Phenotypic Variation between *P. ramorum* isolates from Washington and British Columbia Nurseries

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Introduction

Phytophthora ramorum is an invasive plant pathogen found across North American and European nurseries known to cause Sudden Oak Death as well as many other ramorum-blight related diseases on a wide variety of hosts (USDA APHIS. 2023). Three common lineages of *P. ramorum* include NA1, NA2, and EU1. Within North America, NA1 is the predominant lineage found in nurseries (Grünwald et al. 2012), however, NA2 and EU1 are also found in the region. Additionally, a sexual hybrid of the EU1-NA2 lineages has recently been discovered in North America (Hamelin et al. 2022). Common treatment of *P. ramorum* includes the use of fungicides, such as Metalaxyl-M. Previous research found the standard EC50 (effective concentration resulting ins 50% mortality) for *P. ramorum* against Metalaxyl-M to be around 0.01 ppm (Elliott et al. 2015), however, this study also noted that many isolates appeared to show resistance to the fungicide. In this study, we aim to investigate and compare fungicide resistance and aggressiveness phenotypes of NA1, NA2, EU1, and hybrid *P. ramorum* clonal lineages using isolates from Washington (WA) and British Columbia (BC) nurseries.
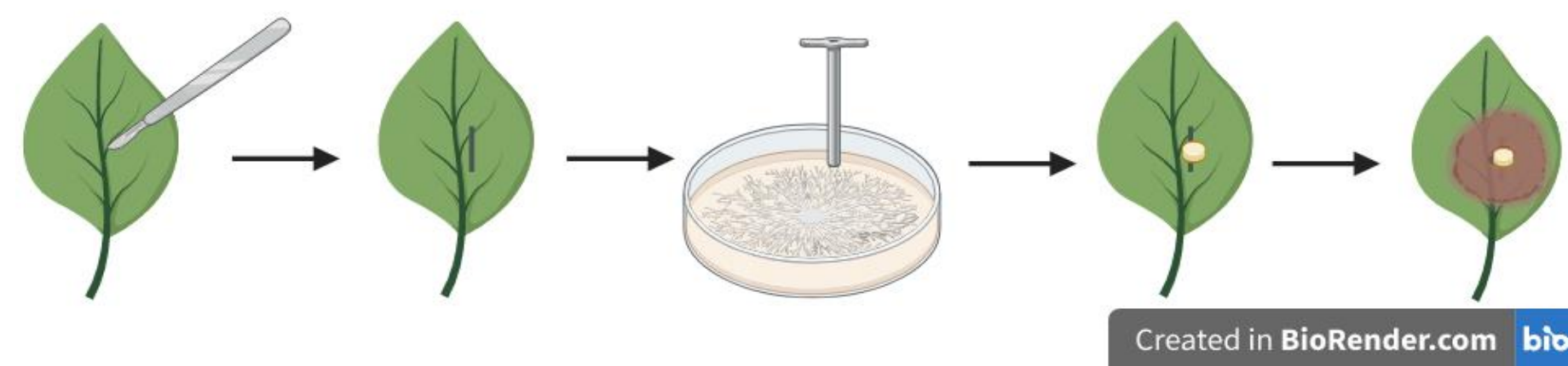
Methods

Aggressiveness Assay:

Figure 1: Aggressiveness assay experimental overview. For Each WA isolate, five Rhododendron leaves were wounded before being inoculated with actively growing mycelium and incubated for 10 days.

