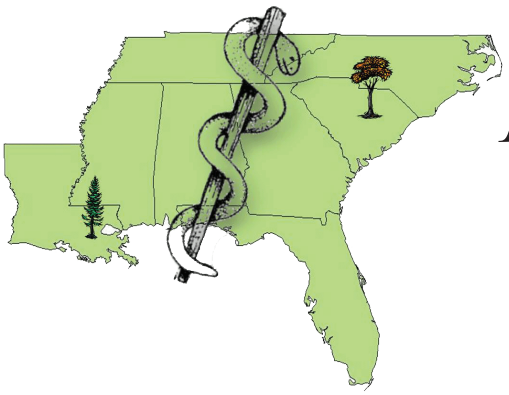

AUBURN UNIVERSITY
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VARIATION IN ROOT LESIONS IN LOBLOLLY PINE (*PINUS TAEDA* L.) FAMILIES AGAINST *LEPTOGRAPHIUM* AND *GROSMANNIA* ROOT INFECTING FUNGI

by
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ABSTRACT
Pine decline has been observed as an emerging forest health problem in the southeastern United States. Complex interactions of biotic and abiotic factors are involved which together predispose, continuously stress and contribute to the decline. Presence of root-feeding beetles and their fungal associates such as *Leptographium* and *Grosmannia* have been consistently reported from the symptomatic stands. A study was conducted on fourteen year old loblolly pine stands at two locations with different open pollinated half sib families to determine the relative resistance to *Leptographium* and *Grosmannia* fungal genera. Inoculations were administered on the roots following an artificial inoculation method. Host responses to the inoculations were recorded as lesion length, lesion width, lesion depth, lesion area and occlusion length eight weeks after inoculations. Roots exhibited dark brown resin filled lesions and occluded tissues. Results indicated that the treatments had variable response with *L. terebrantis* and *G. huntii* causing significantly larger lesions. However, no significant differences were observed in lesion parameters among the fourteen loblolly pine families included in the study.

INTRODUCTION

The southeastern United States is a leading timber producing region of the United States with loblolly pine grown as commercially timber species (Schultz 1997). Loblolly pine is native to the southeastern United States and it is found throughout its range consisting of 14 states from New Jersey to central Florida and west to Texas (Schultz 1997). Loblolly pine has faster growth rate and provides versatile marketable products. It is ecologically important as habitat to a diversity of wildlife and supports the region by economically providing 110,000 jobs and $30 billion to the economy of southeastern United States (Schultz 1997). Loblolly pine is vulnerable to a variety of abiotic and biotic agents such as wind throw, extreme temperatures, lightening damage, drought, flooding, insects and diseases. Reduced growth, tree decline and mortality have been associated with loblolly pine for the past 50 years and have been studied and well documented (Brown and McDowell 1968; Roth and Peacher 1971; Hess et al. 1999). Serious future ecological and economic implications as a result of decline in loblolly pine cannot be neglected.

Decline in loblolly pine was first observed in the Talladega National Forest in the Oakmulgee Ranger District in Central Alabama. Symptoms included thinning crowns, reduced radial growth and root

deterioration in 40 to 50 years old stands (Brown and McDowell 1968). Studies indicated the involvement of unusual physiological and environmental conditions (Roth and Peacher 1971) and other pathogens such as Heterobasidion root rot, *Pythium* species and *Phytophthora cinnamomi* Rands., causal organism of littleleaf disease (Campbell and Copeland, 1954; Brown and McDowell 1968). However, in a 3 year study led by Eckhardt et al. (2007), observations of affected stands showed damage to the lateral roots with recovery of root-feeding bark beetles *Hylastes* species and *Hylobius pales* (Herbst.) and *Pachylobius picivorous* (Germ.) weevil species often accompanied with resin production. The recovery of *Leptographium* species including *L. procerm* (W.B. Kendr.) M.J. Wingf., *G. alacris* (formely *L. serpens*) T.A. Duong, Z.W. de Beer & M.J. Wingf. sp. nov., and *L. terebrantis* S.J. Barras and T.J. Perry have also been reported from the root systems of the infected trees. Recently, *Grosmannia huntii* (R.C. Rob. Jeffr.) Zipfel, Z.W. de Beer & M.J. Wingf (*L. huntii* M.J. Wingfield) has been recovered from the symptomatic loblolly pine roots and the insect vectors involved (Zanzot et al. 2010).

