu, also known as bioavailability (18, 25).

The more alkaline the soil, the higher the solubility of the Cu and therefore the mobility (18, 24, 25). The solubility of Cu minerals in soils decreases in the following order CuCO_3 > $\text{Cu}_3(\text{OH})_2(\text{CO}_3)_2$ (azurite) > $\text{Cu}(\text{OH})_2$ > $\text{Cu}_2(\text{OH})_2\text{CO}_3$ (malachite) > CuO (tenorite) > CuFe_2O_4 (cupric ferrite). Copper sulfates, such as CuSO_4 (chalcocyanite) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (chalcantite), are highly soluble and require high Cu concentrations to form in soils (25). Another aspect that influences Cu mobility is the soil texture, the finer the soil the less mobility the Cu has. The issue of predicting Cu is that there are a large number of factors to consider in the field applications. For example when using a fungicide it is not made of only copper but rather a wide range of chemicals that all interact with the soil and SOM differently (18, 24, 25). Fertilizer, which is high in phosphorus, adsorbs Cu and acts as an immobilizer. However sulfates, which are an essential ingredient in copper salts, increase the solubility of Cu (24). In a study on Cu levels in soil and whether it can be extracted, it was found that on average in acidic soil vineyards the concentration of Cu in 60% of the tests

was over 100mg per kg of soil, compared to 13-24mg per kg that is found naturally occurring in soil (24). It was also found that the 100mg of Cu per kg of soil was not the limit of the possible Cu concentration, and that the soil had significant capacity to adsorb additional Cu (24). However, regardless of the amount of the Cu in the soil, the desorption attempts never amounted to more than 8% of the Cu (24). This points to the irreversibility of the Cu added to the vineyard environment.

Not all Cu-based fungicides are toxically equivalent. The toxicity levels in Cu vary from extremely toxic to moderately toxic to slightly toxic, and lastly to trace amounts of toxicity, **see table 3 for toxicity levels of various fungicides** (18, 24). Cu is a micronutrient and is required in small amounts, $\geq 2\text{mg}$. However, a sudden increase in Cu consumption for humans poses significant health risks, the worst of which is complete organ failure (26). The same can be said for all organisms in the ecosystem who come into contact with the Cu (25, 26). Increased copper levels have negative impacts on microorganism communities, vegetation, and all of the larger living things in the area (13, 18, 25). The impact of toxicity is not limited to the vineyard, as the consumption of the Cu by vegetation, including the grapevines, allows the Cu to be consumed by the creatures that use the vegetation as a food source (13, 18). The Cu can also be transported to surrounding water sources through erosion, which impacts those local ecological communities, as well as moving through the soil to contaminate groundwater, **see table 4 for ecotoxicity impact levels of various fungicide ingredients** (13, 18).

Two additional things to note are that the earthworm communities are highly impacted by high amounts of ecotoxicity, and can therefore act as a bioindicator (18). On a more positive note, some of the microorganisms including fungi have some degree of success with converting Cu-oxychloride into insoluble Cu-oxalate which significantly reduces the toxicity of Cu in soils (18). On a long term scale, there was a study done of an abandoned vineyard specifically examining the impacts of Cu on the local ecological community (27). In that study it was found that the soil at the base of the vines had high amounts of Cu at 1000mg per kg of soil. However, the walkways between the grapevines had low levels of Cu, and while the bioavailability of Cu was high, it seemed as if there was a level of adaptation that had occurred where the species living there could tolerate the Cu levels (27).

