# Arthropod Coocurrence Study

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### March 23, 2016

#### **Tasks**

- Test for number of senescing leaves correlation with Pb
- Think about direct versus indirect genetic effects on community
- Test genotype effect on all species
- Get genetic distance data (RFLP?), maybe ask Zaccheus
- Think about genetic basis of leaf abscision/senescence

# 1 22 Mar 2016

Weighted and probabilistic analyses:

- > ### load pit data
- > source('../src/loadPitdata.R')
- > head(summary(tree.info))

```
leaf.type
                                          leaves
               tree
                             geno
"live:36 " "np12.04: 2 " "1000
                                     " "Min. : 0.00 "
                                :12
                                     " "1st Qu.: 38.75
"sen :36 " "np12.07: 2 " "1008
                                :10
                                     " "Median : 50.00
           "np13.10: 2 " "1017
NA
                                :10
                                    " "Mean : 61.26
           "np2.07 : 2 " "1023
NA
                                :10
           "np2.08 : 2 " "11
                                    " "3rd Qu.: 50.00 "
                                :10
NA
           "np2.10 : 2 " "996
                                :10 " "Max. :464.00 "
NA
```

### > head(summary(tree.arth))

		L	ength	Class	Mode
live	np12.04 10	17 "	16"	"data.frame"	"list"
live	np12.07 10	17 "	16"	"data.frame"	"list"
live	np13.10 10	17 "	16"	"data.frame"	"list"
live	np2.07 100	0 "	16"	"data.frame"	"list"
live	np2.08 996	11	16"	"data.frame"	"list"
live	np2.10 996	11	16"	"data.frame"	"list"

### > head(summary(arth.mats))

			Length	Class	Mode
live	np12.04	1017	"800"	"-none-"	"numeric"
live	np12.07	1017	"800"	"-none-"	"numeric"
live	np13.10	1017	"800"	"-none-"	"numeric"
live	np2.07	1000	"800"	"-none-"	"numeric"
live	np2.08 9	996	"800"	"-none-"	"numeric"
live	np2.10 9	996	"800"	"-none-"	"numeric"

Generate the network using the co-occurrences of each arthropod on a leaf.

```
> library(sna)
> tree.nets <- lapply(arth.mats,coNets)</pre>
> nets.plot <- lapply(tree.nets,rmZeros)</pre>
> par(mfcol=c(8,9),mai=rep(0.1,4))
> for (i in 1:length(nets.plot)){
      if (sum(dim(nets.plot[[i]])) != 0){
          if (tree.info[i,1] == 'sen'){
               vc <- 'red'
          }else{vc <- 'darkgreen'}</pre>
          gplot(nets.plot[[i]],gmode='graph',
                edge.col='darkgrey',vertex.col=vc,
                 vertex.border='lightgrey',
                edge.lwd=nets.plot[[i]], vertex.cex=2)
      }
+ }
>
>
```

>

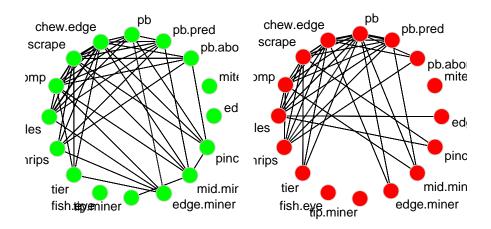
```
> ## Genotype average network
```

```
> ## within each
> liv.nets <- tree.nets[tree.info[,1] == 'live']
> sen.nets <- tree.nets[tree.info[,1] == 'sen']
> for (i in 1:length(liv.nets)){
+    diag(liv.nets[[i]]) <- 0
+    diag(sen.nets[[i]]) <- 0
+ }</pre>
```

