soil systems

MDPI

Article

Appearance and Persistence of Activity in Soil Extracts Increasing Root Rot of American Ginseng (*Panax quinquefolius*) by *Ilyonectria mors-panacis*

Paul H. Goodwin [1,*] and Geovanna Zaro [2]

[1] School of Environmental Sciences, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada
[2] Department of Plant Agriculture, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada; gzaro@uoguelph.ca
* Correspondence: pgoodwin@uoguelph.ca

Abstract: A previous report showed that methanol extracts from soil collected one year after harvesting American ginseng (*Panax quinquefolius*) contained activity that increased root rot caused by *Ilyonectria mors-panacis*. This effect was associated with suppression of the defense responses of *P. quinquefolius*. The activity was undetectable in soil not previously planted with ginseng, and it was hypothesized that it may be a factor in the development of ginseng replant disease (GRD). GRD can persist for 30 or more years and is associated with root rot from *I. mors-panacis*. A survey of activity that increases root rot was made of the soil at different times before and after commercial ginseng root harvesting. No activity that increased root rot from *I. mors-panacis* was detected in the soil of a first American ginseng crop over the three years from planting until prior to harvesting. After harvesting the first crop, no activity was detected during the fall or early spring, but *I. mors-panacis*'s ability to increase root rot was detected in the soil during late spring, when ginseng crop debris from the first crop had almost completely decayed and the soil had warmed. Activity increasing root rot from *I. mors-panacis* was also detected in the soil from 1 to 30 years after ginseng harvesting. These results indicate that activity in soil that increases root rot from *I. mors-panacis* is not detectable until after the crop has been first harvested and then can persist for many years, which is consistent with the long persistence of GRD.

Keywords: ginseng replant disease; root rot; soil sickness

Citation: Goodwin, P.H.; Zaro, G. Appearance and Persistence of Activity in Soil Extracts Increasing Root Rot of American Ginseng (*Panax quinquefolius*) by *Ilyonectria mors-panacis*. *Soil Syst.* **2024**, *8*, 133. https://doi.org/10.3390/soilsystems8040133

Academic Editor: Claudio Cocozza

Received: 26 September 2024
Revised: 10 December 2024
Accepted: 16 December 2024
Published: 18 December 2024

1. Introduction

American ginseng (*Panax quinquefolius*) is an important medicinal herb commercially grown in numerous locations in North America that contains ginsenosides, which can help fight diseases such as dementia, diabetes and cancer [1]. One issue in commercial production is ginseng replant disease (GRD), which results in large numbers of ginseng plants dying when planted in soil previously used for ginseng production even decades earlier [2]. GRD is associated with root rot due to *Ilyonectria mors-panacis*, but even though the fungus causes root rot in the first crop of ginseng, the same pathogen is much more severe when a second crop is grown on the same soil. One suggestion as to why the fungus is far more damaging when the ginseng is replanted is that GRD is caused by the combination of root rot due to *I. mors-panacis* with an unknown host-related factor that must develop in the soil before or relatively shortly after harvesting a first ginseng crop [2].

Extracts from soil where *P. quinquefolius* had been planted one year previously contained compounds that significantly increased root lesion size following inoculation with *I. mors-panacis* as compared to water, which occurred in a dose-dependent manner [3]. In contrast, the application of extracts of soil not planted with ginseng or roots of ginseng did not affect lesion size at any concentration tested. The expression of jasmonate-regulated genes were suppressed after *I. mors-panacis* infection in roots treated with soil extracts

from previously planted ginseng gardens, as compared to roots treated with water or ginseng root extract. This indicates that compounds were present in soil used previously for ginseng production that could suppress elements of the defense response of the roots. Jasmonate is a key plant defense hormone that regulates the triggered expression of defense genes against necrotrophic root rot pathogens [4], and the expression of many deference genes are regulated by jasmonate in ginseng [5]. However, the compounds present in the soil of previously planted ginseng gardens were not found in ginseng root extracts. The origin of these compounds in soil could be related to microbial conversion of ginseng root compounds. Goodwin and Hsiang [6] showed that a bacterium isolated from soil previously used for *P. quinquefolius* production could convert ginseng root compounds in culture into soluble compounds able to suppress the resistance of ginseng roots to rot from *I. mors-panacis*.

While the composition of the compounds that affect gene expression in *P. quinquefolius* roots is unknown, their activity can be detected by showing a significant increase in the root lesion sizes produced by *I. mors-panacis* relative to a control, such as roots treated with water, extracts from soil never planted with ginseng or cell-free supernatants of bacteria in culture media without ginseng root extract [3,5]. Because GRD does not occur in the first ginseng crop even though commercial ginseng typically involves 3–4 years of growth [2], one could hypothesize that any soil compounds associated with GRD should not be found in a first ginseng crop. However, ginseng root compounds such as ginsenosides, phenolics and organic acids continually enter the soil during growth and affect the microbial populations around the roots [7–10]. Thus, it is possible that if such compounds are associated with GRD, they would accumulate even in the soil of a first ginseng crop. GRD can persist for many decades, and therefore one criterion for any soil compound(s) to be associated with GRD is its ability to persist for a long time in soil. While the presence of compounds in soil that can increase the susceptibility of *P. quinquefolius* roots to *I. mors-panacis* has been found, only a soil from one year after harvesting a first crop of ginseng was examined. The goal of this research is to utilize the bioassay for the soil compounds that suppress ginseng root rot resistance to *I. mors-panacis* to assess the appearance and duration of its activity in the soil. Specifically, extracts were examined at multiple time points from soil during a first ginseng crop before harvesting, soil following commercial harvesting of a first crop, and soil previously used for ginseng production many years ago without a subsequent ginseng crop.