EPA toxicity class	Toxicity rating	Characteristic acute toxicity in experimental animals	Example
I	Highly toxic	Oral LD ₅₀ : 0–50 mg kg ⁻¹ Dermal LD ₅₀ : 0–200 mg kg ⁻¹ Inhalation LC ₅₀ : 0–0.2 mg L ⁻¹ Skin/eye irritation: severe	
II	Moderately toxic	Oral LD ₅₀ : > 50–500 mg kg ⁻¹ Dermal LD ₅₀ : > 200–2000 mg kg ⁻¹ Inhalation LC ₅₀ : > 0.2–2.0 mg L ⁻¹ Skin/eye irritation: moderate	Tebuconazole
III	Slightly toxic	Oral LD ₅₀ : 500–5000 mg kg ⁻¹ Dermal LD ₅₀ : > 2000–20,000 mg kg ⁻¹ Inhalation LC ₅₀ : > 2.0–20 mg L ⁻¹ Skin/eye irritation: slight	Metalaxyl, fludioxonil, propiconazole, vinclozolin, cyprodinil, azoxystrobin, tebuconazole
IV	Practically nontoxic	Oral LD ₅₀ : > 5000 mg kg ⁻¹ Dermal LD ₅₀ : > 20,000 mg kg ⁻¹ Inhalation LC ₅₀ : > 20 mg L ⁻¹ Skin/eye irritation: none	Pyrimethanil, propiconazole, vinclozolin, cyprodinil, azoxystrobin, mancozeb

Table 3, shows toxicity levels of various fungicides. (25)

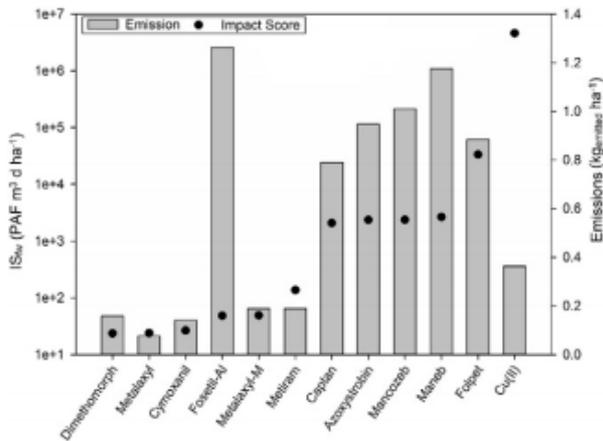


Table 4, shows ecotoxicity impacts of various fungicide ingredients based on the emitted amounts. (18)

Discussion

Survey:

Due to the ongoing pandemic, there were significant barriers to reaching to and receiving responses from the local vineyards. Without the pandemic, it may have been possible to build more of a relationship with each vineyard manager, which would have increased the scope of the questions as well as the likelihood of the vineyard manager’s being more willing to give detailed explanations. It may also have been possible to physically go to each vineyard and try to make direct contact rather than via email. It would also have helped my own personal understanding of the vineyard management if I could see the management practices and variability between vineyards in person. There were three vineyards that responded to me. The sizes and ages of the vineyards were as follows: 5 acres and began viticulture in 1997, 10 acres and began viticulture in 1993, and 30 acres which began

viticulture in 1987. All of these vineyards are extremely young, and small to medium sized, 2 of the 3 vineyards stated that they do in fact have a fungi problem. All 3 vineyards stated that they use fungicides. Perhaps that means 1 of the 3 does not have a fungal problem that impacts the fruit yields due to the fungicides' effectiveness. Pests were by far the largest issue all farmers faced, with birds mentioned by 2 of the 3 vineyards.

Esca, Petri, and Blackfoot diseases:

