Mitigating apple replant disease with biocontrol soil treatments

M. A. A. MECHLER AND J. A. CLINE

Ontario Agricultural College, Department of Plant Agriculture, University of Guelph, Guelph, ON

Outline

- Apple replant disease overview
- Project design and methodology
- Results
- Conclusions

Overview
Apple Replant Disease

- Shift in rhizosphere community
  - Antagonize new trees
- 200 year documentation
- Global issue
  - Unique to each location

(Traquair 1984; Meints & Toma, 2017; Mazzola & Mullinix, 2005; Suszkiw, 2010; Smith, 1995) (Photo Credit: 'Soil microbes', Alice Dohnalkova, 2016)

Apple Replant Disease

- Decreased shoot and root growth
  - Rosette leaf pattern
  - Necrotic roots
- Delay fruit bearing by 2-3 years
- $20-60K/ha loss during an orchard's lifespan

(Rumberger et al, 2004; Granastein & Mazzola, 2001; Winkelmann et al, 2019; Mazzola & Manici, 2012) (Photo Credit: Maik Lucas; Tezar Tantular)

ARD Solutions

Conventional - Chemical Fumigants

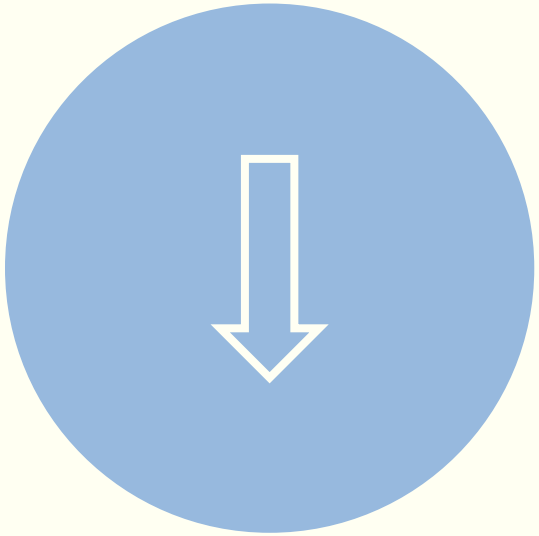
- Limited effectiveness
- Products banned
- Not selective

New Research Priority

- Low impact alternative
- Long-term effectiveness
- Identify specific causes

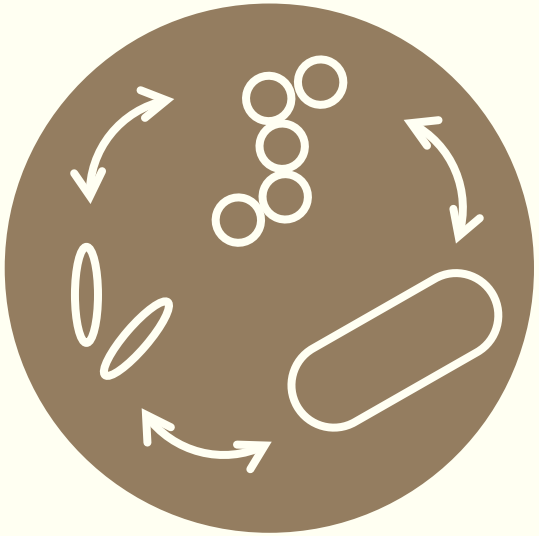
(Granatstein & Mazzola, 2001; Gastroł & Domagala-Światkiewicz, 2015; Yao et al. 2006; Nicola et al. 2017; Agriculture & Agri-Food Canada, 2019)

