Long-Term Monitoring of Disease Progression and the Population Genetics of *Phytophthora ramorum* within SFPUC Watershed

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Berkeley
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San Francisco Public Utility Commission (SFPUC) Peninsula Watershed

23,000 acres in central San Mateo County

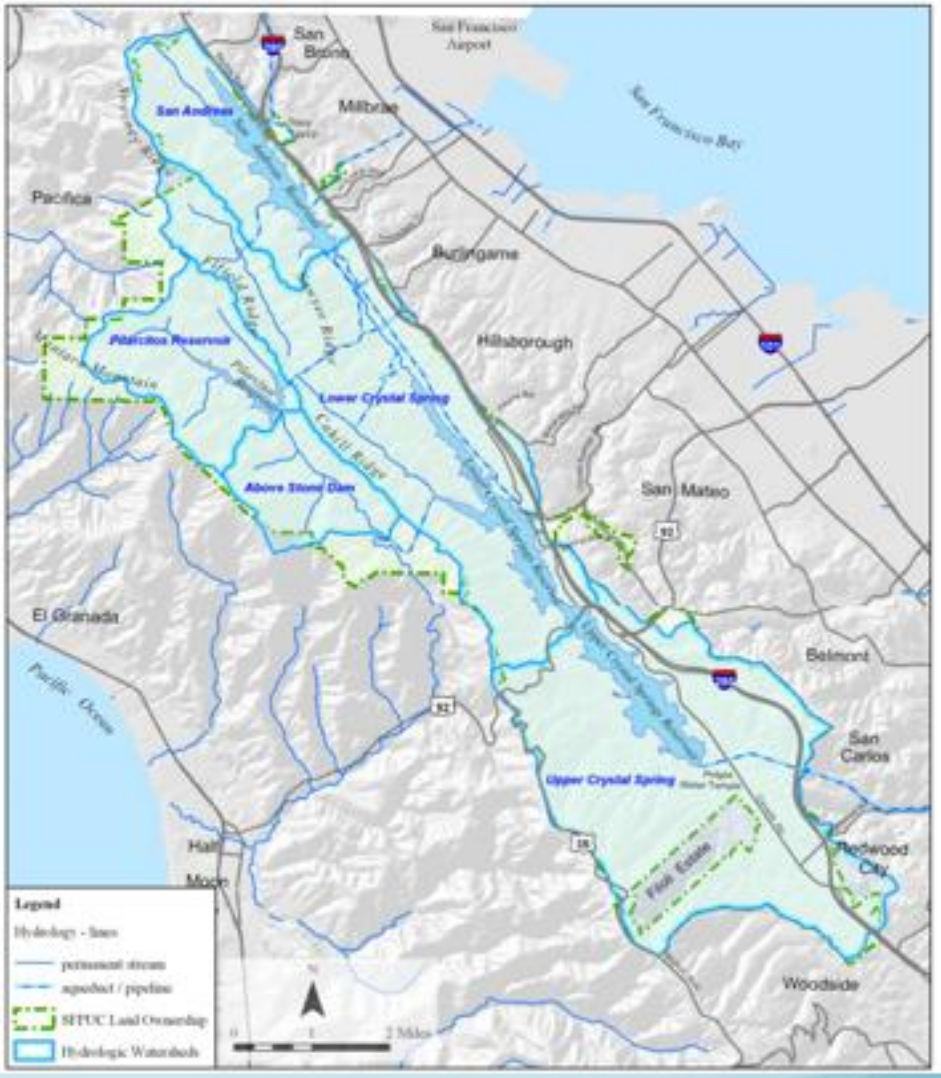
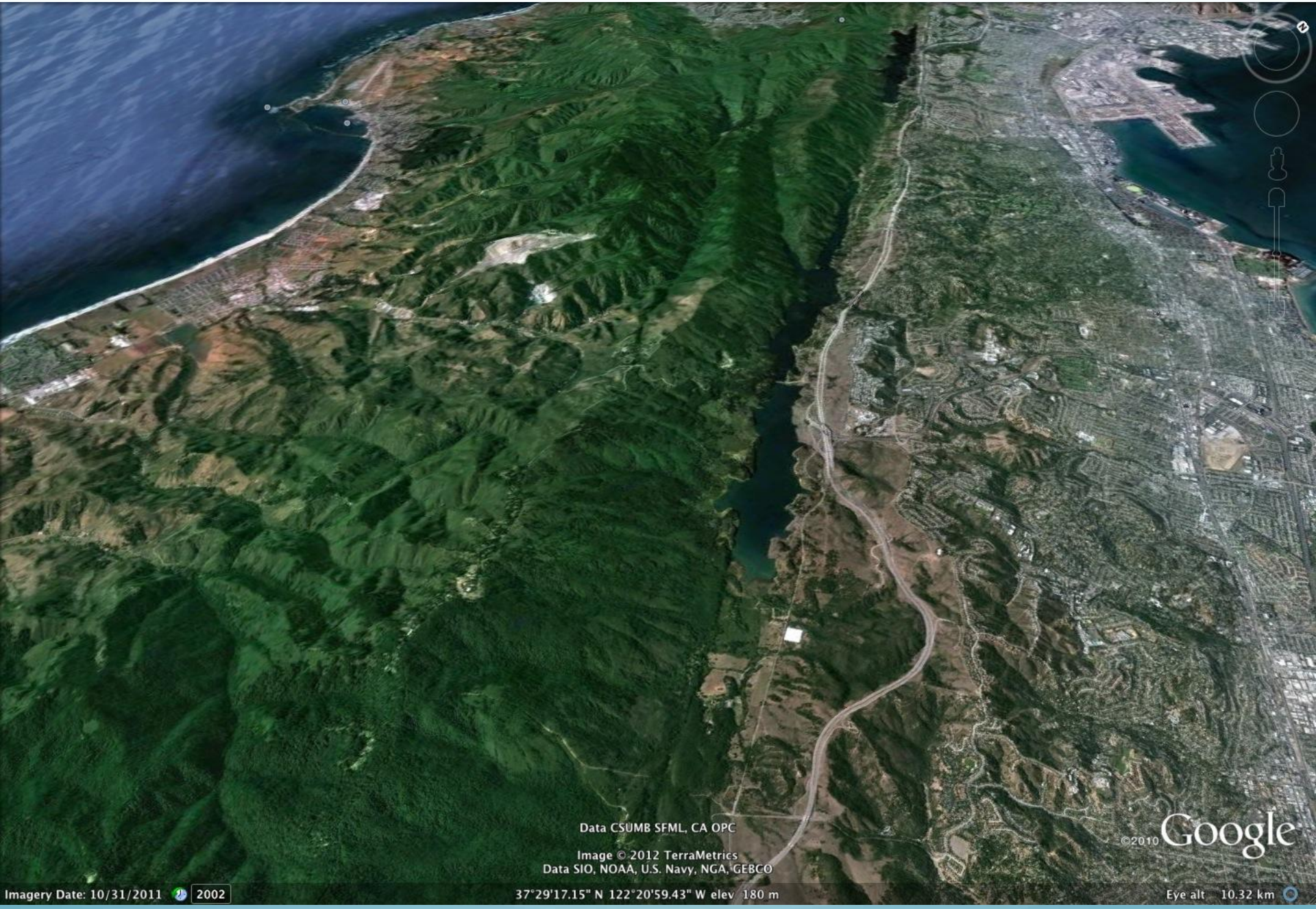


