North American Bramble Growers Research Foundation Annual Progress Report

Interim Report

Fall 2024

Proposal Category: Pest management Strategies – soil borne disease and nematode management

Title: Genomic and phenomic technologies for evaluating and identifying root lesion nematode (*Pratylenchus penetrans*) resistance in red raspberry

Principal Investigators:

Researcher Name	Email	Office Phone
Savannah Phipps[1,2,3]	phippsav@oregonstate.edu	(541)738-4032
Inga Zasada[2]	inga.zasada@usda.gov	(541) 738-4051
Michael Hardigan[1]	Michael.Hardigan@usda.gov	(541) 738-4037
Michael Dossett[5]	MDossett@BCBerryCultivar.com	(604) 309-0048
Wendy Hoashi-Erhardt[4]	wkhe@wsu.edu	(253) 445-4641

Affiliations:

[1]U.S. Department of Agriculture–Agricultural Research Service, Horticultural Crops Production and Genetic Improvement Research Unit, 3420 NW Orchard Avenue, Corvallis, OR 97330

[2]U.S. Department of Agriculture–Agricultural Research Service, Horticultural Crops Disease and Pest Management Research Unit, 3420 NW Orchard Avenue, Corvallis, OR 97330

[3]Department of Botany and Plant Pathology, Oregon State University, 2701 SW Campus Way, Corvallis OR, 97331

[4]Washington State University Puyallup Research and Extension Center, 2606 West Pioneer, Puyallup, WA, 98371-4998

[5]British Columbia Berry Cultivar Development Inc., Abbotsford, BC V2T 1W5, Canada

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Introduction:

Red raspberry growers in the Pacific Northwest face significant pressures from the root lesion nematode, *Pratylenchus penetrans*. Root lesion nematodes (*Pratylenchus* spp.) are one of the most important plant-parasitic nematodes to agricultural crops in the world (Jones et al., 2013). This migratory endoparasite feeds on roots, resulting in damaged vascular tissues and producing the characteristic root lesions that give *Pratylenchus* spp. their common name. The injured roots are inhibited in their uptake of water and nutrients leading to reduced plant vigor and symptoms of stunting and chlorosis.

Growers primarily manage *P. penetrans* through chemical preplant treatments and occasional postplant chemical treatments. New and revised regulations of these chemical treatments make these options increasingly limited to growers. 1,3-dichloropropene is the dominant preplant treatment (Walters et al., 2017), but this method seems to have a short-term effect with nematode population densities rebounding one to three years after application (Bélair, 1991; DeVetter et al., 2018). Other chemicals such as methyl bromide and fenamiphos were once widely used but have now been phased out (Zasada et al., 2010; Zasada & Walters, 2016). Alternative methods such as crop rotation (Forge et al., 2012), soil amendments (Gigot et al., 2013; Forge et al., 2014, 2016) and solarization (Pinkerton et al., 2000, 2002) have been investigated with little success due to limited lifetime control or costs. Development and release of resistant cultivars would aid in relieving the economic and disease pressures.

Resistant cultivars have been developed in breeding programs (Bristow et al., 1980; Vrain & Daubeny, 1984) and are available in germplasm repositories (Zasada & Moore, 2014), but much remains unknown about the genetic loci or mechanisms that confer resistance to *P. penetrans*. Current knowledge suggests *P. penetrans* resistance is a quantitative trait (Vrain et al., 1994), which raises challenges in studying and breeding for this trait. Recent developments in genetic marker analysis methodologies allow for improved prediction and selection for complex traits such as this one. Genomic selection is a modern breeding technique that enables plant breeders to select individuals with desirable genetics at the seedling stage using genomic estimated breeding values (GEBVs) generated from genetic marker information (Meuwissen et al., 2001). This technique has been applied in globally significant crops and demonstrated to be well-suited to accelerate selection of quantitative traits, sometimes with greater efficacy than genomics assisted breeding techniques such as marker assisted selection (Bernardo, 2008; Poland & Rutkoski, 2016).

