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VARIANCE IN TOLERANCE OF DIFFERENT FAMILIES OF LOBLOLLY PINE (*PINUS TAEDA* L.) TO *GROSAMANNIA HUNTII* AND *LEPTOGRAPHIUM TEREBRANTIS*

by
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INTRODUCTION

The southern United States constitutes about 140 billion cubic feet of standing volume of softwood among which pine is a major species. Loblolly pine (*Pinus taeda* L.) is the leading pine species which comprises 50% of the total softwood volume grown in the south (Schultz, 1997; Oswalt et al, 2014). Approximately, one billion seedlings are planted each year (McNabb and Enebak 2008). Thus, loblolly pine plantations have ascendancy over the southern terrain contributing the considerable portion of economy. However, over the past 40 years declining stands of pine have been reported in the south.

Southern pine decline is characterized by short chlorotic needles, sparse crowns, reduced radial growth and premature mortality (Brown & McDowell, 1968). This decline was first observed in loblolly pine in the Oakmulgee Ranger District, Talladega National Forest in 1959 (Brown & McDowell, 1968). Later, several ophiostomatoid fungi were found to be associated with pine decline (Hess et al, 1999; Hess et al, 2002). Root feeding bark beetles like *Hylastes pales*, *Pachylovorus picivorus* and *P nemorensis*, *Hylastes tenuis* and *Dendroctonus terebrantis* were captured from declining stands of loblolly pine. The ophiostomatoid fungi like *Leptographium terebrantis* S.J. Barras &T. J Perry, *Grosmannia huntii* R.C. Rob. Jeffr, *L. procerum* Kendrick M.J. Wingfield and *Grosmannia alacris* T.A. Duong, Z.W. de Beer & M.J Wingfield were isolated from these beetles (Eckhardt et al, 2007).

Study of virulence of these root-infecting ophiostomatoid fungi have been performed in four species of pine and results showed that *G. huntii* is the most virulent (Matusick and Eckhardt 2010). A study of variation in resistance of loblolly pine families to these fungi has been performed in some loblolly pine families and resulted in a wide range of host response (Singh et al, 2014). While we are on the verge of how pine decline can be addressed, it would be appropriate to screen many more families and understand which families of loblolly pine are susceptible and which are tolerant to these fungi associated with pine decline. The objective of this study will be to determine whether there is variation in tolerance of previously unscreened loblolly pine families to two major fungi associated with pine decline. Also, it will help to evaluate the level of susceptibility of these commonly out planted loblolly pine families in the southern United States.

METHODOLOGY

Experimental Design

Artificial inoculation experiments were conducted on loblolly pine seedlings in an outdoor research facility of the Forest Health Dynamics Laboratory, Auburn University. The study was conducted in 33 and 38 different container grown families of loblolly pine in year 2013 and 2014 respectively. However in 2014, four bare root families which were in common to four of the container grown families were also included. These loblolly pine families were chosen as per the most commonly out planted families in the southern United States. Seeds of all families were collected and grown in containers in a forest company nursery for both of the years. After 9 month, the seedlings were properly lifted with intact root and growing medium from individual containers for both the years. Also, 4 bare root families for 2014 were grown in a common nursery bed and seedlings were lifted with their root ball. Each year, each seedling was then planted in trade-gallon pot filled with ProMIx BX® (Premier Tech, Quebec, and Canada) peat-based potting media. The seedlings had a mean stem height of 30 cm and root collar diameter of 4.5 mm. The experimental setup was a randomized complete block design with six blocks/replications. Fungal treatment and family interaction was maintained random. The seedlings were allowed to acclimatize in natural environmental condition for 2 months before inoculation experiment in their respective blocks.

Inoculation of Fungi

Grosamannia huntii (Isolate no. LLP-R-02-100) and *Leptographium terebrantis* (Isolate no. LOB-R-00-805) grown from single spore in asexual stage were used for the inoculation. These fungi were isolated from the primary lateral roots of declining and symptomatic loblolly pine. These fungi were sub-cultured in Malt Extract Agar, 2 weeks before the inoculation date. Then the fungus was inoculated in the root collar area of the seedling 2 cm above the soil line by making a sterile razor cut. A 3mm medium plug with growing fungal mycelium was inoculated in the wound. The wound was wrapped up with the moist cotton balls and sealed with Parafilm®. Initial height and root-collar diameter were measured before the inoculation in all the seedlings.

Lesion Measurement and Fungal Re-isolation

For each year, 52 days after the inoculation, seedlings were destructively harvested and taken to the Forest Health Dynamics Laboratory. Height and root-collar diameter of the seedlings were measured before the harvest. Following harvesting, seedlings were dipped in a solution of FastGreen stain (FastGreen FCF; Sigma Chemical Co., St. Louis, MO, USA) and water in the concentration of 0.25 g/liter and allowed 3 days of capillary action. Then on each seedling the lesion length, lesion width, lesion depth, occlusion length, occlusion depth and occlusion width was measured. Lesions consisted of a dark brown dead tissue section upward and downward from the inoculation points. Occlusion was the blocked vascular tissue lacking capillary action to allow dye to pass through it. To verify the Koch's Postulates, the stem section one centimeter surrounding the lesion was cut and plated in Malt Extract Agar amended with 800mg/l of cyclohexamide and 200 mg/l of streptomycin sulphate. Plates were incubated at room temperature for 14 days.

Statistical Analyses

Mixed-models with family and treatment interaction as random and fungal treatment as fixed effect was performed in SAS 9.4 version using proc-mixed procedure. Graphs were made produced in

STATISTICA 10. Multiple comparison tests were performed using Tukey-Kramer test at a 5% significance level. Due to the large sample size, data was assumed to be normal as explained by the central limit theorem.

RESULTS

Year 2013

Both the fungal treatments caused dark brown lesions in all of the families tested. The fungal re-isolation was 98% to 96% and verified Koch's Postulates. Seedling survival was significantly different among the families (Chi-sq=68.36, *p*<0.0001) and among the treatments (Chi-sq=1419.86, *p*<0.0001).

The length of lesions produced by the wound and wound plus media was significantly shorter than that caused by the fungal treatment. Therefore, the effect of the two fungal treatments were included and the effect of the control were removed from the model. **Figure 1** shows the average lesion length caused by both treatments on the families. There also was radial movement of the lesion was measured as lesion width. Among the two fungi included in the study, *L. terebrantis* caused the longest lesion length. The lesion length produced by *L. terebrantis* was significantly higher than that caused by *G. huntii* (*p*<0.0001) as given in **Table S1**. Similarly, blockage of the vascular tissue (occlusion) also was observed. There was both vertical as well as radial movement of the occlusion. The occlusion length produced by *L. terebrantis* was significantly higher than that caused by *G. huntii* (*p*<0.0001).

Covariance parameter estimates showed that lesion length for families tested were significantly different from zero (Z=0.02). The average overall lesion length and those caused by *G. huntii* and *L. terebrantis* is shown in **Figure 1**, **2,** and **3** respectively. Lesion width was not significantly different from zero (Z=0.19). Similarly, occlusion length (Z=0.35) and occlusion width (Z=0.47) was not significantly different from zero.

