



## Data Article

# Characterization of data observing *Meloidogyne incognita*, *Neofusicoccum parvum*, and *Xylella fastidiosa* infection effects on development of grapevine phenolic compound levels and resistance to subsequent *Neofusicoccum parvum* infections

Christopher M. Wallis

Crop Diseases, Pests and Genetics Research Unit, U.S. Department of Agriculture- Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Ave, Parlier, CA 93648, USA

## ARTICLE INFO

## Article history:

Received 9 November 2023

Revised 9 February 2024

Accepted 4 March 2024

Available online 8 March 2024

Dataset link: [Dataset for Bacterial, Fungal, and Nematode Infection Effects on Development of Grapevine Phenolic Compound Levels and Resistance to Subsequent \*Neofusicoccum parvum\* Infections \(Original data\)](#)

## Keywords:

Bot canker

Fungal trunk disease

Induced defence responses

Phenolics

Plant host resistance

Stilbenoids

*Vitis* spp.

Root knot nematode

## ABSTRACT

Grapevines encounter many different pathogens throughout their lifespans, including the bacterial pathogen *Xylella fastidiosa*, which causes Pierce's disease that results in vascular occlusion and eventual plant host death, the fungal pathogen *Neofusicoccum parvum*, which causes stem cankers that kill individual vines and reduce fruit yields, and the root knot nematode *Meloidogyne incognita*, which destroys root tissues that impacts host vigour. To date, little research has been conducted to examine how one infection could impact subsequent infections by the same or different pathogens despite this is important to ensure healthy vineyards. Therefore, grapevines initially infected with either *X. fastidiosa*, *N. parvum*, or *M. incognita* were subsequently infected with *N. parvum* eight weeks later to observe developing lesion lengths, which were assessed to determine grapevine resistance to infections. Collected data shows that when prior infections were present, the *N. parvum* lesions lengths were smaller. This suggests grapevines had induced resistance to combat infections. Further, defence-associated phenolics were measured by high-performance liquid chromatography

E-mail address: [christopher.wallis@usda.gov](mailto:christopher.wallis@usda.gov)

<https://doi.org/10.1016/j.dib.2024.110301>

2352-3409/Published by Elsevier Inc. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>)

to determine roles in observed resistance to the secondary *N. parvum* infections. Data shows that of the different phenolics examined, only stilbenoids were different due to infections, with lowered levels observed in plants that were infected compared with non-infected controls. These data provide insight into how infections by different pathogens could impact grapevine host resistance to new, subsequent pathogen infections.

Published by Elsevier Inc.

This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>)

## Specifications Table

Subject	Agricultural Sciences, Agronomy and Crop Science
Specific subject area	Plant Pathology, Host-Microbe Interactions, Metabolomics
Data format	Raw and analysed
Type of data	Tables (in .xlsx format): Lesion lengths of <i>N. parvum</i> (mm), mean total phenolic levels (mg/g), mean total flavonoids levels (mg/g), and mean total stilbenoid levels (mg/g); Individual phenolic compounds (mg/g)
Data collection	Lesion length: measured used a ruler of debarked stem segment the was collected around the inoculation site of <i>N. parvum</i> (5 cm above and below the site) Phenolic compound concentrations: methanolic extracts of pulverized stem tissues were injected into a Shimadzu LC20-AD high-performance liquid chromatography system equipped with a Ascentis C18 column and Shimadzu Photodiode Array detector. A water-methanol binary gradient was used for separation, and the 280 nm wavelength was used for quantification. Peaks were identified using commercial standards obtained from Sigma-Aldrich, with conversion made using representative compound standard curves.
Data source location	Institution: U.S. Department of Agriculture- Agricultural Research Service, San Joaquin Agricultural Sciences Center City/Town/Region: Parlier, California Country: United States of America Latitude and longitude: 36°35'46" N; 119°30'47" W
Data accessibility	Repository name: Fig Share Data identification number: 24,093,783.v1 Direct URL to data: <a href="https://doi.org/10.6084/m9.figshare.24093783.v1">https://doi.org/10.6084/m9.figshare.24093783.v1</a>