2. Materials and Methods

2.1. Soil Collection

Soil samples from a first crop of *P. quinquefolius* were collected from around plants in beds of commercial ginseng gardens from Simcoe, ON, Canada (42.84° N, 80.30° W). Each sample of approximately 500 g of soil was collected from a depth up to 30 cm and was thoroughly mixed and sieved (3 × 3 mm grid) to remove any roots and debris. The soil associated with harvesting American ginseng was collected from a 3-year-old commercial ginseng garden in the same area. The number of plants per five 1 m^2 plots within a bed was counted just prior to harvesting. Harvesting was accomplished on 2 October 2018 by mechanically digging 30 cm into each bed, followed by the hand harvesting of the roots. Soil samples were collected at 5 days before harvest (early autumn), 10 dph (days post-harvest) (early autumn), 41 dph (mid-autumn), 73 dph (late autumn), 198 dph (early spring) and 231 dph (late spring). A set of five different 1 m × 1 m plots were used for each time point. Soil samples from fields where *P. quinquefolius* had been known to grow in previous years were collected in midsummer from fields now planted with non-ginseng crops (typically maize or soybean at the time of sampling) and treated as described above. Control soil samples were collected from areas near the sampling sites where *P. quinquefolius* was either not growing or had never been grown previously. All soil samples were stored at −20 °C.

2.2. Soil Extraction

Soil extraction was performed on 200 g of air-dried soil per sample as per Behdarvandi and Goodwin [3]. The soil samples were mixed with 600 mL of 80% methanol (MeOH) and shaken overnight at room temperature (175 rpm) on a gyratory shaker (New Brunswick Co., Edison, NJ, USA). After vacuum filtering through No. 4 qualitative filter paper (Whatman, Maidstone, UK), the methanol was removed by evaporation under vacuum at 40 °C. For the root lesion assay, a 0.1 mg/mL solution was made in distilled sterile (ds) H_2O, centrifuged (Beckman Coulter, High Wycombe, UK) at 10,000 rpm for 5 min, and filtered using a 0.22 µm membrane (Whatman, Maidstone, UK).

2.3. Root Lesion Assay

Three-year-old *P. quinquefolius* roots were obtained from commercial ginseng gardens that used soil not previously used for ginseng production near Simcoe, Ontario. They were rinsed with tap water and stored at 4 °C. Prior to inoculation, the roots were surface-sterilized with 75% ethanol for 10 min and then with 5% bleach (5.25% NlClO) for 5 min. After thorough washing with dsH_2O, holes were created on the roots approx. 1.5 mm wide and 9 mm deep using a sterilized needle. Extracts (15 µL) were added to each hole. After 2 h incubation at room temperature, a 15 µL suspension of 1×10^6 conidia/mL in sdH_2O was placed in each hole, and the roots were incubated in sterile Petri dishes at 22 ± 2 °C. Conidia were harvested from the *I. mors-panacis* isolate IMP.ND4Z15 [11], which was grown on PDA for 4 weeks in the dark. Lesion areas were determined at 12 days post-inoculation by tracing the lesion area on acetate sheets, and the areas were quantified using ImageJ software version 1.22 (https://imagej.net) (accessed between 9 February 2022 and 27 June 2022). There were three replications, with six lesions measured per sample. Data were compared by the analysis of variance (ANOVA) using Minitab, version 16. The means comparison was performed using Fisher's LSD test, with a level of significance at $p = 0.05$. The control roots, which underwent surface sterilization and wounding without pathogen inoculation, did not develop lesions.

3. Results

Lesion sizes caused by *I. mors-panacis* were not significantly different when the roots were treated with control soil extracts (i.e., nearby soil never planted with *P. quinquefolius*) as compared to extracts of soil taken near *P. quinquefolius* roots during the 3 years of growth in a garden never used for ginseng production (Figure 1). This indicates that any compounds in the soil that could decrease the resistance of the roots to infection by *I. mors-panacis* were not detectable in a first crop of *P. quinquefolius*.

Lesion sizes caused by *I. mors-panacis* were not significantly different when the roots were treated with control soil extracts or treated with extracts from soil taken near the roots five days prior to harvest (Figure 2). These were soils of 3-year-old roots, and so, while they are different samples, they are comparable to the soil samples of 3-year-old roots in a first crop of *P. quinquefolius* (Figure 1). The treatment of roots, with extracts from the plots of soil collected at early autumn at 10 dph (mid-October), mid-autumn at 41 dph (mid-November) and early winter at 73 dph (mid-December), did not affect the lesion sizes caused by *I. mors-panacis*. Thus, there was no evidence of detectable levels of compounds in the soil prior to the freezing of the soil that could increase lesion sizes due to *I. mors-panacis* infection. Shortly after the mid-December sampling, the soil froze, and the next sampling was obtained after the soil had thawed in early spring at 198 dph (mid-April). Similarly, there was no evidence that the soil extracts contained compounds able to increase lesion sizes by *I. mors-panacis*. The final soil sampling was in late spring at 231 dph (mid-May), which was just prior to planting of the next crop, maize, in the field where *P. quinquefolius* had been harvested the preceding autumn. In this case, however, the treatment of the roots with extracts from the 231-dph soil resulted in significantly larger *I. mors-panacis* lesions as compared to the treatment of the roots with the control or with the 10 to 198 dph extracts.