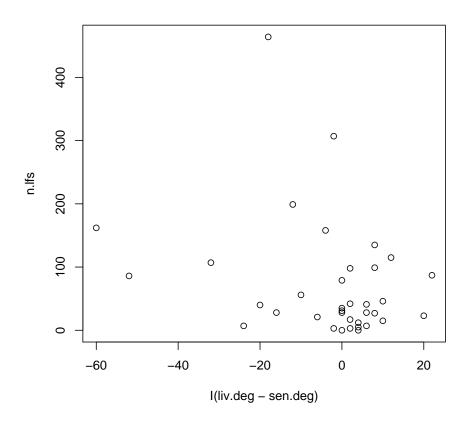
> liv.cen <- unlist(lapply(liv.nets,function(x) centralization(x,FUN='degree')))</pre>

```
> sen.cen <- unlist(lapply(sen.nets,function(x) centralization(x,FUN='degree')))
> t.test(I(liv.cen-sen.cen))
        One Sample t-test
data: I(liv.cen - sen.cen)
t = -1.3324, df = 35, p-value = 0.1914
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
-0.22098995 0.04585768
sample estimates:
 mean of x
-0.08756614
>
  Analyze the mean networks for live and senescent leaves.
> liv.mu <- meanNet(liv.nets)</pre>
> liv.var <- varNet(liv.nets)</pre>
> sen.mu <- meanNet(sen.nets)</pre>
> sen.var <- varNet(sen.nets)</pre>
> par(mfrow=c(1,2),mai=rep(0.1,4))
> gplot(liv.mu,gmode='graph',
        edge.lwd=liv.mu/max(liv.mu)*3, vertex.col='green',
        vertex.border='lightgrey',
        edge.col=grey(liv.var/max(liv.var)), vertex.cex=2,
        mode='circle',displaylabels=TRUE)
```

```
> gplot(sen.mu,gmode='graph',
+ edge.lwd=sen.mu/max(sen.mu)*3,vertex.col='red',
+ vertex.border='lightgrey',
+ edge.col=grey(sen.var/max(sen.var)),vertex.cex=2,
+ mode='circle',displaylabels=TRUE)
```



No relationship between the number of senescing leaves sampled and the difference in degree between networks.



How much variance in network structure does genotype explain?

About 8% of the variation in network structure was attributable to tree genotype. This was primarily due to genetic effects on re-wiring of the network rather than changes to the structure. Leaf senescence strongly impacted network modularity.

- > library(BiodiversityR)
- > dn.tree <- distNet(tree.nets)</pre>
- > adonis(dn.tree~factor(leaf.type)\*factor(geno),data=tree.info, strata=tree.info\$tr

#### Call:

Blocks: strata

Permutation: free

Number of permutations: 999

Terms added sequentially (first to last)

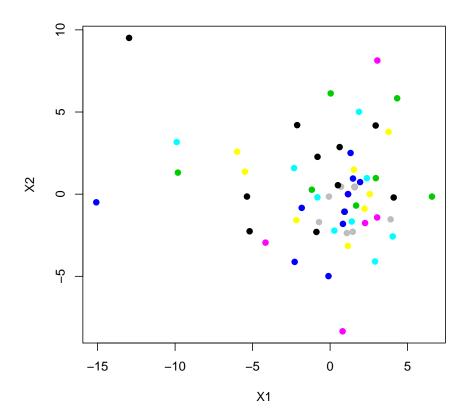
	Df	${\tt SumsOfSqs}$	MeanSqs	F.Model	R2	Pr(>F)	
<pre>factor(leaf.type)</pre>	1	118.53	118.528	5.3315	0.07390	0.001	***
factor(geno)	7	123.52	17.646	0.7937	0.07702	0.005	**
<pre>factor(leaf.type):factor(geno)</pre>	7	116.79	16.684	0.7505	0.07282	0.626	
Residuals	56	1244.97	22.232		0.77626		
Total	71	1603.81			1.00000		

\_\_\_

Signif. codes: 0 âĂŸ\*\*\*âĂŹ 0.001 âĂŸ\*\*âĂŹ 0.01 âĂŸ\*âĂŹ 0.05 âĂŸ.âĂŹ 0.1 âĂŸ âĂŹ 1