The length, width and depth of lesions were found to be affected by treatments as shown by type three fixed effects. A family x treatment interaction was not found to be significant (*p*=0.07) which indicates that an overall ranking of the families can be done on the basis of lesion length as shown in **Table S4**.

Year 2014

In 2014, both the fungi caused dark brown necrotic lesions in all of the seedlings tested. Fungal re-isolation rate was 62% to 82% and verified Koch's postulate. Seedling survival was significantly different among the families (Chi-sq=188.32, *p*<0.0001) but not among the fungal treatments (Chi-sq=4.29, *p*=0.2321).

Similar to year 2013, length of lesion caused by both fungal treatments was significantly longer than those caused by the control treatments as shown by pairwise comparisons test. The effects of both the controls were removed and only the effects of the fungal treatments were included in the model. *Grossmania huntii* produced significantly longer lesion length than *L. terebrantis*

($p<0.0001$). Similarly, length of occlusion caused by *G. huntii* was significantly longer than that caused by *L. terebrantis* ($p<0.0001$).

Lesion length and occlusion length was significantly different among the families as indicated by covariance parameter estimates. However, family x treatment interaction was not found to be statistically significant for both lesion length and occlusion length. Lesion and occlusion length, depth and width were found to be affected by fungal treatments ($p<0.0001$).

Tukey's multiple pairwise comparisons showed that there was no significant difference in lesion length between the bare-root and container grown connector families when treated with both *L. terebrantis* and *G. hunti* as shown in **Figure 4** and **5** respectively. Although, difference was seen among the different families, none of the same two families had significantly different lesion length as shown in **Figure 4** and **5**.

DISCUSSION

The first objective of this study was to scrutinize whether there is variability on tolerance of previously unscreened families of loblolly pine to ophiostomatoid fungi associated with southern pine decline. Our results indicated that there is variance in the tolerance level of different families towards these fungi. Mean lesion length varied significantly among the families but not within the fungal treatments. Even though, all of these genotypes have been planted throughout the southern United States, it is unambiguous from our result that some are more susceptible and some are less susceptible to these fungi. While the screening for tolerance of these families has been conducted in either containerized or bare root families (Singh et al, 2014), this study tried to evaluate the difference in results when using connector bareroot and container grown families in the screening. Our results indicated that there is no significant difference in the lesion length caused by ophiostomatoid fungi within the same bareroot and container grown families. This indicates that lesion length caused by the fungi is similar in seedlings grown by either method.

Among the two fungi tested in the study, *L. terebrantis* and *G. huntii* were found to be more virulent in year 2013 and 2014 respectively as indicated by longer lesion and occlusion length. The results from year 2014 are supported by a previous study done to determine the relative virulence of ophiostomatoid fungi on three pine species. *Grosmannia huntii* was found to be the most virulent species causing the longest lesions in all of the species tested (Matusick and Eckhardt 2010). According to Weather Underground[1], in January 2013 (when seedlings were potted), Auburn Alabama had lowest and and monthly average temperature of 30 ºF and 54 ºF respectively. Whereas, in January 2014, the lowest and average temperature was 9 ºF and 37 ºF respectively which was very low compared to 2013. In addition, seedlings were hit by the winter storm on January 28. The minimum dew point of the month was -9 ºF. Thus, there was a huge weather variation between 2013 and 2014. Therefore, the differences seen between years might be due to family and environment interaction.

Significant differences in lesion length caused by the fungal species were observed among families in both of the years. In year 2013, family L73 had the shortest lesions and families L68 and L66 had the longer lesions when treated with *L. terebrantis*. Whereas, families L51 and L73 had the

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[1] http://www.wunderground.com

shortest lesions and L55, L66 and L67 had the longer lesions when treated with *G. huntii*. In year 2014, families L108 and L99 had shorter lesions and L81 and L91 had longest lesions when treated with *L. terebrantis*. Whereas, L86 and L108 had the shortest lesions and L88 and L91 had the longer lesions when treated by *G. huntii*.

In conclusion, there is variation in susceptibility or tolerance of loblolly families to fungi associated with pine decline. The primary reason for the variation is the family. Since, the tolerance of the families were tested at the premature stage in this study, a separate study is being performed on mature loblolly pine families. Preliminary data suggests that screening families for pine decline associated fungi at the premature and mature stage give similar results (Devkota and Eckhardt 2015). In summary, family differences exist and specific families can perform better in pine decline risk sites.

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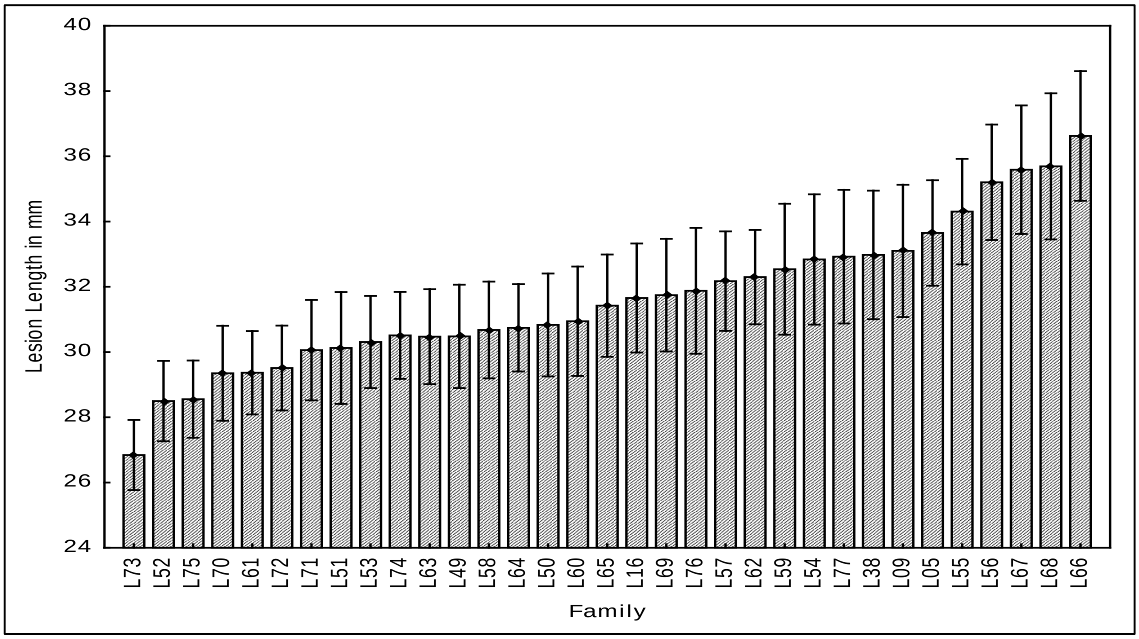

Figure 1. Overall mean lesion length caused by the both the fungal treatments. Current effect: $F_{(32, 2626)}=1.9065$, $p=.00164$

