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Brief Report

Cover Crops as Reservoirs for Young Vine Decline Pathogens

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Abstract: Young vine decline (YVD) is a grapevine trunk disease (GTD) which results in stunted and delayed growth, reduced yield, root necrosis and eventually death of young vines. Given losses associated with root trunk disease, and increasing limits on chemical fungicides, there is a need for sustainable approaches to combat disease; (1) Cover cropping is a commonly used practice in agricultural systems and has potential to reduce disease in vineyards but there is a risk that cover crop species may act as a host for grapevine pathogens, increasing the risk of infection; (2) We tested 25 plant species commonly used in cover crops to assess their potential to act as a host for a Ilyonectria liriodendri, which is a causal agent of young vine decline. We inoculated greenhouse pots with a pathogeninc strain of Ilyonectria and assayed the roots for the presence of the pathogen; (3) Of the 25 cover crops tested, many of the species showed increased root abundance of Ilyonectria, compared to background levels. In particular phacelia (Phacelia tanacetifolia) and buckwheat (Fagopyrum esculentum) showed very high levels of root colonization. (4) This is the first study to our knowledge that highlights the potential of cover crops to soil borne fungal pathogens.

Keywords: Ilyonectria; cover crops; ddPCR; soil fungi; plant pathology; grapevine

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Citation: Rosa, D.; Sharifi, M.; Hart, M.M. Cover Crops as Reservoirs for Young Vine Decline Pathogens. *Agronomy* **2022**, *12*, 2422. https://doi.org/10.3390/agronomy12102422

Academic Editor: Leire Molinero-Ruiz

Received: 30 August 2022
Accepted: 3 October 2022
Published: 6 October 2022

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1. Introduction

Pathogen spillover is a mechanism by which pathogen abundance is increased in a community, leading to disease outbreaks [1]. This occurs in natural and managed ecosystems when pathogens can live asymptomatically in some plants, allowing the abundance of the pathogen to increase to the tipping point of infection for susceptible plant species [2]. In agricultural systems that use cover crops as part of their management, the species included in the cover crop may act as reservoir plants–plants capable of associating with and proliferating a pathogen while remaining largely asymptomatic. However, the capacity for a cover crop species to act for a reservoir species is largely overlooked when growers are selecting cover crop mixes.

Although YVD is a disease complex, the main culprits are fungal pathogens including fungi belonging to the genera Ilyonectria, Dactylonectria, and Cylindrodendrum among others [3,4]. These organisms may be present in soils [5] or enter vineyards via infected nursery material [6]. Fungal spores are easily distributed via contaminated tools, irrigation equipment, and by air from fruiting bodies on decomposing/infected tissue [7]. Causal agents of YVD occur in all major growing areas of the world [8] and although it may start with a few infected vines, the rate of infection will increase as the vineyard ages [9]. Young vine decline continues to contribute to economic losses around the world [10] and currently, options to prevent infection and mitigate decline in vineyards are limited.

Options like fungicide treatment are limited in many countries and are not always effective [11]. Furthermore, most fungicides are designed to combat foliar diseases and not infections in the roots [12]. Since fungicides accumulate in the soil and are considered to be pollutants, legislation in major regions aims to minimize their use as much as possible [13]. This paired with the inclination of consumers to purchase sustainable wine has increased

demand for organic wine production, pressuring growers to use low impact strategies to manage disease [14]. Such strategies include biological control in which organisms that inhibit pathogen growth are introduced into the crop system. A prime example is Trichoderma, a predatorial fungus capable of consuming GTD pathogens and has been studied extensively in the past decade [15–17]. Another approach is establishing groundcover systems or cover crops in the vineyard to suppress pathogens.

Traditionally, cover crops are plants that are grown during the main production season or during off seasons in order to maintain components of soil health which include erosion control, runoff, nutrient management, organic input and maintenance of soil macro and microorganisms [18]. Cover crops can help reduce pathogen pressure through a variety of mechanisms. Brassicaceous cover crops such as white mustard produce antifungal metabolites which can inhibit proliferation of fungi when introduced into the soil [19]. Cover crops also facilitate microbial diversity [20] which could lead to an increase in beneficial/antagonistic microbes such as plant growth promoting rhizobacteria (PGPR) [21] and Arbuscular mycorrhizal fungi [22] increased activity from antagonistic and beneficial microbes could help combat disease in vineyards.