Research Objectives

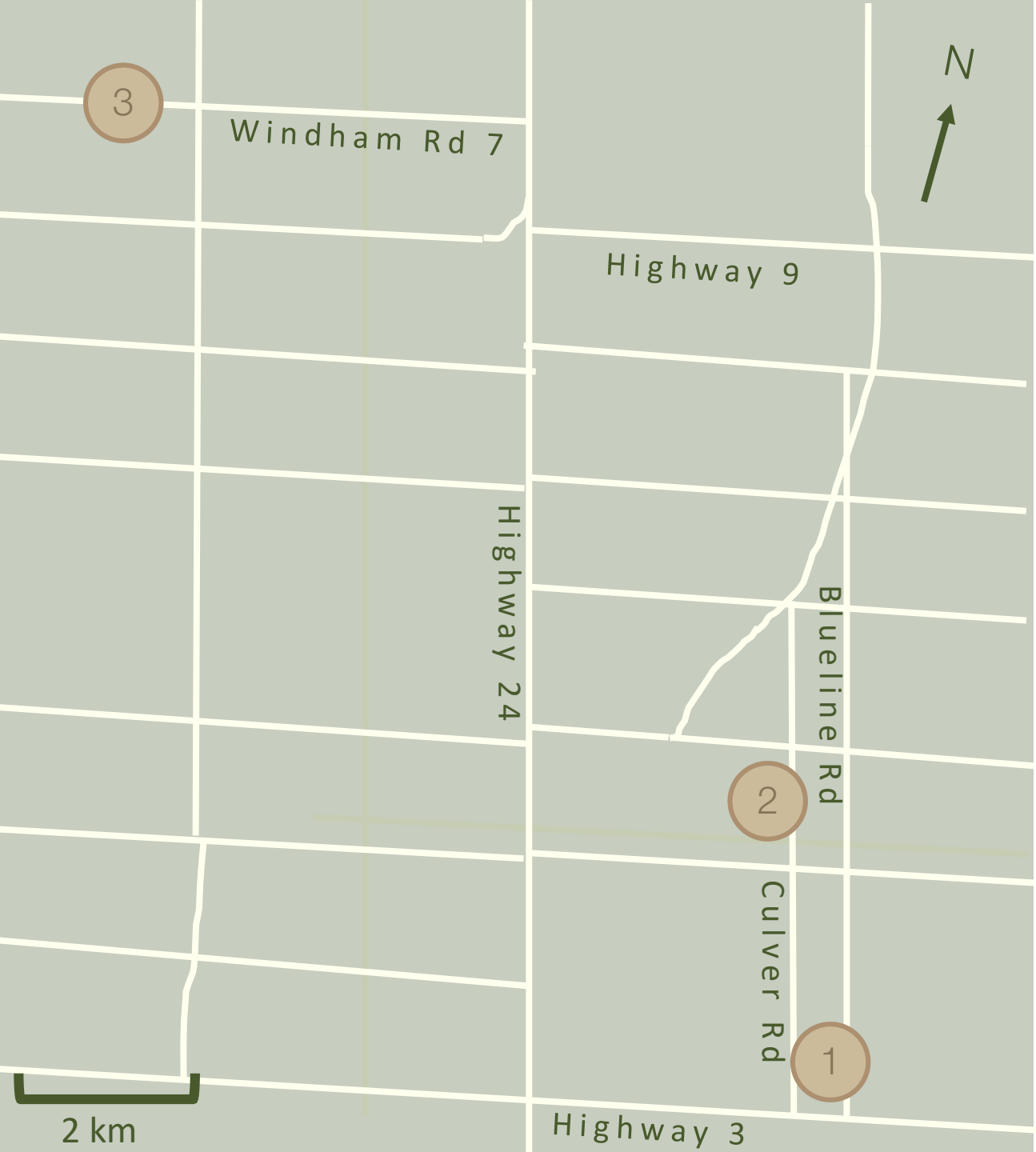


Reduce long-term ARD pressure in Ontario orchards through **biocontrols**

Identify microbes associated with ARD



Characterize the relationship between biocontrols and **rhizosphere microbial species**



Orchard Experiment

- 3 Years
- 3 Orchards in Norfolk County
  - RCBD
- 5 Treatments
  - No additions
  - Chloropicrin

  } Controls
  - Plant Growth Promoting (PGP) Fungi
  - PGP Rhizobacteria
  - PGP Combo

1. Simcoe Research Station

- ‘Aztec Fuji’/M.9 T337
- 2500 trees ha^{-1}
- Vertical axis system
- 12 trees x 5 treatments x 5 blocks (300 experimental units)

2. Schuyler Farms Ltd

- ‘Ambrosia’/M.9 T337
- 1216 trees ha^{-1}
- Free standing system
- 5 trees x 5 treatments x 5 blocks (125 experimental units)

3. Ontario Orchard Supply

- ‘Ambrosia’/B.9
- 4140 trees ha^{-1}
- Vertical axis system
- 5 trees x 5 treatments x 5 blocks (125 experimental units)

Table 1. Application details of initial treatments, applied during planting period