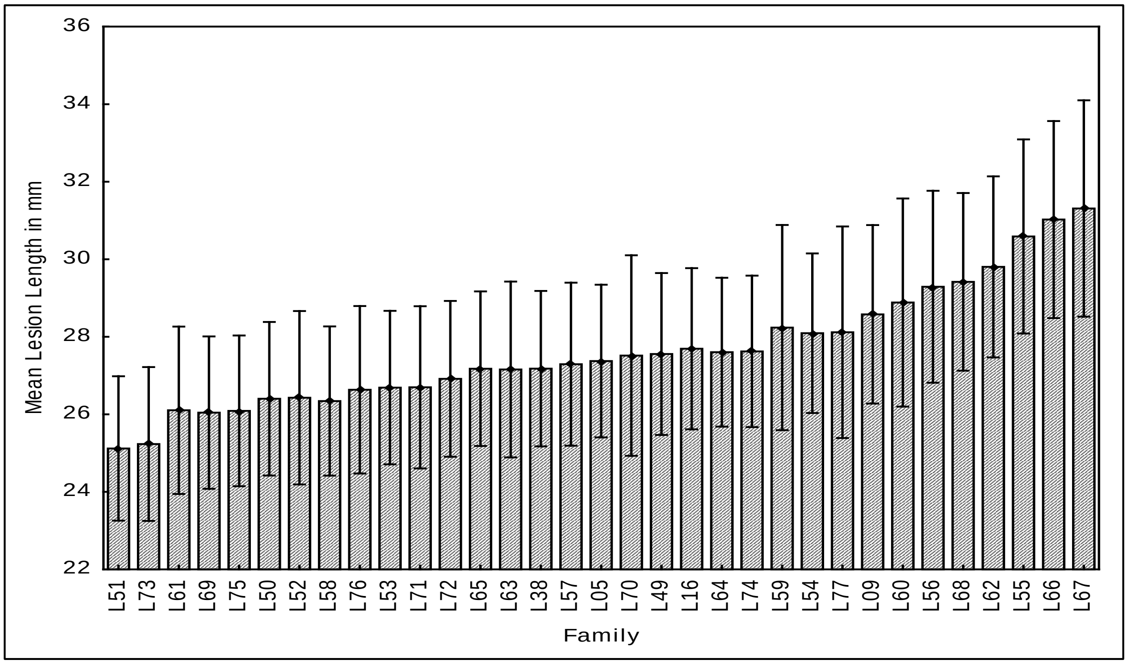

Figure 2. Mean lesion length caused by *G. huntii* in different families Current effect: $F_{(32, 1351)}=.48569$, $p=.99333$

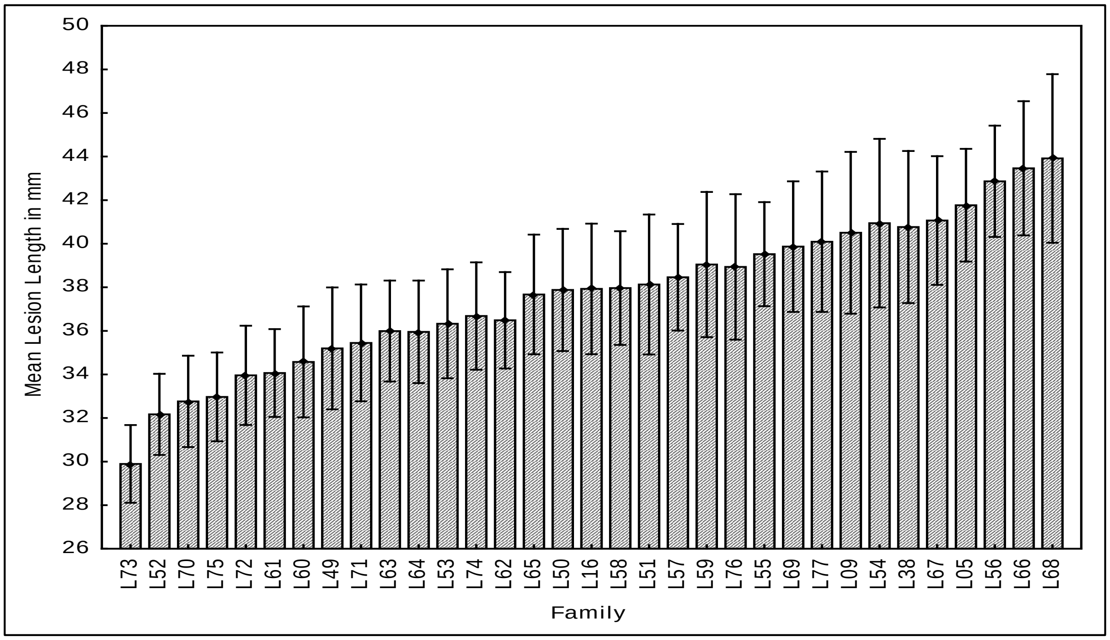

Figure 3. Mean lesion length caused by *L. terebrantis* in different families. Current effect:F $(_{32, 1275})$=1.6162, *p*=.01668

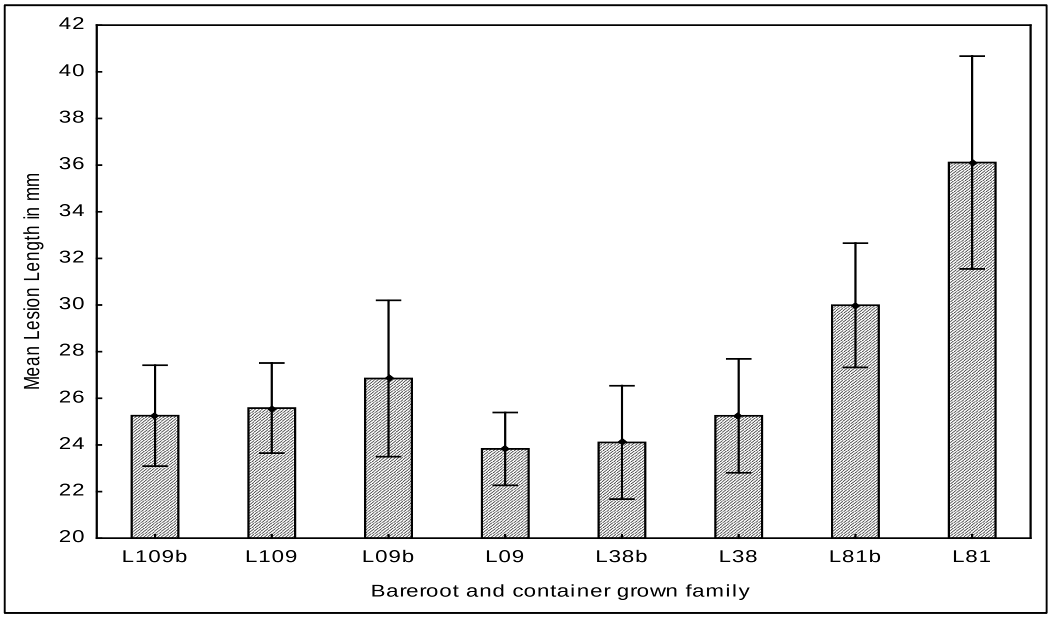

Figure 4. Mean lesion length caused by *L. terebrantis* on bare root and container grown connector families. Current effect Current effect: $F_{(7, 241)}$=2.3885, p=.02226