Another limitation to evaluating individuals for nematode resistance has been the labor intensive and destructive techniques needed to examine *P. penetrans* densities in the roots. High-throughput phenotyping technologies have rapidly advanced alongside genomic technologies and present a more cost and time effective method of assessing individuals. There have been initial investigations into ground-based methods for detecting abiotic and biotic stresses (Williams et al., 2017, 2021, 2023) in red raspberry, but there have been no investigations for use in detecting nematode stress. At this time, accurate, non-destructive methods of phenotyping raspberries for *P. penetrans* resistance are not available. Such methods need to be evaluated before high-throughput phenotyping and genomic selection for this trait will be effective.

This research provides the first application of genomic prediction for quantitative disease resistance in red raspberry and will potentially identify genes associated with *P. penetrans* resistance or tolerance. It will also evaluate high-throughput phenotyping technologies, which will have application not only to raspberry but to other perennial crops. Combined, this information can be used to better inform breeders and reduce development time of more resilient cultivars and encourage genomics- and phenomics-assisted breeding for other difficult or genetically complex traits in perennial crops. This project seeks to i) characterize *P. penetrans* resistance and tolerance phenotypes in a diverse population of 270 red raspberry breeding lines and cultivars, ii) conduct a genome-wide association study (GWAS) and perform genomic prediction of *P. penetrans* resistance using phenotypic and genotypic information, and iii) determine the effectiveness of high-throughput spectral data for assessing *P. penetrans* damage.

Progress to Date:

A diverse panel of 270 red raspberry genotypes composed of released and unreleased genetic material from the USDA-ARS/OSU, Washington State University, British Columbia Berry Cultivar Development Inc. (BCBCDI) breeding programs as well as cultivars from international programs available at the USDA-ARS National Clonal Germplasm Repository were planted in a randomized complete block design with paired non-inoculated and inoculated *P. penetrans* treatments at the Washington State University Northwestern Washington Research and Extension Center in Mount Vernon, Washington (48°26'30.4"N, 122°23'38.1"W) in June 2022. The trial location is on a silt loam soil and records since 2015 have shown it has previously been planted to various grains, vegetables, herbs, and tea. There were 1,916 individual plants in this planting. In the three years since planting, 189 individual plants have died leaving 1,727 plants in field as of August 2024 (Figure 1).

Figure 1: The trial planting in its first year (above) and at the end of the season in its third year (below). Photos by Savannah Phipps.

Aboveground biomass fresh weight for all surviving plants was recorded in 2022, 2023, and 2024 by pruning whole plants to the crown (Figure 2) and then weighing the pruned biomass. Measurements were taken with a tabletop scale in 2022 (Figure 2) and with a hanging scale using a custom hanging arm fitted to a Gator vehicle in 2023 and 2024 (Figure 3).

Figure 2: Above ground fresh weight biomass data collection from 2022. Left: Pruning of plants to the crown. Right: Recording of pruned plant biomass using tabletop scales in 2022. Photos by Savannah Phipps.

Figure 3: Aboveground fresh-weight biomass data collection from 2023. Plants were pruned to the ground and weighed with a hanging scale custom mounted in the back of a Gator. Photo by Shijie Zhang.

In 2023 and 2024, root samples were also collected for *P. penetrans* quantification (Figure 4). *Pratylenchus penetrans* extractions and quantification for the 2024 season are ongoing at the time of this report and will be finished by the end of 2024. Processing and preliminary analysis of aerial and ground-based imaging data is also in progress and expected to be finished in early 2025. Throughout the season high-throughput phenotyping using an AgBOT mk 1 quadcopter equipped with a MicaSense RedEdge-MX multispectral sensor for aerial imaging and an Apple iPhone 14 Pro Max with the AgerPoint Capture app as the ground-based imaging system occurred (Figure 5).

Figure 4: Root sample collection process. Root sampling required digging around the crown of the plant after it was pruned and then collecting fine, fibrous roots into sealable bags. Photos by Mary Peterson.