## 1. Value of the Data

- The dataset provides information on how one pathogen infection in grapevine can alter the course of another infection, specifically *Neofusicoccum parvum*.
- The dataset also quantifies changes in phenolic compound levels that occurs following pathogen infections.
- These data benefit researchers interested in understanding grapevine-pathogen interactions, and those examining multiple infections within the same host plant.
- These data can be reused by other researchers conducting similar plant-microbe interaction research on grapevines, including plants infected by *Neofusicoccum parvum*, *Meloidogyne incognita*, or *Xylella fastidiosa*.

## 2. Objective

Grapevines encounter multiple pathogens throughout their lives, often simultaneously [1,2]. These pathogens include the aggressive, xylem-infecting bacteria *Xylella fastidiosa*, which is

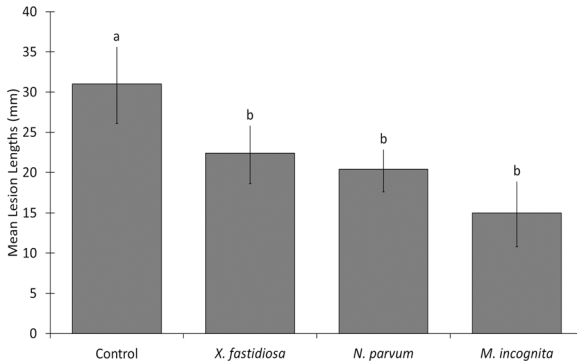
causal agent of Pierce's disease, is spread by vectoring insects, and could result in vine death by clogging the vascular system within a year or two [3,4]. Another pathogen is the chronic fungal pathogen *Neofusicoccum parvum*, which is spread by spore colonization of pruning and other wounds and can kill colonized vines to reduce yields over time [5–7]. A third pathogen is the root-infecting nematode *Meloidogyne incognita*, which reproduces within and feeds upon root tissues to reduce overall host vigor [8,9]. To counter these, growers utilize a variety of pesticides throughout the year to mitigate impacts and maintain fruit yields, but repeated use may build up harmful chemicals in the local environment and negatively impact nearby communities [2]. However, despite the use of pesticides ultimately the combination of infections by multiple pathogens results in loss of vineyard productivity after about 12 years, resulting in the need for growers to replace the vineyard in its entirety [1]. Furthermore, the need to use pesticides can be a financial burden for growers, which necessitates the need to prioritize control of specific pathogens at the expense of others. Knowledge of how these pathogens may predispose grapevines to other pathogens could clarify which to target for control, optimizing management costs. Furthermore, grapevines, like all plants, may utilize induced host defense responses when encountering one pathogen that reduce the success of a subsequent pathogen, and this phenomenon should be considered in vineyard management decisions. Greater knowledge of interactions could result in the development of programs to reduce overall pesticide use and improve crop yields.

However, few studies have examined multiple infections on grapevines to date. Regarding nematodes, co-infection with the soil-borne bacterial pathogen *Rhizoctonia solani* resulted in increased root rotting symptoms, especially if roots were inoculated with the nematode first [10]. Regarding fungal pathogens, another study observed if an infection by one of three different fungal canker pathogens on grapevine stems could affect progression of subsequent infections that occurred on a different branch [11]. Infections of the canker pathogen *Diplodia seriata* were observed to be reduced in vines that had a prior infection, and results suggested this might be due to an increase in phenolic compounds caused by the first infection [11]. Indeed, it is a common response for infections to cause induced changes in host chemistry, especially that of phenolic compounds, which have roles in both structural and chemical defenses against pathogens [12–14]. A recent review has covered different grapevine pathogenic microorganisms and how grapevines response to being infected [15].