Presently, the premature mortality in loblolly pine has been described as a decline disease syndrome that involves complex interactions of abiotic and biotic agents leading to tree mortality. Predisposing factors such as increased slopes, southwest facing aspects, genetics of the genotypes subjects the tree to continuous stress that attracts biotic agents such as root feeding bark beetles and hence ophiostomatoid fungi through their mutualistic role with the bark beetles contribute to the tree decline (Eckhardt et al. 2004a; Eckhardt et al.2004b; Eckhardt and Menard 2008). Several other declines have been reported on confers in the United States with some prominent examples such as red pine (*P. resinosa* Aiton) decline in Wisconsin (Klepzig et al. 1991), ponderosa pine (*P. ponderosa* Laws.) root disease/decline in New Mexico (Livingstone et al. 1983), pole blight of western white pine (*P. monticola* Dougl. Ex D. Don) (Leaphart and Copeland 1957), vascular tissue disruption caused by Procerum root disease in western white pine (Butnor et al. 2000). In all these above described pine syndromes, involvement of ophiostomatoid fungi has been reported either as a causal or contributing factor.

In several studies, artificial inoculations have been conducted on similar sized trees to determine the virulence of ophiostomatoid fungi (Långström et al. 2001; Rice et al. 2007). Fungal inoculations of above-ground parts such as tree stems is appropriate to mimic the bark beetles that naturally attack above ground plant parts and inoculate fungus into the tree stem (Schowalter and Filip 1993). However, in the case of root feeding bark beetles that introduce the fungus into the roots, mature tree root inoculations have been performed and proved appropriate (Wingfield and Knox-Davies 1980; Otrosina et al. 2002; Matusick et al. 2012). Following inoculations, host response is seen in the form of necrotic lesions with darkened phloem tissue and excess resin production at the point of inoculation (Parmeter et al. 1989). Invasion of the fungus is seen as wedge shaped sapwood discoloration with lesions extending longitudinally and laterally from the origin of infection (Bleiker and Uzunovic 2004). Restriction in hydraulic conductance through xylem has been observed in ponderosa pine in response to *Leptographium wageneri* Kendrick. and *Heterobasidion irregulare* nom. nov. Garbelotto & Otrosina (Joseph et al. 1998). Furthermore, diffused symptomatology as decreased hydraulic conductivity, thin crowns, chlorotic foliage and decline was noticed in scots pine (*Pinus sylvestris* L.) when inoculated with *Ophiostoma ips* (Rumb.) Nannf. (Fernandez et al. 2004).

Previous studies have been focused on studying the relative virulence of ophiostomatoid fungi without considering the variability in host genotype resistance. In a study done with two loblolly and two slash pine families, relative virulence of four ophiostomatoid fungi and *H. irregulare* was studied and *G. huntii* was found causing larger lesions. Although, the families were not compared but the genetic differences in one family were held responsible for larger lesions in wound+media control than wound only control (Matusick 2010). This study was conducted to determine the variability symptomatology of loblolly pine genotypes commonly deployed by the timber companies in response to mature tree root inoculations with *Leptographium* and *Grosmannia* root infecting fungi.

METHODS AND MATERIALS

The study was carried out in 14 year old stands at two locations provided by the industry in Georgia and Florida. Two sites were selected from the lower gulf elite population trials planted across the range from Georgia to Mississippi and Texas. Each site consisted of trees from 14 families planted as single tree plots with 15 replications. The families were scattered throughout the trial and each site consisted of 210 trees.

Fungal cultures consisting of single spore isolates maintained in the Forest Health Dynamics Laboratory were used for inoculations. Two weeks before the inoculations the fungi were cultured on 2% malt extract agar (MEA). Four treatments were applied which consisted of two fungal treatments: *G. huntii*, *L. terebrantis* and two controls: wound and wound+media. For each the treatment, two primary lateral roots were carefully excavated on each tree and the fungal species were applied to each root along with a control (Figure 1). About 30 cm (1 ft) space was maintained between the fungal and control treatments. The method given by Wright (1933) was used for root inoculation which consisted of creating a 13 mm wound on the root with a cork borer peeling through the bark to the cambium. After removing the bark plug, a 10 mm plug of mycelium was placed in the wound followed by replacing the bark plug and sealing the wound with duct tape. In the case of wound+media control, sterile media was placed in the wound and in case of wound control, wound was created in the same way but nothing was inserted into the wound. The inoculation points were marked with pin flags and the roots were covered (Figure 2).