> ord.tree <- nmds.min(nmds(dn.tree,2,2),dims=2)</pre>

Using random start configuration

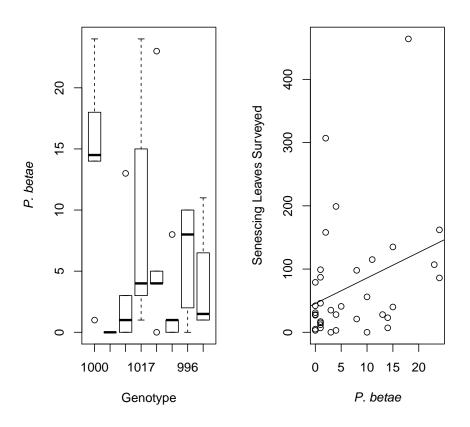


```
> library(bipartite)
> ### centrality
> tree.cen <- unlist(lapply(tree.nets,function(x) centralization(x,FUN='degree')))
> tree.mod <- dget(file='../data/tree.mod') # see src/treeMods.R
> summary(aov(tree.cen~tree+factor(leaf.type)*factor(geno),data=tree.info))
```

```
Df Sum Sq Mean Sq F value Pr(>F)
tree 35 2.9209 0.08345 1.311 0.232
factor(leaf.type) 1 0.1380 0.13802 2.168 0.152
```

```
factor(leaf.type):factor(geno) 7 0.6164 0.08805
                                                   1.383 0.251
Residuals
                               28 1.7824 0.06366
> summary(aov(tree.mod~tree+factor(leaf.type)*factor(geno),data=tree.info))
                               Df Sum Sq Mean Sq F value
                                                           Pr(>F)
                               35 1.0698 0.0306
                                                   0.903 0.616910
tree
factor(leaf.type)
                                1 0.5749 0.5749 16.976 0.000304 ***
factor(leaf.type):factor(geno) 7 0.2974 0.0425
                                                   1.255 0.307767
                               28 0.9482 0.0339
Residuals
Signif. codes: 0 âĂŸ***âĂŹ 0.001 âĂŸ**âĂŹ 0.01 âĂŸ*âĂŹ 0.05 âĂŸ.âĂŹ 0.1 âĂŸ âĂŹ 1
>
   Genotype does not directly influence senescence, but may indirectly influence
senescence through pb.
> summary(aov(I(leaves^0.5)~factor(geno)*pb,
              data=data.frame(tree.info,spp.tot)[tree.info$leaf.type == 'sen',]))
                Df Sum Sq Mean Sq F value Pr(>F)
factor(geno)
                   171.2
                            24.46
                                   1.178 0.3564
                 1
                    98.8
                           98.82
                                   4.760 0.0406 *
pb
factor(geno):pb 6
                   79.5
                           13.25
                                    0.638 0.6985
Residuals
                21
                   436.0
                            20.76
Signif. codes: 0 âĂŸ***âĂŹ 0.001 âĂŸ**âĂŹ 0.01 âĂŸ*âĂŹ 0.05 âĂŸ.âĂŹ 0.1 âĂŸ âĂŹ 1
```

```
> summary(aov(I(pb^0.5) ~ factor(geno),
              data=data.frame(tree.info,spp.tot)[tree.info$leaf.type == 'sen',]))
            Df Sum Sq Mean Sq F value Pr(>F)
factor(geno) 7 30.03
                        4.290
                                2.114 0.0751 .
Residuals
            28 56.81
                        2.029
Signif. codes: 0 âĂŸ***âĂŹ 0.001 âĂŸ**âĂŹ 0.01 âĂŸ*âĂŹ 0.05 âĂŸ.âĂŹ 0.1 âĂŸ âĂŹ 1
> par(mfrow=c(1,2))
> plot(pb~geno,
      data=data.frame(tree.info,spp.tot)[tree.info$leaf.type == 'sen',],
      xlab='Genotype',ylab=expression(italic('P. betae')))
> plot(leaves~pb,
      data=data.frame(tree.info,spp.tot)[tree.info$leaf.type == 'sen',],
       xlab=expression(italic('P. betae')),ylab='Senescing Leaves Surveyed')
> abline(lm(leaves~pb,data=data.frame(tree.info,spp.tot)[tree.info$leaf.type == 'se
```

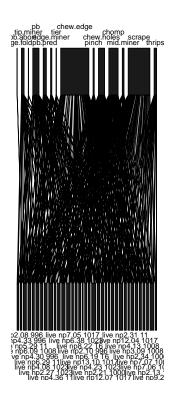


Stand level network modularity

```
> liv.bpn <- spp.tot[tree.info$leaf.type == 'live',]
> sen.bpn <- spp.tot[tree.info$leaf.type == 'sen',]
> liv.modules <- dget('../data/liv.modules')
> sen.modules <- dget('../data/sen.modules')
> par(mfrow=c(1,2))
> plotweb(liv.bpn);title(main='Live')
> plotweb(sen.bpn);title(main='Senescent')
```

Live

### Senescent



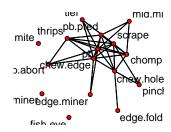


How does the mean interaction network compare to the bipartite to unipartite projection?