For WA isolates, rhododendron leaves were inoculated with *P. ramorum* and incubated (figure 1). Post incubation, the lesion area was measured using ASSESS software (Elliott 2015), and statistical analysis was done via R version 4.3.0 (R Core Team. 2023).
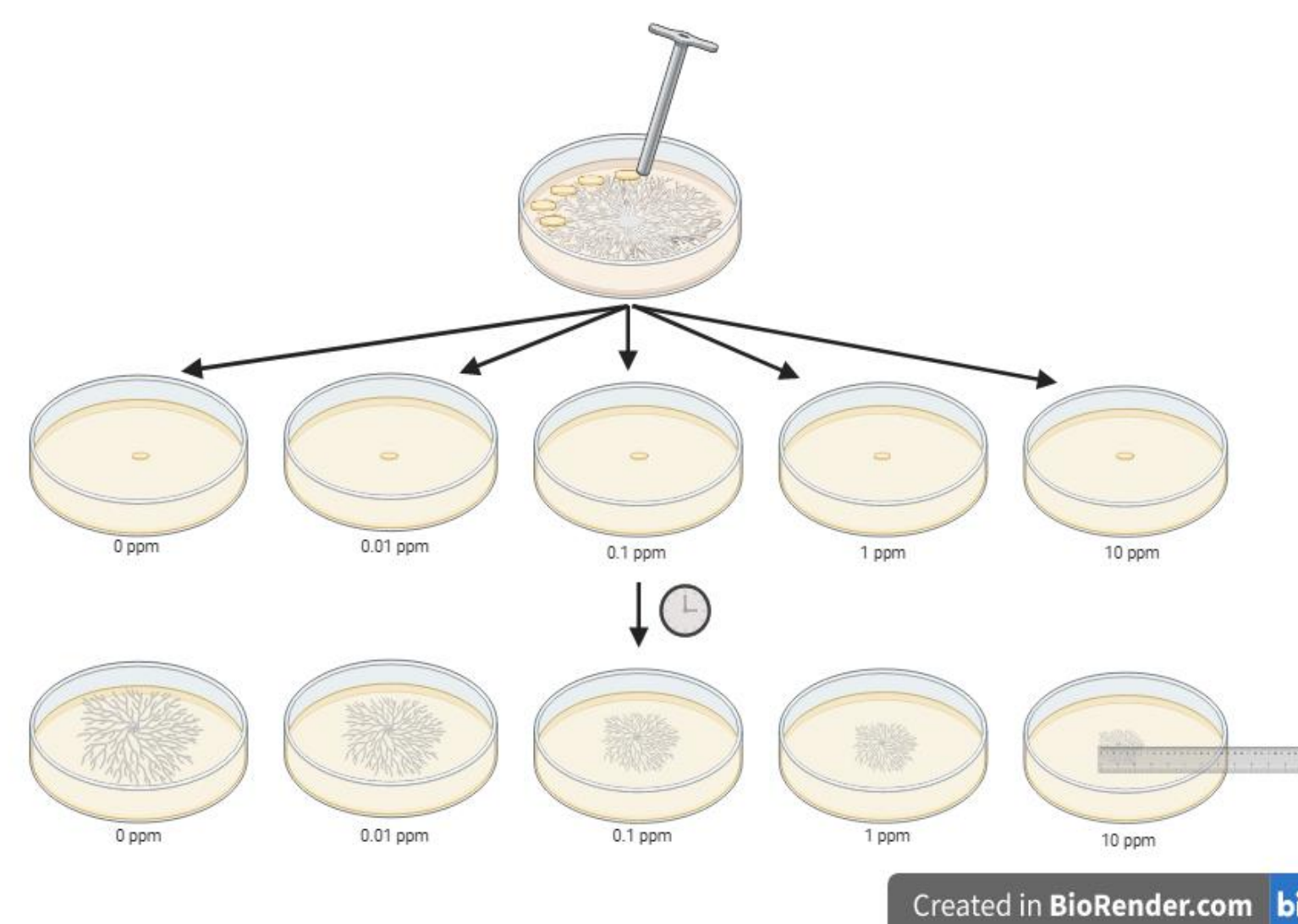
Fungicide Resistance Testing:

Figure 2: Fungicide resistance experimental overview. Actively growing mycelium from each isolate was subcultured onto V8A containing 0, 0.01, 0.1, 1, and 10 ppm Metalaxyl-M. Radial growth of mycelium was measured after 5 and 10 days of incubation.

