The Effect of Black Walnut Phenolics on *Geosmithia morbida* growth

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Introduction

Thousand Canker's Disease

- Black walnut trees are some of the most economically important hardwood trees
- The walnut twig beetle *P. juglandis* infests the trees and introduces its associated fungus *G. morbida*
- This causes thousand cankers disease (TCD) which kills phloem tissue within the tree and cause death and undesirable wood staining

Phenolics

- Phenolics are used by plants to chemically defend themselves against many things including diseases
- Phenolic profiles can differ between trees undergoing stress and between genotypes

Goal

Determine if the phenolic extracts of 6 genotypes of black walnut can alter the growth of *G. morbida* on augmented plates

Figure 1. (left) *P. juglandis* specimen, **(right)** walnut tree affected by thousand cankers disease

Methods and Materials

Tree tissue harvesting

- Trees (n=68) were grown in a greenhouse and half were droughted for a period of ~1 month
  - 6 HTIRC genotypes (8,9,11,12,14,16)
- All trees were then inoculated with *G. morbida*
- Droughted trees were returned to regular watering schedule
- After 1 month, bark tissue samples were harvested and freeze dried

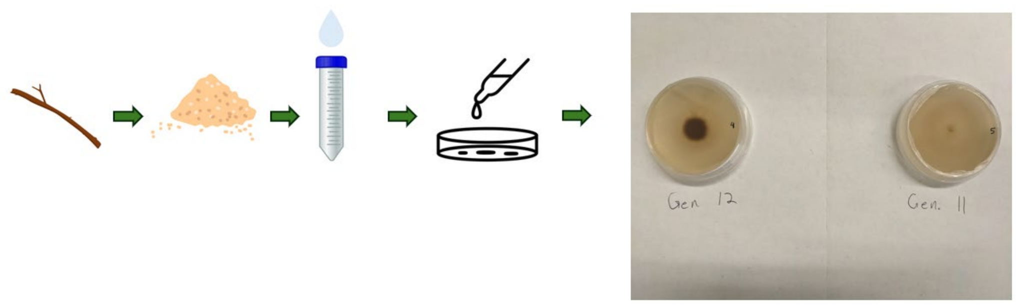


Figure 2. (left) tree wounded and inoculated with *G. morbida*. **(above)** extraction and plate inoculation procedure

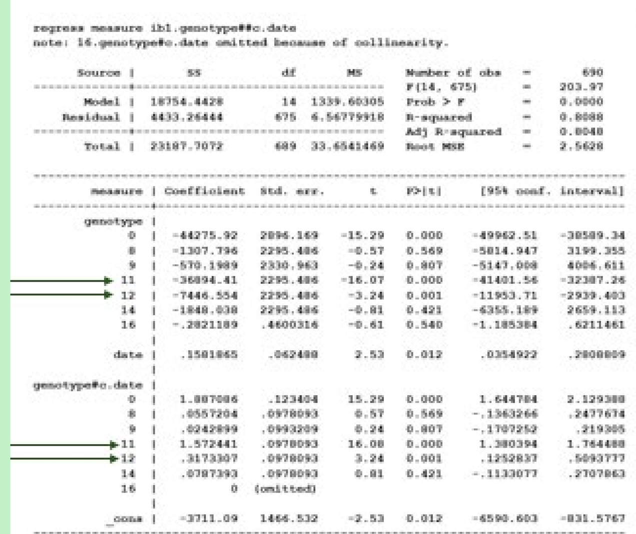
Phenolic extraction and plate inoculation

- Bark was peeled from dried samples and ground in a 60-gauge Wiley mill
- Phenolics extracted from bark using 95% MeOH
- Extracts added to ¼ strength PDA at 2% and plates inoculated
- Controls
  - Genotype 0= ¼ strength PDA
  - Genotype 1= ¼ strength PDA + 95%MeOH
- 2-week growth period with 5 measurement time points
  - Colonies scanned on flatbed scanner and area measured using ImageJ

Statistical analysis

- Used STATA version 18
  - Removed genotype 11 in one analysis due to potential errors in inoculation technique*
  - Ran 2 linear regressions predicting outcome area measurement
    - Genotype and date interaction
      - Compare growth as well as growth rates
    - Watered vs droughted, controlling for date