- > liv.b2u <- t(liv.bpn) %\*% liv.bpn</pre>
- > sen.b2u <- t(sen.bpn) %\*% sen.bpn
- > par(mfrow=c(2,2))
- > liv.coo <- gplot(liv.mu,gmode='graph',displaylabels=TRUE)</pre>
- > sen.coo <- gplot(sen.mu,gmode='graph',displaylabels=TRUE)</pre>
- > gplot(liv.b2u,coord=liv.coo,gmode='graph',displaylabels=TRUE)
- > gplot(sen.b2u,coord=sen.coo,gmode='graph',displaylabels=TRUE)

>









# 2 12 Mar 2014

Summary of analyses below:

- 1. Genotype does not affect SES
- 2. Genotype affects multivariate co-occurrence patterns for live (negative co-occurrences) and senescent (positive co-occurrences) leaves. These data are relativized to co-occurrence maximum and use the Clarke adjustment.

```
> library(vegan)
> pit <- read.csv('~/projects/dissertation/projects/acn/data/arth_cooc_PIT_Lau.csv'
> coMat = function(x,type=c('pos','neg')){
    if (length(type)!=1|all(type!=c('pos', 'neg'))){print('Using positive co-occurre
    if (length(colnames(x))==0){colnames(x) <- paste('sp',1:ncol(x),sep='')}</pre>
    if (type=='neg'){print('Using negative co-occurrence')}
    x \leftarrow sign(x)
   y <- list()
   k <- 0
    for (i in 1:ncol(x)){
+
      for (j in i:ncol(x)){
        k <- k+1
        if (i!=j){
          if (type=='pos'){
            y[[k]] \leftarrow (x[,i]+x[,j])
            y[[k]][y[[k]]!=2] <- 0
            y[[k]] \leftarrow sign(y[[k]])
          }else{
            y[[k]] \leftarrow (x[,i]+x[,j])
            y[[k]][y[[k]]!=1] <- 0
             y[[k]] \leftarrow sign(y[[k]])
          names(y)[k]=paste(colnames(x)[i],colnames(x)[j],sep='_')
        }
      }
```

```
}
+ y=do.call(cbind,y)
    return(y)
+ }
                                            #got through each tree and quantify the n
                                            #get pit info
> pit.env <- pit[,1:6]
                                            #no fungal
> pit.com <- pit[,-1:-7]
> pit.com[is.na(pit.com)] <- 0</pre>
                                            #combine pb.upper and pb.lower and woody
> pit.com[,4] <- pit.com[,4] + pit.com[,6] + pit.com[,8]
> colnames(pit.com)[4] <- 'pb'</pre>
> pit.com <- pit.com[,c(-6,-8)]
>
                                            #combine pb. preds
> pit.com[,5] <- pit.com[,5] + pit.com[,7]</pre>
> pit.com <- pit.com[,-7]
>
                                            #remove pb holes and mite
> pit.com <- pit.com[,c(-6,-17)]
> ###Separate into trees
> pit.com <- split(pit.com,paste(pit.env$tree,pit.env$leaf.type))</pre>
> pit.g <- unlist(lapply(split(pit.env$geno,paste(pit.env$tree,pit.env$leaf.type)),
> pit.lf <- unlist(lapply(split(pit.env$leaf.type,paste(pit.env$tree,pit.env$leaf.t
> pit.cooc <- lapply(pit.com,coMat,type='neg')</pre>
> pit.cooc <- lapply(pit.cooc,function(x) apply(x,2,sum))</pre>
> pit.cooc <- do.call(rbind,pit.cooc)</pre>
```

```
> pit.neg <- pit.cooc
> pit.cooc <- lapply(pit.com,coMat,type='pos')</pre>
> pit.cooc <- lapply(pit.cooc,function(x) apply(x,2,sum))</pre>
> pit.cooc <- do.call(rbind,pit.cooc)</pre>
> pit.pos <- pit.cooc</pre>
> pit.com. <- do.call(rbind,lapply(pit.com,function(x) apply(x,2,sum)))</pre>
> adonis(apply(pit.com.,2,function(x) if (all(x==0)){x}else{x/max(x)})[pit.lf=='liv
> adonis(apply(pit.com.,2,function(x) if (all(x==0)){x}else{x/max(x)})[pit.lf=='sen
> adonis(cbind(pit.cooc[pit.lf=='live',],ds=rep(1,nrow(pit.cooc[pit.lf=='live',])))
> adonis(cbind(pit.cooc[pit.lf=='sen',],ds=rep(1,nrow(pit.cooc[pit.lf=='sen',])))~p
> adonis(cbind(apply(pit.cooc[pit.lf=='live',],2,function(x) if (all(x==0)){x}else{
> adonis(cbind(apply(pit.cooc[pit.lf=='sen',],2,function(x) if (all(x==0)){x}else{x
> barplot(sort(apply(pit.cooc[pit.lf=='live',],2,sum),dec=TRUE)[1:10],las=2)
> barplot(sort(apply(pit.cooc[pit.lf=='sen',],2,sum),dec=TRUE)[1:10],las=2)
>
                                            #ses
> source('~/projects/packages/cooc/src/cooc.R')
> pit.ses. <- lapply(pit.com,function(x) if (length(table(x))<2){NA}else{oecosimu(x)
> pit.ses <- unlist(lapply(pit.ses.,function(x) unlist(x[1])))</pre>
> pit.ses.p <- unlist(lapply(pit.ses.,function(x) unlist(x[3])))</pre>
> pit.ses[is.na(pit.ses)] <- 0</pre>
> summary(aov(pit.ses[pit.lf=='live']~pit.g[pit.lf=='live']))
> summary(aov(pit.ses[pit.lf=='sen']~pit.g[pit.lf=='sen']))
> pit.ses[pit.lf=='live']~pit.g[pit.lf=='live']
```

```
#reml for ses
> source('/Users/Aeolus/projects/packages/ComGenR_development/src/cgREML.R')
> cgREML(pit.ses[pit.lf=='live'],pit.g[pit.lf=='live'])
> cgREML(pit.ses[pit.lf=='sen'],pit.g[pit.lf=='sen'])
> cgREML((pit.ses[pit.lf=='sen']-pit.ses[pit.lf=='live']),pit.g[pit.lf=='sen'])
>
```