Photo courtesy of SFPUC 2011 WEIP report


Data CSUMB SFML, CA OPC
Image © 2012 TerraMetrics
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Imagery Date: 10/31/2011
2002
37°29'17.15" N 122°20'59.43" W elev 180 m
Eye alt 10.32 km

Crystal Springs Reservoir

Coast Live Oak/Bay Laurel Dominated
Warmer/Eastern facing slopes
Older infestation

Pilarcitos Reservoir

Perennial creek
Cooler/wetter microclimate
Douglas Fir/Tan Oak dominated

Benefits of SFPUC as a field site

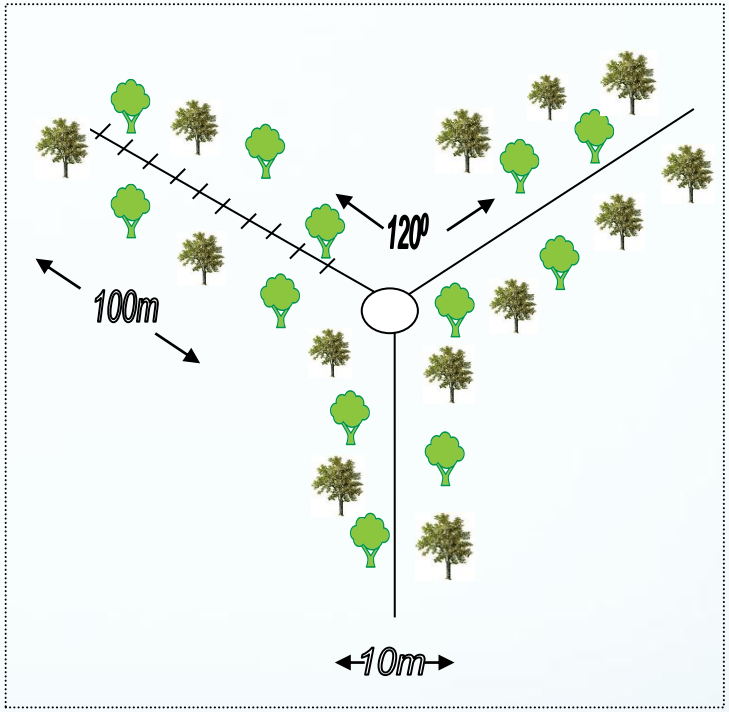
- Closed to the public >100yrs
- Located in a heavily infested/ highly populated area
- Small scale region with contiguous habitat to monitor natural disease progression
- Location of the oldest know infestation in San Mateo County >10yrs
  - Intermediate aged relative to entire CA infestation
- Easily accessible making repeated/exhaustive sampling possible
- No recent fire interaction

