Inferences from a long term *P. ramorum* spore-baiting and bay - thinning study

Garbelotto M., Hayden K., Swain S., and Schmidt D.

Relationship between bays and SOD infected oaks

- Most inoculum produced on bay laurel leaves in mixed evergreen stands (Davidson et al. Phytopathology 2005)
- Tanoak twigs also good sporulators (Davidson et al. 2008)
- Spatial distribution of SOD suggest correlation between oaks and bays (Kelly and Menteemeyer 2002)

Relationship between bays and SOD infected oaks-2

- Infected oaks likely to have an infected bay within 10 m (Swiecki and Bernhardt)
- Spores were trapped in buckets up to 10 m from bay trees using raintraps and a filter approach (Davidson et al. 2005)
- Mascheretti et al. 2008 showed a steep dilution curve of alleles using spatial autocorreletion, that suggests sporangia dilute rapidly but go further than 10m

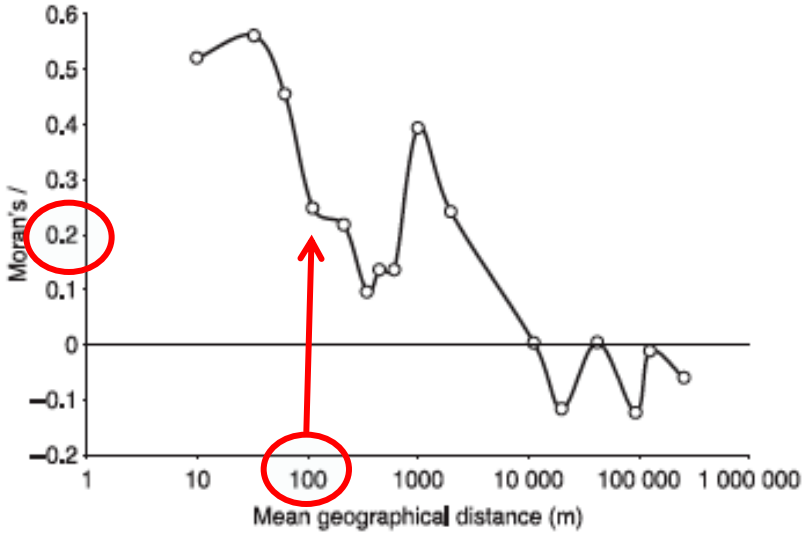


Fig. 4 Spatial autocorrelation analysis of genetic and geographical distance in *Phytophthora ramorum*. Moran's *I*-index, averaged over loci, was calculated from the repeat number at each of four variable microsatellite loci.

The graph above suggests that at least 20% of inoculum reaches 100 m (Mascheretti et al. 2008, Molecular Ecology)