## 3 Feb 2014

Figures

## 4 30 Jan 2014

Continuing to work on script.

Results Summary

- Richness
  - Genotype does not influence richness (Live:  $1.4102840\ 0.2350093$  , Sen:  $1.060422\ 0.303119$  )
  - Or the difference in richness (-2.246907e-09 1.000000e+00)
- Composition

- Leaf type influences composition (Paired t: t=23.7363, df=34, p-value <2.2e-16)
- Genotype does not influence the difference in community composition between leaf types (REML: chi2=0.53,P=0.465)
- Genotype influences community composition for live but not sen leaves (F=1.85,P=0.004,R2=0.28; F=1.22,P=0.176)
- Several genotypes are different (Paired Perm: 1000 vs T6,1008vs1023vsT6, 1023vs11vsT6, 11vs996,996vsT6)

#### • PB effects:

- PB (as percent) decreased from senescent to live leaves by 31% on average (t = -5.5791, df = 34, p-value = 3.036e-06) but this was not different among genotypes (X2=2.809,P=0.0937)
- PB.total Genotype (X2=4.20104879,P=0.0404)
- Percent PB Genotype Live (X2=6.75,P=0.009), Percent PB! Genotype Sen (X2=3.32,P=0.07)
- PB frequencies are different among genotypes (X2 test: 125.62, df=18, P=2.2e-16), frequencies of 0 and 1 PB per leaf are different among genotypes on live leaves (X2=6.701,P=0.0096 and X2=6.27,P=0.012), while 2 PB/leaf for SEN (X2=4.045,P=0.044)
- These species' abundances differ between live and senescent:
  - chew.edge (P=0.00009)
  - scrape (P=0.000)