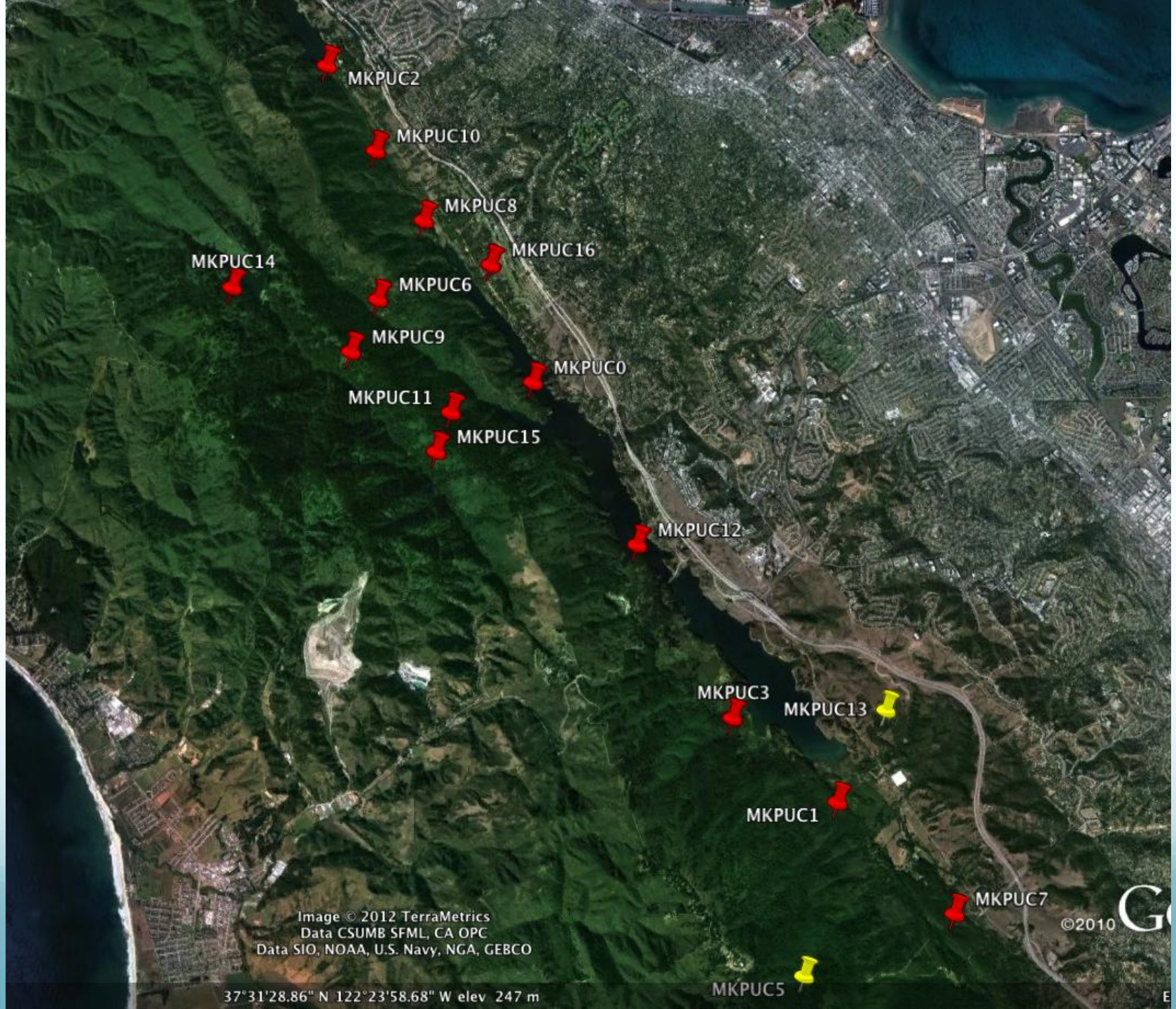
Plot Network

- Established in summer 2008 (pilot study)
- Identified several potential sites in appropriate vegetation types within SFPUC
- Randomly selected **16 plots** based on presence of suitable habitat for *P. ramorum*
  - Bay Laurel, Coast Live Oak, Tan Oak dominated
- Each plot located >2km apart
  - To avoid intermixing of potential pops between plot

Plot design

- Each plot consists of 3 transects 100m long/10m wide
- Tagged each individual Coast Live Oak, Bay Laurel, and Tanoak stem within corridor (~2565 trees)
- Every 10m one Bay Laurel stem was randomly selected for repetitive sampling
- All additional bay trees were tagged to determine overall bay density and DBH was recorded to determine basal area
- In 2009 six plots (3/drainage) were selected for an intensively sampled/soil/leaf comparison study and 3 transects were added to each, these add-ons were used for the duration of the study for a total of 66 possible transects in 16 plots




MKPUC2
MKPUC10
MKPUC8
MKPUC16
MKPUC14
MKPUC6
MKPUC9
MKPUC0
MKPUC11
MKPUC15
MKPUC12
MKPUC3
MKPUC13
MKPUC1
MKPUC7
MKPUC5
Image © 2012 TerraMetrics
Data CSUMB SFML, CA OPC
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
©2010
37°31'28.86" N 122°23'58.68" W elev 247 m