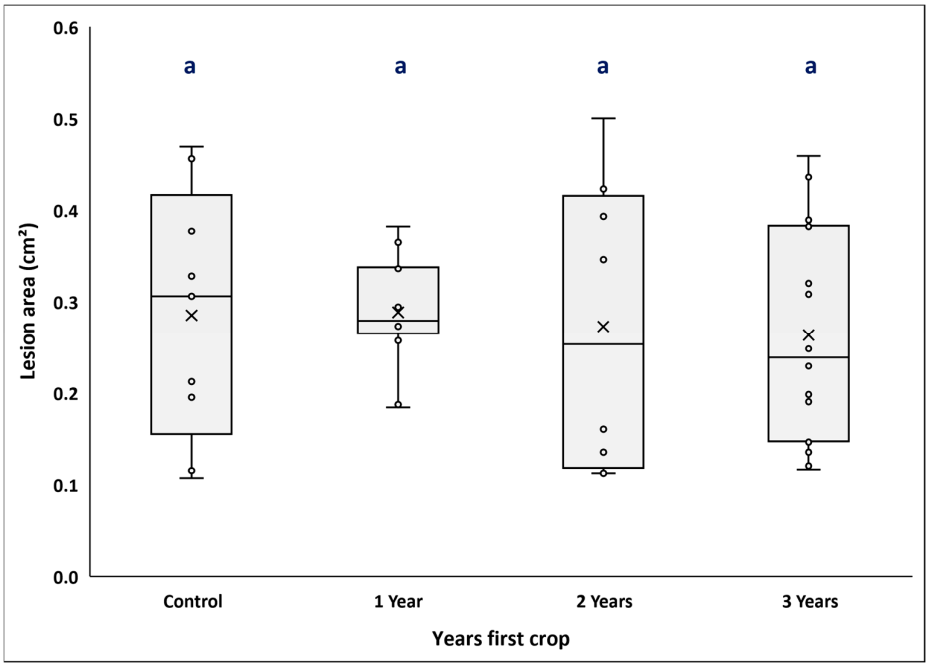

Figure 1. Effect of the extracts from the soil of the first crop of *P. quinquefolius* on the lesion area (cm^2) of detached roots at 12 days post-inoculation with *I. mors-panacis*. X indicates means, and the dots outside the box plot and whiskers indicate outliers. Means comparisons (Fisher's LSD test at $p = 0.05$) are shown above the box plot. The means for the control (no *P. quinquefolius* crop), 1-year, 2-year and 3-year first crops are from 9, 13, 8 and 16 samples, respectively.

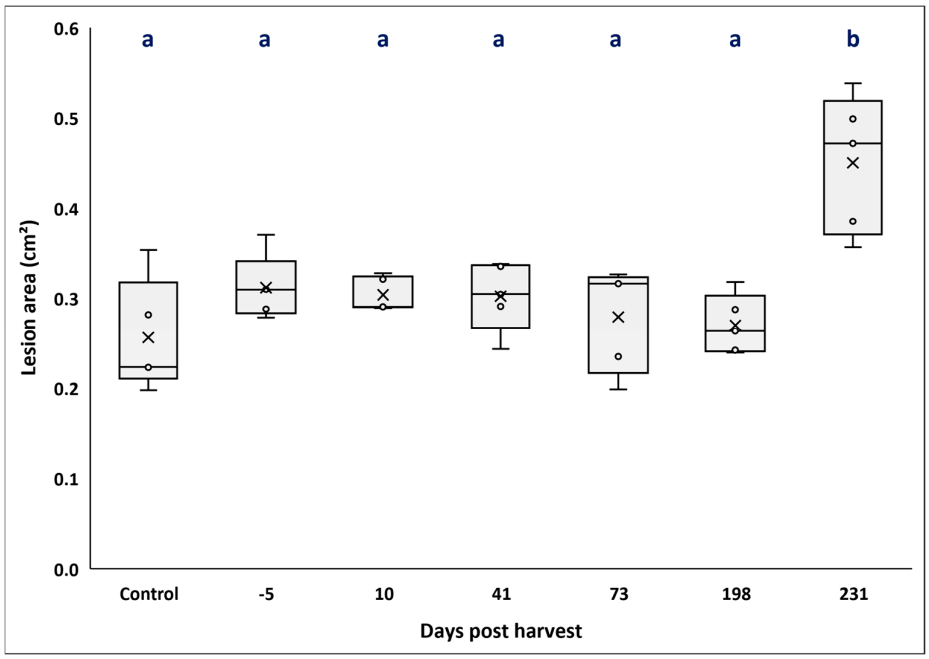

Figure 2. Effect of the extracts from soil just prior (−5 dph) and from 10 to 231 dph following the harvesting of *P. quinquefolius* on the lesion area (cm^2) of the detached roots at 12 days post-inoculation with *I. mors-panacis*. X indicates means, and the dots outside the box plot and whiskers indicate outliers. Means comparisons (Fisher's LSD test at $p = 0.05$) are shown above the box plot. The means for the control (no *P. quinquefolius* crop), −5, 10, 41, 73, 198 and 231 dph are from five samples each.