Although cover crops confer many benefits to grapevines [23], they may be associated with increases in disease. Common cover crops like hairy vetch (Vicia villosa) may facilitate Ilyonectria pathogens if grown in vineyards [24]. Vukicevich et al. [25] found that grapevines grown in soil conditioned by native grasses and forbs were associated with increases in necrotic tissue compared to other groundcover treatments [25]. Likewise, Langenhoven et al. [26] isolated Dactylonectria spp. (black-Foot) and Pythium spp.(crown rot) pathogens from Triticale and ryegrass cover crops [26]. Furthermore, weeds from Spanish vineyards and nurseries tested positive for black-Foot as well as Petri disease pathogens [27]. These studies raise the concern that cover crops act as hosts or maintain an inoculum source in vineyards and nurseries.

Causal agents of YVD are often referred to as generalist, opportunistic [28] and/or weak pathogens [8]. Due to these strategies, YVD pathogens may benefit from root turnover and exudation [29] or even persist inside the living roots of cover crops. It is possible that YVD pathogens could have evolved alongside various plants to enter vascular tissue and survive as endophytes until tissue death, where they would be first in line to decompose the material [30]. This mechanism has not been investigated in a viticultural setting and the priority effects of YVD pathogens on the fungal community is not well studied [31].

If certain cover crops associate with or facilitate grapevine pathogens, they could be detrimental in the vineyard and this would greatly impact how we use cover crops to maintain soil health. In this study, we surveyed native plants as well as commercial cover crop species to determine if they associate with Ilyonectria liriodendri, a widely distributed grapevine trunk pathogen.

2. Materials and Methods

2.1. Plant Material and Soil

This experiment was designed to test the capacity of commonly used cover crops to host a common trunk pathogen. To achieve this goal, we grew only cover crop species in soil that was inoculated with the pathogen. We quantified the amount of inoculum added to each pot so that we could differentiate between positives in the soil that were due to inoculum alone (therefore no hosting capacity of the crop) versus inoculum that had been established in a host. Cover crops (Table 1) were grown in a greenhouse at the Summerland Research and Development Centre (SuRDC), British Columbia, Canada (49°33′57.8″ N 119°38′10.0″ W) from 25 October 2019 to 3 February 2020. The experiment was set up in a randomized complete block design with seven replicates, totaling 175 pots. This room was cooled by a fog system which kept temperatures below 28 °C during the summer months.

Table 1. List of vineyard cover crops that were inoculated with Ilyonectria liriodendri.

N.	Family	Binomial	Commom Name
1	Fabaceae	*Trifolium michelianum*	Balansa clover
2	Fabaceae	*Trifolium alexandrinum*	Berseem clover
3	Fabaceae	*Lotus corniculatus*	Bird's-foot trefoil
4	Polygonaceae	*Fagopyrum esculentum*	Buckwheat
5	Poaceae	*Bouteloua dactyloides*	Buffalo grass
6	Poaceae	*Poa compressa*	Canada bluegrass
7	Asteraceae	*Achillea millefolium*	Common Yarrow
8	Fabaceae	*Trifolium repens*	Crescendo ladino clover
9	Poaceae	*Agropyron cristatum*	Crested Wheatgrass
10	Fabaceae	*Trifolium incarnatum*	Crimson clover
11	Poaceae	*Secale cereale*	Fall rye
12	Fabaceae	*Vicia villosa*	Hairy vetch
13	Poaceae	*Lolium perenne*	Perennial Ryegrass
14	Fabaceae	*Trifolium resupinatum*	Persian clover
15	Boraginaceae	*Phacelia tanacetifolia*	Phacelia
16	Poaceae	*Thinopyrum intermedium*	Pubescent Wheatgrass
17	Poaceae	*Festuca rubra*	Red fescue
18	Poaceae	*Festuca ovina*	Sheep fescue
19	Fabaceae	*Lens culinaris*	Spring lentils
20	Poaceae	*Festuca arundinacea*	Tall fescue
21	Brassicaceae	*Raphanus sativus*	Tillage Radish
22	Fabaceae	*Trifolium repens*	White Clover
23	Brassicaceae	*Sinapis alba*	White Mustard
24	Brassicaceae	*Brassica rapa*	Winfred Brassica
25	Fabaceae	*Pisum sativum*	Winter peas

Soil was collected at SuRDC in September 2019 from field 7, a viticulture research block. This soil is described as a Skaha loamy sand ((Brown Chernozemic soil) (Wittneben 1986; Soil Classification Working Group 1998)), with the following physio-chemical characteristics (0–20 cm depth): conductivity: 33 uS/cm; pH: 6.79; sulphur P-Extr 0.89 ppm; aluminium: 318 ppm; boron: 0.2 ppm; calcium: 768 ppm; copper: 1.68 ppm; iron: 105 ppm; potassium: 119 ppm; magnesium: 89.4 ppm; manganese: 120 ppm; sodium: 3.4 ppm; phosphorus: 30.7 ppm; sulfur: 2.7 ppm; zinc: 1.1 ppm; clay: 5.74%; silt: 10.19% and sand: 84.07%. We chose this soil because it came from a viticulture system, making it the most suitable soil for this study. It had been selected for previous studies largely due being pathogen free, allowing us to use it in manipulative studies with our isolate of *Ilyonectria liriodendra*. Three-litre nursery pots were filled, leaving a four-centimetre gap from the top to retain water, and placed in the SuRDC greenhouse.