Eight weeks following inoculations, the roots were re-excavated, removed from the tree and returned to the laboratory at Auburn University for further processing and measurements. Each root sample was inspected for insect activity and the host response to the inoculations was quantified by measuring the length, width and depth of the infected dark and discolored root tissue. The lesion depth was determined by cutting the root at the inoculation point and measuring the radial discolored sapwood. For determining the occluded root tissue, the root tissue was stained with a solution of FastGreen stain (FastGreen FCF; Sigma Chemical Co.) and water (0.25g/L of water) and the unstained tissue was recorded as occlusion length. Each root lesion margin was traced on transparency sheets and the lesion area determined using a Lasico® Planimeter (Lasico®, Los Angeles, CA). Small pieces of stem tissue from the distal and proximal portions of the inoculation site were plated on malt extract amended with cycloheximide streptomycin (CSMA) media to confirm infection.

The field design was set up as a Randomized Complete Block Design with single tree plots. Each site had 15 blocks with each family having only one tree per block. The response variables to the

inoculations were analyzed using SAS statistical software (SAS Institute, 9.2 ed., Cary, NC). A linear mixed model was fit to the data to compare the treatments and determine the family differences. In the model, the families were tested as a random sample of a breeding population with family x treatment interaction kept as random effects. Site, block and treatment were kept fixed with block effect nested within the sites. Covariance parameter estimates for each response variable such as lesion length, occlusion length, lesion width, lesion depth and lesion area were used to determine the variation among the families and the families were ranked on the basis of general combining ability (GCA) estimates for lesion length. Fungal and control treatments were compared from the type 3 fixed effects.

RESULTS

Inoculation with *L. terebrantis* and *G. huntii* caused resin soaked brown lesions in the loblolly pine roots with extensions to distal and proximal from the inoculation wound (Figure 3). Of the fungi tested on loblolly pine families, *L. terebrantis* caused longer lesion length, occlusion length, lesion width, lesion depth and lesion area followed by *G. huntii* (Table 1). Pairwise comparison between the treatments indicated that lesions produced from *L. terebrantis* were significantly longer, wider and deeper than that of *G. huntii* (Table 2). Both fungal species caused significantly larger lesions than either the wound or wound+media control treatments. However, lesion length, width, depth and area due to the presence of media differed significantly than wound only control (Table 2).

Estimation of covariance parameters indicated that the variation among the families was not significantly different from zero for lesion length ($Z = 0.09$, $P=0.4653$), occlusion length ($Z = 0.06$, $P = 0.4775$), lesion width ($Z = 0.42$, $P = 0.3384$), lesion depth ($Z = 0.40$, $P = 0.3448$) and lesion area ($Z = 0.76$, $P = 0.2248$) (Table 3). Family x treatment interaction was not found to be significant for lesion length, width, depth, occlusion length and lesion area (Table 3). Solutions for Type 3 fixed effects suggested that lesion length, occlusion length, lesion width and lesion area differed significantly between two sites and among the treatments. However, blocks were not found to be significantly different for lesion length, occlusion length and lesion area but were found to be significantly different for lesion width and lesion depth (Table 4). Although the variation among the families was not significantly different, the general combining ability (GCA) estimates were used to rank the families on the basis of lesion length overall and for each fungal species (Table 5). Average lesion length, occlusion length, lesion width and lesion depth for each treatment and for each family are presented in Table 6 and Table 7. Re-isolation percentage of *L. terebrantis* from inoculated loblolly pine roots was 92% while that of *G. huntii* was 58%.

DISCUSSION

Root lesions following inoculations were consistently observed across all treatments including controls on the families tested in the experiment. Dark, discolored resin filled lesions have been observed in previous studies following inoculations with ophiostomatoid species in mature trees (Wingfield 1986; Matusick et al. 2012) (Figure 4). It was apparent that wound and wound+media controls also produced lesions but the lesions due to wound were considerably small than wound+media and fungal treatments. However, lesion morphology due to wound+media control was similar to the fungal species with occasionally greater lesion length than the fungal treatments. Lesions due to controls have been observed in previous studies however with lighter

color due to wound control and pitch filled darker lesions from wound+media (Rice et al. 2007; Matusick et al. 2012).