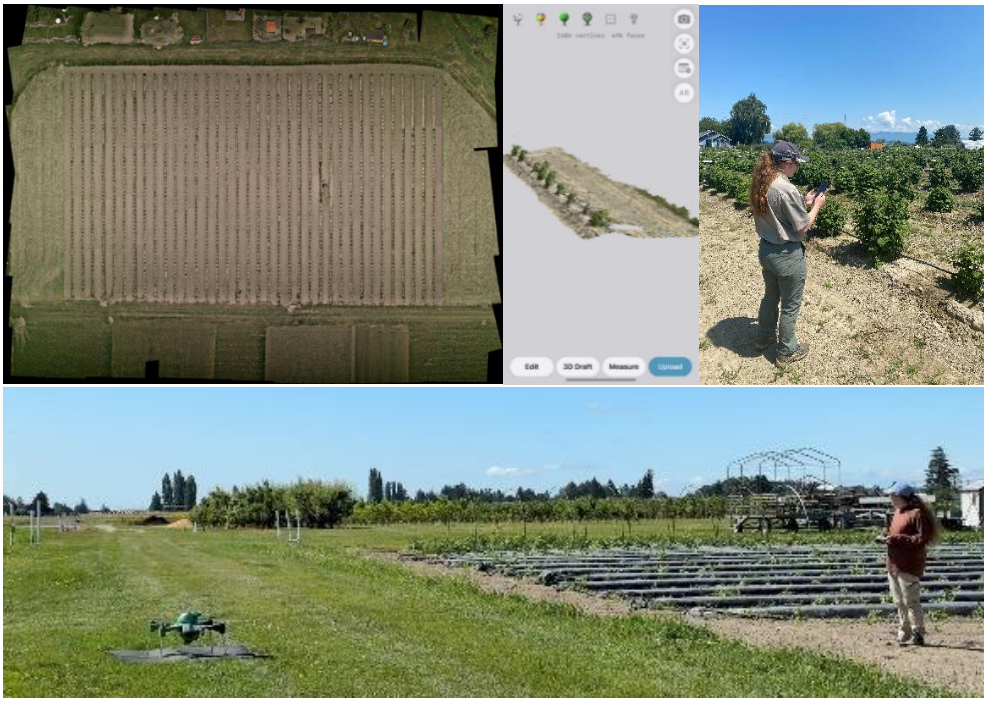

Figure 5: Above left: Constructed orthomosaic of aerial images taken May 5th, 2023. Constructed by Alexander Gregory. Above middle: Unprocessed image capture in the AgerPoint Capture app. Photo by Savannah Phipps. Above right: Savannah Phipps recording images of select genotypes using the AgerPoint Capture app on the iPhone 14 Pro Max. Photo by Hannah Baker. Below: Savannah Phipps preparing for aerial imaging with the AgBOT mk 1 quadcopter equipped with a MicaSense RedEdge-MX multispectral sensor. Photo by Hans Mejia de Leon.

DNA samples from the cultivars in the diversity panel were submitted for whole-genome sequencing performed by Ferris Genomics on their proprietary platform. Sequencing results were received August 15th, 2024. Currently, several samples are being sequenced again due to inadequate sequence coverage.

Preliminary Results:

Initial examination of the phenotype data prior to genetic marker analysis demonstrates differences in fresh weight aboveground biomass and nematode densities between the treatments (Figure 6). Exploration into the aerial imaging data from 2022 suggests a strong correlation between fresh weight aboveground biomass and pixel count indicating that this could be a possible substitute for destructive measurements (Figure 7). Examining the 2023 imaging data from the mobile devices indicates similar trends but requires additional cleanup due misclassification of plants (Figure 8). This is the primary contributor to the few points displayed in the figure provided. Associations with other measurements still need to be examined to determine if there are relationships between image data and nematode parasitism pressure.