A comparison of the average fresh weight of roots per plot at 5 days prior to harvest (−5 dph) and 10 dph showed that approx. 91% of the root biomass was removed by com-

mercial ginseng harvesting (Figure 3). The non-harvested roots were generally small and thus of lower commercial value. Root decay, as indicated by a decline in the fresh weight of the roots sifted from the soil over time, progressed over the fall and early winter with approx. 40% of the fresh weight of the roots (approx. 47 g per plot) decaying between 10 and 73 dph. After the soil thawed in the spring at 198 dph, an additional 62 g fresh root weight per plot had decayed as compared to before the soil's freezing at around 73 dph. Therefore, only approx. 6% of fresh root weight was found in the soil as compared to shortly after harvest (10 dph). Between 198 and 231 dph, approx. 6 g of fresh root weight per plot had further decayed, and by 231 dph, there was just 0.7% of fresh root weight in the soil as compared to 10 dph. Thus, there was no direct correlation between the effect of the soil extract on the *I. mors-panacis* lesion size and the amount of decay of the roots remaining in the soil after harvest. The first detection of such activity was only after almost all of the non-harvested roots had decayed in the soil at 231 dph, which occurred when there were warmer soil temperatures (approx. 10 °C) in the late spring that were sufficient for the seeding of a subsequent crop.

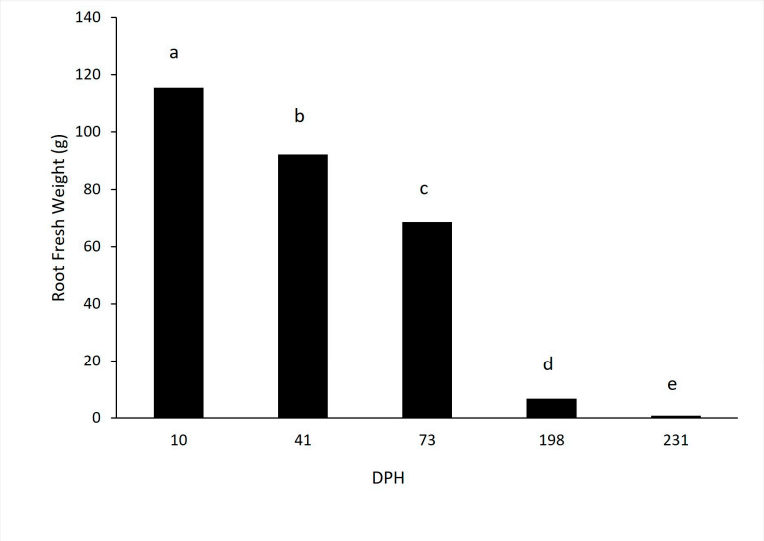

Figure 3. Decay of the non-harvested *P. quinquefolius* roots. Average fresh weight (g) of roots in 1 m^2 × 0.3 m-deep plots from 10 to 231 days post-harvest (dph). Means comparisons (Fisher's LSD test at $p = 0.05$) are shown from five replicate plots per time point. Means with letters in common are not significantly different.

As compared to the treatment of the roots with extracts from the control soil, the *I. mors-panacis* lesion size was significantly higher for the roots treated with extracts from one-year-old soil samples (i.e., soil collected in midsummer the year following the harvesting of *P. quinquefolius*) (Figure 4). This was also true for the effects of the extracts from soils in fields where *P. quinquefolius* had been harvested 3, 10, 20 and even 30 years previously. Only the average lesion size due to *I. mors-panacis* was not significantly different from that of the control where *P. quinquefolius* had been grown 5 years previously, but it was also not significantly different from the lesion sizes of the roots treated with soil extracts at 1, 3, 10, 20 and 30 years after harvesting. This indicates that the compounds first detected during the following spring after harvesting *P. quinquefolius* (Figure 2) were detectable in the soil for many years (up to 30 years) afterward, even though *P. quinquefolius* was never planted again in any of these soils. In addition, the level of activity, as indicated by the relative lesion size due to *I. mors-panacis*, did not decline over the years after harvesting but was comparable between treatment with the extracts of soil at 1 and 30 years following harvesting.