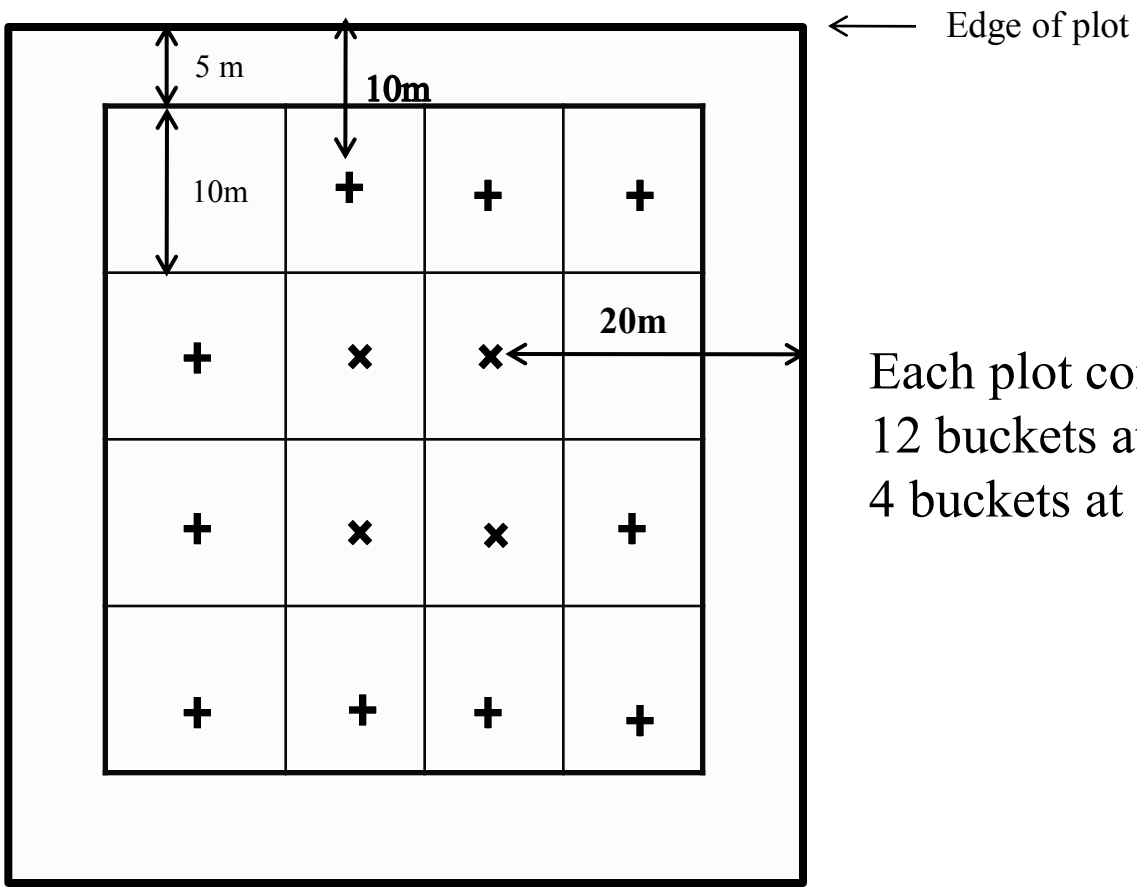
Goals of study

- To test in the field for release of sporangia in presence and absence of rainfall (previous studies had used rainwater)
- To study the environmental factors correlated to sporulation
- To determine how bay removal for 10 and 20m would affect inoculum loads

Materials and Methods

- Eight sites with bays in Soquel Demonstration Forest, Santa Cruz CO.
- Four with tanoaks, four without
- At each site, experimental plots were 50m x 50m subdivided as follows: a 5 m wide edge strip and 16 10x10m squares

A “bait-bucket” each containing one gallon of transported water and five healthy bay leaves were placed in a bucket in the center of each 10x10 square shown in the grid. Water and leaves were changed every 2-3 weeks. Leaves were arranged in a floating “daisy”

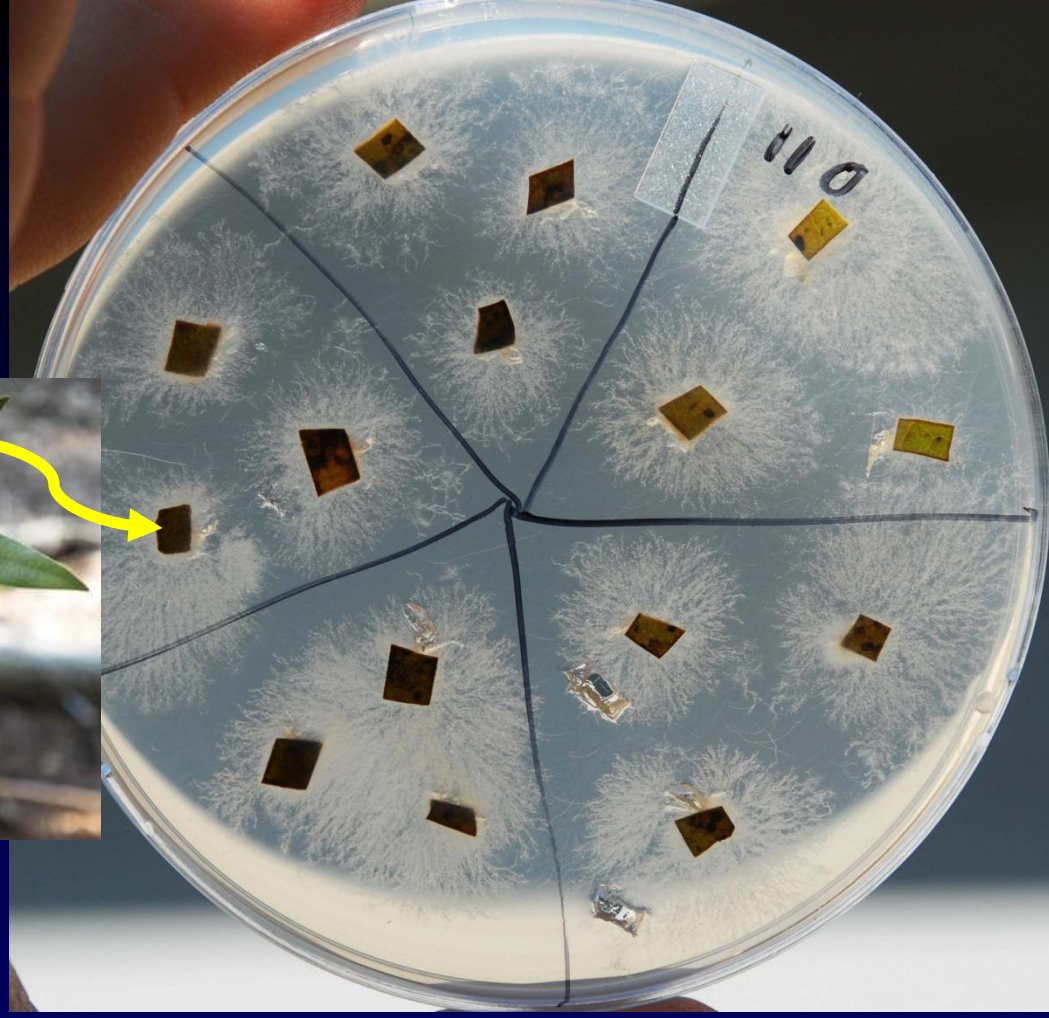


Each plot contains:
12 buckets at least 10 m from edges +
4 buckets at least 20 m from edges ×

8 Experimental Field Sites
16 Buckets / Site
5 Leaves / Bucket
= 640 leaves sampled every 3 weeks all year 2005-2007 and then February to June 2007-2011.