2.2. Pathogen Inoculation and Plant Growing Conditions

Three isolates of *Ilyonectria liriodendri* (SuRDC 340, 60, 393) were introduced to each nursery pot via a 10^6 conidia spore suspension, close to the roots. Each isolate was incubated at 22 °C for one week on 5% potato dextrose agar (PDA) solution. To ensure plates were ready, sporulation was observed with a compound light microscope. Agar plates were flooded with a 1% tween solution which helped free the spores during agitation with a metal utensil. The resulting solution was filtered in a cheese cloth to remove large chunks of agar and hyphae. A hemocytometer was used to make the stock solution and the final concentration was made using the following formula:

$$c1v1 = c2v2$$

where C1V1 = Concentration/amount (start) and Volume (start) C2V2 = Concentration/amount (final) and Volume (final). Then, each pot received 50 mL of inoculum 10 day after seeding.

Each nursery pot was standardised with approximately 10 plants per pot for the duration of the experiment. During the first week, pots were watered by hand with an equal amount of water. Fertilizer supplement was applied once a week during the growing period. Each pot received 50 mL of 20–20–20 fertilizer (Miracle-Gro, Marisville, OH, USA) at the recommended concentration. During harvest, as much soil as possible was washed away from roots with reverse osmosis water. Roots and shoots were put into plastic bags and stored at 4 °C for 24 h until they were dried and weighed.

2.3. Accessing Colonization by I. liriodendra

To determine the extent of colonisation by *I. liriodendri*, we extracted DNA from each cover crop root system and soil. Root samples were collected from each cover crop after growing in soil inoculated with *I. liriodendri* for 3 months. Soil samples were collected from the pot after roots were removed. To quantify the abundance of *I. liriodendri* in each root sample, we used a digital droplet (dd) PCR assay.

At the end of the growing period, approximately five grams of fresh root samples were collected, sub sampled, pooled, and stored at −20 °C until DNA extraction.

Roots were submerged in 10% bleach for 5 min then rinsed with reverse osmosis water three times for one minute. After surface sterilization, roots were crushed with a mortar and pestle in liquid nitrogen. 0.25 g were taken from each sample and loaded into a lysing tube. Roots were lysed at 6.5 m/s and centrifuged for 10 min to facilitate separation of root tissues and nucleic acids.

Root DNA was isolated with the FastDNA Spin Kit for Soil (MPBio ©2018, Irvine, CA, USA) by following manufacturer's instructions. DNA per sample was eluted in 100 µL and, DNA concentration as well as quality was assessed with a nanodrop device 1000c (Thermo Fisher Scientific, Wilmington, NC, USA). DNA was stored at −80 °C until digital PCR amplification.

We used a specific primer/probe assay to amplify the *Ilyonectria* isolates used in the inoculum. This assay targets the beta-tubulin region which is highly conserved region and single copy gene in fungi. The forward primer, 5′-CGAGGGACATACTTGTTTCCAGAG-3′ (Tm 61, GC 60%), reverse 5′-TCAACGAGGTACGCGAAATC-3′ -R (Tm 62, GC 50%), and probe TGTCAAACTCACACCACGTAGGCC (FAM) were designed and tested at the University of British Columbia laboratories [32].

For each 20 µL reaction, 10 µL Supermix (ddPCR Supermix for probes, Bio-Rad Inc., Hercules, CA, USA), 7 µL molecular grade water, 1 µL primers and probe (20× concentration), and 2 µL sample DNA, was used. Droplets were generated manually with the Bio-Rad QX100 Droplet Generator by adding 70 µL of Bio-Rad Droplet Generator Oil for Probes. PCR reactions were completed in the C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) as per following conditions: initial heating at 95 °C for 10 min; denaturation at 94 °C for one minute; annealing at 59 °C for two minutes. Denaturation and annealing steps were repeated for 44 cycles, followed by enzyme inactivation at 98 °C for 10 min.