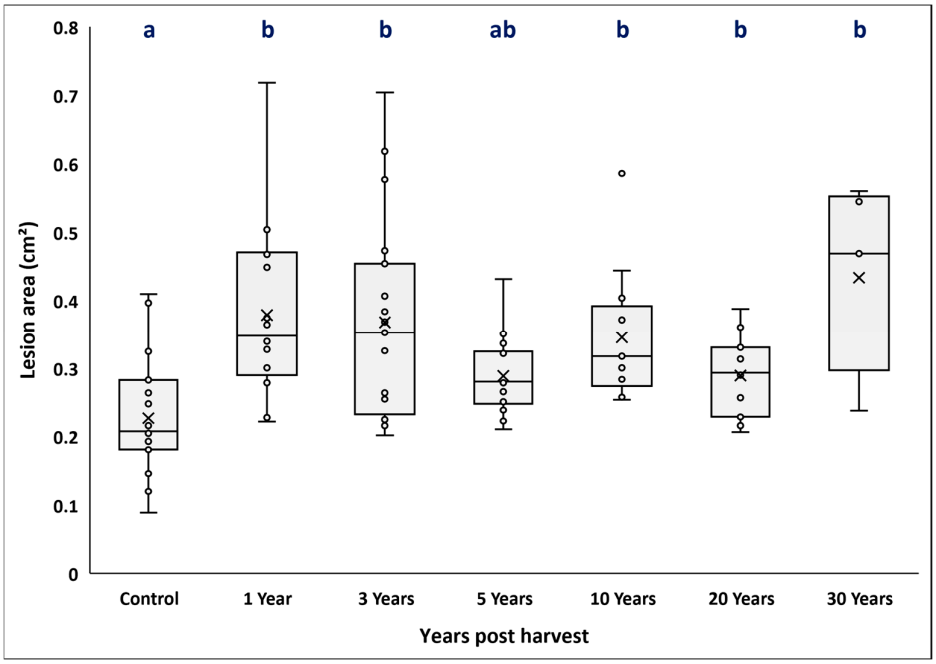

Figure 4. Effect of the extracts from the soil from 1 to 30 years following the harvesting of *P. quinquefolius* on the lesion areas (cm^2) of detached roots at 12 days post-inoculation with *I. mors-panacis*. X indicates means, and the dots outside the box plot and whiskers indicate outliers. Means comparisons (Fisher's LSD test at $p = 0.05$) are shown above the box plot. The means for the control (no *P. quinquefolius* crop), 1 year, 3 years, 5 years, 10 years, 20 years and 30 years post-harvesting are from 19, 17, 23, 16, 13, 11 and 5 samples, respectively. Means with letters in common are not significantly different.

4. Discussion

While there have been a number of hypotheses about the causes of GRD, its ultimate cause is unknown [2]. One proposed mechanism for GRD, which can also be considered a form of soil sickness [12], is that it is due to the synergistic effects of soil-borne pathogens with allelochemicals that have been found in aqueous extracts of ginseng roots, exudates and rhizosphere soil [9]. Researchers have identified many allelochemicals from ginseng, including 11 types of phenolic acids and seven types of ginsenosides, as well as several types of organic acids and organic esters, with phenolic acids and ginsenosides being the most studied [13]. An example of a phenolic allelochemical of ginseng is p-coumaric acid, which inhibits radical growth, photosynthesis and phenylalanine ammonia lyase (PAL) activity [14]. Another example is benzoic acid, which alters the expression of over 6000 ginseng genes, including the upregulation of those that could increase levels of reactive oxygen species (ROS) and the downregulation of those affecting defense responses, such as flavonoid and lignin synthesis [15]. An example of an allelopathic ginsenoside is the ginsenoside Rd that inhibits seed germination and suppressed antioxidant enzyme activities [16]. A second example is a mixture of ginsenosides of lower molecular weight and low polarity (due to the microbial hydrolysis of glycosidic bonds) that inhibits root growth and alters the expression of over 6500 ginseng genes, including those for phenylpropanoid synthesis [17]. While those allelochemicals have been found in ginseng root, it is possible that there are a number of allelochemicals that can undergo transformation by soil microbes into active forms, such as by the oxidation and polymerization of phenolics [18]. Another possible ginseng-related factor in GRD is the unknown compounds extractable by methanol from soil one year following *P. quinquefolius* harvesting that increase lesion sizes by *I. mors-panacis* and suppress gene expression related to jasmonate-regulated root defenses but are undetectable in ginseng roots or soil not previously planted with *P. quinquefolius* [3].

GRD does not occur in the first crop, even though ginseng is grown commercially for 3 to 4 years before harvesting, but is only observed when the soil is replanted with ginseng [2]. Ginsenosides are found in the soil during all years of ginseng cultivation, with increases by the third year [19], and the same is true for phenolic acids in the soil, which are higher at year one than years two or three of ginseng cultivation [20]. Thus, it appears that ginsenosides and phenolics are released into the soil from the roots from the beginning of the growth of ginseng, and there should potentially be allelopathic effects both on the roots that release them and the roots of the adjacent ginseng plants. In contrast, no activity affecting root lesion size by *I. mors-panacis* was detectable in this study for years 1 to 3 of a crop in soil where ginseng had not previously been grown, indicating that the compounds that increase *I. mors-panacis* root rot [3] are not detectable during the growth of a first crop of *P. quinquefolius*. This could be because they are not present in root exudates, they are at too low levels in the root exudates to be detectable in the soil, or they are rapidly degraded upon entering the soil. However, the latter possibility would be inconsistent if they are associated with the known long-term persistence of GRD.