Host reaction in the form of dark resinous lesions following fungal inoculations confirmed the virulence of these species to loblolly pine. Both *G. huntii* and *L. terebrantis* were pathogenic to the pine host which is consistent with previous studies that showed significant larger lesions when inoculated with *Leptographium* and *Grosmannia* species (Matusick et al. 2012). As observed in this study, *L. terebrantis* has been shown to be a moderate to virulent pathogen in previous studies when stem inoculations were performed on mature trees (Raffa and Smalley 1988; Wingfield 1986). *Grosmannia huntii* has been included in virulence trials on loblolly and slash pines recently where it was found causing larger root lesions than other *Leptographium* species including *L. terebrantis* (Matusick et al. 2012). However, *L. terebrantis* was shown to be most virulent to young longleaf pine trees when stem inoculations were performed (Matusick and Eckhardt 2010b). Taken together these trials indicate that *L. terebrantis* is a moderate to severe pathogen of pines.

Damage to pine roots as a result of *Grosmannia* and *Leptographium* infection may potentially affect the activity and reproductive behavior of their root-feeding beetles. Root-feeding beetle and weevil species such as *Hylastes* spp., *H. pales* and *P. picivorous* have been recovered from the symptomatic stands and are considered as potential vectors of these fungal species (Eckhardt et al. 2004b). Considerable damage to the roots in the form of galleries and frass was noticed in this study with no recovery of beetles which indicates that the damage potential of *Grosmannia* and *Leptographium* species is controlled by the beetle vectors (Figure 5).

Pine hosts vary in their response to *Grosmannia* and *Leptographium* species with longleaf pine generally considered as tolerant to these fungal species as compared to loblolly and slash pine (Matusick and Eckhardt 2010a). Moreover, significant variations in lesion length were noticed among the loblolly pine families in a seedling inoculation study (RR2014-06, RR2014-07). However, inconsistent with the seedling inoculations, no significant differences in lesion parameters were seen among the families when mature tree root inoculations were performed. It is probable that family differences in root inoculation trials could be observed in studies with longer durations than the eight weeks tested here. Although not significant, GCA estimates indicated the differences in lesion length among the families included in the study from a breeding population of open pollinated families (Table 5). Further studies are needed to better understand the interactions of *Grosmannia* and *Leptographium* species with mature trees of loblolly pine genotypes and hence discover the role of genetics in *Leptographium* and *Grosmannia* resistance that could be used in seedling deployment strategies to minimize the effects of pine decline.

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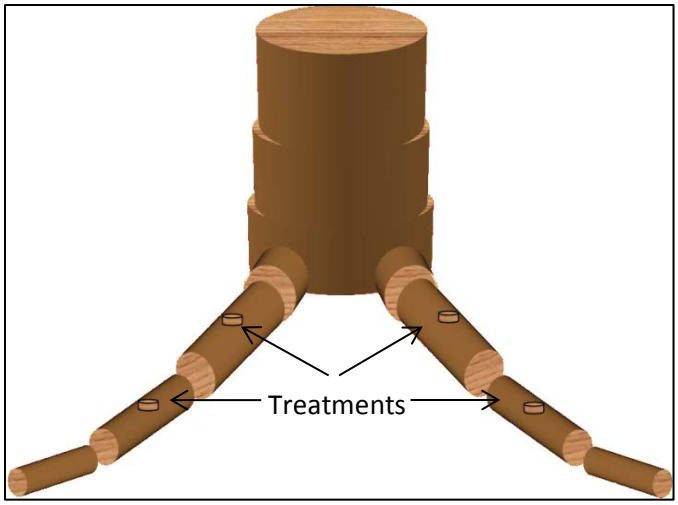

Figure 1. Diagram showing treatments as administered on the tree roots (Matusick 2010).

Figure 2. Roots covered with soil following inoculations with pin flags showing point of inoculations.