Repetitive Sampling Events

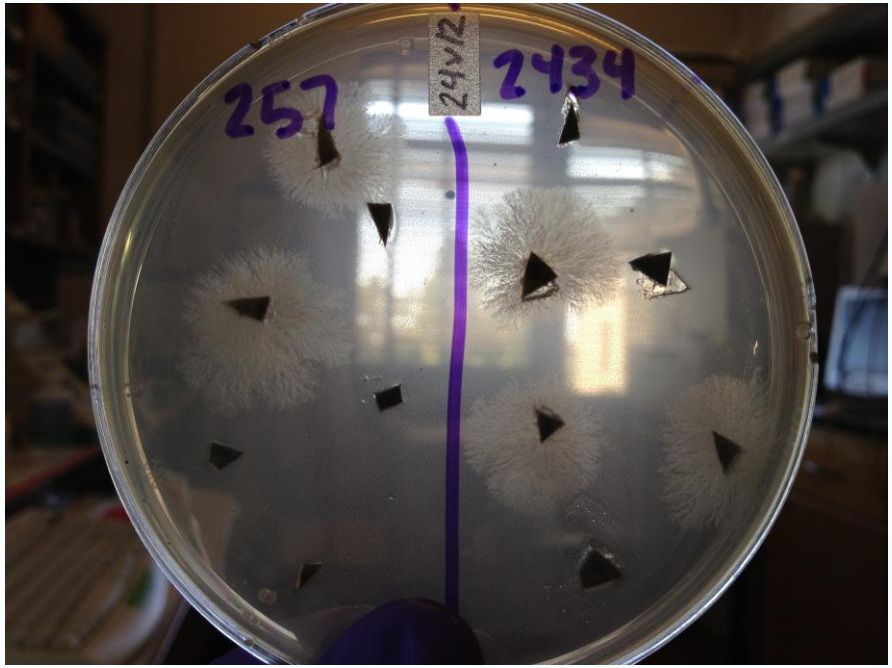
- Bay Laurel sampling was repeated **3 times per year for 3 years** to capture the cyclical life cycle of P. ramorum
  - Winter/Early season (before ideal conditions)
  - Spring/Peak season (warm/wet conditions)
  - Fall/ Late season (after hot/dry conditions)
- The same Bay stems assessed and if symptomatic, sampled up to **9 times** over **3 years** under different climatic conditions
  - Spans dry 2008-2009 period and much wetter 2010-2011

Bay Laurel Sampling

- Every 10m along each transect, Bay stems were assessed for visible symptoms of *P. ramorum*
  - If symptomatic, 6 leaves were collected and plated in selective media within 72 hrs

- All viable isolates genotyped using 6 primer pairs which amplify 10 microsatellite loci
  - Ms18 and 64 (Ivors *et al.*, 2006),
  - Ms39, 43, 45 (Prospero *et al.*, 2007)
  - MsILVO145 (Vercauteren *et al.*, 2010)

- Tissue samples of all symptomatic leaves plated on PARP
- Isolation used to determine culture success at each sampling period throughout the year.
  - Used as a proxy for pathogen viability
- Culture negative leaves undergo diagnostic assay
  - *P. ramorum* - specific nested qPCR