Treatment	Product	Active Ingredient/ Species	Application Method	Application Rate
Control	-	-	-	-
Fumigant	PIC PLUS	Chloropicrin (Cl_3CNO_2)	Shank injected Soil left covered for 7 days, planting will occur an additional 10-14 days later	164 L ha^{-1}
PGP Fungi	Ultrafine Endo	*Glomus intraradices* *G. mosseae* *G. aggregatum* *G. etunicatum*	Root spray	3.12 g/L
	Rootshield Plus	*Trichoderma virens G41* *T. harzianum T22*	Root dip, 3 days before root spray	9 g/L
	Prestop	*Gliocladium catenulatum*	Root spray	5 g/L
PGP Rhizobacteria	Mycostop	*Streptomyces K61*	Root spray	1 g/L
	Serenade Soil	*Bacillus subtilis QST713*	Root spray	0.015 L/ L
PGP Fungi & Rhizobacteria	Ultrafine Endo	*Glomus intraradices* *G. mosseae* *G. aggregatum* *G. etunicatum*	Root spray	3.12 g/L
	Rootshield Plus	*Trichoderma virens G41* *T. harzianum T22*	Root dip, 3 days before root spray	9 g/L
	Prestop	*Gliocladium catenulatum*	Root spray	5 g/L
	Mycostop	*Streptomyces K61*	Root spray	1 g/L
	Serenade Soil	*Bacillus subtilis QST713*	Root spray	0.015 L/ L

Plant Analyses

- **Tree Health**
  - Survival rate
  - Leaf Nutrients
  - Relative circumference growth
  - Chlorophyll fluorescence
  - Rosette leaves
  - Shoot weight
  - Root weight and area

  } Research Station

- **Fruit Development**
  - Bud date
  - Fruitlet count
  - Fruitlet weight

Soil Analyses

- **Bulk Soil**
  - NH_4^+
  - NO_3^-
  - P
  - SOC
- **Rhizosphere**
  - Taxonomic classification and OTU count
  - Shannon diversity
  - Differential Abundance Analysis (phyla and genus level)