We measured droplet fluorescence with the QX 100 Droplet Reader (Bio-Rad, Quantalife software (version 1.7.4) and used FAM-HEX as the fluorescent dye. The threshold was set automatically via the Quantasoft algorithm (Bio-Rad-USA). Data (copy number) for each sample was back calculated to represent the number of copies per gram of soil and root using a formula described in Kokkoris et al. [33].

2.4. Data Analyses

All statistical analyses were performed in R (version 3.6.2) via Rstudio (version 1.2.5033) (R Core Team 2019). Digital PCR data (copies per gram of soil and root) were fitted to a generalized linear mixed-effects model with block as a random factor using the *lme4* package (1.1-21). Soil and root data were analyzed separately using Type II analysis of variance in the *car package* (3.0-6). Tukey's honest significance test in the *emmeans* package (1.4.3.01) was used for post hoc comparisons. All plots were created in *ggplot2* (3.2.1).

3. Results

3.1. Abundance of Ilyonectria in Roots

After a brief growth period in the greenhouse, *Ilyonectria liriodendri* was isolated from the roots of various cover crops, in which copy number was significantly different among treatment groups ($p = 2.2 \times 10^{-16}$). Phacelia roots had the largest presence of *Ilyonectria* DNA, averaging 10,569 copies per gram of root followed by Buckwheat and common yarrow with 4817 and 1621 copies per gram, respectively, (Figure 1). The only cover crop that did not yield any pathogenic DNA was Crescendo ladino clover. This cover crop treatment was not significantly different from the others (see Appendix A for copy number summary statistics).

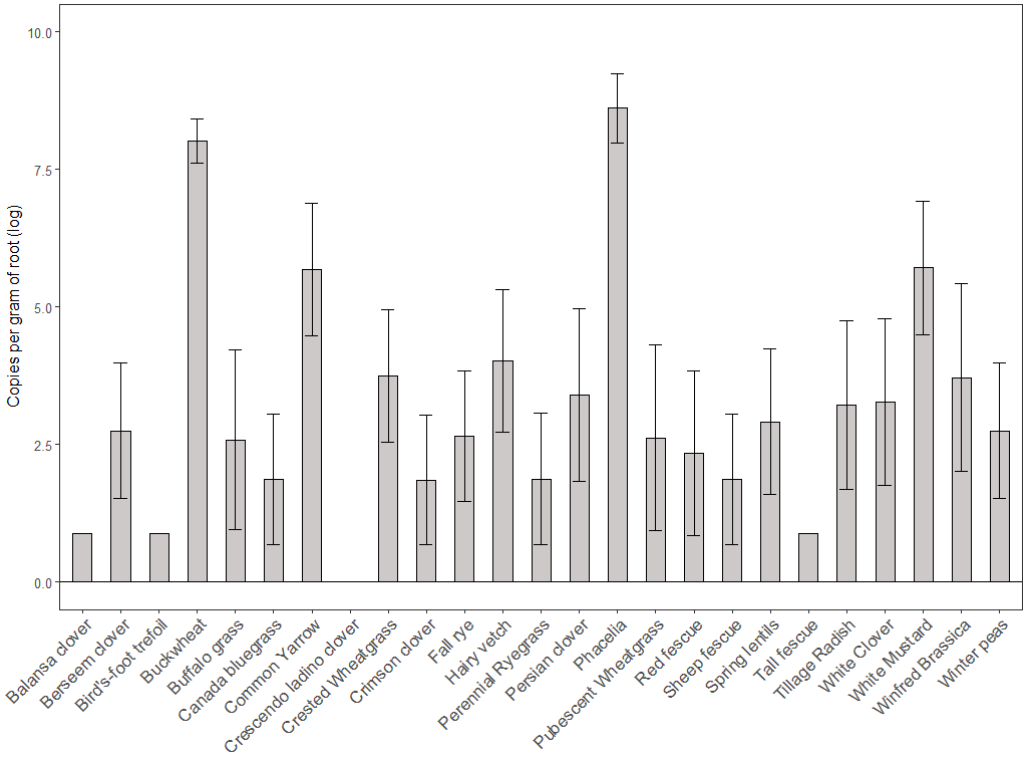

Figure 1. Log concentration of *Ilyonectria* DNA (copy number per gram of root) isolated from surface sterilized cover crop roots grown for three months. Log-transformed data are displayed. Dotted line represents the amount of inoculum added to each pot, for comparison.

3.2. Abundance of Ilyonectria in Soil

Similar patterns were observed in DNA isolated from soil samples. Overall analysis of variance resulted in a significant difference in *Ilyonectria* copy number between cover crop treatments ($p = 2.2 \times 10^{-16}$). As expected, soil conditioned by phacelia contained the highest amount of pathogenic DNA with 3384 copies per gram. Surprisingly, Persian clover soil yielded the second highest concentration at 1564 copies per gram of soil followed by Buckwheat with 1466 copies per gram (Figure 2). *Ilyonectria* DNA was recovered from all soil samples (Appendix A).