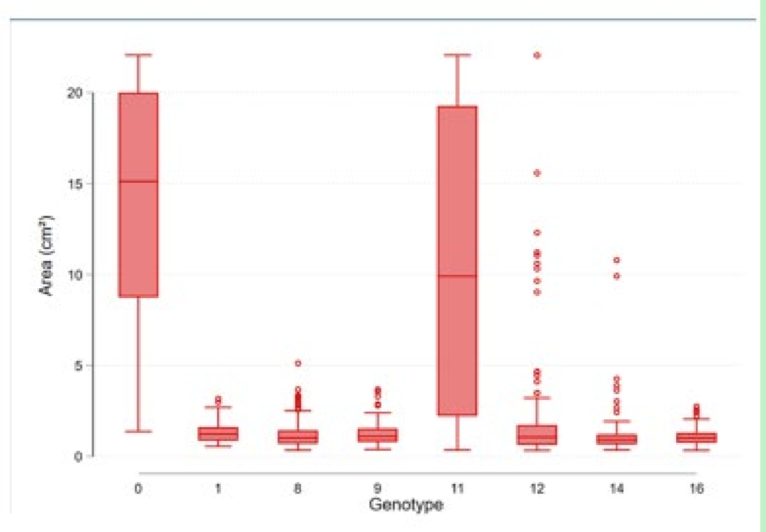
Results



regress measure ib1.genotype##c.date
note: 16.genotype#c.date omitted because of collinearity.

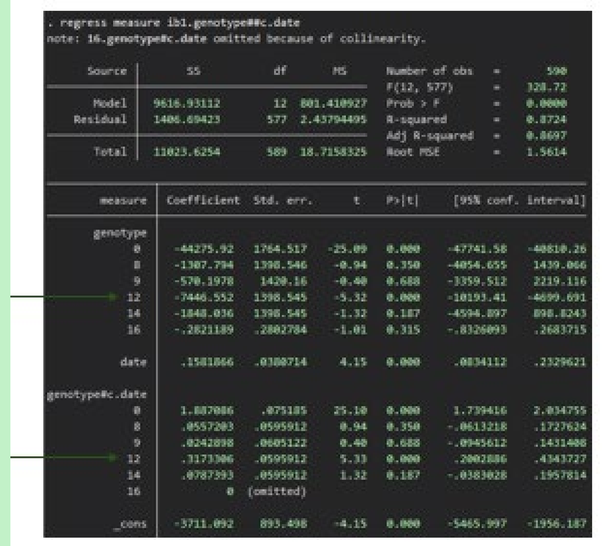
Source	SS	df	MS			
Model	18754.4428	14	1339.60305	Number of obs	=	690
Residual	4433.26444	675	6.56779918	F(14, 675)	=	203.97
				Prob > F	=	0.0000
				R-squared	=	0.8088
				Adj R-squared	=	0.8048
Total	23187.7072	689	33.6541469	Root MSE	=	2.5628

measure	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
genotype						
0	-44275.92	2896.169	-15.29	0.000	-49962.51	-38589.34
8	-1307.796	2295.486	-0.57	0.569	-5814.947	3199.355
9	-570.1989	2330.963	-0.24	0.807	-5147.008	4006.611
11	-36894.41	2295.486	-16.07	0.000	-41401.56	-32387.26
12	-7446.554	2295.486	-3.24	0.001	-11953.71	-2939.403
14	-1848.038	2295.486	-0.81	0.421	-6355.189	2659.113
16	-.2821189	.4600316	-0.61	0.540	-1.185384	.6211461
date	.1581865	.062488	2.53	0.012	.0354922	.2808809
genotype#c.date						
0	1.887086	.123404	15.29	0.000	1.644784	2.129388
8	.0557204	.0978093	0.57	0.569	-.1363266	.2477674
9	.0242899	.0993209	0.24	0.807	-.1707252	.219305
11	1.572441	.0978093	16.08	0.000	1.380394	1.764488
12	.3173307	.0978093	3.24	0.001	.1252837	.5093777
14	.0787393	.0978093	0.81	0.421	-.1133077	.2707863
16	0	(omitted)				
_cons	-3711.09	1466.532	-2.53	0.012	-6590.603	-831.5767



Results 1. (left) linear regression output for analysis including genotype 11. **(right)** overall area measure of fungal growth by genotype.