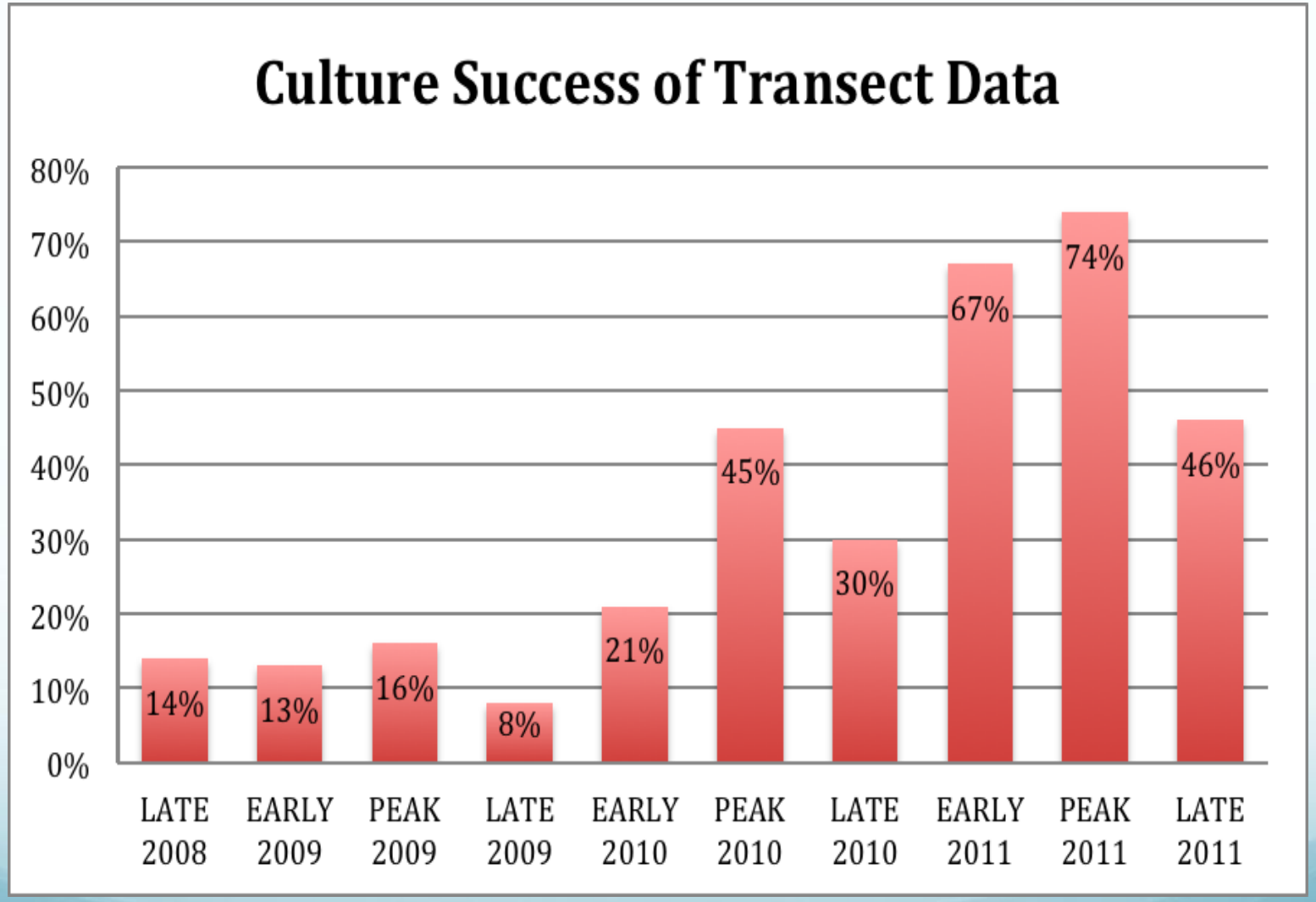


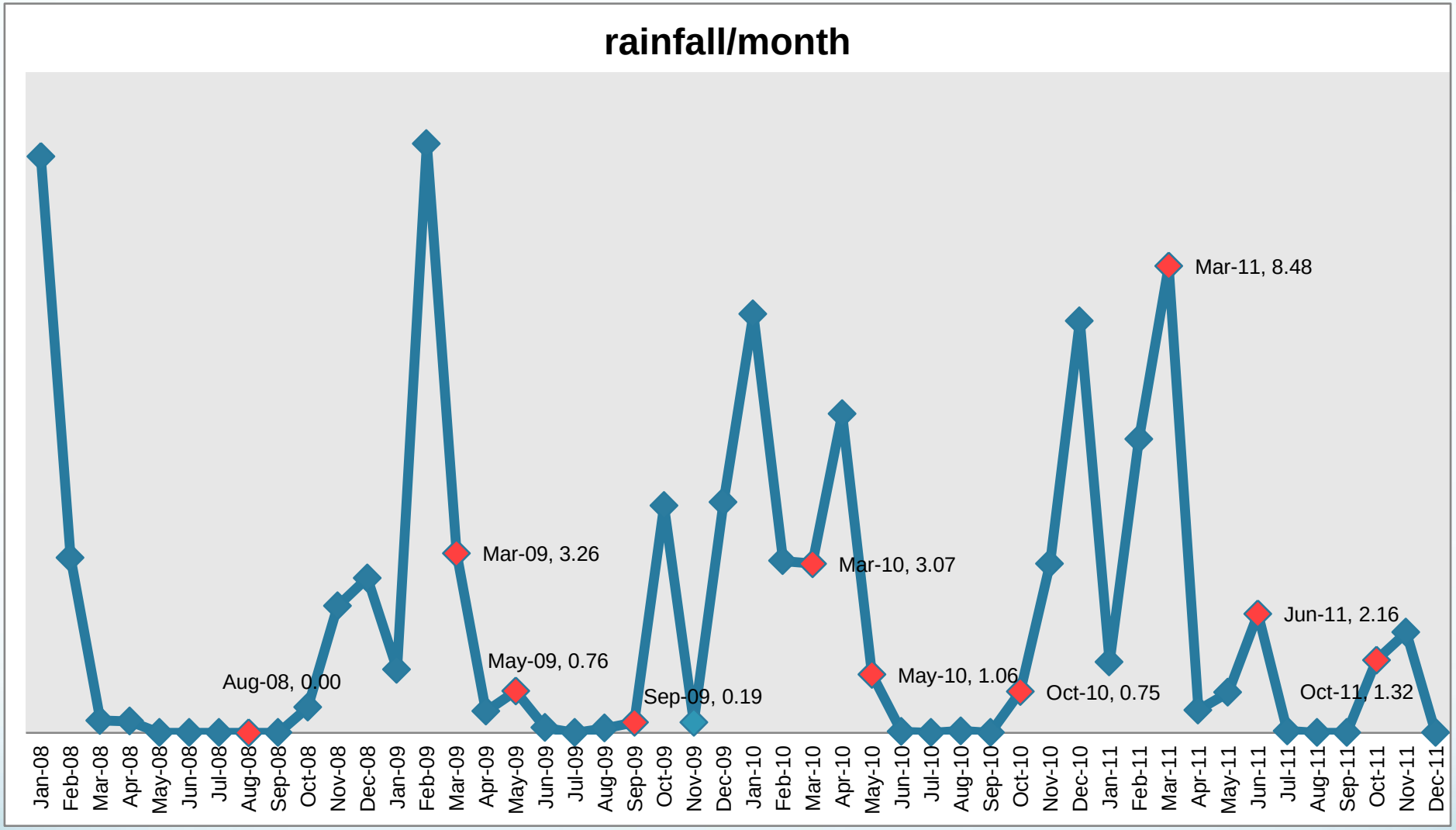
This process yields three possible outcomes

- Culture +/PCR +
- Culture -/ PCR + Is *P.ramorum* is present and simply dormant?
- Culture -/ PCR - ***

***Because only convincingly symptomatic leaves are collected…

- Are other *Phytophthora* species with identical visual symptoms present?
  - Subject to *P. nemarosa* and *P. pseudosyringae* specific assay to determine presence/absence


Culture Success of Transect Data
80%
70%
60%
50%
40%
30%
20%
10%
0%
14%
13%
16%
8%
21%
45%
30%
67%
74%
46%
LATE 2008
EARLY 2009
PEAK 2009
LATE 2009
EARLY 2010
PEAK 2010
LATE 2010
EARLY 2011
PEAK 2011
LATE 2011


rainfall/month
Mar-11, 8.48
Mar-09, 3.26
Mar-10, 3.07
Jun-11, 2.16
May-09, 0.76
Aug-08, 0.00
May-10, 1.06
Sep-09, 0.19
Oct-10, 0.75
Oct-11, 1.32
Jan-08
Feb-08
Mar-08
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Oct-11
Nov-11
Dec-11