P. ramourm isolates were plated onto fungicide-amended media and radial growth was measured (figure 2). Statistical analysis was done using R version 4.3.0 (R Core Team 2023). R package "ec50estimator" (Alves. 2020) was used for EC50 calculations
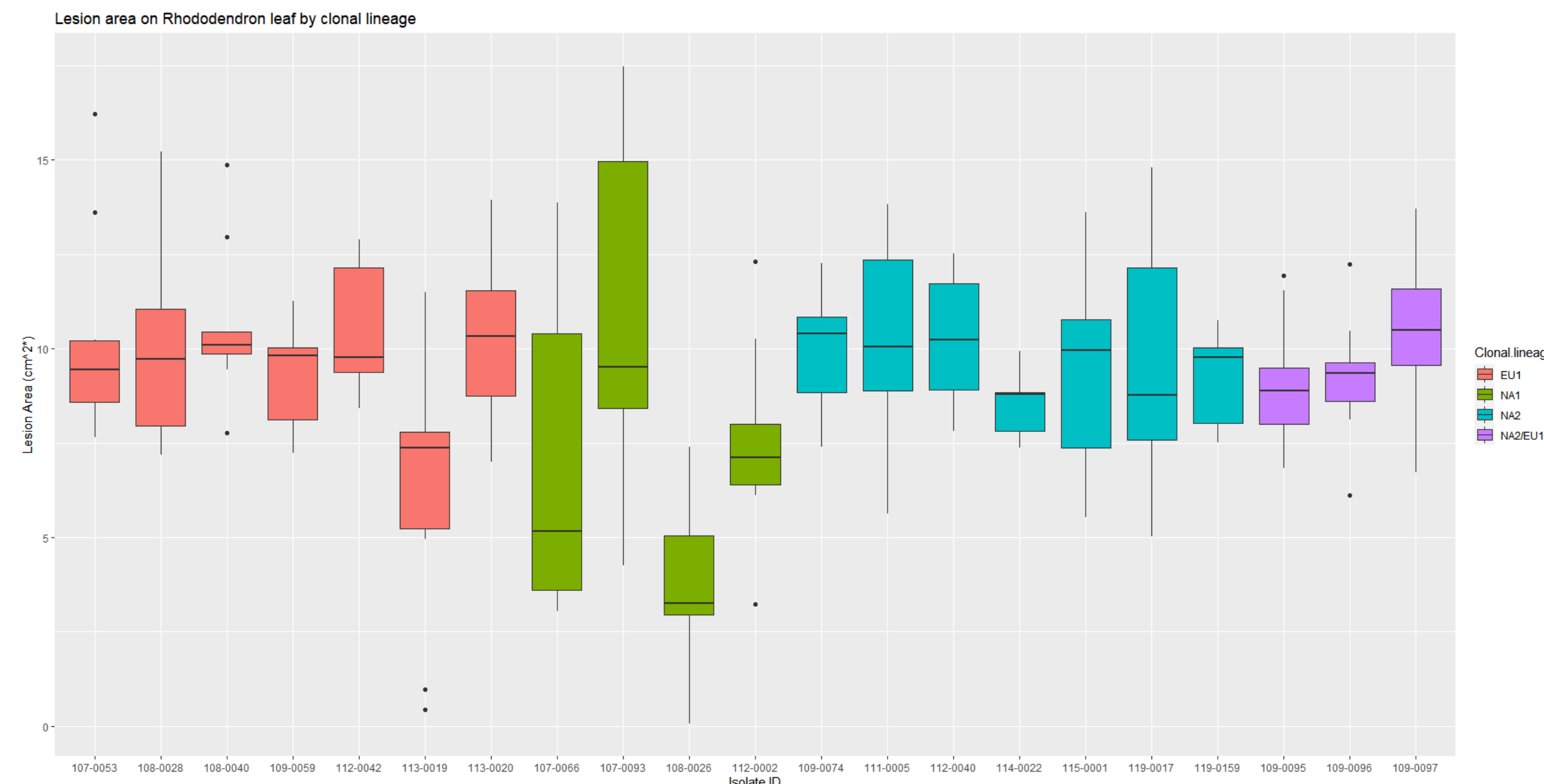
Results

Aggressiveness Assay:

Figure 3: Boxplot of ANOVA results on WA aggressiveness assay results. Each boxplot represents the lesion area range of each isolate. Isolate IDs are on the x-axis, and lesion area in cm^2 is on the y-axis. Isolate IDs are grouped by associated clonal lineage: Red boxes represent EU1, Green boxes represent NA1, Blue boxes represent NA2, and purple boxes represent EU1-NA2 hybrids. Graph generated in R version 4.3.0 (R Core Team. 2023). Significant differences were found in the lesion area by isolates (p=0) and lineage (p=0). Additionally, Significant differences were found in lesion area between NA1 and EU1 (p=0.0003), NA1 and NA2 (p=0.0002), and NA1 and EU1-NA2 hybrids (p=0.006)

Fungicide Resistance:

Clonal Lineage	Origin	Isolate ID	EC50	Clonal Lineage	Origin	Isolate ID	EC50	Clonal Lineage	Origin	Isolate ID	EC50
EU1	WA	107-0053	0.0002	EU1	BC	122-0042	0.0034	NA2	WA	119-0017	0.0309
EU1	WA	108-0028	0.0023	EU1	BC	122-0043	<0.01	NA2	WA	119-0159	0.0064
EU1	WA	108-0040	0.7062	EU1	BC	122-0047	0.0060	NA2	BC	122-0035	0.0034
EU1	WA	109-0059	0.0016	EU1	BC	122-0049	0.0092	NA2	BC	122-0044	0.0046
EU1	WA	112-0042	0.9689	NA1	WA	107-0093	0.0126	NA2	BC	122-0048	0.0095
EU1	WA	113-0019	0.0043	NA1	WA	108-0021	0.0037	EU1-NA2	WA	109-0096	0.0090
EU1	WA	113-0020	<0.01	NA1	WA	108-0026	0.7062	EU1-NA2	WA	109-0097	0.0057
EU1	BC	122-0032	0.0029	NA1	WA	112-0002	0.0193	EU1-NA2	BC	122-0033	0.4609
EU1	BC	122-0036	<0.01	NA2	WA	111-0005	0.0074	EU1-NA2	BC	122-0034	0.8186
EU1	BC	122-0038	0.0019	NA2	WA	112-0040	0.0111	EU1-UK3	BC	122-0037	<0.01
EU1	BC	122-0040	0.0044	NA2	WA	114-0022	0.0121	EU1-UK3	BC	122-0039	<0.01
EU1	BC	122-0041	0.0004	NA2	WA	115-0001	0.0106				

Table 1: EC50 values for WA and BC isolates against Metalaxyl-M. EC50 values of "<0.01" indicate that the EC50 values were too low to calculate effectively. Where EC50 values are indicated as "N/A" EC50 values could not be estimated. Five isolates showed EC50 values exceeding 0.1 ppm against Metalaxyl-M. No significant differences were found between EC50 values by clonal lineage.

Cluster analysis:

Sample ID	Clonal Lineage	Nursery	Lesion area cm^2	EC50	Cluster
107-0053	EU1	N35	10.237	2E-04	1
108-0028	EU1	N40	9.977	0.002	1
109-0059	EU1	N43	9.304	0.002	1
107-0093	NA1	N35	11.073	0.013	1
111-0005	NA2	N39	10.207	0.007	1
112-0040	NA2	N26	10.272	0.011	1
114-0022	NA2	N47	8.554	0.012	1
115-0001	NA2	N47	9.484	0.011	1
119-0017	NA2	N54	9.815	0.031	1
119-0159	NA2	N32	9.164	0.006	1
109-0096	NA2/EU1	N45	9.21	0.009	1
109-0097	NA2/EU1	N45	10.443	0.006	1
108-0026	NA1	N44	3.852	0.706	2
113-0019	EU1	N1	6.265	0.004	3
112-0002	NA1	CA	7.517	0.019	3
108-0040	EU1	TF41	10.591	0.706	4
112-0042	EU1	N41	10.509	0.969	4