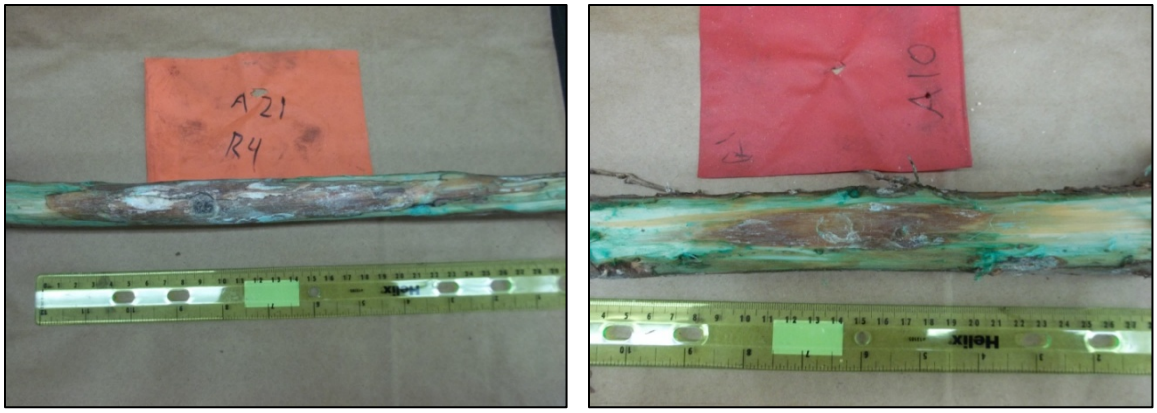

Figure 3. Lesions on the roots following inoculations with *Leptographium terebrantis* (left) and *Grosmannia huntii* (right).

Figure 4. Resin filled lesions on the roots following inoculations with *G. huntii* (left) and *L. terebrantis* (right).

Figure 5. Root-feeding beetle galleries and resin seen on the roots

Table 1. Least square means of treatments for lesion length, occlusion length, lesion width and lesion depth for loblolly pine families.

Variable	Treatment	Estimate	Std. Error	DF	t Value	Pr > |t|
Lesion Length	LT	13.5583	0.1934	1339	70.1	<.0001
	GH	11.1729	0.192	1339	58.2	<.0001
	WM	7.1301	0.1943	1339	36.69	<.0001
	W	5.2018	0.1942	1339	26.79	<.0001
Occlusion length	LT	13.7053	0.1836	1324	74.67	<.0001
	GH	11.389	0.1807	1324	63.01	<.0001
	WM	7.1749	0.1826	1324	39.28	<.0001
	W	5.2025	0.1823	1324	28.54	<.0001
Lesion Width	LT	4.8947	0.03855	1337	126.98	<.0001
	GH	4.4909	0.03813	1337	117.78	<.0001
	WM	3.9262	0.03885	1337	101.07	<.0001
	W	3.6599	0.03879	1337	94.34	<.0001
Lesion Depth	LT	3.0315	0.04612	1337	65.74	<.0001
	GH	2.6205	0.04573	1337	57.3	<.0001
	WM	1.9101	0.04634	1337	41.22	<.0001
	W	1.4531	0.04626	1337	31.41	<.0001
Lesion Area	LT	3.5278	0.05133	1275	68.73	<.0001
	GH	2.8795	0.05080	1275	56.69	<.0001
	WM	1.7660	0.05213	1275	33.88	<.0001
	W	1.1516	0.05135	1275	22.43	<.0001

Note: P- value <0.05 shows significant differences at alpha=0.05
GH- *Grosmannia huntii*, LT- *Leptographium terebrantis*, W- Wound, WM- Wound+Media

Table 2. Differences in least square means of treatments for lesion length, occlusion length, lesion width and lesion depth for loblolly pine families.