The harvesting of *P. quinquefolius* typically involves a machine digger cutting the soil just below the roots (approx. 30 cm), and then the roots are manually removed from the loosened soil [21]. However, some roots are not harvested and thus remain in the soil, mostly smaller pencil-shaped roots which are of lower value [22]. Although over 90% of the fresh root weight was harvested in this study, there still were a notable number of roots that remained in the soil to decay. The roots decayed relatively rapidly following harvest, as evidenced by a reduction in the fresh weight of the roots sieved from the soil during the autumn, but the soil extracts from that period showed no detectable activity of *I. mors-pancis*-increased root rot relative to the control. By the time the ground thawed in the following spring (198 dph), almost 95% of the non-harvested roots had decayed, also without detectable activity in the soil extracts that could increase root rot from *I. mors-pancis*. Similarly, soluble extracts from *P. quinquefolius* roots were not able to increase root rot or reduce jasmonate-related gene expression during infection by *I. mors-panacis* [3]. The first timepoint with detectable activity of increased root rot from *I. mors-panacis* was late spring (231 dph), when only a relatively small number of roots had decayed since 198 dph. However, a major difference between 198 and 231 dph was the warming of the soil. At 198 dph, the soil had recently thawed and was relatively cold, but by 231 dph, the soil was sufficiently warm to plant maize. Possibly, the warmer soil in late spring combined with the decayed ginseng roots may be important for the production of the compounds that can increase root rot from *I. mors-panacis*. This could be related to the ability of soil bacteria to convert compounds released into the soil from decaying ginseng roots into compounds that could increase root rot from *I. mors-panacis*. It was reported that *P. plecoglossicida* produced compounds in pure culture that increased *Ilyonectria* root rot but only in media containing ginseng root extract [6]. Soil temperature is an important factor in the dynamics of soil microbial activity [23], and soil bacteria can be greatly inhibited by low temperatures, with growth rates reaching near zero at 0 °C [24]. In addition, the ability of soil microbes to convert soil organic matter is more sensitive to soil warming as compared to other parameters such as total rhizomicrobial soil respiration [25]. Therefore, one possibility for the lack of activity increasing *Ilyonectria* root rot in the soil extracts at 198 dph was that the soil was not yet sufficiently warm enough to allow certain soil bacteria, such as *P. plecoglossicida*, to efficiently convert ginseng root compounds from the decayed roots into the compounds able to increase *Ilyonectria* root rot.

Another feature of GRD is that it can persist in soil for many decades [2]. Allelochemicals, however, do not normally persist long in agricultural soils once the plants producing them have been removed. For example, one of the more long-lasting allelochemicals in soil is the oxidized hydrojuglone of walnut that can remain in the soil for over a year after the removal of a tree [26]. However, it can be readily decayed by soil bacteria, such as *Pseudomonas*, which found in the soil under walnut trees [27]. Ginsenosides and pheno-

lic compounds are relatively short-lived in soil [28,29], and thus the persistence of those allelochemicals of ginseng is not consistent with the very long duration of GRD.

This study showed that the extracts of soils up to 30 years following *P. quinquefolius* harvesting have the activity to increase *Ilyonectria* root rot lesion sizes as compared to a control, which is consistent with the long-term persistence of GRD. The most persistent organic molecules in soil are considered to be synthetic molecules with non-polar bonds, such as the polycyclic aromatic hydrocarbons, benzo[ghi]perylene from coal and coronene from carpathite, which have half-lives of over 9 years in soil [30]. However, polar synthetic compounds can also be persistent in soil, such as a number of polar pesticides and their transformation products that can be detected even after 10 years in soil [31]. While plant-based compounds are considered relatively short-lived in soil, some can have long-term persistence in soil. An examination of soil organic compounds in four European long-term bare fallow sites without new organic inputs for up to 53 years showed that some plant materials that are typically considered to be persistent, such as cutin, suberin and lignin-related compounds, declined substantially over time, whereas long-chain alkanes from plants remained almost constant or increased [32]. Based on the relative abundance of N-containing compounds, the authors [32] concluded that an enrichment of microbially derived compounds occurred over the fallow period, indicating the continuing microbial metabolism of organic materials even without further inputs from plants. The persistence of plant compounds in soil was believed to be related to differences in their chemical traits/recalcitrance, interactions with the soil mineral matrix, or a combination of those factors. Many studies of GRD emphasize phenolics and ginsenosides in the soil [13]. However, there are many other compounds released from ginseng roots, such as 47 different liposoluble components found in the rhizosphere soil of *P. notoginseng* that were composed of various esters, steroids, alkanes, benzene derivatives, alkenes, terpenoids phenanthrene, alcohol and aldehyde, and some of those, like the alkanes, cyclopentadecane, nonacosane and the benzene D-α-tocopherol, were present only in soils with previous ginseng cultivation [33]. Nonacosane was one of 37 oils found in *P. quinquefolius* roots [34]. Thus, one possibility is that decaying ginseng roots could contain and then release potentially highly persistent compounds into soil, such as alkanes or other compounds. Further work is needed to determine the nature of the compounds in previously used *P. quinquefolius* soil that contains increased root rot from *I. mors-panacis*, as well as their persistence and their interactions with microbes and soil temperatures in the soil matrix. The preliminary HPLC and mass spectrometry analysis indicates that the compounds able to increase root rot from *I. mors-panacis* are neither ginsenosides nor phenolic acids [35].