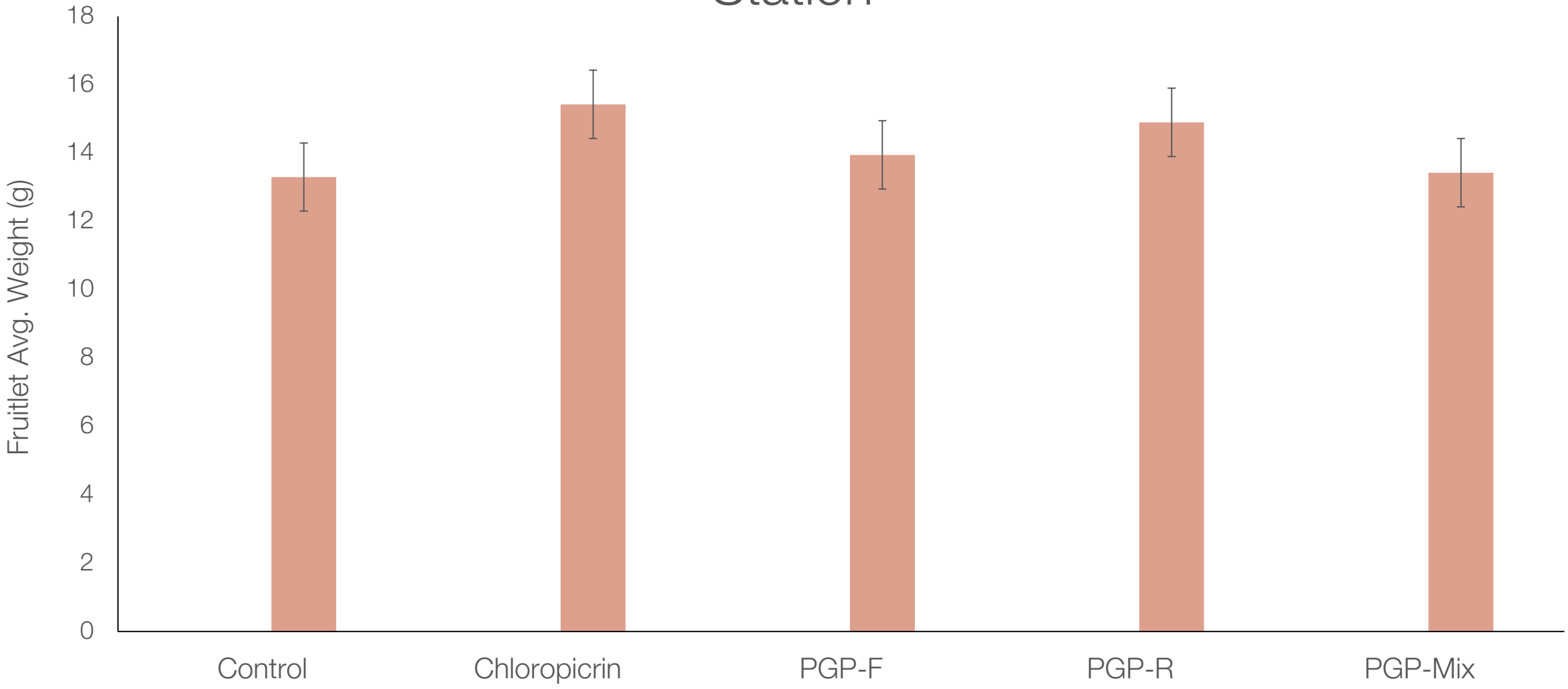
Results

Table 2. Treatment effect on plant growth at the Simcoe Research Station

Treatment	2021				2022	
	Mean Shoot Weight (g)		Mean Root Weight (g)		Avg. Fruitlet Weight (g)	
Control	236.5	ab	137.2	ab	13.30	b
Chloropicrin	307.0	a	150.0	ab	15.43	a
PGP - Fungi	218.1	b	129.7	b	13.95	b
PGP - Rhizobacteria	251.9	ab	180.6	a	14.90	ab
PGP - Mix	222.8	ab	132.2	ab	13.43	b
P	0.0350		0.0288		0.0262	

2021 Biomass Weights at Simcoe Research Station

2022 Fruitlet Average Weight at Simcoe Research Station



p=0.0262

Table 3. Treatment effect on plant growth at Ontario Orchard Supply

Treatment	2021		2022	
	Rel. Circ. Growth %		Fruit Count. Avg/tree	
Control	7.47	bc	5.314	b
Chloropicrin	20.95	a	11.33	a
PGP - Fungi	6.42	c	9.18	ab
PGP - Rhizobac	16.67	ab	6.59	ab
PGP - Mix	16.60	ab	9.48	ab
P	<0.0001		0.0426	

Table 4. Treatment effect on plant growth at Schuyler Farm Ldt

Treatment	2021		2022	
	Rel. Circ. Growth %		Rel. Circ. Growth %	
Control	12.4	ab	49.8	a
Chloropicrin	16.7	ab	55.0	a
PGP - Fungi	19.2	a	46.3	a
PGP - Rhizobac	10.8	b	48.3	a
PGP - Mix	14.6	ab	48.7	a
P	0.0369		0.2609	

Conclusion – Soil & Tree Metrics

Simcoe Station

- Minimal soil changes
- PGP-Rhizobacteria
  - Improved plant growth
  - As effective as chloropicrin
- PGPs-Fungi
  - No improved growth

Schuyler Farm Ldt

- No soil changes
- PGP-Rhizobacteria
  - No improved plant growth
- PGPs-Fungi
  - Initially improved growth
  - Not sustained

Ontario Orchard Supply

- No soil changes
- PGP-Rhizobacteria
  - Improved plant growth
  - As effective as chloropicrin
- PGPs-Fungi
  - No improved growth

Table 5. Treatment effect on microbial phyla diversity at the Simcoe Research Station

Treatment	2020				2021			
	Fungi Shannon Diversity		Bacteria Shannon Diversity		Fungi Shannon Diversity		Bacteria Shannon Diversity	
Control	0.501	a	0.854	a	0.864	ab	0.833	ab
Chloropicrin	0.724	a	0.907	a	1.031	a	0.787	ab
PGP - Fungi	0.639	a	0.742	a	0.938	ab	0.673	b
PGP - Rhizobacteria	0.781	a	0.795	a	0.801	ab	0.905	ab
PGP - Mix	0.775	a	0.654	a	0.588	b	0.994	a
P	0.0504		0.0876		0.0185		0.0360	

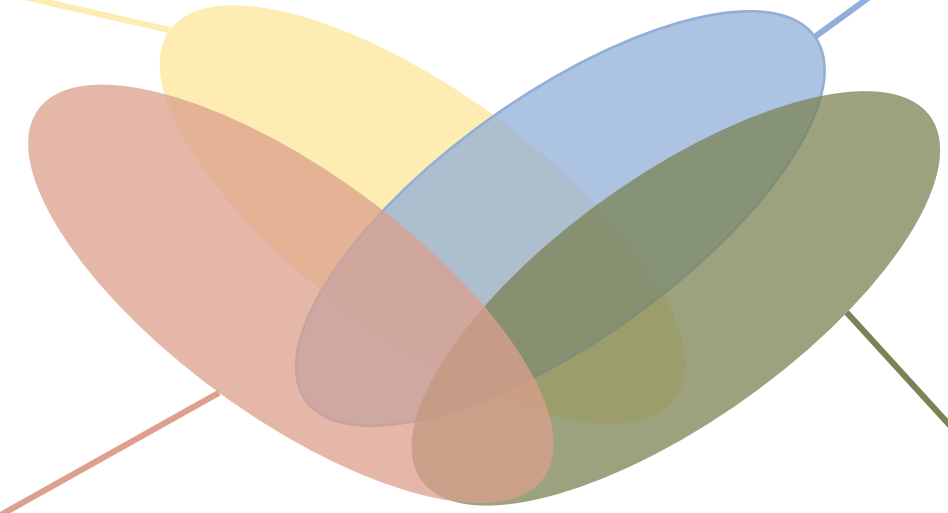
Fall 2020 Bacterial Differential Abundance Analysis at Simcoe Research Station