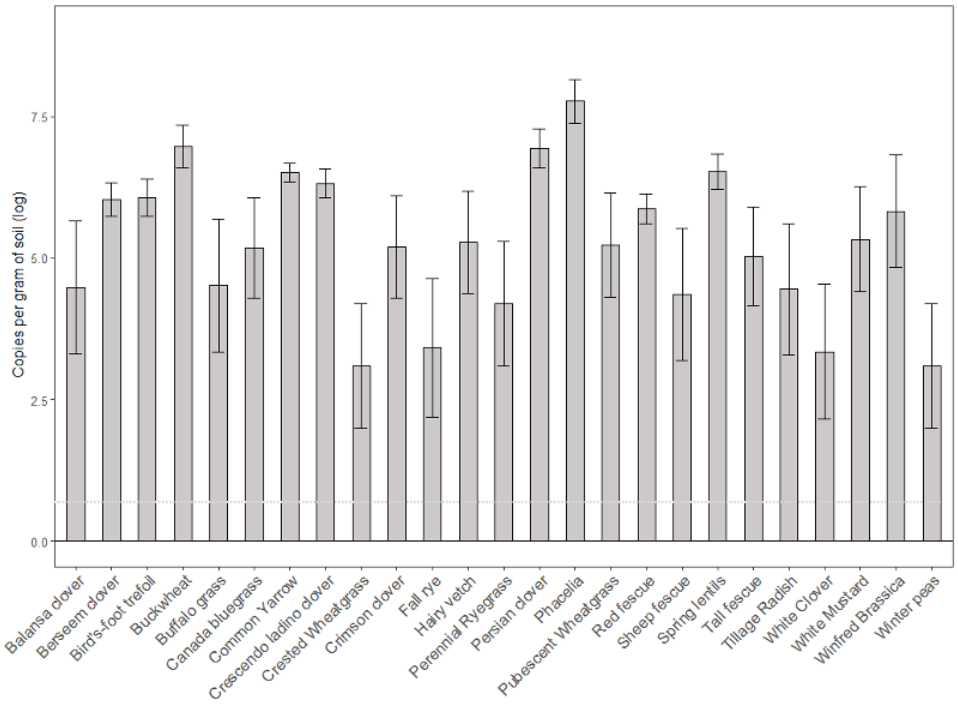

Figure 2. **Log** concentration of *Ilyonectria* DNA (copy number per gram of soil) isolated from soil conditioned by each cover crop after a three-month growth period. Data is shown in the log transformation. Dotted line represents the amount of inoculum added to each pot, for comparison.

4. Discussion

This study shows that cover crops used in perennial agriculture can act as alternate hosts for a common grapevine pathogen. In our study, some cover crop speciessignificantly increased the abundance of *Ilyonectria* spp. in both roots and soil.

In itself this is not surprising; *Ilyonectria liriodendri* has a cosmopolitan distribution, having been isolated from soils in the Americas, Europe, and Oceania [34,35]. Moreover, this pathogen is present in multiple perennial cropping systems including apple [5], cherry [36], tea [37], and avocado [38] which highlights its generalist nature as a pathogen. Unlike previous studies, our work shows that the pathogen can infect non-crop species, across a wide taxonomic distribution. Given that the plants in our study are commonly used as cover crops in areas where *Ilyonectria* spp. are a significant pathogen, growers should consider the ability of cover crops to act as a reservoir for pathogens when selecting candidate species.

Two plants in particular have the potential to greatly amplify the abundance nof *Ilyonectria* spp. in soil. Phacelia is a genus native to the Americas belonging to *Boraginaceae*, which are classified as asterids [39]. *Phacelia tanacetifolia* is grown extensively arable crop rotations to condition soil structure, especially in sandy loam soils [40–42]. This is the first study to our knowledge that shows the ability of *P. tanacetifolia* to associate with *Ilyonectria* spp. Previous studies show that phacelia has the capacity to host other fungal pathogens (*Sclerotinia minor* [43], *Rhizoctonia solani* [44]). *Ilyonectria robusta* has been isolated from the roots of *Taraxacum officinale* which is also a perennial asterid [45].

