Evaluating Hybrid Poplar Clones Developed by the Univ. of Minnesota's Natural Resource Research Institute in Michigan's Upper Peninsula: 7th-year Results

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Executive Summary

This report is a more complete description of the analyses performed to create the identically titled poster which Ray Miller presented at the 2016 Short Rotation Woody Crops Operations Working Group Meeting in Ft. Pierce, FL. Additional analyses of 2015 survival and variation among taxa for growth and disease characters which were not covered in the poster are presented here. The plantings reported on here are part of a larger network of clone tests established by the Univ. of Minnesota's Natural Resource Research Institute (NRRI). All clones were developed by the NRRI's breeding program which retains all proprietary rights to this material.

Over 110 hybrid poplar clones (representing four taxa) developed by the NRRI were established at two sites in Michigan's Upper Peninsula. One set of clones was established in a single planting at Michigan State University's Forest Biomass Innovation Center (FBIC) near Escanaba, MI. A second set was planted at FBIC and Skandia, MI. Height and DBH were measured annually after a three year establishment period. Incidence of *Septoria* leaf spot (SLS), *Melampsora* rust (MEL), and *Marssonina* leaf spot (MLS) were also scored in Years 3-7. Survival was tallied in 2015.

Differences among clones were significant in each of the five years of measurement for both height and DBH. Broad-sense heritability estimates (H^2) tended to increase over the measurement period, and ranged from 0.22 for height to 0.48 for DBH in Year-7. The magnitude of H^2 estimates warrants continued selection and breeding efforts. However, the selection process will have to account for clone X site interactions which were significant for each year of measurement, and were approximately equal to clone effects in Year-7.

There were significant differences among taxa for Year-7 DBH and 2015 survival (the most current growth and survival data) in all three plantings. Both taxa comprised of *P. trichocarpa* x *deltoides* backcrosses ranked lowest for growth and survival in all three plantings.

MEL infection rates in both clone sets were lower than infection rates of SLS and MLS. Of the three disease characters, clone effects were most frequently significant for MLS (six of seven planting x year of measurement combinations), with SLS and MEL less frequently significant (four of seven and three of seven planting x measurement year combinations, respectively). Of the three disease characters measured MLS appears to be the most prevalent in the three plantings.

There were no significant differences among taxa in MEL in all three plantings across all years of measurement. However, differences in SLS and MLS infection rates among taxa were significant in each planting for one or more years of measurement. *P. trichocarpa* x *deltoides* backcrosses tended to suffer higher SLS and MLS infection rates at FBIC than other taxa, although this relationship did not hold at Skandia. Although the data presented here are far from conclusive, the low rankings of *P. trichocarpa* x *deltoides* backcrosses for growth and survival and their higher SLS and MLS infection rates at FBIC suggest this taxa may not be suitable for short-rotation biofuel plantings in Upper Michigan.

Significantly negative Spearman rank-order correlations indicate that MLS and SLS infection rates negatively affected height and DBH in both FBIC plantings, but were not significantly correlated with growth characters at Skandia. Significant positive correlations between MLS and SLS 2013 infection rates across all three plantings suggest the potential for combined selection for these two characters.

Plantation Establishment

Over 110 hybrid poplar clones were established at two sites in Michigan's Upper Peninsula. One set of clones was established in 2008 in a single planting (identified as FBIC08) at MSU's Forest Biomass Innovation Center (FBIC) near Escanaba, MI. A second set of clones was planted at FBIC and Skandia, MI in 2009, and are identified as FBIC09 and Skandia09 respectively. Four taxa were represented across the three plantings. A single clone of an additional taxa (*P. nigra x maximowiczii)* was included in FBIC08 as a control. The number of clones representing each taxa across the three plantings is given in Table 1. The 2008 and 2009 clone sets had only 14 clones in common. The distribution of clones across the plantings is given in Table 1a in the Appendix.

Plantings were established in weed-free cultivated fields using actively growing 4" mini-cuttings produced in the NRRI greenhouse, and planted in a randomized block design with 6 replications of single-tree plots at each site. Weed control was accomplished using a combination of mechanical and herbicide treatments for the first two years after planting. General characteristics of the FBIC and Skandia sites are given in Table 2.

Table 1. Number of clones representing each of four hybrid poplar taxa in three Upper Michigan plantings of two NRRI poplar clone trials.