GRAND TOTAL LEAVES USED
39680

SOD Spore Collection and Baiting

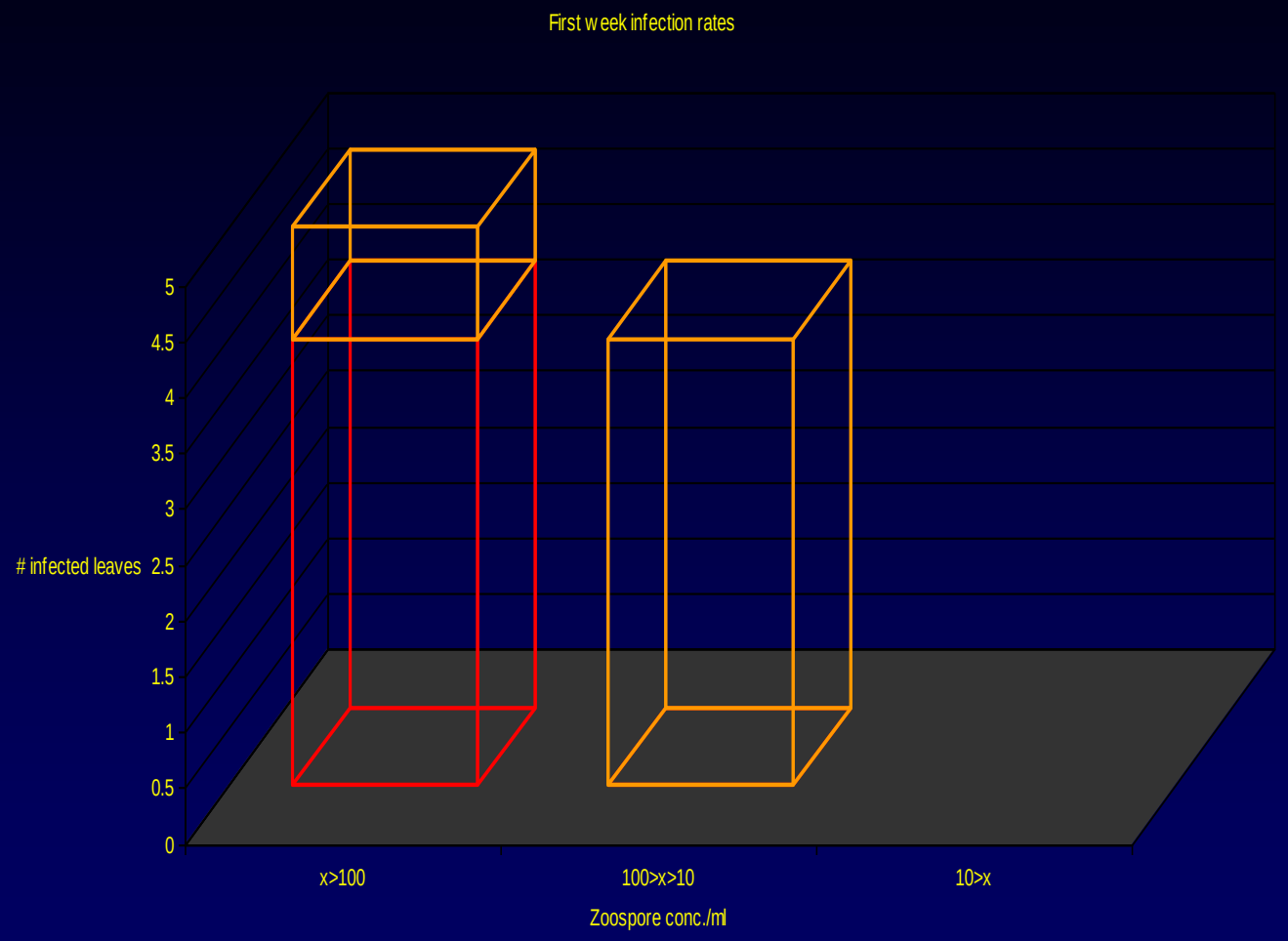


P. ramorum spores are baited with bay laurel leaves in water and plated on PARP medium

What are we testing for?

- Presence of sporangia *and* their release
- Successful *infection* of baits
- Biologically relevant and closer to actual plant infection than simple count of sporangia
- Is assay quantitative?