Of the three fungal diseases, Esca and Petri are thought to have existed since the beginning of viticulture. However, due to the similarities in symptoms between the Blackfoot disease and Petri it seems likely that the pathogen affecting the vine was always assumed to be Petri when it could have been either Blackfoot or Petri (7, 8, 10, 11, 12, 21). All three fungal pathogens are a combination of multiple fungi that have a parasitic relationship with the host plant wherein the fungi consume nutrients intended for the host until it perishes (10, 11). The diseases are formed by groups of fungi that have adapted not only to work as a team, but also to thrive in our agricultural systems, in this case in vineyards (12). Having multiple species of fungi working together makes the diseases able to adapt to vastly different landscapes, which is proven by them existing in every region where grapes are grown globally (12, 20). The lack of research done on the topic has been extremely limiting, as there are few answers on the specifics of each disease other than locations of occurrences, and visible symptoms. Things like the individual species that form each disease, how those species work together, what each of their roles are, and how to treat them; are all still a work in progress (7, 8, 10, 11, 12, 21). There is also a large amount of discrepancy between studies on which fungal species compose each disease, and what determines those differences (7, 8, 10, 11, 12, 21). There is also evidence that points to significant increases in the incidences of these diseases (10, 11, 26). With the impacts of climate change becoming increasingly relevant to crop growth, it seems that the fungal diseases are no different and that these increases in occurrences are a direct result of climate change (26).

Treatment:

Cu based fungicides are an essential part of viticulture (13, 14). Cu based fungicides are permitted to be used in organic vineyards and have a large variety of chemical potential allowing them to be used effectively for a wide range of harmful fungi (12, 14). Biocontrol agents do not act as quickly as chemical fungicides, so Cu based fungicides have the added

benefit of working quickly too (20, 25). Since there is a lack of research on the cause of the three diseases, and well as their specific functions; targeting the fungi and effectively treating them has not been confirmed to be widely successful with either copper based fungicides or alternative treatments, **see image 5 for a visual representation of infection potential in a grapevine** (12). However there have been a number of studies done on different methods of treatment for Petri, Esca, and Blackfoot (7, 20, 25).

The HWT treatments seems to be the simplest and most cost effective of the methods outlined, although it is specifically for young vines in the nursery and the treatment only targets pathogens that have found their way into the root system (20). It has not been tested on adult vines nor has it been tested on pruning wounds (20). The chemical baths also had some success, but seem to make the most sense being applied to grafting, wherein the new limb is soaked in specific chemicals and then attached to the vine (20, 23, 25). Pruning wounds seem to be best treated with a preventative biocontrol measure which is a paste of a fungal species named *Trichoderma*, which also has properties that speed up the growth of the host in the area the fungi are applied (20, 23, 25). Lastly, a biofumigation control agent, mustard, was tested in New Zealand which decreased the amount of harmful fungi in the soil. This has potential to be grown as a cover crop to protect the vines before they are planted and can continue to protect them through their lives (20, 25).

There are a number studies that have been completed that test various treatments to the diseases, however since none of them have been replicated sufficiently it is hard to say how effective each treatment is (20, 25). There also have not been any treatments within the scope of my research that have successfully treated any vine with symptoms of any of the diseases. In addition, there was nothing that I read about the transmission potential of any of the three diseases, and then determining the speed of the transmission would be crucial in determining the best treatment methods; as well as potentially a clue to better understand how the diseases work.