Bay Infection Summary

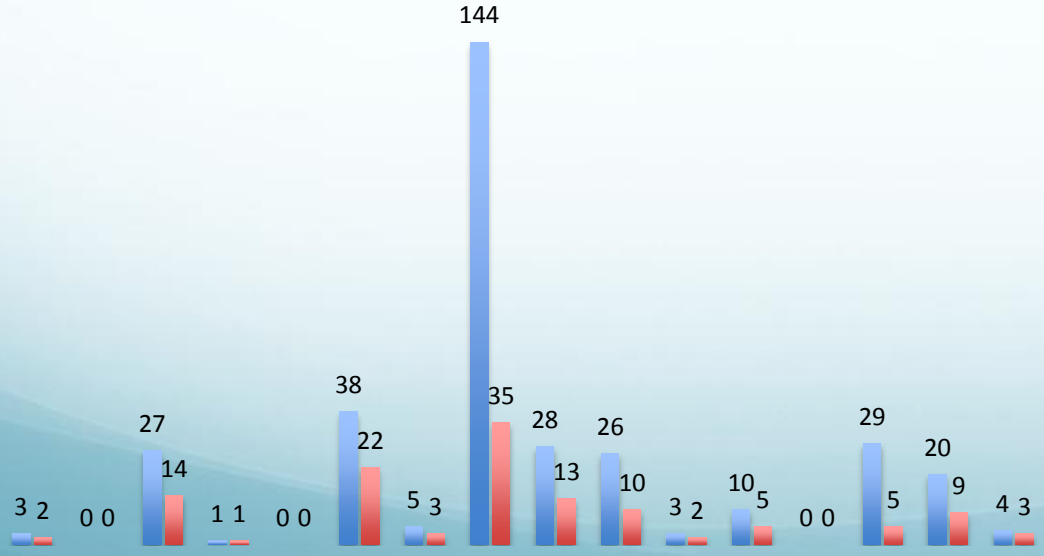
	2009	2010	2011	TOTAL
#Bays Assessed	~1500	~1500	1125	
# Sampled	1454	1436	1082	3972
# Isolates	254	338	677	1269
%Sampled	0.97	0.95	0.96	
%Isolated	0.17	0.23	0.63	
# MLG recovered	53	77	TBD	

** 2009 and 2010 have a higher # of trees assessed due to soil/leaf comparison study

MLG summary

	2009		2010		2011	
plot	# isolates	# MLG	# isolates	# MLG	# isolates	# MLG
0	2	2	3	2	40	TBD
1	0	0	0	0	7	TBD
2	15	12	27	14	69	TBD
3	9	6	1	1	29	TBD
5	0	0	0	0	0	TBD
6	24	15	38	22	52	TBD
7	12	5	5	3	14	TBD
8	116	21	**144**	**35**	156	TBD
9	17	10	28	13	68	TBD
10	27	12	26	10	72	TBD
11	6	2	3	2	13	TBD
12	18	6	10	5	10	TBD
13	0	0	0	0	0	TBD
14	3	3	**29**	**5**	44	TBD
15	3	2	20	9	83	TBD
16	2	1	4	3	20	TBD
	254		338		677	

2010 MLG Diversity by Plot



MLG summary cont..

	2009	2010	2011	TOTAL
# isolates	254	338	677	1269
#MLG	57	77	TBD	125 so far

MLG	Freq overall
87	11
38	12
105	15
117	15
17	16
109	17
120	17
111	18
59	25
70	26
47	33
13	34
26	**44**
64	**46**
76	**47**
52	**84**
37	**105**
48MLGs	**<10 times**
60 MLGs	**singletons**

Very large data set for such a small region, when compared to # of isolates from entire Ca infestation

Oak sampling

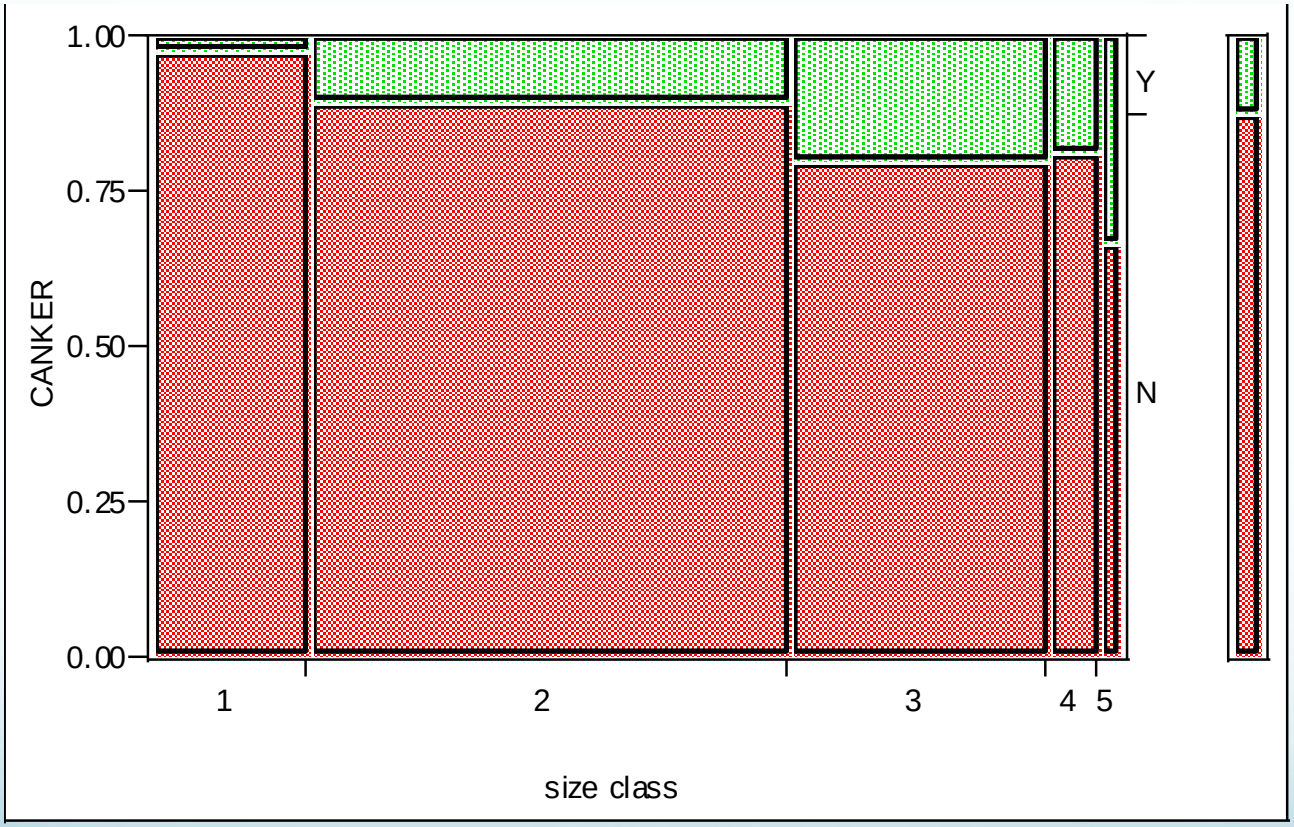
- All Coast Live Oaks >1000 were assessed once per year for **5 years** (2008-2012)
  - Presence of bleeding cankers (height and aspect)
  - Canopy dieback
  - Beetle damage
  - Hypoxylon
  - Proximity to infected bay stem
  - DBH
- Cambial tissue of nearly all bleeding cankers was sampled each year