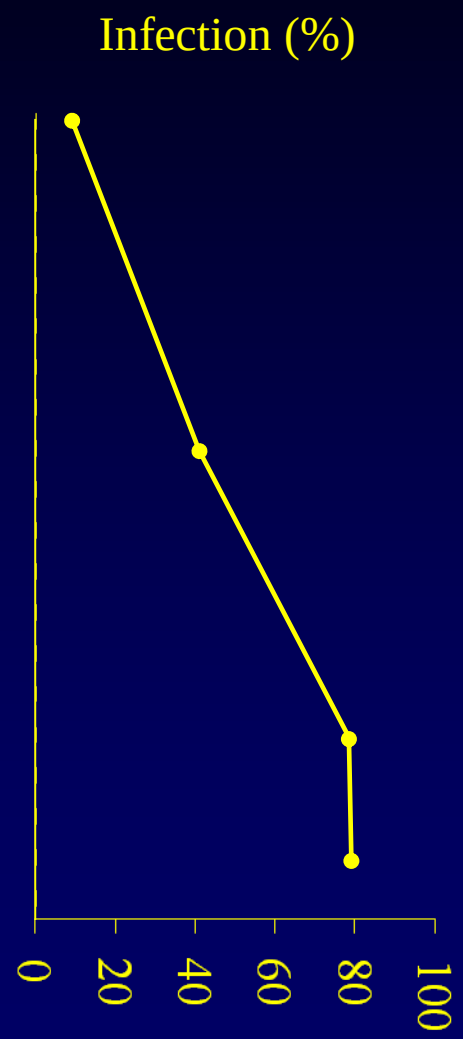
Calibration trial results

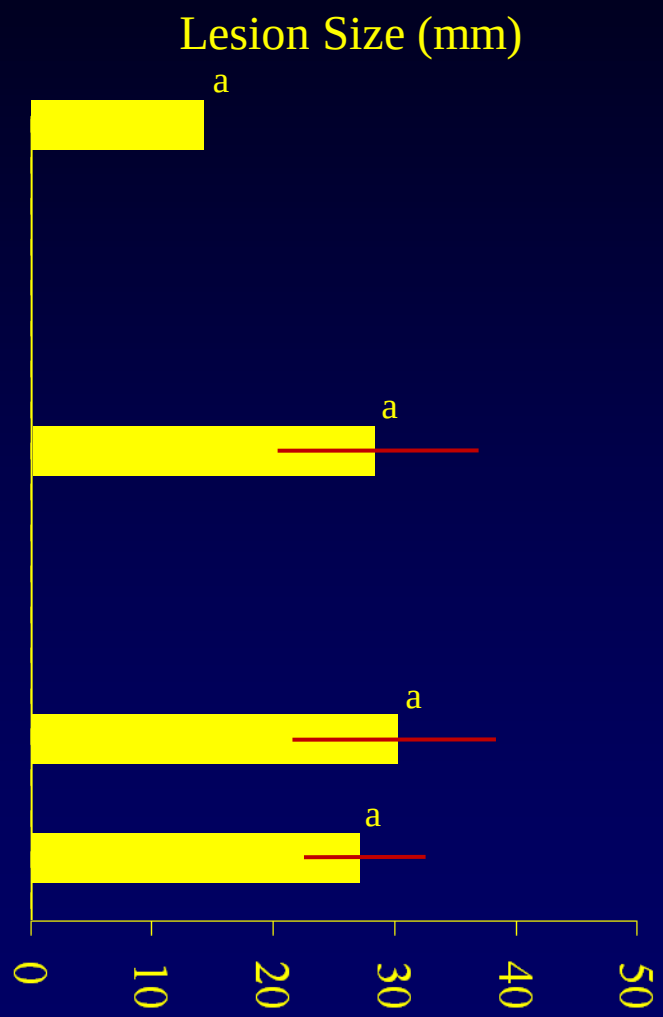


Assay is semiquantitative: 4 and 5 leaves infected indicate high inoculum level, 2-3 medium, 0-1 low

We inoculated oaks using low, medium, and high inoculum using a resin concave "inoculator"