Chloropicrin v Control

- Acinetobacter
- Allo-Neo-Para-Rhizobium
- Arthrobacter
- Bromate-reducing bacterium WC21
- Cellulomonas
- Comamonas
- Flavobacteriacea bacterium SAP834.2
- Pseudomonas
- Rhizobium cellulosilyticum
- Salmonella enterica ssp. serovar
- Stenotrophomonas rhizophila
- Yersinia pestis 2944

PGP-F v Control

- Acinetobacter
- Acinetobacter sp. IGCAR-9/07
- Bromate-reducing bacterium WC21
- Cellulomonas
- Chyrseobacterium taihuense
- Chyrseobacterium taiwanense
- Enterobacter cloacae
- Enterobacter cloacae sp. dissolvens
- Enterobacter UCD-UG_FMILLET
- Kluyvera ascorbate
- Rhizobium cellulosilyticum
- Stenotrophomonas rhizophila
- Yersinia pestis 2944



PGP- R v Control

- Acinetobacter
- Acinetobacter sp. IGCAR-9/07
- Arthrobacter
- Bacillus subtillis
- Bacillus subtillis ssp. spizizer
- Bacillius sp. RPT0001
- Chyrseobacterium
- Chyrseobacterium taihuense
- Phycicoccus
- Psuedomonas sp.
- Terrabacter
- Yersinia pestis 2944

PGP- Mix v Control

- Acinetobacter sp. IGCAR-9/07
- Acinetobacter
- Bacillus cereus
- Bacillus sp. Kaza-34
- Bacillus subtillis
- Bacillus subtillis ssp. spizizer
- Bacillius sp. RPT0001
- Bacillus velezensis
- Chyrseobacterium taiwanense
- Psuedomonas
- Yersinia pestis 2944

Ecological Role

Beneficial Neutral Pathogenic

Fall 2021 Bacterial Differential Abundance Analysis at Simcoe Research Station

Chloropicrin v Control

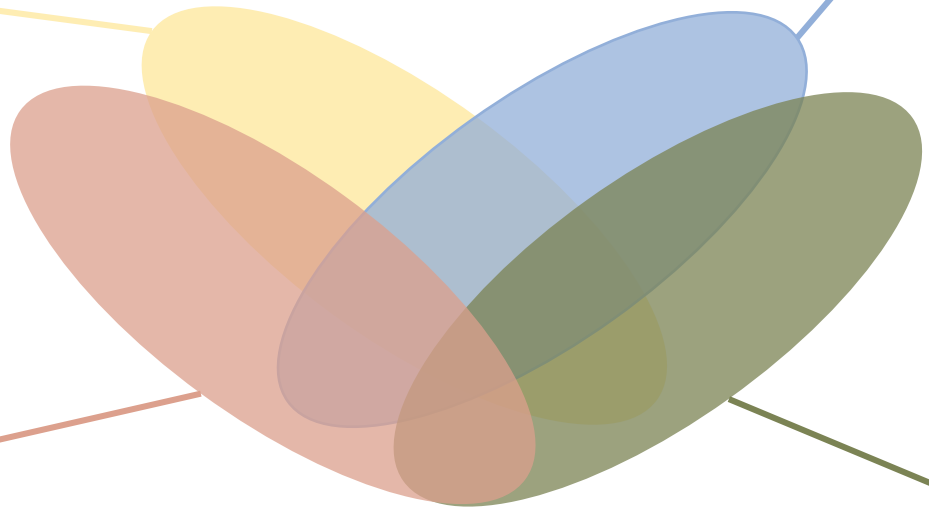
- Acinetobacter
- Allo-Neo-Para-Rhizobium
- Bacillus sp
- Chyrseobacterium
- Citrobacter sp. SRC_NBB2
- Staphylococcus aureus

PGP-F v Control

- Acinetobacter
- Allo-Neo-Para-Rhizobium
- Chyrseobacterium
- Citrobacter sp. SRC_NBB2
- Citrobacter sp. TNT4
- Kineosporia
- Lactococcus
- Pantoea ananatis
- Pantoea sp. TNT6
- Rhizobium etli 8C-3
- Staphylococcus aureus
- Yersinia pestis 2944

PGP-R v Control

- Acinetobacter
- Allo-Neo-Para-Rhizobium
- Bacillus sp.
- Bacillus cereus
- Chyrseobacterium
- Chyrseobacterium taihuense
- Citrobacter sp. SRC_NBB2
- Citrobacter sp. TNT4
- Cryptosporangium
- Lactococcus
- Staphylococcus aureus
- Streptomyces
- Streptomyces flaveus
- Terrabacter