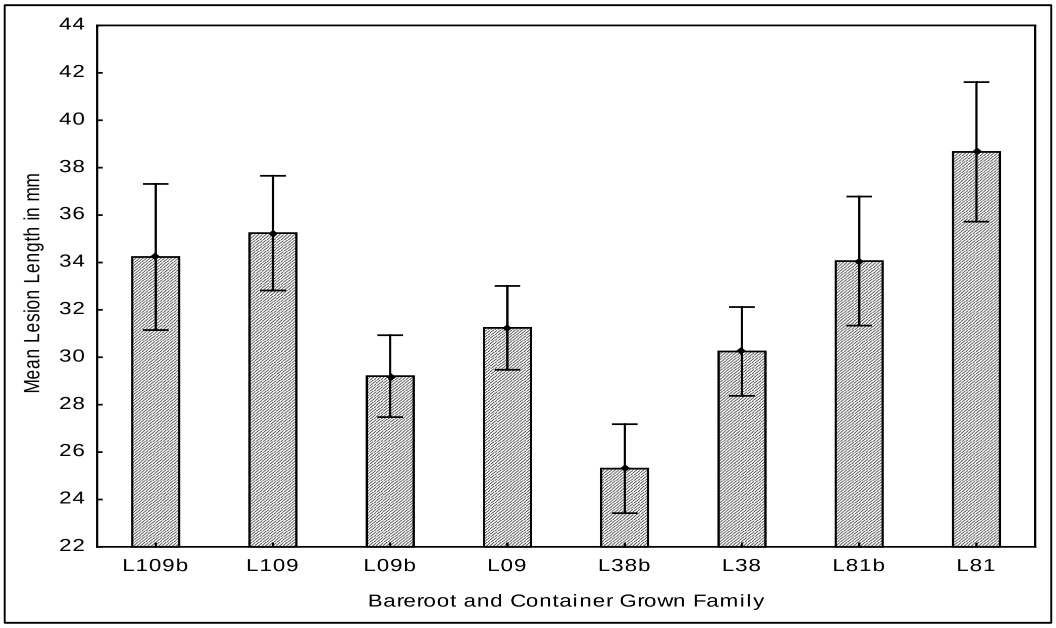

Figure 5. Overall mean lesion length produced by *G. huntii* on bare root and container grown connector families. Current effect: $F_{(7, 258)}$=2.5042, p=.01662

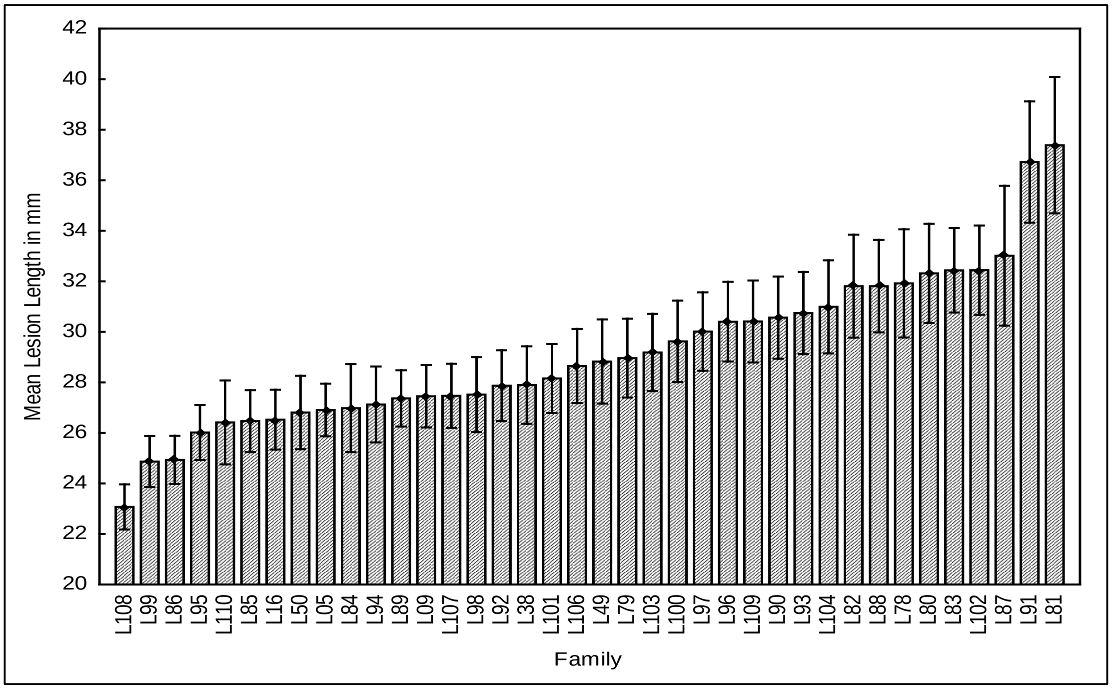

Figure 6: Overall mean lesion length caused by both the fungal treatments on families. Current effect. F $_{(37,2910)}$=3.4841, *p*=<0.0001

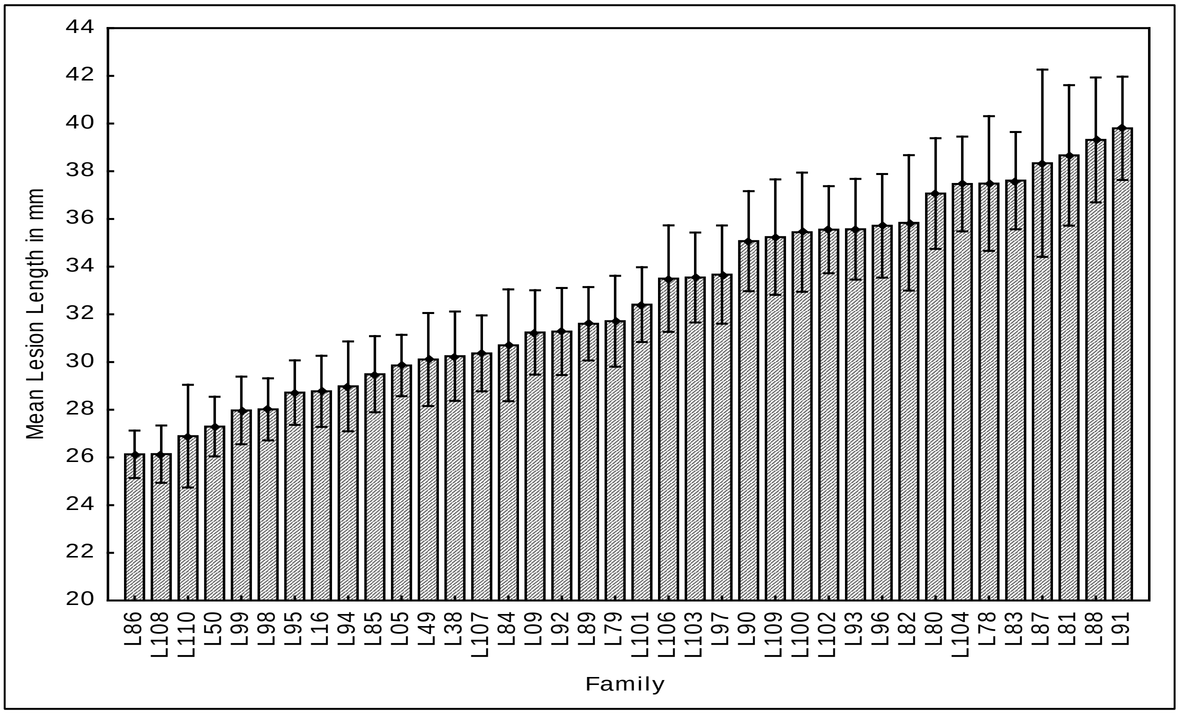

Figure 7: Mean lesion length by *G. huntii* on different family (Current effect: $F_{(37,\ 1446)}$=3.8184, p=.00000)