- chomp (P=0.00013)
- chew.holes (P=0.00553)
- tier (P=0.01134)
- mid.miner (P=0.00553)
- pinch (P=0.00683)
- pb (P=0.00060)
- Co-occurrence patterns
  - Live SES is lower than sen ses (Live: SES=-7.865,P=0.001; Sen: SES=-4.447,P=0.001)
  - No genotype effect on co-occurrence patterns within trees (X2=0,P=1)
  - Co-occurrence network structure different between live and sen (QAP: z=7.454,P<0.0001).
- Nestedness
- At the tree scale, both live and sen are nested for r00 and r0
- At the genotype scale, live is nested for r00 and both for r0

# 5 29 Jan 2014

Analyze data for the following:

- Genetic effect on P. betae
- Genetic effect on composition
- Genetic effect on SES

- Co-occurrence network structure
- Nestedness for live and senesced

## 6 4 Dec 2013

Notes from meeting with Tom:

- P. betae phenology likely done by late June (see Williams and Whitham)
- Test for number of senescing leaves correlation with Pb
- Think about direct versus indirect genetic effects on community
- Test genotype effect on all species
- Get genetic distance data (RFLP?), maybe ask Zaccheus
- Think about genetic basis of leaf abscision/senescence

## 7 2 Dec 2013

Results of analysis of liv and sen

- Networks are sparse but show distinct differences, need to figure out how to compare models
- Co-occurrence
- $\bullet$  Co-occurrence increases after senescence (t = 2.1951, df = 30, p-value = 0.03603)

- Genotype effect live: geno.ses 6 22.46 3.743 1.006 0.441
- $\bullet$  Genotype effect sen: geno.ses 6 33.59 5.599 4.699 0.002 \*\*geno.ses 6 33.59 5.599 4.699 0.002 \*\*
- Genotype mean ses shift from some positive to all negative going from sen to liv
- Greater percent pemphigus on sen than live (t = 5.4687, df = 34, p-value = 4.226e-06)
- No difference in fungus (t = -1.8687, df = 34, p-value = 0.07031)

#### **Dataset Alterations**

- 1. Check the following
  - (a) np5.5 doesn't exist but "NP6-5 1023 1 M"
  - (b) np13.10 1017 $_live -$  "235NP13 1010171M" np2.41023 $_live -$  "199NP2 41000C0F"
- (c) "np3.36  $1000_live$ " "np3.3611 $_sen$ " "168NP3 3610000F" np4.231023 $_sen$  "147NP4 2310231M"
- (2) np5.5 does not exist in the pit dataset, changing to np6.5
  - 3. np3.36 is genotype 1000, changing in dataset from 11 to 1000 for sen
  - 4. np2.4 is 1000, but there is no np2.4 in the senescing dataset, make np2.4 into np4.23.
  - 5. np13.10 missing in senescing dataset, make np13.10 sen zero.

6. All passed the following test:

all(sort(unique(paste(test[[1]]\$geno,test[[1]]\$tree)))==sort(unique(paste(te

Notes from samples:

- NP2-10 996 tree: almost all leaves from one branch, which appeared to be dying back.
- NP8.22 T6 tree: "weird"
- NP6.05 1023 tree: check tree number. Could be NP5.05.
- np2.21 1000 tree: pair of green leaves with galls and leaf tier
- np4.23 1023 tree: should it be np4.27?
- np5.25 996 tree: yellow leaf with gall tied to one green leaf (no gall), which fell apart
- np3.12 1000 tree: fungal branch infection on most branches. Most leaves from 3 branches
- np3.36 11 tree: 1000? check litter and garden info

## 8 26 Nov 2013

# 9 15 Nov 2013

• Wait to process litter leaves, they are mixed with other leaves and would need to be sorted

- Moving them into Bio340
- Transporting senescing leaves back to Cambridge

# 10 11 Nov 2013

- Group woodies with non-woody
- How do you handle the absence of senescent leaves?
- What about the affect of Pemphigus loads on individual leaves or on trees?