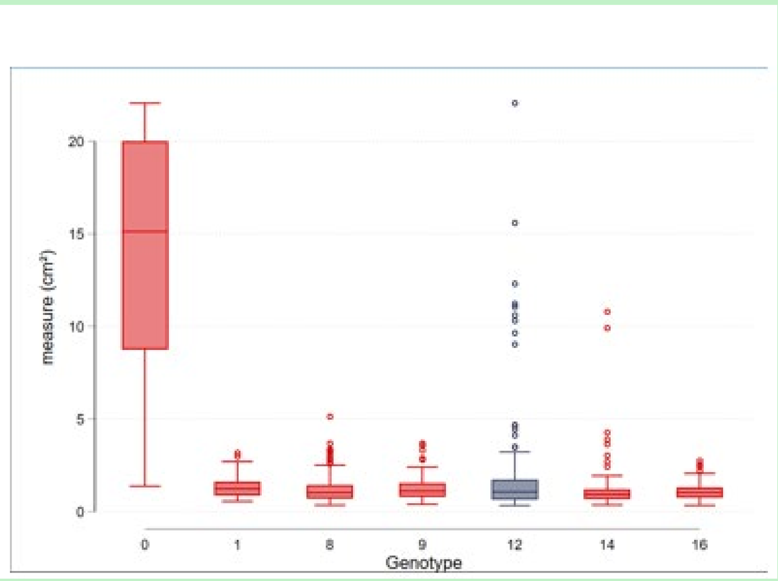
Results 2. (left) linear regression output for analysis excluding genotype 11. **(right)** overall area measure of fungal growth by genotype, with the significant genotype 12 highlighted

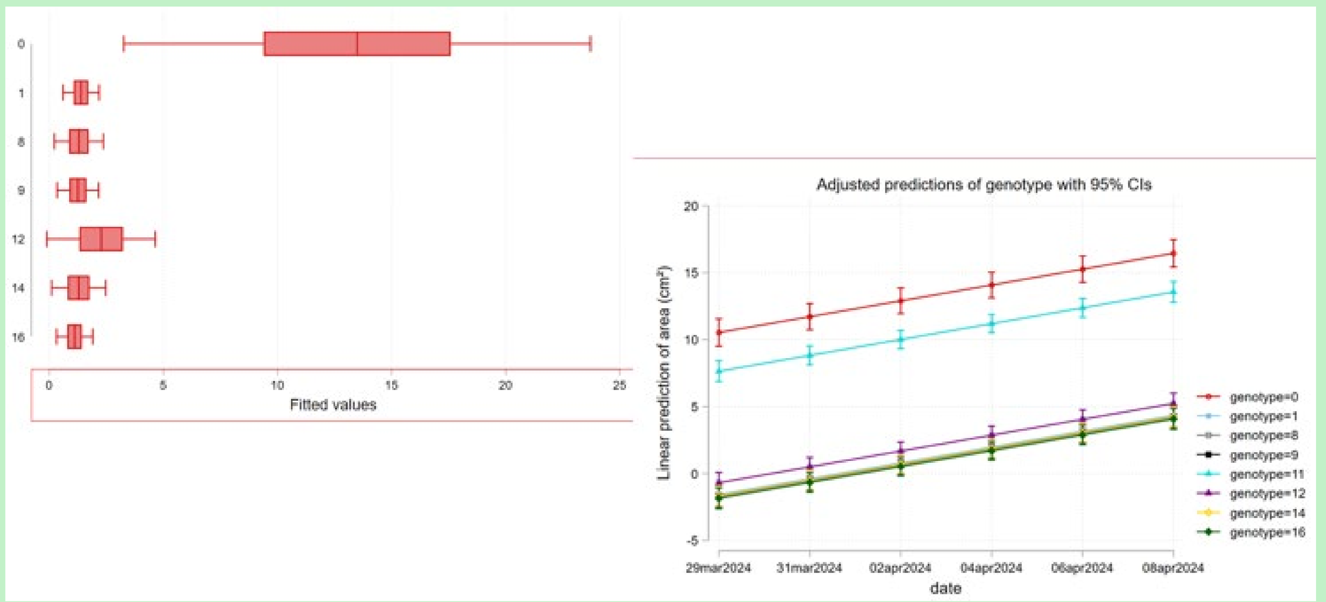


. regress measure ib1.genotype##c.date
note: 16.genotype#c.date omitted because of collinearity.