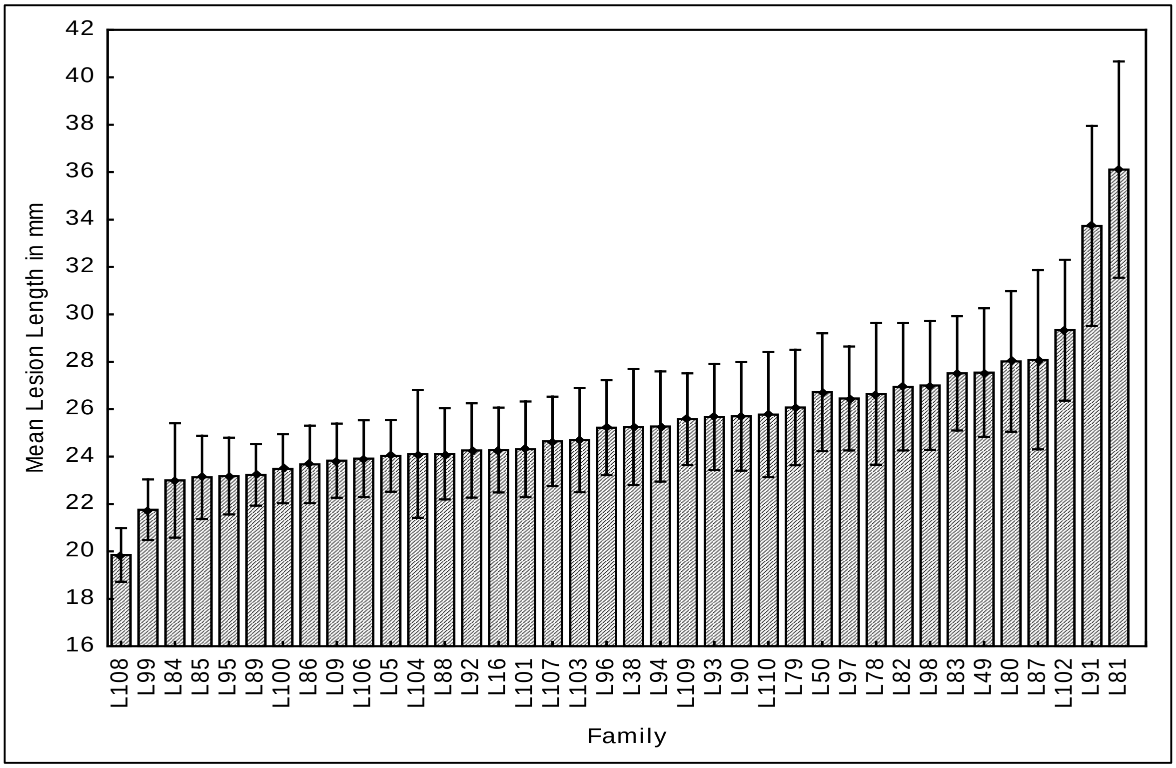

Figure 8: Mean Lesion length caused by *L. terebrantis* on different families. Current effect: $F_{(37,\ 1428)}$=1.4567, p=.03840

Table 1. Covariance parameter estimates form mixed-model (Year 2013)

Variable	Cov Parm	Estimate	Std. Error	Z Value	Pr > Z
Lesion length	Fam	2.88	1.42	2.03	0.02
	Fam*TRT	1.74	1.16	1.50	0.07
	Residual	116.24	3.26	35.70	<0.0001
Lesion width	Fam	0.03	0.03	0.88	0.19
	Fam*TRT	0.01	0.04	0.31	0.38
	Residual	6.53	0.18	35.69	<0.0001
Occlusion length	Fam	5.80	15.94	0.36	0.35
	Fam*TRT	28.33	20.41	1.39	0.08
	Residual	915.17	39.02	23.45	<0.0001
Occlusion width	Fam	0.01	0.16	0.08	0.47
	Fam*TRT	0.14	0.21	0.65	0.26
	Residual	10.33	0.46	22.37	<0.0001

Table 2. Type three fixed effects from mixed-model. Initial root collar diameter (RCD) was used as covariate (Year 2013)

Variable	Effect	DF	F Value	Pr>F
Lesion length	RCD	1	1.68	0.195
	Block	5	44.74	<0.0001
	TRT	1	369.20	<0.0001
	Block*TRT	5	33.74	<0.0001
Lesion width	RCD	1	29.10	<0.0001
	Block	5	22.08	<0.0001
	TRT	1	323.42	<0.0001
	Block*TRT	5	17.57	<0.0001
Lesion depth	RCD	1	9.53	<0.0020
	Block	5	4.43	<0.0005
	TRT	1	17.83	<0.0001
	Block*TRT	5	0.31	<0.90
Occlusion length	RCD	1	0.35	<0.5558
	Block	5	20.91	<0.0001
	TRT	1	16.99	<0.0001
	Block*TRT	5	9.40	<0.0001
Occlusion width	RCD	1	3.04	0.08
	Block	5	10.71	<0.0001
	TRT	1	4.75	0.03
	Block*TRT	5	2.58	0.02
Occlusion depth	RCD	1	67.86	<0.0005
	Block	5	4.99	0.003
	TRT	1	62.25	<0.0001
	Block*TRT	5	1.85	0.13

Table 3. Covariance parameter estimates from mixed model (Year 2014)

Parameter	Covariance Parameter	Estimate	Standard Error	Z values	Pr>Z
Lesion length	Fam	6.1631	2.1626	2.85	0.0022
	Fam*treatment	1.4593	1.2580	1.16	0.1230
	Residual	149.07	3.9379	37.85	<0.0001
Occlusion Length	Fam	7.3414	3.4887	2.13	0.0166
	Fam*treatment	4.5952	2.8944	1.59	0.0562
	Residual	289.50	7.6896	37.65	<0.0001

Table 4. Type three fixed effects from mixed model (Year 2014)