Variable	Trt	Trt	Estimate	Std Error	DF	t Value	Pr > |t|
Lesion Length	GH	LT	-2.3854	0.2702	1339	-8.83	<.0001
	GH	W	5.9712	0.2724	1339	21.92	<.0001
	GH	WM	4.0428	0.2712	1339	14.91	<.0001
	LT	W	8.3566	0.2729	1339	30.62	<.0001
	LT	WM	6.4282	0.2722	1339	23.61	<.0001
	W	WM	-1.9283	0.2732	1339	-7.06	<.0001
Occlusion Length	GH	LT	-2.3163	0.2560	1324	-9.05	<.0001
	GH	W	6.1865	0.2566	1324	24.11	<.0001
	GH	WM	4.2141	0.2556	1324	16.49	<.0001
	LT	W	8.5028	0.2581	1324	32.94	<.0001
	LT	WM	6.5304	0.2577	1324	25.35	<.0001
	W	WM	-1.9724	0.2571	1324	-7.67	<.0001
Lesion Width	GH	LT	-0.4037	0.05273	1337	-7.66	<.0001
	GH	W	0.8310	0.05339	1337	15.56	<.0001
	GH	WM	0.5647	0.05304	1337	10.65	<.0001
	LT	W	1.2347	0.05354	1337	23.06	<.0001
	LT	WM	0.9684	0.05335	1337	18.15	<.0001
	W	WM	-0.2663	0.05364	1337	-4.96	<.0001
Lesion Depth	GH	LT	-0.4110	0.06313	1337	-6.51	<.0001
	GH	W	1.1674	0.06364	1337	18.34	<.0001
	GH	WM	0.7104	0.06337	1337	11.21	<.0001
	LT	W	1.5784	0.06380	1337	24.74	<.0001
	LT	WM	1.1214	0.06366	1337	17.61	<.0001
	W	WM	-0.4570	0.06386	1337	-7.16	<.0001
Lesion Area	GH	LT	-0.6483	0.06808	1275	-9.52	<.0001
	GH	W	1.7279	0.06848	1275	25.23	<.0001
	GH	WM	1.1135	0.06874	1275	16.20	<.0001
	LT	W	2.3762	0.06874	1275	34.57	<.0001
	LT	WM	1.7618	0.06916	1275	25.47	<.0001
	W	WM	-0.6144	0.06927	1275	-8.87	<.0001

Note: P- value <0.05 shows significant differences at alpha=0.05
GH- *Grosmannia huntii*, LT- *Leptographium terebrantis*, W- Wound, WM- Wound+Media

Table 3. Covariance Parameter Estimates are presented. Variation among families is not significantly different from zero for lesion length, and occlusion length. Treatment x family interaction is non-significant.

Variable	Cov Pram	Estimate	Std. Error	Z Value	Pr>Z
Lesion Length	Family	0.005374	0.06168	0.09	0.4653
	Trt x Family	0.1644	0.1216	1.35	0.0882
	Residual	8.5194	0.3361	25.34	<.0001
Occlusion Length	Family	0.002917	0.05171	0.06	0.4775
	Trt x Family	0.1425	0.1060	1.34	0.0894
	Residual	7.6081	0.3017	25.22	<.0001
Lesion Width	Family	0.000920	0.002207	0.42	0.3384
	Trt x Family	0	.	.	.
	Residual	0.4807	0.01867	25.74	<.0001
Lesion Depth	Family	0.001428	0.003575	0.40	0.3448
	Trt x Family	0.008169	0.006340	1.29	0.0988
	Residual	0.4843	0.01909	25.36	<.0001
Lesion Area	Family	0.003830	0.005066	0.76	0.2248
	Trt x Family	0.008199	0.007531	1.09	0.1381
	Residual	0.5710	0.02308	24.73	<.0001

Note: P- value <0.05 shows significant differences at alpha=0.05

Table 4. Type 3 Tests of Fixed Effects (F tests) suggest significant and non-significant effects for lesion length, lesion width, lesion depth and occlusion length.

Variable	Effect	Num DF	Den DF	F Value	Pr>F
Lesion length	Diameter	1	1339	22.04	<.0001
	Site	1	1339	28.87	<.0001
	Trt	3	1339	385.74	<.0001
	Block(Site)	28	1339	1.19	0.2268
Occlusion Length	Diameter	1	1324	27.95	<.0001
	Site	1	1324	35.25	<.0001
	Trt	3	1324	450.96	<.0001
	Block(Site)	28	1324	1.23	0.1913
Lesion Width	Diameter	1	1337	22.04	<.0001
	Site	1	1337	16.81	<.0001
	Trt	3	1337	215.30	<.0001
	Block(Site)	28	1337	1.98	0.0018
Lesion Depth	Diameter	1	1337	34.46	<.0001
	Site	1	1337	0.02	0.9015
	Trt	3	1337	245.10	<.0001
	Block(Site)	28	1337	1.55	0.0343
Lesion Area	Diameter	1	1275	14.38	0.0002
	Site	1	1275	19.05	<.0001
	Trt	3	1275	484.19	<.0001
	Block(Site)	28	1275	1.21	0.2051

Note: P- value <0.05 shows significant differences at alpha=0.05

Table 5. Families ranking for Lesion Length (estimate) overall and across all the four treatments.