		Planting ID		
Taxa	**Abbr.**	**FBIC08**	**FBIC09**	**Skandia09**
P. deltoides x maximowiczii	DxM	3	5	5
P. deltoides x nigra	DxN	44	37	37
P. deltoides x (P. trichocarpa x deltoides)	DxTD	2	16	16
(P. trichocarpa x deltoides) x deltoides	TDxD	3	11	11

Table 2. Site description of two NRRI poplar clone trials in Upper Michigan.

Site Conditions	**FBIC**	**Skandia**
North Latitude	45.7686	46.3619
West Longitude	87.2007	87.2442
Growing Degree Days (base 10^{o} C)	1685	1593
Soil Series	Onaway fine sandy loam	Munising fine sandy loam

Characters Measured

Height and DBH were measured in Years 3-7. Survival was scored in 2015 which was Year-7 for the 2009 plantings and Year-8 for FBIC08. Incidence of *Septoria* leaf spot (SLS), *Melampsora* rust (MEL), *Marssonina* leaf spot (MLS) were also scored in Years 3-7 using the scale outlined in Table 3.

Table 3. Abbreviations and description of index used to score infection rates of three leaf diseases in three Upper Michigan plantings of two NRRI poplar clone trials.

Disease	Abbr.	Index
Melampsora leaf spot	MEL	1= none-trace
Marssonina leaf spot	MLS	2= some (10-30% of foliage)
Septoria leaf spot	SLS	3= heavy (>30% of foliage)

Analysis Methods

Growth Characters

Single-site analyses of variance were conducted on height and DBH data for each year of measurement at each site to test the significance of variation among clones using *SAS, Proc GLM.* Single-site broad-sense heritability estimates (H^2) were computed using variance component estimates generated by *SAS, Proc Varcomp (method=reml)* for each year of measurement using the following formula:

$$H^2 = \frac{var(clone)}{var(clone) + var(error)}$$

A two-site analysis of variance was conducted on height and DBH data for Years-3,5 and 7 in the 2009 plantings to test the significance of site, clone, and site*clone effects using *SAS, Proc GLM. SAS, Proc Varcomp* was used to generate variance component estimates of these effects, which were used to compute the percent of total variation represented by each effect. These percentages reflect to the relative magnitude of each effect, with special attention given to clone and clone*site effects. Two-site broad-sense heritabilities were estimated using the following formula:

$$H^2 = \frac{var(clone)}{var(clone) + var(clone * site) + var(error)}$$

The growth performance of taxa was examined by performing analyses of variance and Tukey HSD tests on each site's Year-7 DBH, the most current growth character common to all three plantings (*SAS, Proc GLM*). The percentage of the total variation for each term in the model in Year-7 DBH was also computed using *SAS, Proc Varcomp*.

Differences in 2015 survival (i.e. Year-8 survival in FBIC08 and Year-7 survival in FBIC09 and Skandia) among clones and among taxa were tested for significance using *SAS, Proc Glimmix* which is designed to analyze binary data. If significant variation among clone or taxon means for 2015 survival was found, mean comparison tests (Tukey-Kramer adjusted least squares means) were computed.

Disease Characters

Due to their non-parametric nature, Kruskal-Wallis tests (*SAS, Proc Npar1way*) were used to test for significant differences among clones and taxa for disease infection rates in years 2010-2013. Disease data were grouped by year of measurement rather than years in the field (as I did for height and DBH above), because I reasoned the amount of inoculum, especially at a single site (i.e. FBIC), would be more strongly linked to a given year than a given age. Spearman rank-order correlations (*SAS, Proc Corr*) were computed using clone means in each planting to examine the relationships between clone performance for each of the growth and disease characters. This process was repeated using taxon means to examine the relationship between taxon performance for each of the growth and disease characters.

Results and Discussion

Growth Characters

Plantation means for all measured characters are given in Table 4. Growth and survival means are based on Year-7 data while disease means are based on 2015 data. Skandia09 had dramatically lower survival and growth than both of the FBIC plantings which were comparable in growth and survival. Weed control at Skandia was problematic and a contributing factor to poor growth and survival at the site. In contrast, means for disease characters were quite uniform across all three plantings.

Table 4. Plantation means for growth characters (Year-7 performance) and disease characters (2015 performance) in three plantings of two NRRI clone trials planted in Upper Michigan.