Therefore, data were obtained to examine how distinct pathogen classes, either the bacterial pathogen *X. fastidiosa*, the fungal pathogen *N. parvum*, or the root-knot nematode *M. incognita*, could alter grapevine phenolic compounds and affect the progression of second, subsequent infections by *N. parvum*. This was part of a larger effort to understand interactions between multiple pathogens as mediated through grapevine hosts, which should provide clarity in how plants cope with the multiple infections over time. Knowledge gained could be used to adjust overall vineyard management strategies and demonstrate the usefulness of induced grapevine host defense responses in limiting disease development.

### 3. Data Description

When plants were infected with *X. fastidiosa*, *N. parvum*, or *M. incognita* previously, the lesion lengths of subsequent *N. parvum* inoculations were reduced compared to plants that were previously uninfected ( $F_{3, 40} = 10.716$ ;  $P < 0.001$ ) (Fig. 1). There was a significant block effect of year, with greater lesion lengths observed in 2018 compared to 2019 ( $F_{1, 40} = 119.815$ ;  $P < 0.001$ ). The previous pathogen infections likely caused shifts in overall host physiology to lead to these results, as the second infections by *N. parvum* were started distally (on a different organ) from where the initial inoculations were performed. A few different hypotheses could explain the observations. First, the infections by *X. fastidiosa*, *N. parvum*, or *M. incognita* might have impacted grapevine vigour by reducing the ability of the grapevines to maintain homeostasis and capacity to produce photosynthate. An overall reduction of resources available to the second pathogen would lead to reduced development [11]. A second hypothesis would be the *N. parvum* lesion



**Fig. 1.** Mean ( $\pm$ SE) *N. parvum* lesion lengths. Plants were previously infected with no pathogens, *X. fastidiosa*, another *N. parvum* infection, or *M. incognita*. Different letters represent mean separations due to Tukey HSD tests. In addition, all pathogen infected plants had lesions that significantly differed ( $P < 0.05$ ) from the non-infected plants in pairwise Student's *t*-tests.

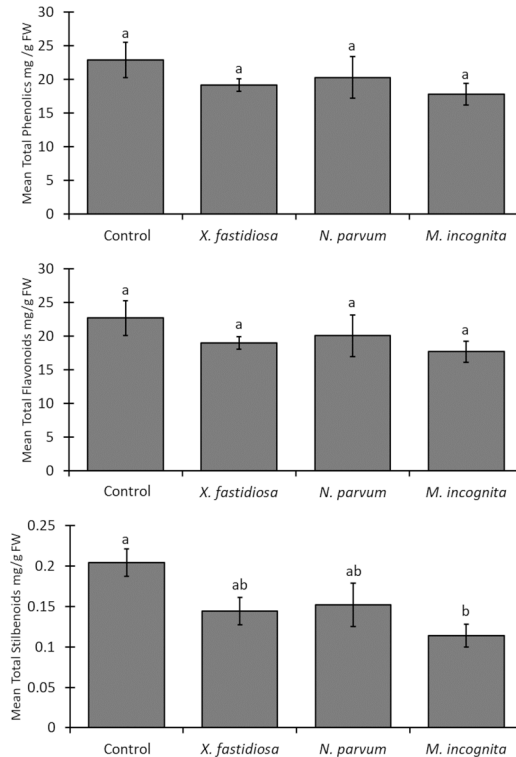
lengths were affected by metabolites produced by the other pathogens (*X. fastidiosa*, the prior *N. parvum* infection, or *M. incognita*). However, knowledge of metabolites produced by these plant pathogens is lacking and a target for future research.