Oak Infection Summary

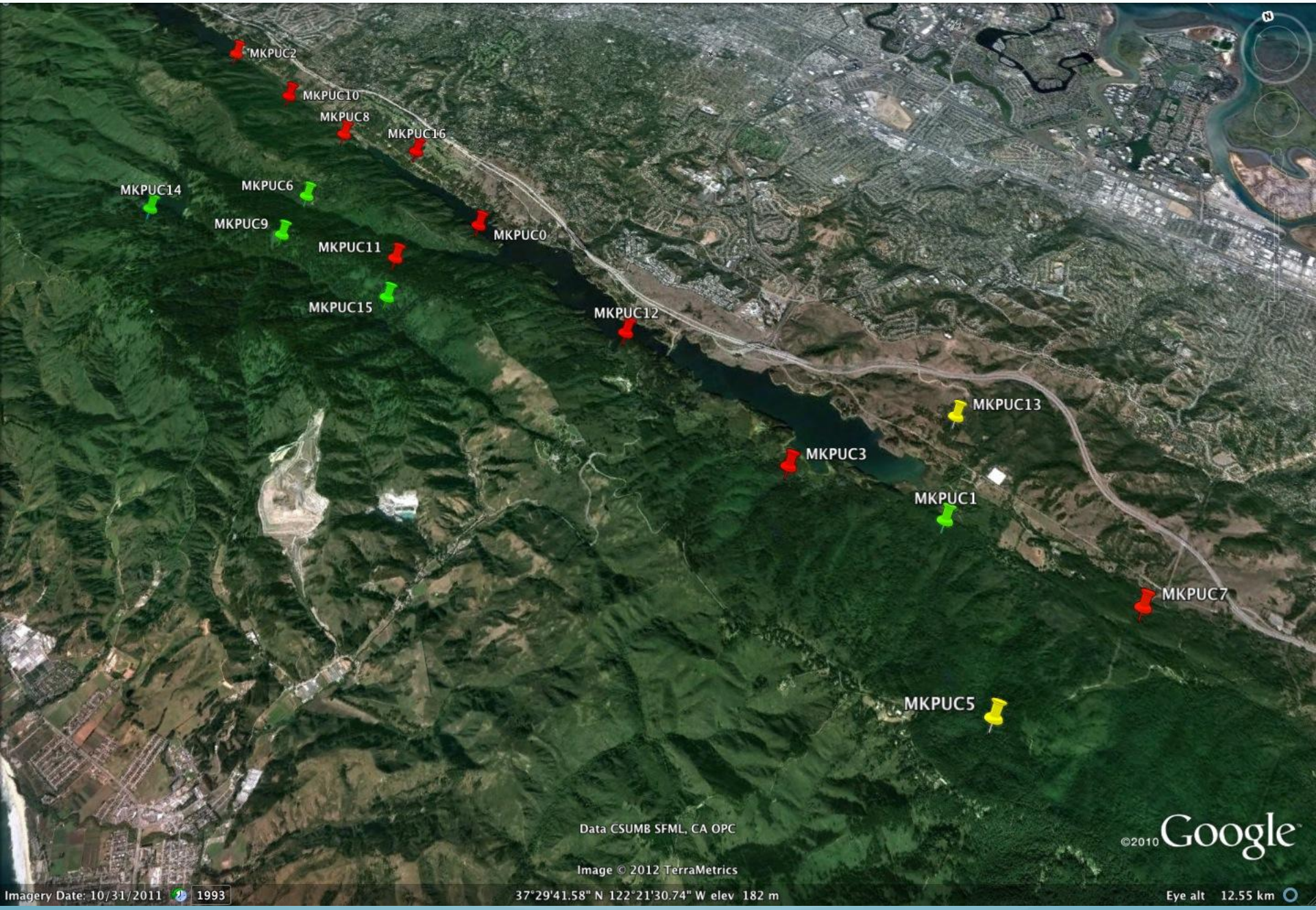
	2008	2009	2010	2011	2012
# Oaks Assessed	952	952	952	952	952
# Dead	53	91	122	144	165
# Cankered/ Alive	97	94	70	49	68
#Cankered/ Dead	28	43	89	111	121
Yearly Infection Overall %	13.1%	14.4%	16.7%	16.8%	19.9%
Yearly Mortality Rate %		3.99%	3.26%	2.31%	2.21%
# Newly Cankered		24	7	12	9