Buckwheat *Fagopyrum esculentum*) also augmented the concentration of *Ilyonectria* spp. far greater than background levels. It is commonly used in cover crops due to its rapid establishment, weed suppression, pollinator species, and ability to extract phosphorus [18]. Unlike phacelia, buckwheat is a native to Southeast Asia [46] which makes plant provenance an unlikely explanation for why these two cover crops are the most likely to act as reservoir hosts for *Ilyonectria* spp. However, buckwheat does meet the criteria outlined in Cronin et al. [47] in which ideal reservoir hosts grow rapidly, have a short lifespan, and have high phosphorus concentrations in their tissues. Previous studies show that buckwheat is prone to damping off and root rot by *fusarium* spp. [48] and *Rhizoctonia* spp. [49].

More recently, Zini et al. isolated *Fusarium incarnatum-equiset* from germinated buckwheat seeds [50]. Considering these findings, it is not surprising that *Ilyonectria spp* were isolated from buckwheat roots and that this species could act as a reservoir host for grapevine pathogens.

Many other crop species in our study increased pathogen incidence in roots, but to a lesser degree. This was particularly true for many brassica species (Tillage radish, White Clover, White Mustard, Winfred Brassica and Persian Clover). The levels of *Illyonectria spp* in the roots of these crops are surprising since brassicas are well known for their fungicidal properties [51] and are used by growers specifically to reduce fungal pathogens in the soil [52]. Of these, only Persian Clover had elevated soil concentrations of *Ilyonectria*. Thus, cover crops may host pathogens asymptomatically in the growing season, but unless the plants are mulched into the soil, they may have limited biofumigant properties.

Most of our cover crops showed little to no ability to associate with *Ilyonectria* spp. We could not detect any *Ilyonectria spp* in Crescendo Ladino Clover roots, while Balansa clover, Birdsfoot Trefoil and Tall Fescue had levels that were not different from zero. In areas where *Illyonectria* spp. is a problem, these taxa may be good candidates to prevent outbreaks.

It is important to note that the behaviour of *IIlyonectria* spp. in our study may have been affected by resident soil microbes, as microbial communities can influence eachtother through a variety of different mechanisms including competition, facilitation. Thus, our results reflect a specific set of conditions and microbial community. To fully understand the risk of these cover crop species to act as pathogen reservoirs, future analyses must be conducted in under different soil and growing conditions. This study provides an excellent basis on which to develop future work.

5. Conclusions

While the benefits of cover crops are many, including improved soil nutrients, water relations and soil stability, they may not be universally beneficial. Here, we showed that commonly used cover crops may have the ability to increase the abundance of grapevine pathogens by acting as an alternate host. In areas where soil borne disease is a problem, the choice of cover crop may make the difference between pathogen suppression and outbreak. In this survey phacelia and buckwheat were found to act as reservoir hosts for *Ilyonectria liriodendri*. For a grower dealing with YVD, using phacelia and buckwheat in a cropping mixture may increase the abundance of the pathogen, leading to disease outbreak under the right conditions.

Author Contributions: Conceptualization D.R..; methodology, D.R., M.S. and M.M.H.; validation, M.M.H. and M.S.; formal analysis, D.R. investigation, D.R.; data curation, D.R.; writing—original draft preparation, M.M.H.; writing—review and editing, M.M.H.; supervision, M.S.; project administration, M.M.H. and M.S.; funding acquisition, M.M.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by AAFC Going Forward Wine Cluster (MMH) and partially by Organic Science Cluster 3 (M.S.), which was supported by the AgriScience Program under Agriculture and Agri-Food Canada's Canadian Agricultural Partnership investment.

Acknowledgments: Summerland Research and Development greenhouse facility we used in this study. We would like to acknowledge the technical support by Bill Rabie, Lana Fukumoto and greenhouse staff.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