Source	SS	df	MS			
Model	9616.93112	12	801.410927	Number of obs	=	590
Residual	1406.69423	577	2.43794495	F(12, 577)	=	328.72
				Prob > F	=	0.0000
				R-squared	=	0.8724
				Adj R-squared	=	0.8697
Total	11023.6254	589	18.7158325	Root MSE	=	1.5614

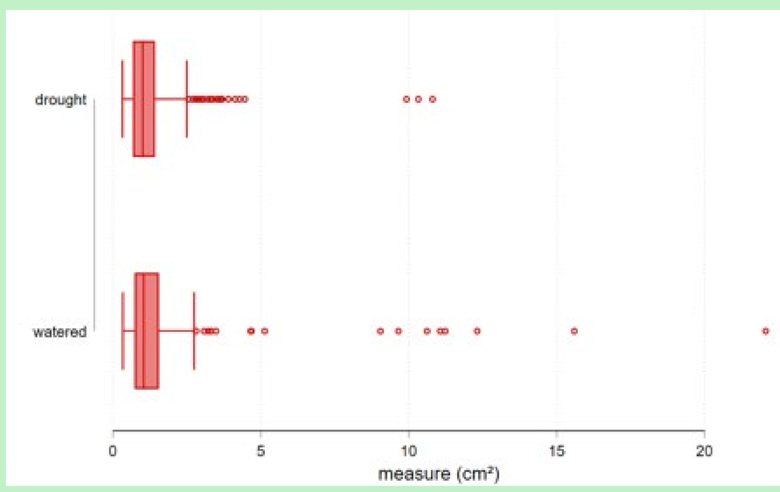
measure	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
genotype						
0	-44275.92	1764.517	-25.09	0.000	-47741.58	-40810.26
8	-1307.794	1398.546	-0.94	0.350	-4054.655	1439.066
9	-570.1978	1420.16	-0.40	0.688	-3359.512	2219.116
12	-7446.552	1398.545	-5.32	0.000	-10193.41	-4699.691
14	-1848.036	1398.545	-1.32	0.187	-4594.897	898.8243
16	-.2821189	.2802784	-1.01	0.315	-.8326093	.2683715
date	.1581866	.0380714	4.15	0.000	.0834112	.2329621
genotype#c.date						
0	1.887086	.075185	25.10	0.000	1.739416	2.034755
8	.0557203	.0595912	0.94	0.350	-.0613218	.1727624
9	.0242898	.0605122	0.40	0.688	-.0945612	.1431408
12	.3173306	.0595912	5.33	0.000	.2002886	.4343727
14	.0787393	.0595912	1.32	0.187	-.0383028	.1957814
16	0	(omitted)				
_cons	-3711.092	893.498	-4.15	0.000	-5465.997	-1956.187

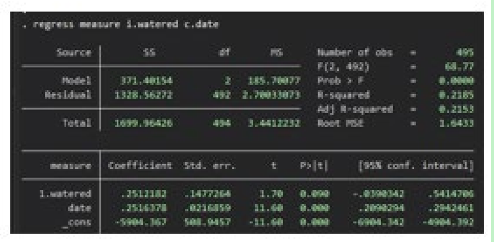




Results 3. (left) Predicted values of fungal growth area by genotype. **(right)** predictions for growth rate over time by genotype

Results 4. (left) comparison of overall drought vs well-watered fungal area measure. **(right)** linear regression for drought status and area measure