Oak infection increases with DBH

The proportion of cankers increases with each size class, yet the number of trees present in each size class decreases



Size class
1= 0-20cm
2= 20-40cm
3= 40-60cm
4= 60-80cm
5= >80cm
Right Margin
Y= canker
N=no canker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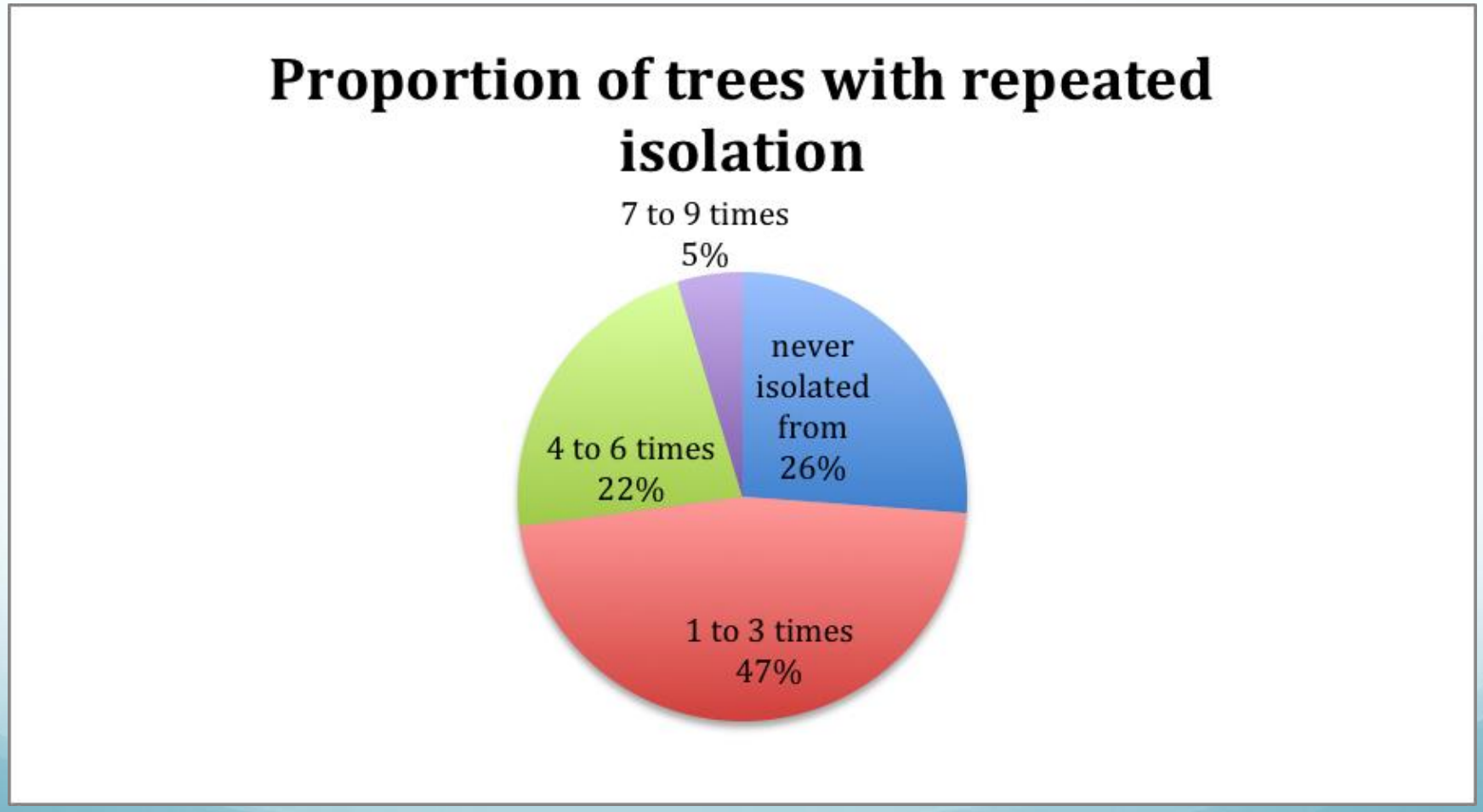
Imagery Date: 10/31/2011
1993
37°29'41.58" N 122°21'30.74" W elev 182 m
Eye alt 12.55 km

Additional environmental variables

- Tree level
  - Slope
  - Aspect
  - Elevation
  - Canopy Cover
- Plot Level
  - Daily Temps
  - Species Composition
  - Levels of poison oak

Repeat Growers “hotspots”



What factors are driving hotspots?

- Sites harbor viable individuals during unfavorable conditions
  - dry Fall 2009 sampling event with lowest culture success
  - All located in same plots with high OAK infection
- Are these sites acting as source populations for subsequent sporulation events?
- What environmental factors can be tied to thee spots?
- Targets for management/bay removal

Thank you!