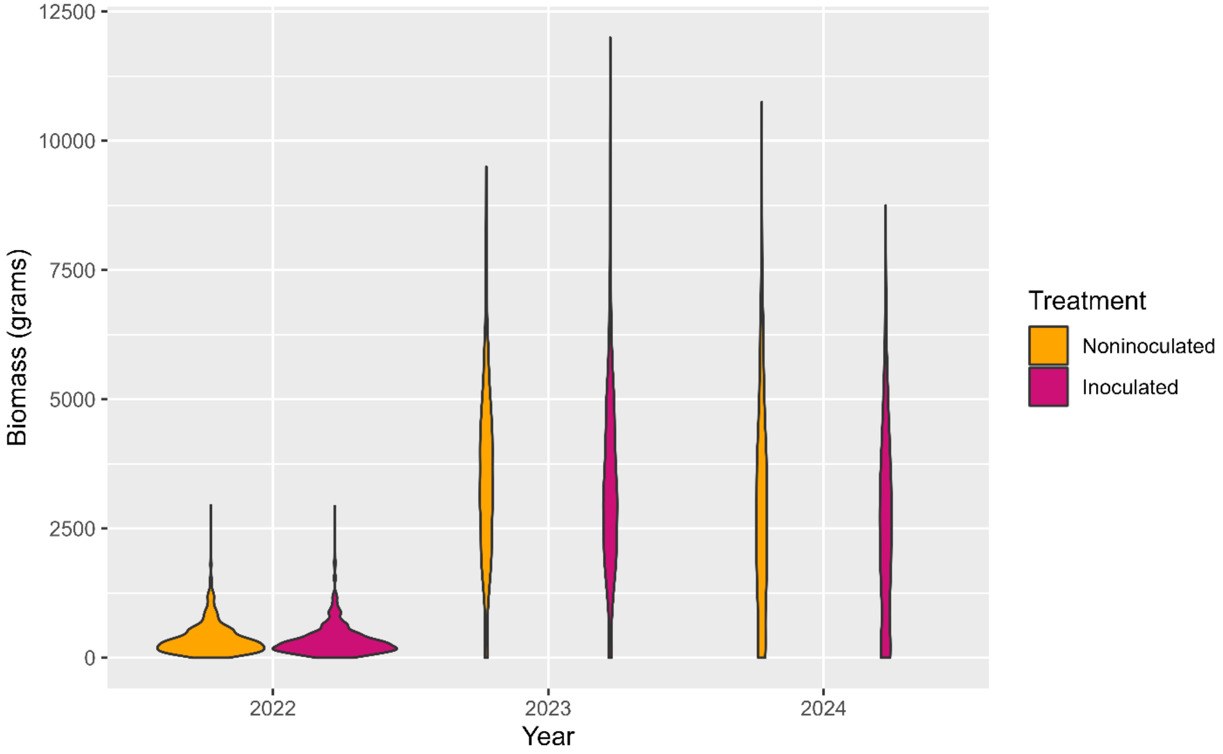

Figure 6: Distribution of fresh weight aboveground biomass across the study years.