Character	FBIC08	FBIC09	Skandia09
Survival (%)	0.92	0.92	0.64
Height (ft)	21.1	17.3	6.0
DBH (in)	3.2	3.1	1.1
MEL	1.0	1.0	1.2
MLS	2.0	1.9	1.9
SLS	1.8	1.6	1.4

Variation among clones for height and DBH was significant at each site for each year of measurement (Pr>F=.01). Single-site H^2 estimates (Table 5) tended to increase over time, with the exception of height in Skandia09 which remained relatively consistent over the years of measurement. H^2 estimates for the 2009 set of clones (FBIC09 and Skandia09) were comparable and differed by 0.05 or less over the last two years of measurement. The single planting of the 2008 clone set (FBIC08) had consistently higher H^2 estimates, particularly for DBH, than the 2009 clone set, which indicates stronger clone effects for growth in the 2008 clones. In general, the magnitude of single-site H^2 estimates should support selection efforts for height and DBH given reasonable selection differentials.

Table 5. Single-site broad-sense heritability estimates for three plantings of two NRRI clone trials planted in Upper Michigan.

		Broad-sense heritabilities Years in field					
Planting	**Character**	**3**	**4**	**5**	**6**	**7**	**8**
FBIC08	Height	0.19	0.27	0.27	0.27	0.32	
	DBH		0.34	0.38	0.42	0.48	0.44
FBIC09	Height	0.10	0.15	0.20	0.24	0.22	
	DBH	0.13	0.17	0.23	0.24	0.26	
Skandia09	Height	0.31	0.29	0.21	0.29		
	DBH	0.15	0.21	0.22	0.28	0.28	

Two-site analysis of variance of height and DBH in FBIC09 and Skandia09 plantings revealed significant clone and clone x site effects for Years-3, 5, and 7. The magnitude of clone and clone x site effects relative to total variation was small, with each effect representing only 1% to 4% of the total variation

(Table 6). The relative magnitude of clone and clone x site effects was nearly equal for height and DBH in Years-3, 5 and 7. The importance of clone x site effects is evident in the decreased H^2 estimates for the two-site analysis (Table 6). Comparing height and DBH H^2 estimates in the single- versus two-site analyses reveals reductions of more than 50% for both characters in nearly every year examined (the sole exception is Year-3 height). These reductions are primarily due to the inclusion of clone x site effects in the computation of two-site H^2 estimates. Significant clone x site effects are commonly observed in Michigan hybrid poplar trials and will seriously impede future selection efforts unless a set of broadly adapted clones can be identified or clones can be successfully matched to specific site types.

Table 6. Analysis of variance results, percent of total variation, and broad-sense heritability estimates (H^2) for Years 3, 5 and 7 growth characters in two plantings of an NRRI clone trial in Upper Michigan.

		Year-3				Year-5				Year-7	
Source of Variation	DF	Height		DBH		Height		DBH		DBH	
		Var%	Pr > F	Var%	Pr > F	Var%	Pr > F	Var%	Pr > F	Var%	Pr > F
Site	1	0.84	0.0001	0.75	0.0001	0.87	0.0001	0.79	0.0001	0.68	0.0001
Block(site)	10	0.01	0.0001	0	0.0346	0.01	0.0001	0	0.0106	0.01	0.0057
Clone	69	0.02	0.002	0.01	0.0458	0.01	0.0174	0.02	0.0405	0.04	0.0052
Clone X site	69	0.01	0.0315	0.02	0.5575	0.02	0.0001	0.03	0.0178	0.04	0.0193
Error		0.12		0.22		0.09		0.16		0.23	
H^2		0.11		0.06		0.09		0.09		0.14	

The disparity between the growing conditions at FBIC and Skandia is evident in the magnitude of site effects. Site effects ranged from 68% (Year-7 DBH) to 87% (Year-5 height) of the total variation, and dwarfed all other model effects including the error term (Table 6). The significance of clone x site effects reported above was almost certainly exacerbated by the disparity in growing conditions between the two sites.

Differences among taxa for Year-7 DBH were significant in all three plantings and the percent of total variation attributable to taxon effects ranged from 7% to 20% (Table 7). Both taxa comprised of *P. trichocarpa* x *deltoides* backcrosses (TDxD and its reciprocal DxTD) were at the bottom of the rankings for Year-7 DBH in all three plantings (Table 8). Taxa with *P. maximowiczii* as a parent (NxM and DxM) consistently led the rankings but were not significantly different from DxN clones (Table 8).

Table 7. Percentage of total variation attributable to taxon effects, and F-test probabilities for variation among taxa for Year-7 DBH in three plantings of two NRRI poplar clone trials in Upper Michigan.