A third hypothesis to explain reduction in the secondary *N. parvum* infections may be there was an increased systemic production of plant host defences, due to processes such as systemic acquired resistance or induced systemic resistance [13]. This would involve the *de novo* production of phenolic and other compounds. Thus, the levels of phenolic compounds were quantified via high-performance liquid chromatography. The levels of stem total phenolics and total flavonoid levels did not statistically differ ( $P > 0.05$ ) due to initial infections (Fig. 2) or experiment replicate (year). However, previous infections, regardless from which pathogen, had significantly lowered total stilbenoid levels than non-infected controls ( $F_{3,39} = 5.331$ ;  $P = 0.004$ ) (Fig. 2). Only stilbenoid levels were different than control levels using Tukey HSD multiple comparison tests. However, pairwise comparisons using Student's *t*-tests revealed lowered stilbenoid levels in *X. fastidiosa*, *N. parvum*, and *M. incognita* infected plants than controls. Stilbenoids also were significantly greater in 2019 than 2018 ( $F_{1,39} = 11.404$ ;  $P = 0.002$ ). The role of stilbenoids is generally associated only with providing the plant defense against microorganisms with no other roles established [13].

Based on these findings, it appears unlikely that systemic induction of stilbenoids was involved in the observed reductions of lesion lengths. Rather, the first two hypotheses, or a combination of them, could explain these results. Indeed, a reduction in photosynthate due to reduced vigor would not only potentially reduce resources that the *N. parvum* could feed upon, but also result in fewer resources for the plant to invest in defense-related metabolites.

#### 4. Experimental Design, Materials and Methods

All grapevine plants utilized in this experiment were potted in 20 L pots with field-collected autoclaved sandy loam mixture and maintained in a controlled greenhouse environment with a 16-hour light cycle, a set temperature at approximately 26 °C, and watered to field capacity once per week. In two separate experimental replicates (in May 2018 and May 2019), a total of up to six 'Cabernet Sauvignon' grapevines each, arranged in a randomized complete block design, were left as healthy controls, inoculated with the bacterial pathogen *X. fastidiosa*, inoculated with the fungus *N. parvum*, or inoculated with *M. incognita*. *X. fastidiosa* inoculations were performed by the pin-prick method [3], which consisted of making five wounds on the grapevine stem 5 cm from the rootstock-scion junction with a 16-gauge needle, and then pipetting a slurry of bac-



**Fig. 2.** Mean total phenolic, flavonoid, or stilbenoid levels ( $\pm$ SE) taken before subsequent *N. parvum* inoculations. Plants were either non-inoculated or infected with *X. fastidiosa*, *N. parvum*, or *M. incognita*. Different letters represent mean separations due to Tukey HSD tests. In addition, all pathogen infected plants had stilbenoid levels that significantly differed ( $P < 0.05$ ) from the non-infected plants in pairwise Student's *t*-tests.

teria (roughly 10,000 CFU per mL) as an approximately 10  $\mu$ L droplet with a 24-gauge needle. Infections of *X. fastidiosa* were confirmed by ELISA using a kit and protocol provided by Agdia Incorporated (Elkhart, Indiana, USA). *N. parvum* inoculations consisted of taking a 10 mm colonized agar plug into a 12 mm wound on the grapevine stem 5 cm from the rootstock-scion junction and wrapping in parafilm [16]. Infections were confirmed by observing lesion length development and reisolating *N. parvum* from the inoculation site at the end of the study. *M. incognita* soil inoculations were performed by diluting eggs in water to a concentration of approximately 250 eggs/mL, and then, following mixing by test tube inversion, pipetting 1 mL into each of four holes made in the soil around the pot in which the grapevines were planted. Infections were confirmed by harvesting roots at the end of the study to observe the presence of root knots and the presence of J2 stage root knot nematodes within the soil.