Family	Estimate	Rank	Estimate	Rank GH	Estimate	Rank LT
L-7	-0.022	1	-0.3354	1	-0.273	2
L-30	-0.022	2	-0.2874	2	-0.436	1
L-53	-0.014	3	-0.07579	7	-0.231	3
L-23	-0.014	4	0.06251	11	-0.206	4
L-18	-0.005	5	-0.1846	5	-0.029	8
L-37	-0.002	6	-0.1855	4	-0.074	6
L-1	-0.001	7	-0.03086	8	0.2711	13
L-6	-0.00095	8	-0.01837	9	0.0264	9
L-16	0.0087	9	-0.159	6	0.2066	11
L-13	0.0092	10	0.5948	14	-0.097	5
L-51	0.0095	11	0.03192	10	0.2165	12
L-40	0.012	12	0.4601	13	-0.034	7
L-8	0.013	13	-0.2248	3	0.4833	14
L-52	0.0287	14	0.3524	12	0.1755	10

Note: P- value <0.05 shows significant differences at alpha=0.05

Table 6. Summary statistics for lesion length, occlusion length, lesion width and lesion depth.

Variable	Trt	N	Mean	SD	Minimum	Maximum
Lesion Length	LT	342	195.1	103.7	13	590
(cm)	GH	351	133	79.6	16	510
	WM	336	59.3	55.2	12	315
	W	344	30.6	25	9	235
Occlusion Length	LT	342	207.2	105.1	13	590
(cm)	GH	351	139.3	78.8	16	510
	WM	335	60.2	56.9	12	315
	W	344	30.7	25.5	9	235
Lesion Width	LT	342	24.9	9.3	10	57
(cm)	GH	351	21	8.6	9	85
	WM	335	15.8	5.2	4	60
	W	343	13.5	3.6	5	38
Lesion Depth	LT	341	9.7	3.9	0	25
(cm)	GH	351	7.2	2.9	0	30
	WM	335	4.3	3.2	0	23
	W	344	2.7	2.2	0	12
Lesion Area	LT	327	44.5	37.2	2.6	331.1
	GH	340	23.2	20.3	1.1	160.6
	WM	314	9.3	11.3	0.6	77.8
	W	335	4.2	5.0	0.7	55.5

Table 7. Average lesion length, occlusion length, lesion width and lesion depth for each family.

Family	Lesion length	Lesion Width	Occlusion Length	Lesion Depth
L-1	172.3(100.7)	22.8(9.4)	181.9(100.9)	8.3(3.5)
L-6	161.6(98.5)	22.1(8.0)	172.1(99.4)	8.6(3.7)
L-7	145.2(104.0)	21.7(7.7)	153.9(106.2)	8.2(3.0)
L-8	174.7(99.5)	22.6(8.5)	182.5(109.1)	8.7(4.1)
L-13	185.7(95.6)	23.2(9.7)	193.4(94.2)	7.8(3.4)
L-16	167.0(101.5)	23.4(10.3)	174.0(104.0)	8.5(3.7)
L-18	155.5(98.0)	20.7(6.1)	167.9(100.7)	8.3(4.0)
L-23	161.7(113.9)	23.2(11.6)	168.2(112.6)	8.8(4.6)
L-30	130.8(75.5)	23.3(10.1)	158.6(86.1)	8.3(4.2)
L-37	152.5(86.7)	24.6(9.4)	156.3(84.4)	8.8(4.2)
L-40	176.1(93.8)	24.2(8.9)	184.3(95.3)	8.8(3.3)
L-51	168.2(101.6)	23.5(8.8)	171.7(102.9)	8.0(3.1)
L-52	182.2(92.0)	22.7(7.2)	193.5(88.8)	8.6(2.5)
L-53	156.4(90.2)	23.5(12.0)	164.4(91.0)	8.1(4.0)

Note: Means followed by standard deviations in parenthesis