Source of Variation	FBIC08		FBIC09		Skandia09	
	Var%	Pr > F	Var%	Pr > F	Var%	Pr > F
Taxon	0.20	0.0041	0.07	0.0012	0.14	0.0025
Replicate	0.01	NS	0.00	NS	0.13	0.0001
Error	0.79		0.93		0.73	

Table 8. Tukey HSD test results for Year-7 DBH of taxa in three plantings of two NRRI poplar clone trials in Upper Michigan.

FBIC08						FBIC09					Skandia09				
Tukey Grouping			Mean (in)	N	Taxon	Tukey Grouping		Mean (in)	N	Taxon	Tukey Grouping		Mean (in)	N	Taxon
	A		4.03	6	NxM		A	3.55	26	DxM		A	1.42	18	DxM
B	A		3.63	24	DxM	B	A	3.20	186	DxN	B	A	1.13	149	DxN
B	A	C	3.25	245	DxN	B		2.77	67	DxTD	B		0.84	27	TDxD
B		C	2.31	13	TDxD	B		2.74	51	TDxD	B		0.84	32	DxTD
		C	2.06	5	DxTD										

Although the 2015 survival of individual clones ranged from 0% to 100% none of these differences were found to be statistically significant in any of the three plantings. This is likely due to the relatively small number (six) of ramets per clone at each site. In contrast, differences in 2015 survival among taxa were significant in all plantings (Pr >F =0.006 or higher). Rankings for 2015 survival followed a pattern similar to Year-7 DBH, with TDxD and DxTD clones at the bottom of the rankings (Table 9). The DxN clones survived significantly better than all other taxa in Skandia09 and better than TDxD and DxTD clones in both FBIC plantings. Survival was analyzed as binary data, and this analysis appears to be more sensitive to group size than analyses of continuous data (e.g. DBH data). For example, differences among clones for Year-7 DBH were significant, while clonal differences for 2015 survival, although they ranged from 0-100%, were not statistically significant.

Table 9. Results of mean comparison tests (Tukey-Kramer adjustment of least squares means) for 2015 survival of taxa in three plantings of two NRRI poplar clone trials in Upper Michigan.

FBIC08					FBIC09					Skandia09				
Tukey Grouping		N planted	2015 surv (%)	Taxon	Tukey Grouping		N planted	2015 surv (%)	Taxon	Tukey Grouping		N planted	2015 surv (%)	Taxon
A		276	0.89	DxN	A		222	0.84	DxN	A		222	0.67	DxN
A	B	24	1.00	DxM	A	B	30	0.87	DxM		B	30	0.60	DxM
A	B	6	1.00	NxM		B	66	0.77	TDxD		B C	66	0.41	TDxD
	B	18	0.72	TDxD		B	96	0.70	DxTD		C	96	0.33	DxTD
	B	12	0.42	DxTD										

Disease Characters

Kruskal-Wallis tests revealed significant differences among clones for MLS in six of the seven year of measurement x planting combinations (Table 10). Clone effects were less frequently significant for SLS (significant in four of seven year x planting combinations) and MEL (significant in three of seven year x planting combinations). The more frequent significance of clone effects for MLS, coupled with the fact that plantation means for MLS generally trended higher than plantation means for MEL and SLS, indicates that MLS is more prevalent in these plantings than MEL and SLS.

Table 10. Plantation means and results of Kruskal-Wallis tests for differences among clones for disease infection rates in three plantings of two NRRI poplar clone trials in Upper Michigan (Pr > Chi Square).

		FBIC08 (df=55)		FBIC09 (df=69)		Skandia09 (df=68)	
Disease	**Yr of meas.**	**Mean**	**Prob>Chi Sq**	**Mean**	**Prob>Chi Sq**	**Mean**	**Prob>Chi Sq**
MEL	2013	1.0	0.0204	1.0	NS	1.1	0.0192
	2012	0.1	NS*	0.4	0.0052		
	2010	0.0	NI**	0.0	NI		
SLS	2013	1.8	0.0001	1.6	0.0001	1.3	0.0001
	2012	0.0	NI	0.4	NS		
	2010	2.2	0.0001	2.4	NS		
MLS	2013	1.9	0.0006	1.9	0.0003	1.9	0.0001
	2012	2.1	0.0001	1.2	NS		
	2010	2.2	0.0001	0.6	0.0003		