Two months after the initial inoculation treatments, all vines received a *N. parvum* inoculation performed as described above. For plants initially infected with either *X. fastidiosa* or *N. parvum*, these *N. parvum* inoculations were performed on a different branch than the first inoculations. At the same time as the secondary inoculations of *N. parvum*, tissue samples were collected for chemical analyses. This involved collecting stem samples from 15 cm to 20 cm above the rootstock-scion union on an opposite branch from the initial infection (or equivalent locations for controls). All collected tissues were flash-frozen in liquid nitrogen and stored at  $-20^{\circ}\text{C}$  until processing. Furthermore, the inoculation success of the first inoculation treatments was determined at this time by counting living nematodes in the soil samples (the presence of J2 juvenile nematodes as the result of successful *M. incognita* colonization), observing the de-

velopment of initial *N. parvum* infection lesion lengths (with those exceeding 12 mm of growth and having visible pycnidia formation considered successful infections), or observing *X. fastidiosa* infection success by using drop digital PCR with *Xylella* specific primers on the stem tissues as collected for chemistry above [14]. In all cases, the initial inoculation treatments were successful.

Two months after the second inoculations, *N. parvum* lesions were measured as a proxy for infection success. *N. parvum* inoculations had the inoculated stems harvested and lesion lengths were measured by debarking and measuring lengths via a ruler, both apically and basally from the initial inoculation site.

Phenolic compounds were quantified by using the methods of Wallis et al. [16] and Wallis and Chen [17]. Solvents were obtained by Thermo-Fisher Scientific (Waltham, MA, USA) and standards from Sigma-Aldrich (St. Louis, MO, USA) unless specified. Both the frozen stem and root samples were pulverized with a mortar and pestle in liquid nitrogen and had a 0.10 g aliquot weighed out into 1.5 mL centrifuge tubes and then extracted overnight at 4 °C in methanol. Tissue remaining following centrifugation the following morning was re-extracted in 0.5 mL of methanol as well, with this second extract combined with the first to yield a total of 1.0 mL methanol extract. A Shimadzu (Columbia, MD, USA) LC-20AD pump based liquid chromatograph equipped with Supelco Ascentis RP-18 (Sigma-Aldrich) column and a Shimadzu PDA-20 photodiode array detector was employed to conduct high-performance liquid chromatography (HPLC) on 50 µL of the methanol extract per sample. Standards identified certain compounds by matching retention times, with other compounds putatively identified via liquid chromatography-mass spectrometry using a Shimadzu LCMS2020 system running similar conditions to the HPLC. Commercially available standard compounds from the same phenolic subclass, specifically procyanidin B2 for proanthocyanins, catechin for flavan-3-ols, quercetin glucoside for flavonoid glycosides, and resveratrol for stilbenoids, were run to make standard curves to convert peak areas into mg/g fresh weight.

JMP statistics version 17 (SAS Institute, Inc., Cary, NC, USA) was used to conduct all statistical tests with  $\alpha = 0.05$ . Analyses of variance were used to compare fungal lesion lengths and levels of total phenolics, total flavonoids, or total stilbenoids (the sum of individual phenolics comprising these groupings) between initial inoculation treatments, with experimental year as a block factor and follow-up Tukey HSD tests to compare differences among all treatment means. Furthermore, to examine differences between each individual infection treatment with controls alone, Student's *t*-tests were used.

## Limitations

Not applicable.

## Ethics Statement

Data collected did not involve human subjects, animal experiments, or data collected from social media platforms.

## Data Availability

[Dataset for Bacterial, Fungal, and Nematode Infection Effects on Development of Grapevine Phenolic Compound Levels and Resistance to Subsequent \*Neofusicoccum parvum\* Infections \(Original data\) \(Fig Share\)](#)

## CRedit Author Statement

**Christopher M. Wallis:** Conceptualization, Methodology, Data curation, Validation, Writing – original draft, Writing – review & editing.