Variables	Effect	Num DF	Den DF	F value	F value Pr>F
Lesion length	RCD	1	2864	6.98	0.0083
	Block	5	2864	117.11	<0.0001
	TRT	1	2864	179.62	<0.0001
	Block*TRT	5	2864	115.03	<0.0001
Lesion Width	RCD	1	2864	137.60	<0.0001
	Block	5	2864	74.62	<0.0001
	TRT	1	2864	75.75	<0.0001
	Block*TRT	5	2864	10.29	<0.0001
Lesion depth	RCD	1	2864	68.03	<0.2426
	Block	5	2864	16.98	<0.0001
	TRT	1	2864	35.98	<0.0001
	Block*TRT	5	2864	53.15	<0.0001
Occlusion length	RCD	1	2833	13.02	0.0003
	Block	5	2833	187.50	<0.0001
	TRT	3	2833	295.73	<0.0001
	Block*TRT	15	2833	245.55	<0.0001
Occlusion width	RCD	1	2833	266.42	<0.0001
	Block	5	2833	234.15	<0.0001
	TRT	3	2833	574.56	<0.0001
	Block*TRT	15	2833	174.19	<0.0001
Occlusion depth	RCD	1	2832	177.16	<0.0001
	Block	5	2832	147.25	<0.0001
	TRT	3	2832	427.68	<0.0001
	Block*TRT	15	2832	161.18	<0.0001

Supplementary Report (For Year 2013)

Table S1. Pairwise comparisons for lesion length

Effect	TRT	Estimate	Standard Error	Adj P
TRT	GH vs LT	-10.7437	0.4512	<.0001
TRT	GH vs W	3.084	0.4455	<.0001
TRT	GH vs WM	2.9476	0.4462	<.0001
TRT	LT vs W	13.8277	0.4496	<.0001
TRT	LT vs WM	13.6913	0.4504	<.0001
TRT	W vs WM	-0.1364	0.4446	0.99

Table S2. Pairwise comparisons for occlusion length

Effect	TRT	Estimate	Standard Error	Adj P
TRT	GH vs LT	-25.7428	1.1918	<.0001
TRT	GH vs LT W	8.7539	1.1747	<.0001
TRT	GH vs WM	7.8741	1.1768	<.0001
TRT	LT vs W	34.4967	1.187	<.0001
TRT	LT vs WM	33.6169	1.1892	<.0001
TRT	W vs WM	-0.8798	1.1721	0.8764

Table S3. Mean lesion length and occlusion length (overall and across each fungus)

Fam	Lesion Length			Occlusion Length		
	Overall Mean	**Mean(GH)**	**Mean(LT)**	**Overall Mean**	**Mean GH**	**Mean LT**
L05	32.80(12.35)	25.53(4.52)	40.25(13.4)	52.91(26.39)	58.15(46.87)	51.33(17.19)
L09	32.13(15.10)	26.67(8.17)	38.61(18.65)	59.78(42.3)	58.43(48.59)	60.62(39.16)
L16	30.75(12.37)	25.86(6.35)	36.17(15)	59.86(38.16)	45.22(17.92)	62.79(40.65)
L38	32.13(16.09)	25.38(5.79)	39.22(20.05)	51.7(23.76)	37.319(15.65)	58.1(24.16)
L49	29.98(11.65)	26.41(5.78)	33.55(14.67)	51.32(26.11)	49.23(30.97)	51.86(25.34)
L50	29.93(11.59)	24.63(5.83)	36.12(13.5)	48.85(17.41)	41.21(10.32)	52.28(19.02)
L51	29.24(13.30)	23.39(4.77)	36.38(16.61)	50.21(32.01)	31.76(6.88)	61.28(36.02)
L52	27.64(8.30)	24.65(9.02)	30.57(6.37)	42.97(14.82)	34(11.06)	47.65(14.55)
L53	29.39(9.53)	24.88(5.22)	34.53(10.73)	55.69(36.72)	36.31(10.36)	58.67(38.49)
L54	31.90(15.30)	26.31(6.79)	39.07(19.74)	55.9(28.37)	48.12(28.5)	60.65(28.02)
L55	33.48(12.68)	28.83(11.54)	38.02(12.2)	52.18(30.62)	46.27(35.62)	55.94(26.88)
L56	34.39(14.32)	27.54(11.49)	41.41(13.62)	72.58(47.22)	60.53(34.7)	77.88(51.52)
L57	31.31(11.41)	25.4(5.91)	36.94(12.55)	57.31(33.35)	40.61(8.5)	64.73(37.54)
L58	29.81(10.96)	24.689(6.68)	36.21(11.94)	50.56(23.75)	38.02(13.62)	57.45(25.24)
L59	32.02(14.47)	26.22(10.53)	38.35(15.64)	51.41(20.37)	42.89(23.07)	55.46(18.15)
L60	30.45(12.58)	27.03(12.47)	33.78(11.92)	65.57(46.13)	57.76(32.47)	68.58(50.66)
L61	28.48(8.40)	24.18(6.32)	32.47(8.18)	53.23(29.63)	70.75(60.15)	48.69(13.26)
L62	31.47(10.92)	28.11(10.67)	34.91(10.02)	62.96(42.13)	55(31.45)	68.53(48.24)
L63	29.95(9.67)	25.99(8.17)	34.24(9.43)	43.72(18.5)	34.4(13.08)	46.32(19.15)
L64	29.89(9.42)	25.9(5.88)	34.29(10.64)	52.39(32.5)	40.49(21.8)	57.84(35.43)
L65	30.53(11.52)	25.33(4.89)	36.01(13.82)	46.8(16.79)	39.62(13.7)	50.11(17.28)
L66	35.84(16.73)	29.27(11.95)	42.09(18.3)	69.37(52.83)	42.31(23.32)	82.89(58.32)
L67	34.80(16.65)	29.57(14.14)	39.67(17.47)	67.83(36.84)	83.96(49.43)	61.15(28.65)
L68	34.88(18.88)	27.67(9.73)	42.45(22.94)	55.32(27.73)	39.02(16.25)	65.42(28.82)
L69	30.86(13.24)	24.22(4.72)	38.22(15.63)	56.27(41.30)	35.95(13.04)	65.65(46.48)
L70	28.48(10.67)	25.68(11.81)	31.14(8.8)	44.84(14.03)	35.07(5.82)	48.66(14.51)
L71	29.18(11.53)	24.93(7.41)	33.77(13.37)	47.69(36.76)	36.52(12.84)	52.61(42.66)
L72	28.61(8.39)	25.06(5.05)	32.24(9.57)	51.09(26.3)	41.53(15.19)	54.28(28.63)
L73	26.30(5.70)	24(4.1)	28.35(6.17)	40.3(18.21)	51.72(26.79)	34.59(8.32)
L74	29.96(7.39)	26.53(4.97)	34.61(7.65)	48.72(24.16)	41.03(13.57)	53.72(28.28)
L75	27.68(7.37)	24.35(5.77)	31.27(7.27)	48.76(29.95)	41.12(17.96)	52.58(34.14)
L76	31.02(15.77)	24.82(7.69)	37.38(19.16)	56.69(33.97)	32.68(12.13)	68.18(35.18)
L77	32.90(16.43)	27.71(13.41)	38.49(17.68)	55.98(36.27)	47.82(32.55)	60.4(38.06)

*Note: Mean followed by standard deviation in parenthesis

Table S4. Estimate and rank of lesion length (overall and among the fungus)