sample.ID	block	cover.crop	root.positives	soil.positives	root.per.gram	soil.per.gram
1	1	Balansa clover	0	0	0	0
26	2	Balansa clover	0	2	0	370.37037
51	3	Balansa clover	0	1	0	172.413793
76	4	Balansa clover	0	6	0	1034.48276
101	5	Balansa clover	1	0	200	0
126	6	Balansa clover	0	6	0	1034.48276
151	7	Balansa clover	3	600		
2	1	Berseem clover	0	4	0	714.285714
27	2	Berseem clover	0	1	0	172.413793
52	3	Berseem clover	2	1	400	178.571429
77	4	Berseem clover	1	6	192	1153.84615
102	5	Berseem clover	0	3	0	517.241379
127	6	Berseem clover	1	1	192	217.391304
152	7	Berseem clover	4	740.740741		
3	1	Bird's-foot trefoil	1	1	200	178.571429
28	2	Bird's-foot trefoil	0	1	0	192.307692
53	3	Bird's-foot trefoil	0	11	0	1964.28571
78	4	Bird's-foot trefoil	0	3	0	576.923077
103	5	Bird's-foot trefoil	0	3	0	576.923077
128	6	Bird's-foot trefoil	0	1	0	192.307692
153	7	Bird's-foot trefoil	3	625		
4	1	Buckwheat	29	8	6042	1428.57143
29	2	Buckwheat	82	14	15769	2800
54	3	Buckwheat	10	9	1923	1875
79	4	Buckwheat	13	4	2500	689.655172
104	5	Buckwheat	9	1	1667	178.571429
129	6	Buckwheat	5	3	1000	600
154	7	Buckwheat	14	2692.30769		
5	1	Buffalo grass	1	6	1667	1111.11111
30	2	Buffalo grass	0	2	0	416.666667
55	3	Buffalo grass	2	0	3333	0
80	4	Buffalo grass	0	3	0	652.173913
105	5	Buffalo grass	0	2	0	434.782609
130	6	Buffalo grass	0	0	0	0
155	7	Buffalo grass	2	400		
6	1	Canada bluegrass	0	2	0	416.666667
31	2	Canada bluegrass	0	1	0	227.272727
56	3	Canada bluegrass	0	0	0	0
81	4	Canada bluegrass	0	3	0	625
106	5	Canada bluegrass	2	4	400	714.285714
131	6	Canada bluegrass	1	1	185	185.185185
156	7	Canada bluegrass	4	714.285714		
7	1	Common yarrow	0	4	0	714.285714
32	2	Common yarrow	2	6	400	1153.84615
57	3	Common yarrow	23	2	4600	370.37037
82	4	Common yarrow	5	2	1000	416.666667
107	5	Common yarrow	5	3	1000	625
132	6	Common yarrow	2	5	345	1086.95652
157	7	Common yarrow	4	689.655172		
8	1	Crescendo ladino	0	1	0	178.571429
33	2	Crescendo ladino	0	2	0	384.615385
58	3	Crescendo ladino	0	3	0	576.923077
83	4	Crescendo ladino	0	3	0	500
108	5	Crescendo ladino	0	8	0	1379.31035

sample.ID	block	cover.crop	root.positives	soil.positives	root.per.gram	soil.per.gram
133	6	Crescendo ladino	0	5	0	1041.66667
158	7	Crescendo ladino	3	555.555556		
9	1	Crested wheatgrass	1	1	208	200
34	2	Crested wheatgrass	1	1	714	172.413793
59	3	Crested wheatgrass	0	0	0	0
84	4	Crested wheatgrass	0	1	0	200
109	5	Crested wheatgrass	1	2	200	370.37037
134	6	Crested wheatgrass	1	0	200	0
159	7	Crested wheatgrass	0	0		
10	1	Crimson clover	0	0	0	0
35	2	Crimson clover	0	3	0	600
60	3	Crimson clover	0	1	0	200
85	4	Crimson clover	2	7	400	1400
110	5	Crimson clover	0	3	0	517.241379
135	6	Crimson clover	1	2	172	384.615385
160	7	Crimson clover	1	185.185185		
11	1	Fall rye	1	0	192	0
36	2	Fall rye	0	6	0	1071.42857
61	3	Fall rye	1	1	200	166.666667
86	4	Fall rye	0	0	0	0
111	5	Fall rye	1	2	208	370.37037
136	6	Fall rye	0	2	0	370.37037
161	7	Fall rye	0	0		
12	1	Hairy vetch	2	4	357	689.655172
37	2	Hairy vetch	0	2	0	312.5
62	3	Hairy vetch	0	3	0	576.923077
87	4	Hairy vetch	2	1	400	217.391304
112	5	Hairy vetch	6	0	1034	0
137	6	Hairy vetch	1	2	200	416.666667
162	7	Hairy vetch	5	961.538462		
13	1	Perennial ryegrass	1	1	185	192.307692
38	2	Perennial ryegrass	0	0	0	0
63	3	Perennial ryegrass	2	4	417	740.740741
88	4	Perennial ryegrass	0	0	0	0
113	5	Perennial ryegrass	0	2	0	434.782609
138	6	Perennial ryegrass	0	2	0	416.666667
163	7	Perennial ryegrass	1	217.391304		
14	1	Persian clover	0	6	0	1200
39	2	Persian clover	0	2	0	384.615385
64	3	Persian clover	5	5	962	862.068966
89	4	Persian clover	1	10	192	1923.07692
114	5	Persian clover	0	33	0	5500
139	6	Persian clover	20	3	3846	576.923077
164	7	Persian clover	3	500		
15	1	Phacelia	18	2	3750	370.37037
40	2	Phacelia	99	8	19038	1428.57143
65	3	Phacelia	2	14	385	2500
90	4	Phacelia	69	10	13269	2083.33333
115	5	Phacelia	15	26	3125	5200
140	6	Phacelia	124	18	23846	3103.44828
165	7	Phacelia	45	9000		
16	1	Pubescent wheatgrass	0	0	0	0
41	2	Pubescent wheatgrass	0	1	0	185.185185
66	3	Pubescent wheatgrass	5	8800	833.333333	