5. Conclusions

A survey of *P. quinquefolius* soils showed that the compounds capable of increasing lesion sizes due to *I. mors-panacis* in the methanol extracts of soil after *P. quinquefolius* harvest cannot be detected in the soil of the first crop of *P. quinquefolius* or in the soil following ginseng harvesting up to early spring of the following year. However, it can be detected in the soil in late spring during the year after harvesting and then in the soil for up to 30 years after *P. quinquefolius* has been grown. These traits are consistent with the development and duration of GRD. In addition, the bioassay for this activity could be a way to screen soils for their potential to develop GRD before ginseng is planted.

Author Contributions: Conceptualization, P.H.G.; methodology, P.H.G.; investigation, P.H.G. and G.Z.; resources, P.H.G.; data curation, P.H.G.; writing—original draft preparation, P.H.G. and G.Z.; writing—review and editing, P.H.G.; visualization, P.H.G.; supervision, P.H.G.; project administration, P.H.G.; funding acquisition, P.H.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the authors upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Mancuso, C.; Santangelo, R. *Panax ginseng* and *Panax quinquefolius*: From pharmacology to toxicology. *Food Chem. Toxicol.* **2017**, *107*, 362–372. [CrossRef] [PubMed]
2. Westerveld, S.M.; Shi, F. The history, etiology, and management of ginseng replant disease: A Canadian perspective in review. *Can. J. Plant Sci.* **2021**, *101*, 886–901. [CrossRef]
3. Behdarvandi, B.; Goodwin, P.H. Effect of soil and root extracts on the innate immune response of American ginseng (*Panax quinquefolius*) to root rot caused by *Ilyonectria mors-panacis*. *Plants* **2023**, *12*, 2540. [CrossRef]
4. Okubara, P.A.; Paulitz, T.C. Root defense responses to fungal pathogens: A molecular perspective. *Plant Soil* **2005**, *274*, 215–226. [CrossRef]
5. Lee, O.R.; Sathiyaraj, G.; Kim, Y.J.; In, J.G.; Kwon, W.S.; Kim, J.H.; Yang, D.C. Defense genes induced by pathogens and abiotic stresses in *Panax ginseng* CA Meyer. *J. Ginseng Res.* **2011**, *35*, 1–11. [CrossRef]
6. Goodwin, P.H.; Hsiang, T. Cell-free extracts of the ginseng soil bacterium *Pseudomonas plecoglossicida* promote suppression of resistance of American ginseng (*Panax quinquefolius*) to root rot caused by *Ilyonectria mors-panacis*. *Biology* **2024**, *13*, 671. [CrossRef]
7. Shi, H.; Yang, J.; Li, Q.; PinChu, C.; Song, Z.; Yang, H.; Luo, Y.; Liu, C.; Fan, W. Diversity and correlation analysis of different root exudates on the regulation of microbial structure and function in soil planted with *Panax notoginseng*. *Front. Microbiol.* **2023**, *14*, 1282689. [CrossRef]
8. Sun, J.; Yang, J.; Zhao, S.; Yu, Q.; Weng, L.; Xiao, C. Root exudates influence rhizosphere fungi and thereby synergistically regulate *Panax ginseng* yield and quality. *Front. Microbiol.* **2023**, *14*, 1194224. [CrossRef]
9. Yang, L.; Yu, L.; Sun, Z.; Zhang, T.Y.; Zhang, Y.; Yang, L.M. Allelopathic effects of organic acids and saponins in ginseng root exudates on pathogenic and biocontrol bacteria. *J. Agric. Sci. Technol.* **2022**, *24*, 145–155.
10. Goodwin, P.H. The rhizosphere microbiome of ginseng. *Microorganisms* **2022**, *10*, 1152. [CrossRef]
11. Behdarvandi, B.; Hsiang, T.; Valliani, M.; Goodwin, P.H. Differences in saprophytic growth, virulence, genomes, and secretomes of *Ilyonectria robusta* and *I. mors-panacis* isolates from roots of American ginseng (*Panax quinquefolius*). *Horticulturae* **2023**, *9*, 713. [CrossRef]
12. Huang, L.F.; Song, L.X.; Xia, X.J.; Mao, W.H.; Shi, K.; Zhou, Y.H.; Yu, J.Q. Plant-soil feedbacks and soil sickness: From mechanisms to application in agriculture. *J. Chem. Ecol.* **2013**, *39*, 232–242. [CrossRef] [PubMed]
13. Xu, Y.G.; Liu, H.J.; Zhang, K.M.; Zhu, S.S.; Yang, M. Allelopathic plants: 28. Genus *Panax* L. *Allelopath. J.* **2020**, *51*, 21–40. [CrossRef]
14. Jiao, X.L.; Bi, X.B.; Gao, W.W. Allelopathic effects of p-coumaric acid on American ginseng and its physiological mechanism. *Acta Ecol. Sin.* **2015**, *35*, 3006–3013.
15. Wu, B.; Long, Q.L.; Gao, Y.; Wang, Z.; Shao, T.W.; Liu, Y.N.; Li, Y.; Ding, W.L. Comprehensive characterization of a time-course transcriptional response induced by autotoxins in *Panax ginseng* using RNA-Seq. *BMC Genomics* **2015**, *16*, 1010. [CrossRef]
16. Shen, Y.L.; Fan, M.L.; Li, G.; Li, Q.; Meng, X.R.; Wang, E.P.; Chen, C.B. Allelopathic effect of ginsenoside rd on ginseng seeds and seedlings. *Appl. Ecol. Environ. Res.* **2023**, *21*, 59–72. [CrossRef]
17. Wang, R.; Zhou, T.; Wang, Y.; Dong, J.; Bai, Y.; Huang, X.; Chen, C. Exploring the allelopathic autotoxicity mechanism of ginsenosides accumulation under ginseng decomposition based on integrated analysis of transcriptomics and metabolomics. *Front. Bioeng. Biotechnol.* **2024**, *12*, 1365229. [CrossRef]
18. Jilani, G.; Mahmood, S.; Chaudhry, A.N.; Hassan, I.; Akram, M. Allelochemicals: Sources, toxicity and microbial transformation in soil—A review. *Ann. Microbiol.* **2008**, *58*, 351–357. [CrossRef]
19. Fang, J.; Xu, Z.F.; Zhang, T.; Chen, C.B.; Liu, C.S.; Liu, R.; Chen, Y.Q. Effects of soil microbial ecology on ginsenoside accumulation in *Panax ginseng* across different cultivation years. *Ind. Crops Prod.* **2024**, *215*, 18637. [CrossRef]
20. An, S.; Wei, Y.; Li, H.; Zhao, Z.; Hu, J.; Philp, J.; Ryder, M.; Toh, R.; Li, J.; Zhou, Y.; et al. Long-term monocultures of American ginseng change the rhizosphere microbiome by reducing phenolic acids in soil. *Agriculture* **2022**, *12*, 640. [CrossRef]
21. Ontario Ministry of Agriculture, Food and Rural Affairs. *Guide to Ginseng Production*; Queen's Printer for Ontario: Toronto, ON, Canada, 2015.
22. Suárez, I.D.S.; Valliani, M.; Hsiang, T.; Goodwin, P.H. Decay of root debris after harvesting American ginseng (*Panax quinquefolius*) and changes in soil chemistry and microbiology. *Soil Syst.* **2023**, *7*, 108. [CrossRef]
23. Chernov, T.I.; Zhelezova, A.D. The dynamics of soil microbial communities on different timescales: A review. *Eurasian Soil Sci.* **2020**, *53*, 643–652. [CrossRef]
24. Pietikäinen, J.; Pettersson, M.; Bååth, E. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiol. Ecol.* **2005**, *52*, 49–58. [CrossRef]
25. Zhu, B.; Cheng, W. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. *Glob. Change Biol.* **2011**, *17*, 2172–2183. [CrossRef]
26. Willis, R.J. *Juglans* spp., juglone and allelopathy. *Allelopath. J.* **2000**, *7*, 1–55.