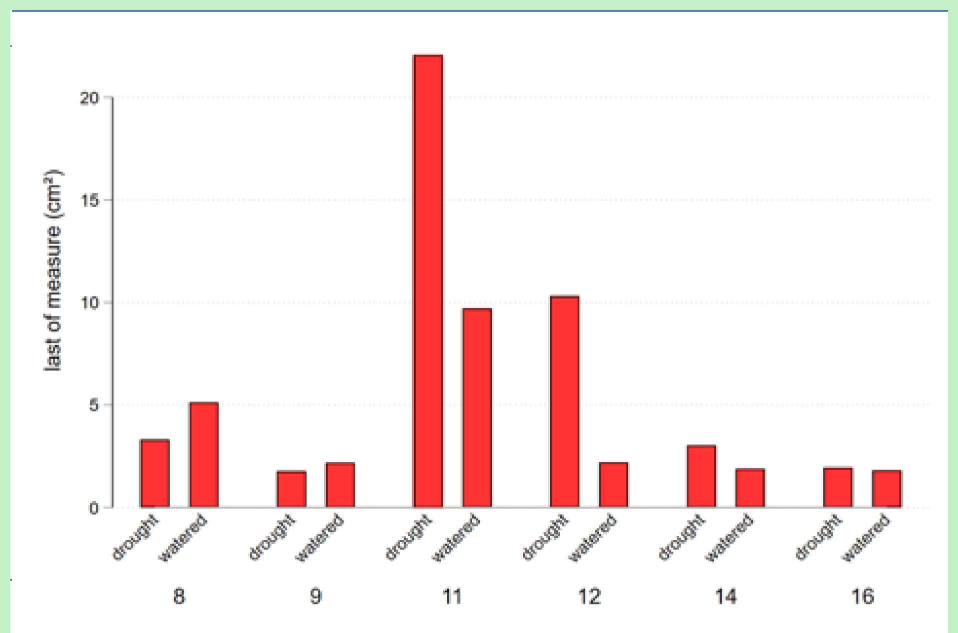




. regress measure i.watered c.date

Source	SS	df	MS			
Model	371.40154	2	185.70077	Number of obs	=	495
Residual	1328.56272	492	2.70033073	F(2, 492)	=	68.77
				Prob > F	=	0.0000
				R-squared	=	0.2185
				Adj R-squared	=	0.2153
Total	1699.96426	494	3.4412232	Root MSE	=	1.6433

measure	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
1.watered	.2512182	.1477264	1.70	0.090	-.0390342	.5414706
date	.2516378	.0216859	11.60	0.000	.2090294	.2942461
_cons	-5904.367	508.9457	-11.60	0.000	-6904.342	-4904.392



Results 5. Final area measure of each genotype by watering status

Discussion

- Genotype 11 was significantly different from the rest but must be excluded in case of technical error suggested by its close similarities to the control and its phenotypic differences
- Genotype 12 produced a significant decrease in fungal growth when compared to MeOH when including date (p<0.00), potentially suggesting resistance due to phenolics
- Genotype 12 also grew at a slightly faster pace than the MeOH controls
- Droughted tree phenolics were not significantly associated with changes in growth when compared to watered trees, controlling for the date.
  - Likely due to chance as literature on the effects of drought on tree phenolics is well established.
  - Could also be due to return to watered status
  - Fungal growth area between drought and well watered trees was inconsistent in genotypes, with some having more growth in watered than droughted

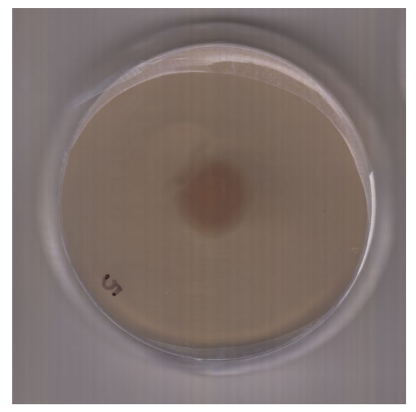


Figure 3. (left) final scan of genotype 16 Drought showing typical growth pattern **(right)** final scan of genotype 11 Drought showing atypical growth morphology

Conclusions

- Based on these findings, growers may consider growing and cross-breeding genotype 12 trees in areas susceptible to TCD
- Much future research is needed to further support this research including but not limited to the following projects:
  - Re-plate all genotypes so that further analysis can be run to account for morphological differences in the fungal growth on genotype 11 plates
  - Quantify the phenolics present in each tree genotype
  - Compare results to previous work measuring the canker sizes in same trees

References

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