Only obtained infections at high inoculum levels



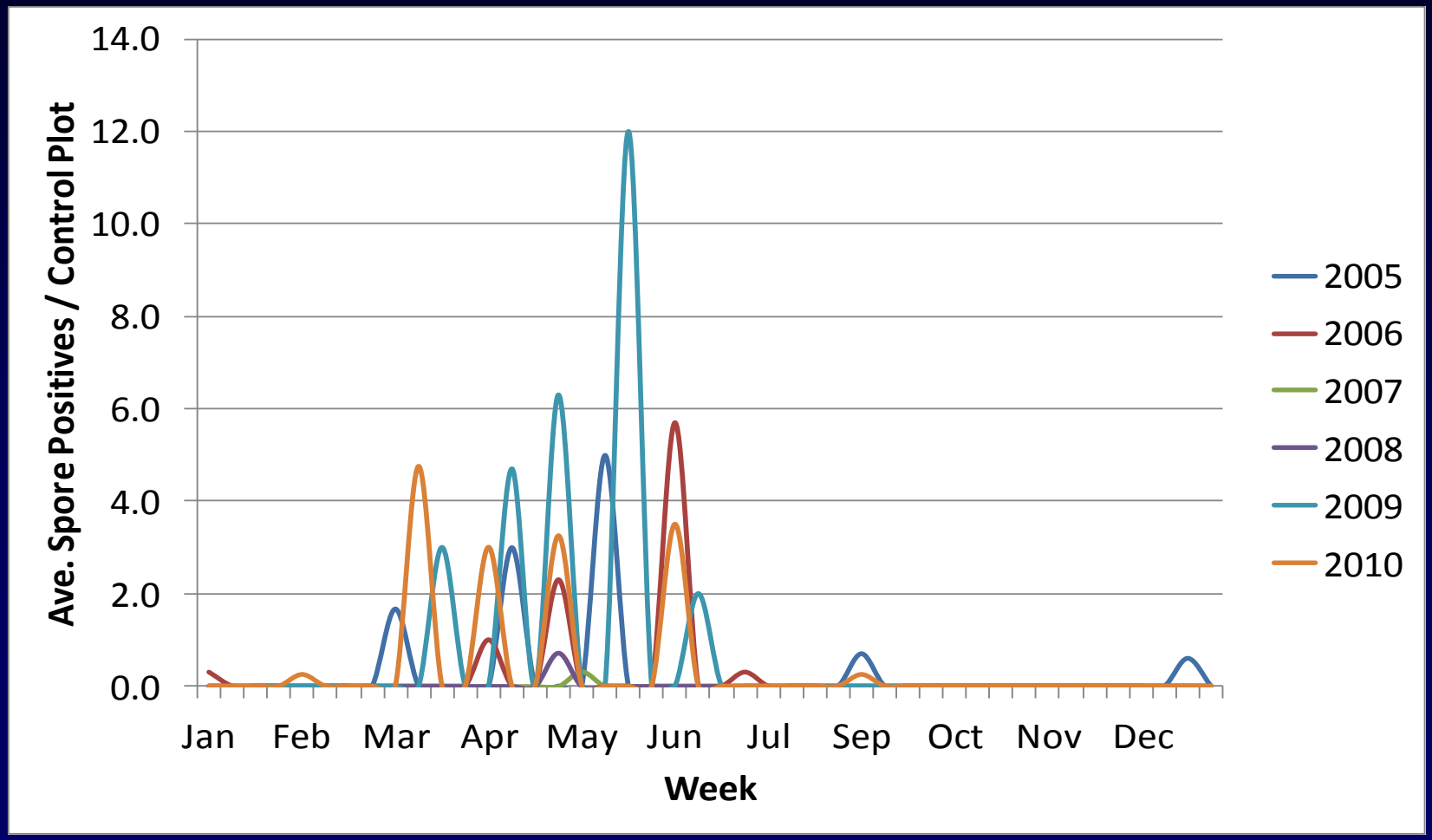


Experimental details and timeline

- 2005 Baseline, no treatment (wet year)
- 2006 Baseline, no treatment (wet year)
- 2007 Treatment (dry year) All bays removed in 4 plots
- 2008 Treatment (drought) Bays kept out
- 2009 Treatment (average rainfall) Bays kept out
- 2010 Treatment (average rainfall) Bays kept out
- 2011 Treatment (wet) Bays kept out- Analyses
- 2012-on Treatment : let bays come back in