27. Schmidt, S.K. Degradation of juglone by soil bacteria. *J. Chem. Ecol.* **1988**, *14*, 1561–1571. [CrossRef]
28. Rabas, A.; Colo, A.; Kaberi, K.; Ivanov, D.A.; Bernards, M.A. The properties of ginsenosides in ginseng garden soil: Accumulation, persistence and behaviour. *Plant Soil* **2024**. [CrossRef]
29. Otte, B.A.; Rice, C.P.; Davis, B.W.; Schomberg, H.H.; Mirsky, S.B.; Tully, K.L. Phenolic acids released to soil during cereal rye cover crop decomposition. *Chemoecology* **2020**, *30*, 25–34. [CrossRef]
30. Wild, S.R.; Berrow, M.L.; Jones, K.C. The persistence of polynuclear aromatic hydrocarbons (PAHs) in sewage sludge amended agricultural soils. *Environ. Pollut.* **1991**, *72*, 141–157. [CrossRef]
31. Chiaia-Hernandez, A.C.; Keller, A.; Wächter, D.; Steinlin, C.; Camenzuli, L.; Hollender, J.; Krauss, M. Long-term persistence of pesticides and TPs in archived agricultural soil samples and comparison with pesticide application. *Environ. Sci. Tech.* **2017**, *51*, 10642–10651. [CrossRef]
32. Barré, P.; Quénéa, K.; Vidal, A.; Cécillon, L.; Christensen, B.T.; Kätterer, T.; Macdonald, A.; Petit, L.; Plante, A.F.; van Oort, F.; et al. Microbial and plant-derived compounds both contribute to persistent soil organic carbon in temperate soils. *Biogeochemistry* **2018**, *140*, 81–92. [CrossRef]
33. Qiao, Y.J.; Zhang, J.J.; Shang, J.H.; Zhu, H.T.; Wang, D.; Yang, C.R.; Zhang, Y.J. GC-MS-based identification and statistical analysis of liposoluble components in the rhizosphere soils of *Panax notoginseng*. *RSC Adv.* **2019**, *9*, 20557–20564. [CrossRef] [PubMed]
34. Smigielski, K.; Dolot, M.; Raj, A. Composition of the essential oils of ginseng roots of *Panax quinquefolium* L. and *Panax ginseng* CA Meyer. *J. Essent. Oil Bear. Plants* **2006**, *9*, 261–266. [CrossRef]
35. Bernards, M.A. (Western University, London, ON, Canada). Personal communication, 2024.