PGP-Mix v Control

- Actinobacter calcoaceticus
- Acinetobacter
- Allo-Neo-Para-Rhizobium
- Bacillus
- Bacillus sp.
- Bacillus cereus
- Bacillus subtillis
- Citrobacter sp. SRC_NBB2
- Citrobacter sp. TNT4
- Comamonas
- Kineosporia
- Kluyvera ascorbate
- Lactococcus
- Rhizobium etli 8C-3
- Staphylococcus aureus

Ecological Role

Beneficial Neutral Pathogenic

Conclusion – Rhizosphere Microbial Community

PGP- Fungi

- Biocontrol species did not persist
- Decreased bacterial diversity
- Increased fungal diversity

PGP - Bacteria

- Biocontrol species persisted
- Increased bacterial diversity
  - More beneficial
- No impact on fungal diversity

PGP - Mix

- Bacterial species persisted
- Increased bacterial diversity
  - More beneficial
- Decreased fungal diversity

Acknowledgements

UNIVERSITY of GUELPH

ONTARIO APPLE GROWERS

THE NORFOLK FRUIT GROWERS ASSOCIATION

CANADIAN AGRICULTURAL PARTNERSHIP

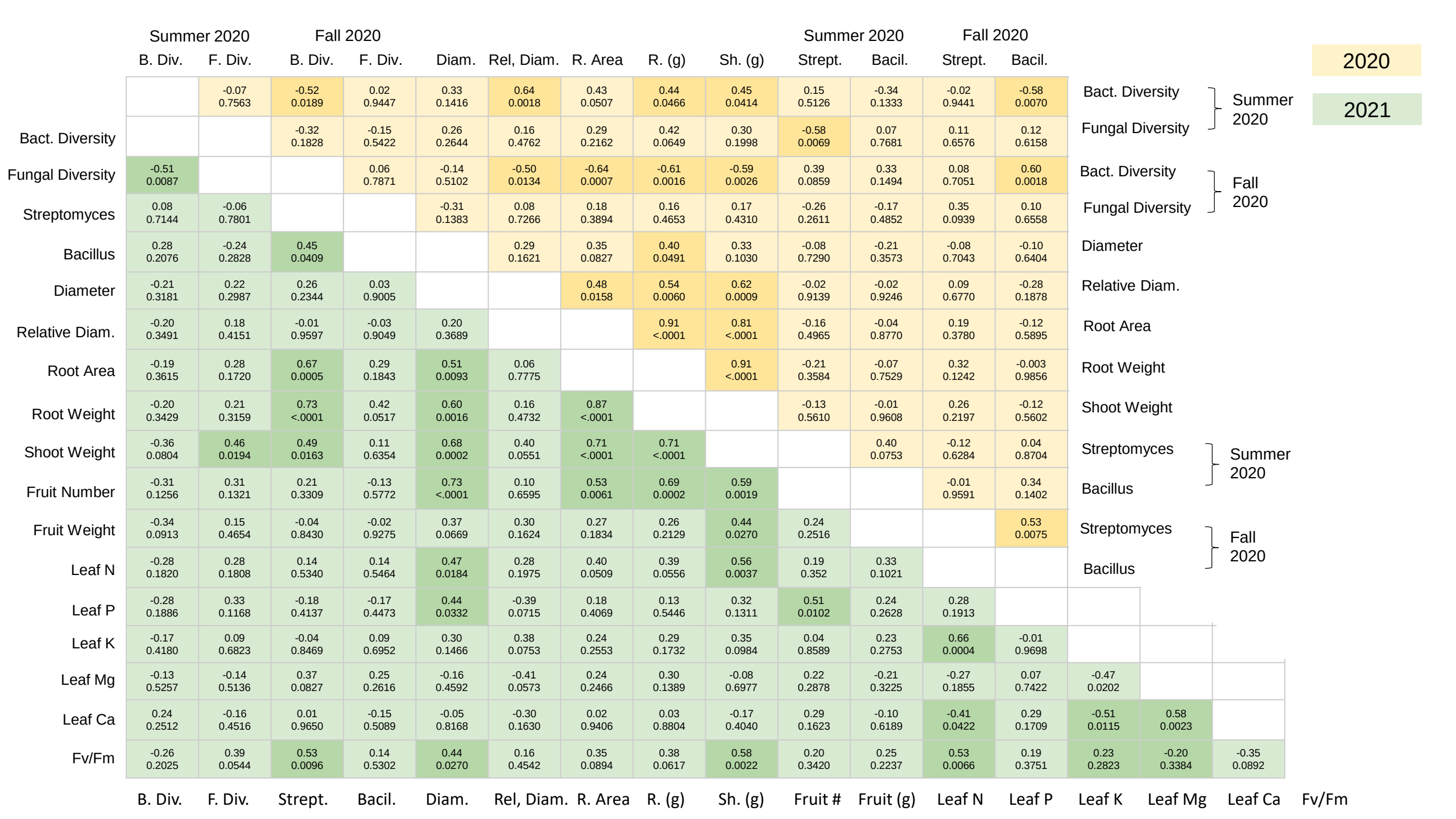
Ontario


Questions?