*NS = Not significant at α=0.05

**NI = No infection.

Kruskal-Wallis tests found no significant differences among taxa for MEL in each year of measurement across all three plantings (Table 11). Differences among taxa for SLS and MLS were significant five of the six year x planting combinations, and four of seven year x planting combinations respectively. Despite the significant differences among taxa for SLS and MLS, it is difficult to draw firm conclusions regarding the relative performance of the taxa because of unequal group sizes (which precludes mean comparison testing of non-parametric data) and no clear patterns across the plantings and years of measurement. Table 2a in the Appendix lists the taxa rankings for SLS and MLS for each year x planting combination in which taxa effects were significant. There is a trend for DxTD and TDxD clones to incur higher SLS and MLS infection rates than the other taxa in the two FBIC plantings, but this trend is not evident in the Skandia planting. Given that DxTD and TDxD clones were at the bottom of the taxon rankings for Year-7 DBH (Table 8) and survival (Table 9) the data from these two trials do not bolster the case for the use of *P. trichocarpa x deltoides* backcrosses in Upper Michigan.

Table 11. Results of Kruskal-Wallis test for differences among taxa for disease infection rates in three plantings of two NRRI clone trials in Michigan's Upper Peninsula.

Disease	**Yr of meas.**	**FBIC08**	**FBIC09**	**Skandia09**
MEL	2013	NS	NS	NS
	2012	NS	NS	
	2010	NI	NI	
SLS	2013	0.0002	0.0001	0.0024
	2012	NI	0.0032	
	2010	0.0001	NS	
MLS	2013	0.0002	0.0001	0.0001
	2012	0.0001	NS	
	2010	NS	NS	

Within-site Spearman rank-order correlations of clone means revealed significant positive correlations between MLS and SLS infection rates in all three plantings (Table 12). MEL correlations were omitted from Table 12 because the only significant MEL correlation was with SLS in Skandia09 (r=0.30). There were significant negative correlations between SLS and MLS, and height and DBH in FBIC08. In FBIC09 only SLS was negatively correlated with clone performance for height and DBH. In Skandia09 MLS and SLS were not correlated with either growth character (Table 12). The lack of significant correlations between disease and growth characters at Skandia may be a product of poorer growing conditions in Skandia09 which limited growth more than disease infection rates. The positive correlations between MLS and SLS in all three plantings suggests the potential for combined selection for these two characters.

Table 12. Spearman rank-order correlations for clone means for 2013 disease and growth traits in three plantings of two NRRI clone trials in Upper Michigan. All correlations significant at α=0.05 shaded yellow.

	FBIC08 (n=56)			FBIC09 (n=70)			Skandia09 (n=69)		
Disease	SLS	MLS	DBH	SLS	MLS	DBH	SLS	MLS	DBH
MLS	0.72			0.55			0.49		
DBH	-0.45	-0.38		-0.32	-0.06		-0.01	0.09	
HT	-0.34	-0.33	0.88	-0.27	-0.03	0.93	0.01	0.18	0.71

Conclusions

Although there were consistently significant clone effects for height and DBH, and single-site H^2 estimates were moderate, strong clone x site effects reduced two-site H^2 estimates by 50% or more. We have observed significant clone x site effects in other Michigan hybrid poplar trials, and unless a set of broadly adapted clones can be identified, or clones can be successfully matched to specific site types, clone x site effects will seriously impede future selection efforts to improve hybrid poplar for use in Michigan.

P. trichocarpa x deltoides backcrosses (TDxD and DxTD) were at the bottom of taxa rankings for Year-7 DBH and 2015 survival; they also tended to have higher infection rates of SLS and MLS in both FBIC plantings (Table 2a). While by no means conclusive, the data from the two NRRI clone trials presented here do not support using *P. trichocarpa x deltoides* backcrosses in short-rotation biofuel plantings in Upper Michigan.

Of the three disease characters, clone effects were most frequently significant for MLS (six of seven planting x year of measurement combinations), with SLS and MEL less frequently significant (four of seven and three of seven planting x measurement year combinations, respectively). Of the three disease characters measured MLS appears to be the most prevalent in the three plantings.

There were no significant differences among taxa in MEL infection rates in all three plantings across all years of measurement. However, differences in SLS and MLS infection rates among taxa were significant in each planting for one or more years of measurement.