#### Senescent Leaf Data Collection Notes

- Double check and correct np3.36 genotype from Master Sheet
- Pb.upper = gall opening is on the upper side of the leaf
- Fungal = necrotic tissue
- Think about fungal from previous observations, maybe just call it necrosis

## 11 7 Nov 2013

- Running genotype sensitivity analysis
- Not significantly different when removing just one genotype
- Try removing more genotypes

# 12 6 Nov 2013

- 1. Decide on species groups
- 2. Re-run analyses
- 3. Collect senescing and leaf litter data
- 4. Identify to species using Randy's notebook
- 5. Look into Gina's time paper
- 6. Serphid and Chanamyid flies

## 13 5 Nov 2013

Running preliminary analyses.

- 1. Stand level networks (structure)
- 2. Tree level co-occurrence patterns (significant genotype effect)

## 14 Before 15 Oct 2013

### 14.1 Tasks

- Photograph representatives of all leaf mods
- Measure average specific leaf area for each shoot
- Weigh leaf mods where possible

- Run co-occurrence analyses on leaf modifiers
- Run analysis of genotypic effect on leaf mod co-occurrence patterns
- Add covariates to genetic model

#### **Process Summary**

• ONC Co-Occurrence 2012

Did a preliminary analysis of the co-occurrence data with tree level co-occurrence patterns among genotypes

Significant genotype pattern of observed C-scores

Average dp networks "look" different

Can still average among shoots prior to analysis

Need to check effects of the number of leaves sampled

- Art's 2004-2006 data
- Gina's 2002-2003 data

#### Summary

• Study the cooccurrence patterns of leaf modifying arthropods on different genotypes of Narrowleaf Cottonwood (Populus angustifolia).

#### 14.2 Meta-data

• Leaves collected from trees of known genotype in the north garden of the Ogden Nature Center on 4 May 2012.

- Between 3 and 4 branches were cut from 15-20 feet on the west aspect of trees.
- Branches were stored in water until the leaf shoots were removed from each branch.
- Branches were between 0.5 and 1 cm in diameter
- Small (0.25 cm) green leafhoppers abundant
- Leaves were stored in ziplock bags until processed ( 1 week)
- Leaf modifiers were scored at the leaf scale, keeping track of the identity of each shoot
- Small, young/juvenile leaves close to the apical meristem were not surveyed, as they would not have equal exposure time to arthropods
- NOTE: N4.30 P. betae tends to be higher up on the leaf above the junction of the leaf and the petiole
- NOTE: In future studies, you can write the shoot number on the leaves, and possibly the leaf number
- NOTE: Specimen N2-9 had many instances of some kind of black rot in the petioles that almost always coincided with pemphigus galls and sometime phylocolpa. It also occurred in petioles of adjacent stems without pemphigus when there was a high enough load. It seemed to grow both out of the gall and up from the stem into the petiole. Possibly an effect of nutrient dynamics?

### 14.3 PIT Tree Level Co-occurrence Data Aug 2012

### 14.4 Methods

- 1. 20 shoots collected from between 2 and 3 meters height
- 2. leaves were collected haphazardly from around the entire tree
- 3. shaded leaves were avoided
- 4. abscising leaves were avoided
- 5. 5 leaves per shoot (n=10) for each tree starting from the bottom of the whorl at the first full sized leaf
- 6. The following leaf modifiers were assessed:
  - p.upper (number): Pemphigus opening on upper side of leaf
  - p.lower (number): Pemphigus opening on lower side
  - p.nec (number): Pemphigus with necrosis
  - p.hole (number): Pemphigus with chow hole
  - p.woody (number): woody Pemphigus gall (likely a different species, check senescent leaves for opening location)
  - p.open (number): Pemphigus that has been opened
  - chew.edge (presence): chewing at the edge
  - chew.holes (presence): chewing holes not at edge
  - lace (presence): lacey chewing
  - fish.eye (presence): hole with necrosis surrounding

- tip.miner (presence): mining at the tip
- edge.miner (presence): mining at the edge
- fungal (presence): fungal necrosis
- chomp (presence): large removal of tissue
- pinch (presence): pinching at the edge of the leaf
- thirps (presence): thrip damage (golden-brown hue with exuvia and frass)