Page Links

References

•Agriculture and Agri-Food Canada. 2019. Crop Profile for Apple in Canada, 2016: Fourth Edition.
•Gąstoł, M. and Domagała-Świątkiewicz, I. 2015. Mycorrhizal Inoculation of Apple in Replant Soils – Enhanced Tree Growth and Mineral Nutrient Status. Acta Sci. Pol. Hortorum Cultus. 14: 17–37.
•Granatstein, D. and Mazzola, M. 2001. Alternatives to Fumigation for Control of Apple Replant Disease in Washington State Orchards.
•Lucas, M., Balbín-Suárez, A., Smalla, K. and Vetterlein, D. 2018. Root growth, function and rhizosphere microbiome analyses show local rather than systemic effects in apple response to replant disease soil. PLoS ONE. 13(10): 1-21.
•Mazzola, M. and Gu, Y.H. 2000. Impact of wheat cultivation on microbial communities from replant soils and apple growth in greenhouse trials. Phytopathology, 90(2): 114-119.
•Mazzola, M. and Mullinix, K. 2005. Comparative field efficacy of management strategies containing Brassica napus seed meal or green manure for the control of apple replant disease. Plant Disease. 89: 1207-1213.
•Meints, H. and Toma, A. 2017. Apple Replant Disease – Theory versus practice, an overview of known controlling methods. thatchtec.
•Nicola, L., Turco, E., Albanese, D., Donati, C., Thalheimer, M., Pindo, M., Insam, H., Cavalieri, D. and Pertot, I. 2017. Fumigation with dazomet modifies soil microbiota in apple orchards affected by replant disease. Appl. Soil Ecol. 113: 71–79.
•Rumberger, A., Yao, S., Merwin, I. A., Nelson, E. B. and Thies, J. E. 2004. Rootstock genotype and orchard replant position rather than soil fumigation or compost amendment determine tree growth and rhizosphere bacterial community composition in an apple replant soil. Plant Soil. 264: 247–260.
•Smith, T.J. 1995. Orchard Update: Washington State University Coop. Ext. Bull. September issue, Pullman, WA.
•Suszkiw, J. 2010. Biocontrol Bacteria Hold Their Own to the Benefit of Apples. Agricultural Research Magazine, USDA.
•Traquair, J. A. 1984. Etiology and control of orchard replant problems: A review. Can. J. Plant Pathol. 6: 54–62.
•Winkelmann, T., Smalla, K., Kanfra, X., Meyhöfer, R., Reim, S., Schmitz, M., Wrede, A., Zühlke, S., Grunewaldt, J., Weiß, S. and Schloter, M. 2019. Apple Replant Disease: Causes and Mitigation Strategies. Current Issues in Molecular Biology. 30: 89–106.
•Yao, S., Merwin, I. A., Abawi, G. S. and Thies, J. E. 2006. Soil fumigation and compost amendment alter soil microbial community composition but do not improve tree growth or yield in an apple replant site. Soil Biology and Biochemistry. 38: 587–599.