Fam	Overall Estimate	Rank	Estimate LT	Rank	Estimate GH	Rank
L73	-2.9315	1	-2.1358	1	0.3593	27
L52	-1.9889	2	-1.4714	2	0.2662	26
L75	-1.7656	3	-1.1474	4	0.07754	19
L61	-1.588	4	-0.8976	6	-0.06473	14
L70	-1.5451	5	-1.3601	3	0.4238	29
L72	-1.3018	6	-0.906	5	0.1172	21
L71	-0.8816	7	-0.554	11	0.01972	18
L53	-0.6454	8	-0.2726	13	-0.1185	13
L64	-0.6002	9	-0.6086	10	0.2449	24
L49	-0.5801	10	-0.7578	8	0.4063	28
L51	-0.5069	11	0.365	21	-0.6722	2
L60	-0.4789	12	-0.7809	7	0.4907	30
L63	-0.4183	13	-0.3701	12	0.1167	20
L58	-0.3356	14	0.1816	18	-0.385	7
L50	-0.3178	15	0.1953	19	-0.3879	6
L65	-0.1056	16	-0.02479	15	-0.0392	16
L57	-0.02435	17	0.2659	20	-0.2806	10
L74	-0.00209	18	-0.2315	14	0.2302	23
L76	0.07086	19	0.4471	22	-0.4042	5
L69	0.1071	20	0.7775	25	-0.7126	1
L16	0.1756	21	0.1133	16	-0.00696	17
L62	0.1788	22	-0.6579	9	0.7663	33
L38	0.6166	23	0.7821	27	-0.4084	4
L59	0.7743	24	0.7807	26	-0.3114	9
L54	0.8677	25	0.886	29	-0.3602	8
L05	1.0237	26	1.1173	30	-0.497	3
L77	1.1636	27	0.5309	23	0.1742	22
L09	1.2418	28	0.8081	28	-0.05559	15
L55	1.2895	29	0.1725	17	0.6089	32
L56	1.8906	30	1.2861	32	-0.1403	12
L67	1.9654	31	0.628	24	0.563	31
L68	2.1677	32	1.5938	33	-0.2802	11
L66	2.4846	33	1.2454	31	0.2602	25

Table S5. Re-isolation percentage of families (overall and across each fungus)

Family	**% Re-isolation (Overall)**	**% Re-isolation LT**	**% Re-isolation GH**
L05	96.25	97.50	92.69
L09	95.76	96.97	92.31
L16	96.10	94.60	95.12
L38	97.53	97.50	95.24
L49	97.56	97.57	95.24
L50	97.47	97.30	95.35
L51	96.30	97.30	93.33
L52	95.35	97.73	90.70
L53	96.15	94.60	95.24
L54	95.47	96.98	92.86
L55	95.12	97.62	90.24
L56	97.56	97.57	95.24
L57	98.77	97.62	97.50
L58	98.78	97.30	97.83
L59	95.76	97.14	91.90
L60	98.77	97.62	97.50
L61	96.34	97.68	92.50
L62	96.43	95.24	95.35
L63	96.20	97.37	92.86
L64	90.36	95.00	84.10
L65	94.94	94.88	92.69
L66	96.39	95.35	95.12
L67	96.43	95.46	95.12
L68	95.07	92.50	95.24
L69	92.41	92.11	90.48
L70	97.59	95.35	97.57
L71	92.69	92.50	90.70
L72	96.25	95.00	95.12
L73	97.76	97.88	95.35
L74	96.00	90.63	97.73
L75	97.62	97.57	95.46
L76	96.34	97.57	92.86
L77	91.47	90.91	89.74

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Table S6. Pairwise comparison for lesion length

Effect	Treatment	Estimate	Standard Error	Adj P
TRT	GH vs LT	7.1035	0.4979	<.0001
TRT	GH vs W	14.8868	0.4956	<.0001
TRT	GH vs WM	14.9228	0.4973	<.0001
TRT	LT vs W	7.7832	0.4966	<.0001
TRT	LT vs WM	7.8193	0.4983	<.0001
TRT	W vs WM	0.03609	0.496	0.9999

Table S7: Pairwise comparison for occlusion length

Effect	Treatment	Estimate	Standard Error	Adj P
	GH vs LT	13.2551	0.6666	<.0001
TRT	GH vs W	29.1919	0.6657	<.0001
TRT	GH vs WM	28.0168	0.6667	<.0001
TRT	LT vs W	15.9368	0.668	<.0001
TRT	LT vs WM	14.7617	0.6689	<.0001
TRT	W vs WM	-1.1751	0.668	0.2933

Table S8. Mean lesion and occlusion length produced in the families (overall and among the fungal treatments)

Family	Lesion Length			Occlusion Length		
	Overall Mean	Mean GH	Mean LT	Overall Mean	Mean GH	Mean (LT)
L05	26.91(9.46)	29.86(8.24)	24.03(9.78)	36.17(17.52)	43.85(13.21)	28.66(18.09)
L09	27.45(11.71)	31.24(11.73)	23.83(10.59)	36.63(18.48)	44.79(15.93)	29.01(17.56)
L100	29.62(14.05)	35.44(15.61)	23.49(8.86)	36.59(18.72)	45.27(16.37)	27.44(16.71)
L101	28.15(12.21)	32.41(9.66)	24.31(13.09)	39.66(20.21)	49.18(16.52)	31.07(19.53)
L102	32.44(16.19)	35.55(11.83)	29.33(19.25)	42.02(23.18)	49.55(15.01)	34.49(27.32)
L103	29.18(13.06)	33.55(11.46)	24.7(13.22)	38.14(20.16)	47.59(16.32)	28.42(19.25)
L104	30.99(14.96)	37.47(11.57)	24.11(15.23)	35.96(21.82)	45.36(16.66)	25.31(22.31)
L106	28.65(13.2)	33.5(14.12)	23.91(10.38)	35.2(17.66)	42.61(15.8)	27.97(16.49)
L107	27.47(11.56)	30.36(10.18)	24.64(12.22)	37.24(20.36)	44.54(13.57)	30.12(23.33)
L108	23.07(8.09)	26.14(7.78)	19.85(7.17)	28.51(14.58)	34.87(11.86)	21.83(14.29)
L109	30.41(15.55)	35.24(16.41)	25.58(13.11)	36.1(20.86)	42.61(19.68)	29.15(20.03)
L110	26.42(12.96)	26.89(12.75)	25.77(13.48)	30.66(16.68)	31.36(13.39)	29.69(20.69)
L16	26.53(10.72)	28.77(9.54)	24.28(11.46)	37.06(21.52)	40.08(12.35)	34.03(27.69)
L38	27.89(14.13)	30.25(12.57)	25.25(15.44)	33.24(20.28)	38.39(19.89)	27.44(19.34)
L49	28.83(14.33)	30.11(11.87)	27.55(16.5)	32.25(17.59)	34.19(12.83)	30.32(21.33)
L50	26.81(12.16)	27.29(7.18)	26.38(15.4)	30.2(16.95)	33.58(10.63)	27.18(20.75)
L78	31.92(18.42)	37.49(16.94)	26.65(18.42)	35.44(23.52)	44.03(19.16)	27.54(24.61)
L79	28.96(14.13)	31.71(12.35)	26.07(15.42)	36.97(20.94)	42.64(15.34)	31.02(24.34)
L80	32.31(17.55)	37.07(14.31)	28.02(19.2)	41.49(25.41)	51.14(18.99)	32.54(27.47)
L81	37.39(21.59)	38.67(16.66)	36.11(25.81)	43.78(27.02)	45.85(22.09)	41.55(31.72)
L82	31.81(16.28)	35.84(16.79)	26.95(14.47)	37.61(19.43)	44.29(15.74)	29.54(20.63)
L83	32.44(15.14)	37.61(12.88)	27.51(15.62)	40.68(22.6)	50.74(15.76)	31.11(24.09)
L84	26.98(13.27)	30.7(12.84)	22.99(12.76)	34.69(19.37)	42.08(14.56)	26.46(20.97)
L85	26.47(10.98)	29.49(10.34)	23.13(10.82)	38.11(19.21)	44.25(13.62)	31.31(22.18)
L86	24.93(8.61)	26.13(6.45)	23.67(10.34)	34.36(17.33)	36.94(11.64)	31.67(21.61)
L87	33.01(24.29)	38.34(23.88)	28.08(23.91)	41.87(28.72)	49.56(25.58)	34.94(29.92)
L88	31.81(16.46)	39.31(16.76)	24.12(12.16)	41.7(23.18)	52.11(20.58)	31.04(20.92)
L89	27.37(10.02)	31.6(9.74)	23.23(8.53)	37.03(18.21)	45.15(13.02)	28.92(19.16)
L90	30.56(14.46)	35.07(13.42)	25.7(14.11)	41.28(20.97)	47.73(18.52)	33.94(21.42)
L91	36.72(20.24)	39.8(12.82)	33.73(25.32)	47.79(32.23)	52.9(16.85)	42.83(41.85)
L92	27.87(11.73)	31.28(10.95)	24.26(11.59)	35.75(17.5)	42.75(11.5)	28.34(19.76)
L93	30.75(14.54)	35.57(13.52)	25.68(13.98)	37.22(20.62)	43.69(18.31)	30.59(20.97)
L94	27.13(13.43)	28.98(11.92)	25.27(14.7)	34(19.95)	38.92(15.19)	29.09(22.93)
L95	26.02(9.86)	28.72(8.77)	23.18(10.24)	36.13(18.52)	42.79(12.82)	29.13(21.02)
L96	30.4(14.22)	35.71(13.74)	25.22(12.82)	39.45(19.87)	48.97(16.74)	30.16(18.37)
L97	30.01(13.62)	33.67(12.7)	26.45(13.69)	38.67(20.67)	45.79(15.72)	31.56(22.69)
L98	27.52(13.2)	28.02(8.23)	27(16.96)	32.31(18.36)	33.14(10.82)	31.42(24.01)
L99	24.87(9.14)	27.97(9.08)	21.76(8.18)	33.75(17.11)	42.41(13.92)	25.08(15.66)