Table 2: Table of preliminary cluster analysis results. Only WA isolates with definitive lesion area and fungicide resistance data were used. Lesion area and EC50 data were standardized in R version 4.3.0 (R Core Team. 2023) prior to running cluster analysis. "TF41" indicates that the sample came from a landscaping site that had plants from N41.
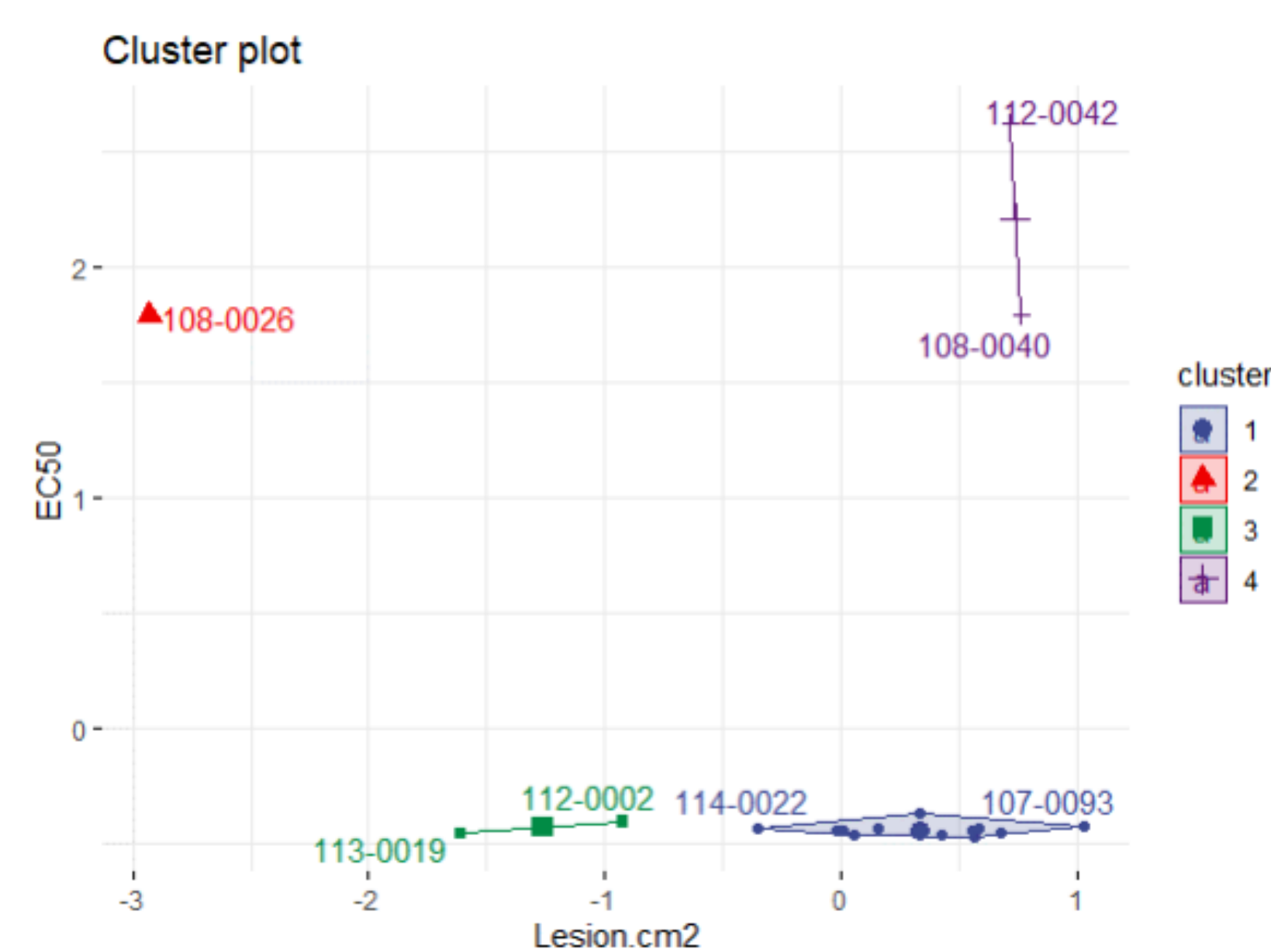

Figure 4: Preliminary cluster analysis comparing Metalaxyl-M EC50 values and lesion area data for WA. Plot generated used data from table 2. Graph created using R version 4.3.0 (R Core Team. 2023).

Conclusions

- Within WA isolates, the NA1 lineage showed the most variance in lesion area compared to the other lineages studied, indicating that this lineage displays the widest range of aggressiveness of the studied lineages

- Five of the isolates used in this study showed an EC50 value greater or equal to 0.1 ppm as opposed to the 0.01 ppm standard. No significant differences in EC50 values were found across the different lineages of *P. ramorum,* likely due to high variability within each lineage. This preliminary data suggests that *P. ramorum* is developing a resistance to Metalaxyl-M, however, clonal lineage is not a determining factor in this resistance development.

- Both isolates with the highest aggressiveness and Metalaxyl-M resistance originated from the same nursery location (table 2).

Future Directions

- Further analysis into isolates with EC50s too low to calculate is needed in order to generate accurate EC50 values. Having accurate EC50 calculations will allow for more accurate testing of variance between clonal lineages.

- Fungicide resistance tests using Dimethomorph, Mandipropamid, and Fosetyl-Al fungicides to further test for fungicide resistance within clonal lineages

- Once EC50 values for all isolates against all fungicides have been determined, further cluster analysis can be performed to compare fungicide resistance and aggression of *P. ramorum* clonal lineages.

References