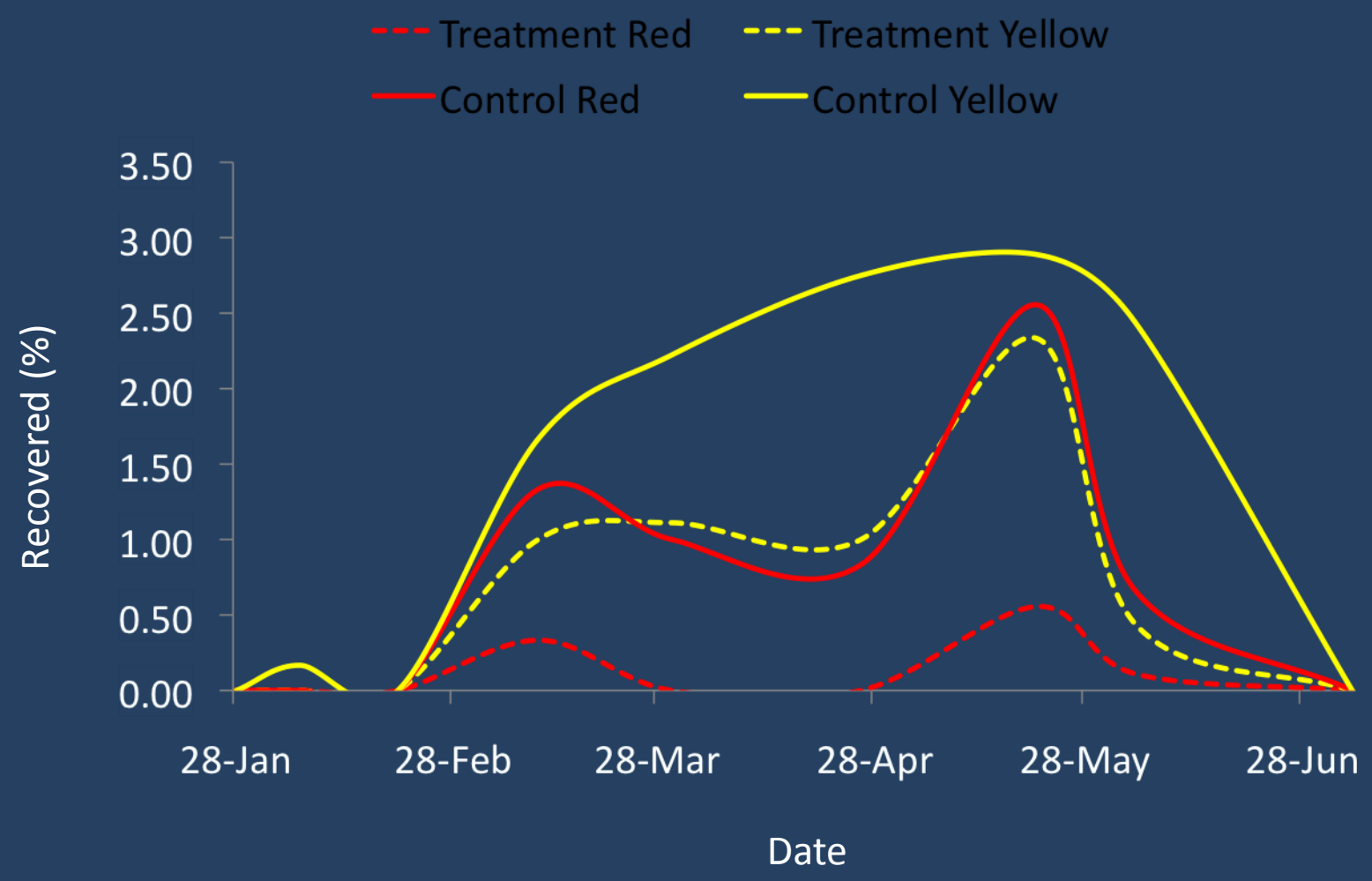
Average number of positive buckets (1-5 leaves infected) in control plots between 2005 and 2010:

Infections of bait leaves only occur in Spring (sampling not limited by presence of rain), and it varies by year