*Note: Mean followed by standard deviation in parenthesis

Table S9. Family estimate and ranking for lesion length (overall and across both the treatments)

Family	Estimate	Overall Rank	Estimate	GH Rank	Estimate	LT Rank
L108	-4.2968	1	-0.6585	5	-0.3589	6
L86	-3.1182	2	-1.0034	2	0.265	30
L99	-3.0851	3	-0.4963	9	-0.2342	10
L95	-2.3474	4	-0.4748	10	-0.08105	21
L85	-2.1383	5	-0.3111	14	-0.1952	12
L16	-1.9243	6	-0.5992	8	0.1436	27
L110	-1.8808	7	0.3901	32	-0.8355	4
L05	-1.5823	8	-0.3761	12	0.001443	22
L92	-1.5651	9	-0.2118	15	-0.1588	16
L94	-1.3367	10	-0.6174	6	0.3009	31
L50	-1.2819	11	-0.939	3	0.6354	35
L89	-1.1694	12	0.08441	22	-0.3613	5
L98	-1.1372	13	-1.0526	1	0.7834	37
L107	-1.1259	14	-0.3137	13	0.04709	24
L84	-0.8759	15	-0.08691	17	-0.1205	20
L09	-0.8437	16	-0.02733	18	-0.1725	13
L38	-0.7575	17	-0.4106	11	0.2312	29
L101	-0.717	18	0.000344	19	-0.1701	14
L49	-0.3822	19	-0.5993	7	0.5088	34
L79	-0.2689	20	-0.2081	16	0.1444	28
L106	-0.1474	21	0.3304	26	-0.3653	4
L100	0.01321	22	0.6748	33	-0.6717	2
L103	0.399	23	0.254	24	-0.1595	15
L97	0.4919	24	0.04023	21	0.07624	25
L90	0.9976	25	0.4722	29	-0.236	9
L93	1.0203	26	0.4492	28	-0.2076	11
L96	1.0613	27	0.5885	31	-0.3372	7
L109	1.3123	28	0.6224	32	-0.3116	8
L82	1.6993	29	0.2966	25	0.1057	26
L88	1.7616	30	1.3647	38	-0.9475	1
L104	1.8676	31	0.9579	37	-0.5157	3
L80	2.2601	32	0.4924	30	0.04278	23
L78	2.2762	33	0.6954	35	-0.1565	17
L83	2.3535	34	0.6878	34	-0.1305	19
L87	2.395	35	-0.1431	18	-0.1431	18
L102	2.4152	36	0.1282	23	0.4437	33
L91	4.5296	37	0.3629	27	0.7096	36
L81	5.1283	38	0.008896	20	1.2054	38

Table S10. Re-isolation and survival percentage

Family	% Re-isolation Total	% Re-isolation LT	% Re-isolation GH	% Survival
L05	71.43	71.43	71.43	98.82
L09	69.23	68.09	70.45	94.92
L100	67.11	64.86	69.23	98.75
L101	63.75	59.52	68.42	97.65
L102	80.95	71.43	90.48	98.24
L103	73.97	72.22	75.68	98.82
L104	69.70	71.88	67.65	95.88
L106	62.96	73.17	52.50	98.24
L107	73.49	73.17	73.81	98.82
L108	67.47	65.85	69.05	99.41
L109	71.74	76.09	67.39	97.46
L110	73.77	73.08	74.29	89.41
L16	63.41	58.54	68.29	99.41
L38	64.71	60.00	68.89	93.91
L49	77.33	75.68	78.95	94.67
L50	67.61	68.42	66.67	88.17
L78	68.00	63.16	72.97	93.49
L79	68.29	65.00	76.19	98.82
L80	64.20	64.29	64.10	98.82
L81	76.92	84.85	68.75	97.46
L82	71.88	62.07	80.00	94.08
L83	72.29	78.57	65.85	99.41
L84	77.59	82.14	73.33	99.41
L85	67.50	68.42	66.67	99.41
L86	63.41	60.00	66.67	97.63
L87	84.42	85.00	83.78	98.22
L88	77.78	82.50	73.17	98.82
L89	74.07	75.61	72.50	99.41
L90	78.48	76.32	80.49	98.82
L91	69.01	69.44	68.57	99.41
L92	77.14	79.41	75.00	98.82
L93	72.50	74.36	70.73	98.82
L94	74.07	77.50	70.73	98.82
L95	78.05	75.00	80.95	98.82
L96	68.29	63.41	72.50	99.41
L97	75.32	74.36	76.32	98.82
L98	75.95	79.49	72.50	96.45
L99	71.95	70.73	73.17	99.41