sample.ID	block	cover.crop	root.positives	soil.positives	root.per.gram	soil.per.gram
91	4	Pubescent wheatgrass	0	1	0	200
116	5	Pubescent wheatgrass	0	2	0	333.333333
141	6	Pubescent wheatgrass	4	3	769	517.241379
166	7	Pubescent wheatgrass	9	1500		
17	1	Red fescue	0	1	0	208.333333
42	2	Red fescue	3	3	600	555.555556
67	3	Red fescue	0	4	0	869.565217
92	4	Red fescue	0	1	0	156.25
117	5	Red fescue	0	2	0	370.37037
142	6	Red fescue	11	1	2115	178.571429
167	7	Red fescue	4	666.666667		
18	1	Sheep fescue	0	3	0	600
43	2	Sheep fescue	1	1	185	185.185185
68	3	Sheep fescue	0	0	0	0
93	4	Sheep fescue	0	0	0	0
118	5	Sheep fescue	0	2	0	400
143	6	Sheep fescue	2	1	400	185.185185
168	7	Sheep fescue	11	2115.38462		
19	1	Spring lentils	0	5	0	925.925926
44	2	Spring lentils	1	2	192	416.666667
69	3	Spring lentils	0	2	0	416.666667
94	4	Spring lentils	1	12	200	2222.22222
119	5	Spring lentils	0	3	0	652.173913
144	6	Spring lentils	5	7	1000	1521.73913
169	7	Spring lentils	1	192.307692		
20	1	Tall fescue	0	4	0	869.565217
45	2	Tall fescue	0	1	0	200
70	3	Tall fescue	0	0	0	0
95	4	Tall fescue	0	1	0	208.333333
120	5	Tall fescue	0	2	0	416.666667
145	6	Tall fescue	1	3	192	750
170	7	Tall fescue	1	172.413793		
21	1	Tillage radish	0	0	0	0
46	2	Tillage radish	1	0	192	0
71	3	Tillage radish	1	6	200	1034.48276
96	4	Tillage radish	0	3	0	500
121	5	Tillage radish	0	2	0	333.333333
146	6	Tillage radish	31	3	6200	535.714286
171	7	Tillage radish	2	357.142857		
22	1	White clover	0	4	0	833.333333
47	2	White clover	3	0	600	0
72	3	White clover	0	2	0	370.37037
97	4	White clover	0	1	0	208.333333
122	5	White clover	1	1	179	227.272727
147	6	White clover	17	0	3269	0
172	7	White clover	0	0		
23	1	White mustard	0	2	0	400
48	2	White mustard	4	1	741	217.391304
73	3	White mustard	7	10	1346	2000
98	4	White mustard	1	0	192	0
123	5	White mustard	5	2	1000	434.782609
148	6	White mustard	20	3	4000	535.714286
173	7	White mustard	2	400		

sample.ID	block	cover.crop	root.positives	soil.positives	root.per.gram	soil.per.gram
24	1	Winfred brassica	0	2	0	384.615385
49	2	Winfred brassica	8	8	1667	1333.33333
74	3	Winfred brassica	0	3	0	652.173913
99	4	Winfred brassica	2	4	370	714.285714
124	5	Winfred brassica	0	11	0	2115.38462
149	6	Winfred brassica	40	6	7692	1000
174	7	Winfred brassica	0	0		
25	1	Winter peas	2	1	400	200
50	2	Winter peas	1	0	192	0
75	3	Winter peas	0	0	0	0
100	4	Winter peas	0	0	0	0
125	5	Winter peas	1	1	185	227.272727
150	6	Winter peas	0	1	0	166.666667
175	7	Winter peas	2	322.580645		
176	1	Exp control background inoculant level			55556	6.70804154
176	2	Exp control background inoculant level			55556	6.70804154
176	3	Exp control background inoculant level			55556	6.70804154
176	4	Exp control background inoculant level			55556	6.70804154
176	5	Exp control background inoculant level			55556	6.70804154
176	6	Exp control background inoculant level			55556	6.70804154
176	7	Exp control background inoculant level			55556	6.70804154

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