	B. Div.	F. Div.	Strept.	Bacil.	Diam.	Rel, Diam.	R. Area	R. (g)	Sh. (g)	Fruit #	Fruit (g)	Leaf N	Leaf P	Leaf K	Leaf Mg	Leaf Ca	Fv/Fm
Bact. Diversity		-0.07 0.7563	-0.52 0.0189	0.02 0.9447	0.33 0.1416	0.64 0.0018	0.43 0.0507	0.44 0.0466	0.45 0.0414	0.15 0.5126	-0.34 0.1333	-0.02 0.9441	-0.58 0.0070				
Fungal Diversity			-0.32 0.1828	-0.15 0.5422	0.26 0.2644	0.16 0.4762	0.29 0.2162	0.42 0.0649	0.30 0.1998	-0.58 0.0069	0.07 0.7681	0.11 0.6576	0.12 0.6158				
Streptomyces	-0.51 0.0087			0.06 0.7871	-0.14 0.5102	-0.50 0.0134	-0.64 0.0007	-0.61 0.0016	-0.59 0.0026	0.39 0.0859	0.33 0.1494	0.08 0.7051	0.60 0.0018				
Bacillus	0.08 0.7144	-0.06 0.7801			-0.31 0.1383	0.08 0.7266	0.18 0.3894	0.16 0.4653	0.17 0.4310	-0.26 0.2611	-0.17 0.4852	0.35 0.0939	0.10 0.6558				
Diameter	0.28 0.2076	-0.24 0.2828	0.45 0.0409			0.29 0.1621	0.35 0.0827	0.40 0.0491	0.33 0.1030	-0.08 0.7290	-0.21 0.3573	-0.08 0.7043	-0.10 0.6404				
Relative Diam.	-0.21 0.3181	0.22 0.2987	0.26 0.2344	0.03 0.9005			0.48 0.0158	0.54 0.0060	0.62 0.0009	-0.02 0.9139	-0.02 0.9246	0.09 0.6770	-0.28 0.1878				
Root Area	-0.20 0.3491	0.18 0.4151	-0.01 0.9597	-0.03 0.9049	0.20 0.3689			0.91 <.0001	0.81 <.0001	-0.16 0.4965	-0.04 0.8770	0.19 0.3780	-0.12 0.5895				
Root Weight	-0.19 0.3615	0.28 0.1720	0.67 0.0005	0.29 0.1843	0.51 0.0093	0.06 0.7775			0.91 <.0001	-0.21 0.3584	-0.07 0.7529	0.32 0.1242	-0.003 0.9856				
Shoot Weight	-0.20 0.3429	0.21 0.3159	0.73 <.0001	0.42 0.0517	0.60 0.0016	0.16 0.4732	0.87 <.0001			-0.13 0.5610	-0.01 0.9608	0.26 0.2197	-0.12 0.5602				
Fruit Number	-0.36 0.0804	0.46 0.0194	0.49 0.0163	0.11 0.6354	0.68 0.0002	0.40 0.0551	0.71 <.0001	0.71 <.0001			0.40 0.0753	-0.12 0.6284	0.04 0.8704				
Fruit Weight	-0.31 0.1256	0.31 0.1321	0.21 0.3309	-0.13 0.5772	0.73 <.0001	0.10 0.6595	0.53 0.0061	0.69 0.0002	0.59 0.0019			-0.01 0.9591	0.34 0.1402				
Leaf N	-0.34 0.0913	0.15 0.4654	-0.04 0.8430	-0.02 0.9275	0.37 0.0669	0.30 0.1624	0.27 0.1834	0.26 0.2129	0.44 0.0270	0.24 0.2516			0.53 0.0075				
Leaf P	-0.28 0.1820	0.28 0.1808	0.14 0.5340	0.14 0.5464	0.47 0.0184	0.28 0.1975	0.40 0.0509	0.39 0.0556	0.56 0.0037	0.19 0.352	0.33 0.1021						
Leaf K	-0.28 0.1886	0.33 0.1168	-0.18 0.4137	-0.17 0.4473	0.44 0.0332	-0.39 0.0715	0.18 0.4069	0.13 0.5446	0.32 0.1311	0.51 0.0102	0.24 0.2628	0.28 0.1913					
Leaf Mg	-0.17 0.4180	0.09 0.6823	-0.04 0.8469	0.09 0.6952	0.30 0.1466	0.38 0.0753	0.24 0.2553	0.29 0.1732	0.35 0.0984	0.04 0.8589	0.23 0.2753	0.66 0.0004	-0.01 0.9698				
Leaf Ca	-0.13 0.5257	-0.14 0.5136	0.37 0.0827	0.25 0.2616	-0.16 0.4592	-0.41 0.0573	0.24 0.2466	0.30 0.1389	-0.08 0.6977	0.22 0.2878	-0.21 0.3225	-0.27 0.1855	0.07 0.7422	-0.47 0.0202			
Fv/Fm	0.24 0.2512	-0.16 0.4516	0.01 0.9650	-0.15 0.5089	-0.05 0.8168	-0.30 0.1630	0.02 0.9406	0.03 0.8804	-0.17 0.4040	0.29 0.1623	-0.10 0.6189	-0.41 0.0422	0.29 0.1709	-0.51 0.0115	0.58 0.0023		
	-0.26 0.2025	0.39 0.0544	0.53 0.0096	0.14 0.5302	0.44 0.0270	0.16 0.4542	0.35 0.0894	0.38 0.0617	0.58 0.0022	0.20 0.3420	0.25 0.2237	0.53 0.0066	0.19 0.3751	0.23 0.2823	-0.20 0.3384	-0.35 0.0892	