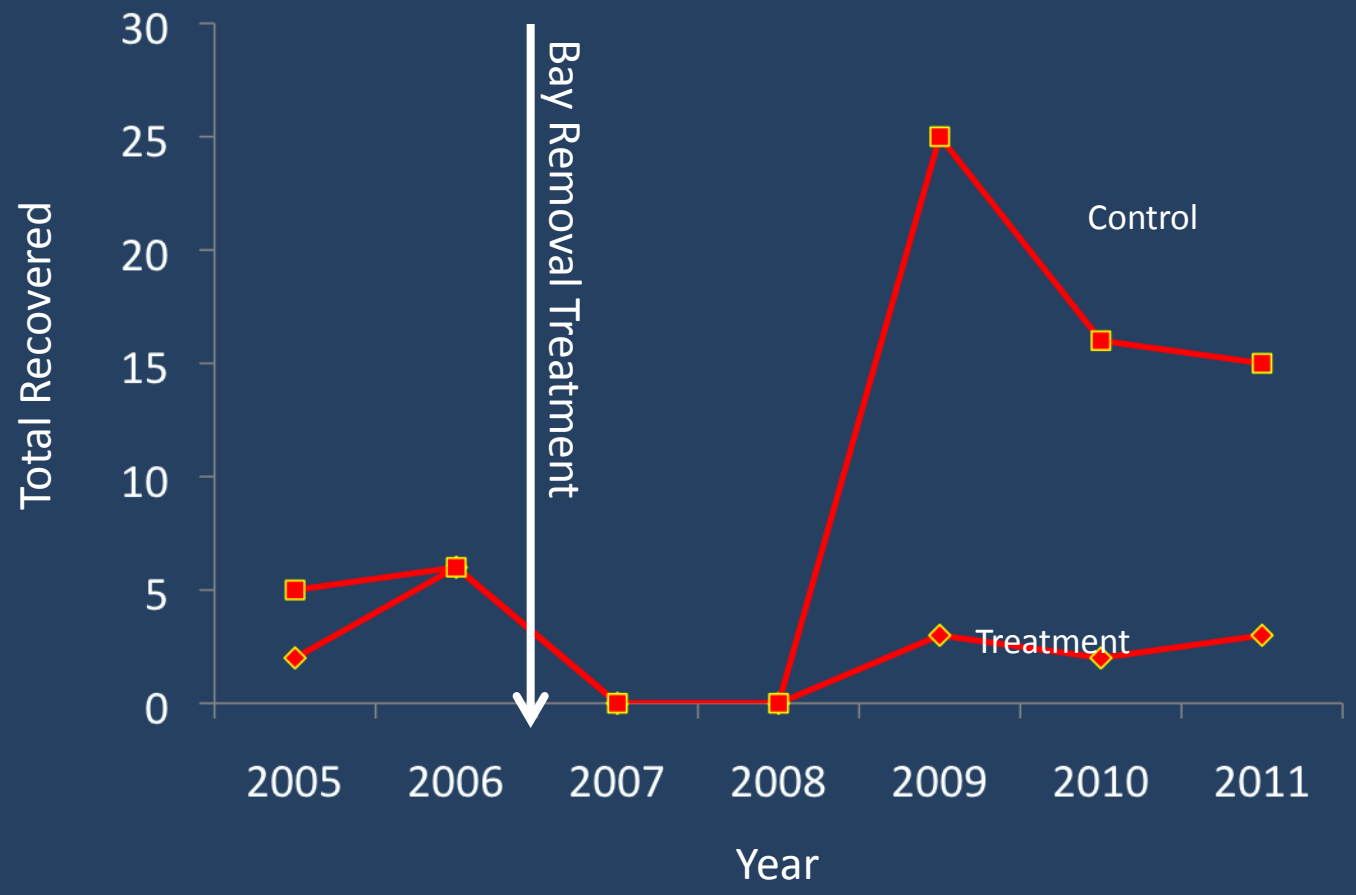


Sporangia levels increase with time but effect of temperature or of build-up?

Amount of rainfall biggest driver: threshold effect at 7 mm



SDSF Bay Removal Experiment Sum of Red Buckets per Year – Laurel Treatments + 2 LT



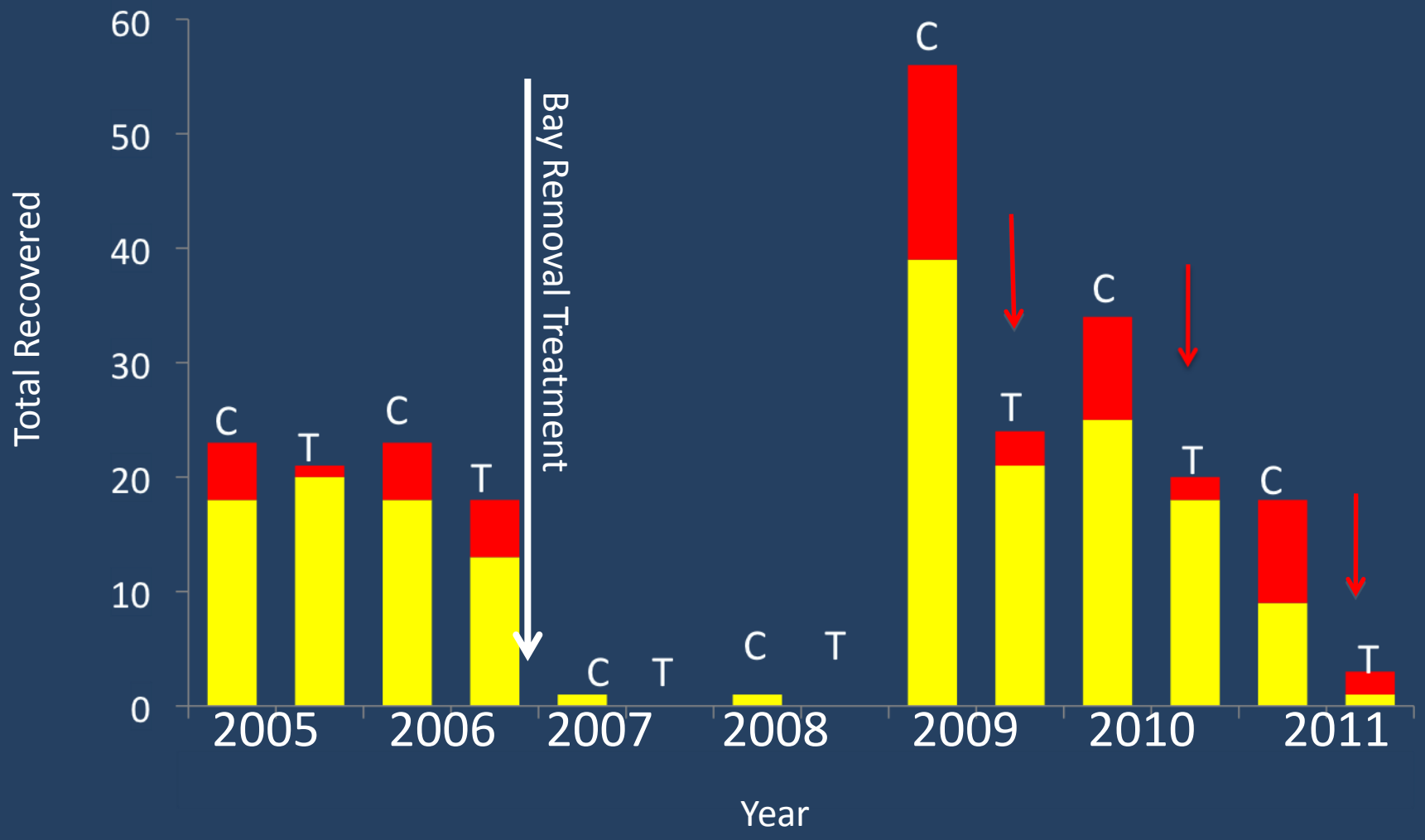
Comparison between treated and control plots using a GLM shows:

- Reduction of inoculum in treated plots: P=0.003
- Reduction of **high** inoculum in treat: P= 0.006
- End effect of treatment was equal for **high inoculum** whether tanoaks were present or absent

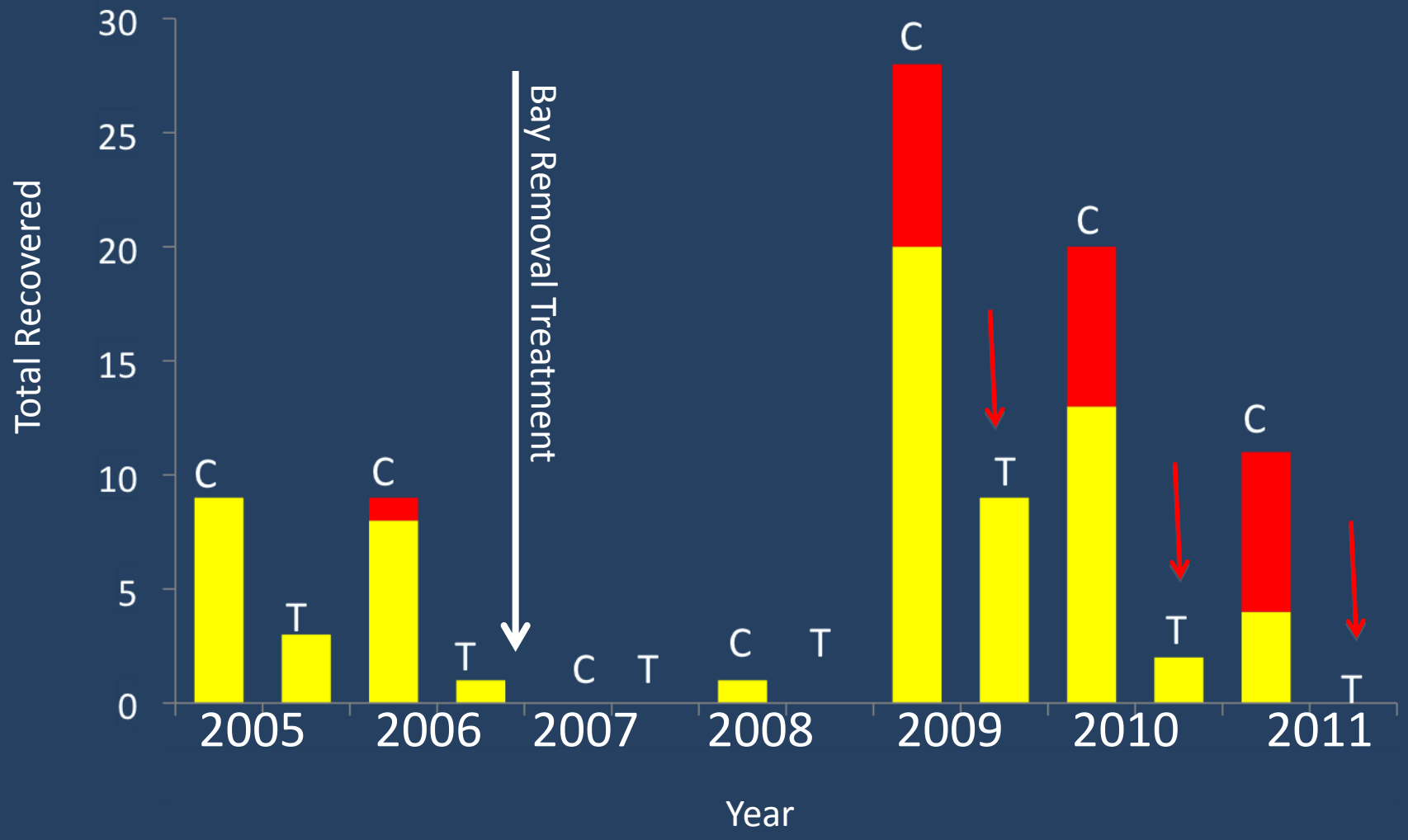
Comparison between 10m and 20m bay clearing, matched pairs analysis after treatment

- 10m clearing had a higher incidence of high levels of inoculum than 20m (P=0.002)

Red indicates incidences of high inoculum with 10 m clearing (T after bay removal= red arrows)



No instances of high inoculum with 20 m clearing (red arrows)



Conclusions:

1- We have shown that high inoculum is necessary for infection of oaks

2- We have shown that infection of leaves (on water surfaces) occurs only during the Spring. Rainfall is necessary, and warmer temperatures appear to increase infection levels

3- Bay removal for 10 and 20 m significantly reduced inoculum but did not eliminate it, sporangia move further than 10 m!

4- High inoculum necessary to infect oaks is significantly reduced by 10 m bay thinning and eliminated by 20 m clearing, independeent of presence of tanoaks. For very large oaks, 20 m removal may be recommended

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