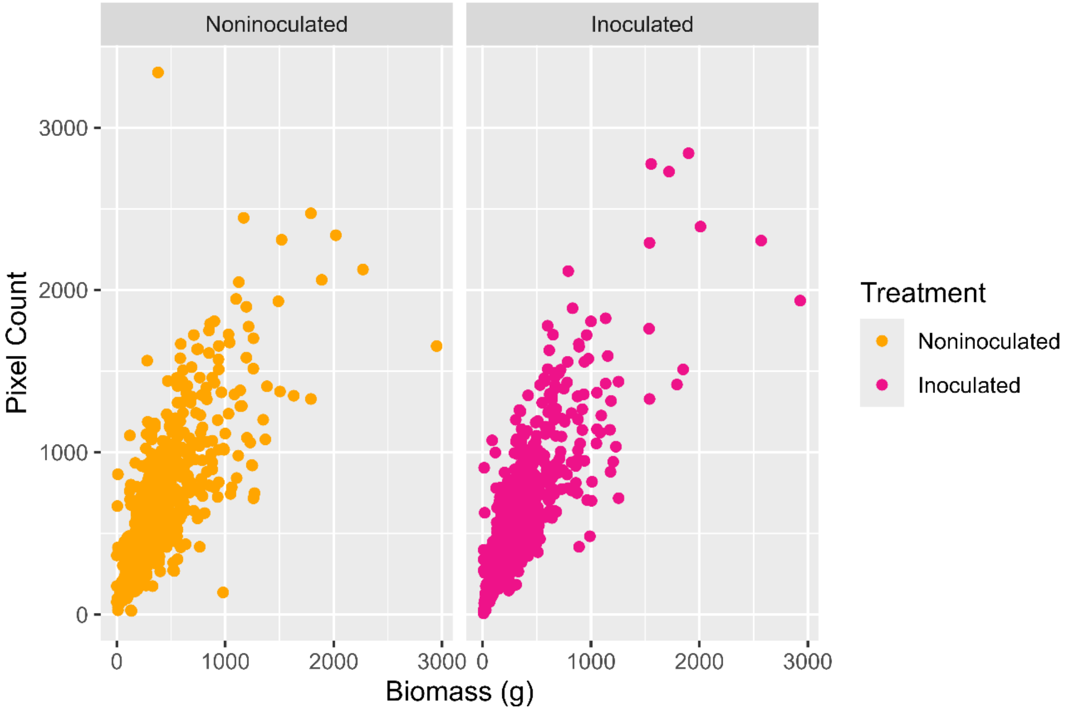

Figure 7: Correlation between aboveground biomass and pixel counts recorded by the drone in September 2022.

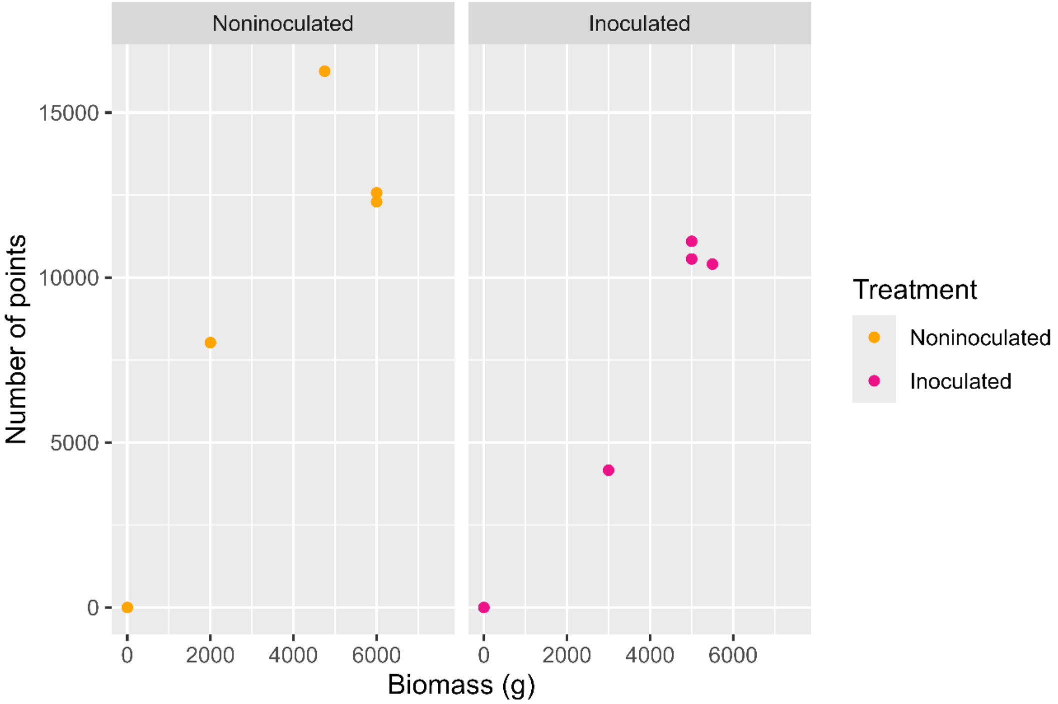

Figure 8: Correlation between aboveground biomass and LiDAR points generated by the AgerPoint Capture app in September 2023.

Future Work:

After receiving the updated sequencing results, a GWAS will be conducted to identify potential genetic markers for resistance. Genomic prediction models will then be developed with the intention of generating GEBVs for nematode resistance. Single and multiyear data will be incorporated into both procedures to better examine any patterns that may be present. Results from these analyses should be available in the Spring 2025 and will be publicly available in a peer-reviewed journal.

The aerial and mobile imaging data will be processed in 2025 and analyzed for correlations to the biomass and nematode density data. Similar GWAS and genomic prediction procedures will be implemented to also assess the ability for these technologies to contribute to genetic studies should the examined correlations be strong. The results of this study will be made publicly available in a peer-reviewed journal in late 2025.

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