MLS and SLS infections negatively impacted height and growth in both FBIC plantings, but were not significantly correlated with growth characters at Skandia. Significant positive correlations between MLS and SLS 2013 infection rates across all three plantings suggest the potential for combined selection for these two characters.

Appendix

Table 1a. Distribution of clones across three plantings of two NRRI hybrid poplar clone trials in Upper Michigan.

Clone ID	FBIC08	FBIC09	Skandia09
21700	1	1	1
23300	1	1	1
99001111	1	1	1
99007108	1	1	1
99007115	1	1	1
99008070	1	1	1
99037049	1	1	1
99038013	1	1	1
99038022	1	1	1
99038036	1	1	1
99059016	1	1	1
99059043	1	1	1
22033018		1	1
22090013		1	1
23001 03057		1	1
23001 03071		1	1
23057 32006		1	1
23059 32018		1	1
23070 02099		1	1
23070 27001		1	1
23070 33014		1	1
23071 33042		1	1
23076 21006		1	1
23079 17041		1	1
23079 17047		1	1
23079 17069		1	1
99037017		1	1
99037046		1	1
99037051		1	1
99038002		1	1
22700	1	1	1
99008080	1	1	1
NM6	1		
22007002		1	1
22021008	1		
22021009	1		
22021018	1		
22021048	1		
22021051	1		
22057002	1		

22057006	1		
22057033		1	1
22091022	1		
23001 04014		1	1
23010 11022		1	1
23010 11024		1	1
23014 11066		1	1
23054 30001		1	1
23060 26041		1	1
23060 26063		1	1
23070 27023		1	1
23070 27061		1	1
23070 33024		1	1
23071 33040		1	1
23071 33057		1	1
23074 01609		1	1
23074 16002		1	1
23074 16008		1	1
23074 16037		1	1
23074 37038		1	1
23076 01391		1	1
23076 01395		1	1
23076 18011		1	1
23076 18019		1	1
23076 20012		1	1
23076 20056		1	1
34400		1	1
41700	1		
52124074		1	1
52124105		1	1
52124144		1	1
52124230		1	1
9732-11	1		
9732-19	1		
9732-24	1		
9732-31	1		
99002003		1	1
99002017		1	1
99002026		1	1
99002030		1	1
99007071	1		
99007087		1	1
99007116	1		
99008002	1		
99022069		1	1

99038003	1		
99059019	1		
99059066		1	1
99105008	1		
99105088	1		
DN5			
N944-4		1	1
152x11861	1		
22057030	1		
22057032	1		
22066086	1		
22066094	1		
22069011	1		
22090032	1		
22091021	1		
24400	1		
31500	1		
502.37	1		
6300	1		
99037039	1		
99037044	1		
99037053	1		
99038005	1		
99038012	1		
99098008	1		
DN164	1		
DN2	1		
NC14106	1		
Total	56	70	70

Table 2a. Taxon means and rankings for SLS and MLS infection rates in three plantings of two NRRI hybrid poplar clone trials in Upper Michigan.

		FBIC08			FBIC09			Skandia09		
Disease	Yr. of Meas.	Mean	N	Taxon	Mean	N	Taxon	Mean	N	Taxon
SLS	2013	2.3	10	DxTD	2.1	74	DxTD	1.7	24	DxM
		1.9	17	TDxD	1.8	56	TDxD	1.6	67	DxTD
		1.9	261	DxN	1.4	29	DxM	1.3	41	TDxD
		1.8	6	NxM	1.4	200	DxN	1.3	183	DxN
		1.5	24	DxM						
	2012				0.6	86	DxTD			
					0.4	61	TDxD			
			NI*		0.4	215	DxN			
					0.4	29	DxM			
					0.3	3	NxN			
	2010	3.0	21	DxM						
		2.8	9	DxTD						
		2.1	190	DxN		NS**				
		1.7	15	TDxD						
		1.0	6	NxM						
MLS	2013	2.3	10	DxTD	2.3	74	DxTD	2.0	183	DxN
		2.0	17	TDxD	2.0	29	DxM	1.8	24	DxM
		2.0	261	DxN	2.0	56	TDxD	1.7	67	DxTD
		1.9	24	DxM	1.8	200	DxN	1.5	41	TDxD
		1.7	6	NxM						
	2012	2.7	17	TDxD						
		2.7	10	DxTD						
		2.2	6	NxM		NS				
		2.1	267	DxN						
		1.9	24	DxM						
	2010									
			NS			NS				

*NI = No infection

**NS = Not significant at α=0.05