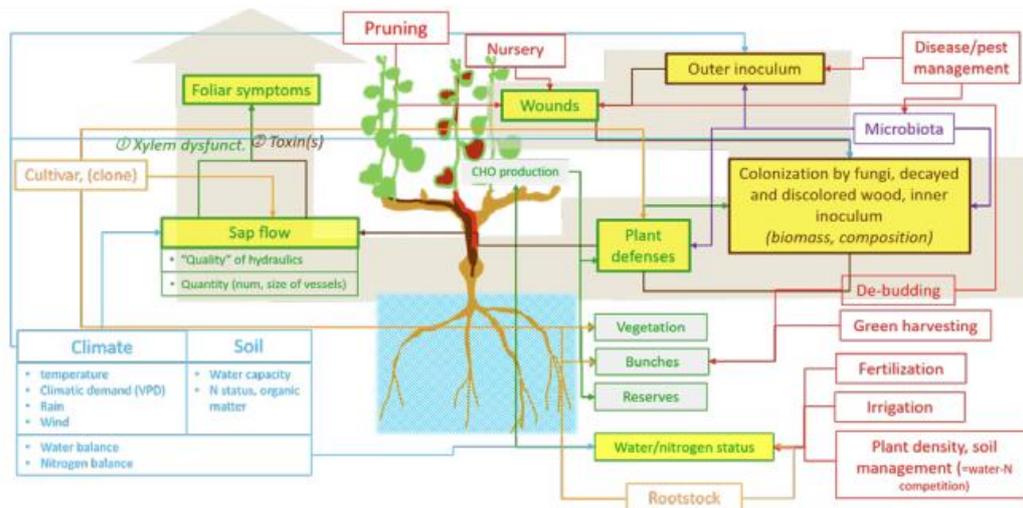


Image 5, shows the complexity of infection on a single grapevine. (12)

Ecotoxicity of Cu:

Cu is a low mobility element, and easily accumulates to harmful levels. The same adaptability of Cu that makes it easy to work with also creates significant issues when introduced to field conditions (13, 18, 24, 25). Cu is adsorbed by the SOM when it makes its way to the soil, and depending on the soil's properties the Cu will behave differently (24, 25). The toxicity levels vary, along with mobility; this makes predicting the behavior of Cu extremely difficult as it is dependent on local factors (24, 25). Each different trait that occurs when Cu is put in a specific situation present a range of possibilities and challenge, (24, 25). Since Cu is so variable, depending on its solubility it can end up in groundwater sources, or if it is in soils with a lower pH, it will become less mobile and more susceptible to erosion, which will carry the Cu into the surrounding water sources (18, 24, 25). The bioavailability of Cu in the area has significant potential negative impacts on the local organisms (13, 18). The ability of the soil to almost limitlessly adsorb Cu, creates significant potential for the surrounding vegetation and water sources to uptake the Cu (18, 25). The Cu that is now in those sources will impact the creatures that consume them. A straight forward example is that the grapevines have the capacity to uptake Cu and then that is partially accumulated into the fruit which is then consumed by a variety of animals including humans (18, 26). Relatively small amounts of Cu have negative impacts on humans if ingested suddenly. Those impacts in severe cases include total organ failure (26). Unfortunately, there are not a lot of studies on the long term health implications of Cu. There is data suggesting that a sudden increase of Cu in a diet has negative impacts, but there is a capacity to adapt to long term slight increases of

Cu into the diet (26). There are also different toxicity levels of Cu depending on the chemical variation. Interestingly enough the ingredient Captan; which is found in in some copper based fungicides, is only slightly to moderately toxic, and it has had good results when used in the chemical bath studies as a treatment to various fungal pathogens (18, 24, 25).

Conclusion

None of the vineyards from the surveys have issues with the specific fungi mentioned in this paper. However the conclusion I drew from the available data is that all three fungal pathogens, Petri, Blackfoot, and Esca, are currently active in British Columbian vineyards, and the scale of those occurrences are increasing (28). An additional factor is drought caused by climate change may further increase the range of these pathogens, as has been noted in California (20, 28). The consistent issue across all areas this review covers is that there is a significant lack of information. It was surprising to find that even in the aspects related to human health there is a large gap in knowledge, where the long term effects of Cu on health are unknown. Even the harmful amounts are not agreed upon within the medical community (26). This presents an issue for policy makers, who want to make laws limiting the use of Cu based fungicides, and may create a barrier for researchers who need funding for their studies (18). However, if fungal pathogens in vineyards continue to outpace modern technological adaptations in management practices, significant economic losses in vineyards and potentially other agriculturally based fields will provide the pressure needed to get funding on these projects. On a long term scale there is evidence that suggests that while desorption of Cu has not been successful, bioremediation is an effective treatment for contaminated soils in vineyards (27). This evidence can be seen in the natural occurring microorganisms that have the ability to convert Cu-oxychloride into insoluble Cu-oxalate which significantly reduces the toxicity of Cu in soils (18).

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