## Acknowledgements

The work was funded by allocated funds to the San Joaquin Valley Agricultural Sciences Center, U.S. Department of Agriculture- Agricultural Research Service, Project # 2034-22000-012-00D. The author thanks Mala To, Justin King, Ben Tanielian and Nalong Mekdara for their technical assistance in this work. The author also thanks Andreas Westphal from the University of California, Parlier, CA, for providing root knot nematodes for these experiments. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

- [1] C. Bertsch, M. Ramírez-Suero, M. Magnin-Robert, P. Larignon, J. Chong, E. Anou-Mansour, et al., Grapevine trunk diseases: complex and still poorly understood, *Plant Pathol.* 62 (2013) 243–265.
- [2] M. Keller, *The Science of Grapevines: Anatomy and Physiology*, Academic Press, San Diego, CA, USA, 2010.
- [3] D.L. Hopkins, A.H. Purcell, *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases, *Plant Dis.* 86 (2002) 1056–1066.
- [4] J. Rapicavoli, B. Ingel, B. Blanco-Ulate, D. Cantu, M.C. Roper, *Xylella fastidiosa*: an examination of a reemerging plant pathogen, *Mol. Plant Pathol.* 19 (2018) 786–800.
- [5] J.R. Úrbez-Torres, W.D. Gubler, Pathogenicity of Botryosphaeriaceae species isolated from grapevine cankers in California, *Plant Dis.* 93 (2009) 584–592.
- [6] R. Travadon, P.E. Rolshausen, W.D. Gubler, L. Cadle-Davidson, K. Baumgartner, Susceptibility of cultivated and wild *Vitis* spp. to wood infection by fungal trunk pathogens, *Plant Dis.* 97 (2013) 1529–1536.
- [7] K. Baumgartner, V. Hillis, M. Lubell, M. Norton, J. Kaplan, Managing grapevine trunk diseases in California's southern San Joaquin Valley, *Am. J. Enol. Viticul.* 70 (2019) 267–276.
- [8] C. Gutierrez-Gutierrez, J.E. Palomares-Rius, R.M. Jimenez-Diaz, P. Castillo, Host suitability of *Vitis* rootstocks to root-knot nematodes (*Meloidogyne* spp.) and the dagger nematode *Xiphinema index*, and plant damage caused by infections, *Plant Pathol.* 60 (2011) 575–585.
- [9] M. Abdel-Sattar, A.H. Haikal, S.E. Hammad, *Meloidogyne incognita* population control and nutritional status and productivity of Thompson seedless grapevines managed with different treatments, *PLoS ONE* 15 (2022) e0239993.
- [10] G.E. Walker, Effects of *Meloidogyne* spp. and *Rhizoctonia solani* on the growth of grapevine rootings, *J. Nematol.* 29 (1997) 190–198.
- [11] C.M. Wallis, Z. Gorman, E.R.A. Galarneau, K. Baumgartner, Mixed infections of fungal trunk pathogens and induced systemic phenolic compound production in grapevines, *Front. Plant Sci.* 3 (2022) 1001143.
- [12] R.A. Dixon, Natural products and plant disease resistance, *Nature* 411 (2001) 843–847.
- [13] C.M. Wallis, E.R.A. Galarneau, Phenolic compound induction in plant-microbe and plant-insect interactions: a meta-analysis, *Front. Plant Sci.* 11 (2020) 580753.
- [14] C.M. Wallis, A.R. Zeilinger, A. Sicard, D.J. Beal, M.A. Walker, R.P.P. Almeida, Impact of phenolic compounds on progression of *Xylella fastidiosa* infections in susceptible and PdR1-locus containing resistant grapevines, *PLoS ONE* 15 (2020) e0237545.
- [15] G. Armijo, R. Schlechter, M. Agurto, D. Munoz, C. Munez, R. Arce-Johnson, Grapevine pathogenic microorganisms: understanding infection strategies and host response scenarios, *Front. Plant Sci.* 7 (2016) 382.
- [16] C. Wallis, A. Eyles, B. McSpadden Gardener, R. Hansen, D. Cipollini, D.A. Herms, et al., Systemic induction of phloem secondary metabolism and its relationship to resistance to a canker pathogen in Austrian pine, *New Phytol.* 177 (2008) 767–778.
- [17] C.M. Wallis, J. Chen, Grapevine phenolic compounds in xylem sap and tissues are significantly altered during infection by *Xylella fastidiosa*, *Phytopathol.* 102 (2012) 816–826.