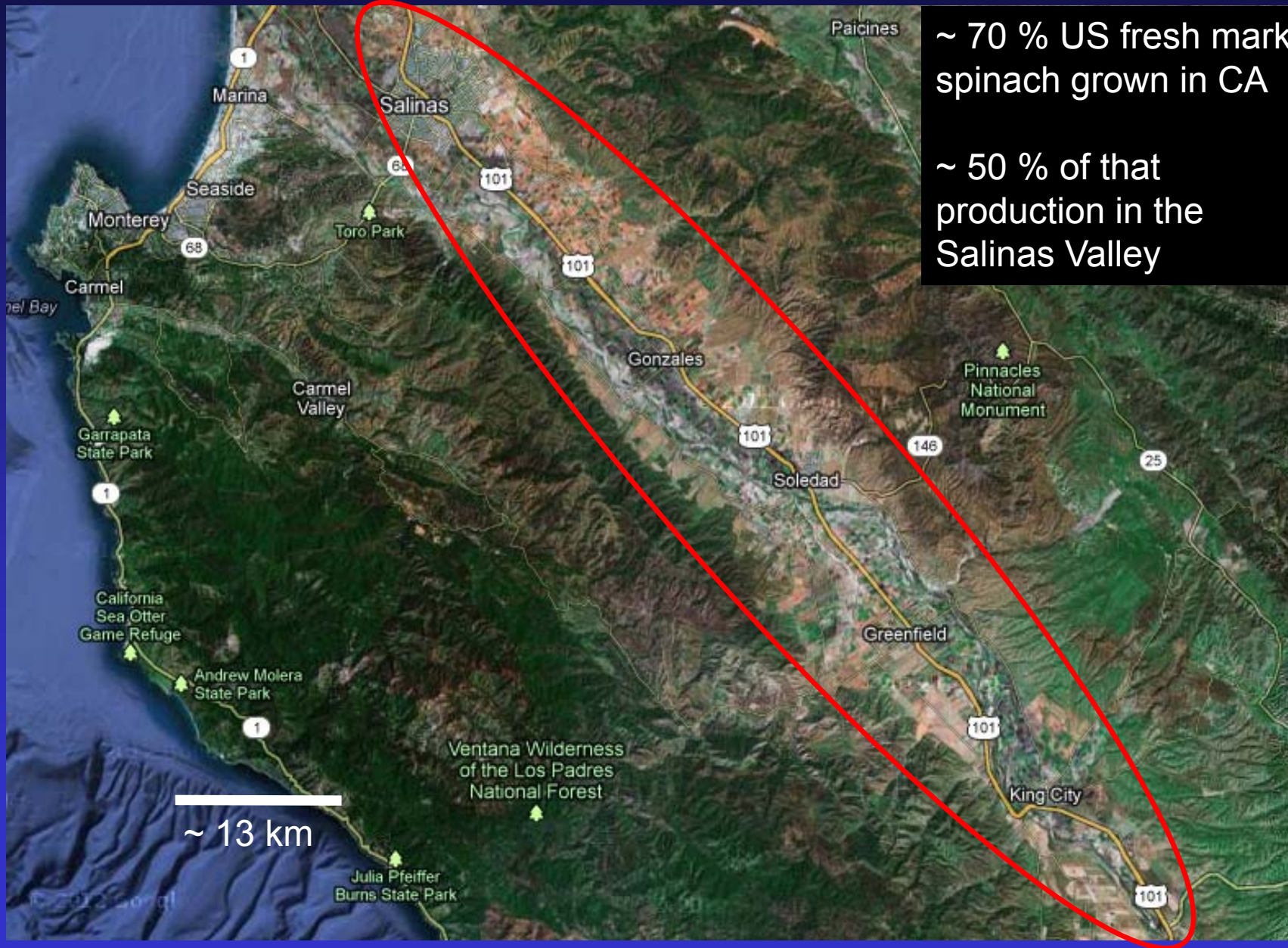


# Quantification of airborne *Peronospora* for downy mildew disease warning

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Salinas, CA



# The Salinas Valley, California



~ 70 % US fresh market  
spinach grown in CA

~ 50 % of that  
production in the  
Salinas Valley



# Downy mildew on spinach

*Peronospora effusa* (*Peronospora farinosa* f. sp. *spinaciae*)



Symptoms: Chlorotic spots on top of leaves.

Signs: typically grey-brownish downy masses of spores on the underside of leaf

# Downy mildew on spinach

*Peronospora effusa*

## Objectives:

1. Develop an assay (qPCR) for detection and quantification of DNA from airborne *Peronospora effusa*.
2. Validate the assay in the field.
3. Assess levels of *Peronospora effusa* DNA associated with disease development in a field plot.

*Peronospora effusa*/spinach



*Peronospora schachtii*/chard





# TaqMan assay to distinguish *Peronospora effusa* from related species (18S rRNA gene target)

AS1 →

Probe  
Pef1/Pbe1 ←

← PeR1  
← PbR1

*P. effusa*/AF528560.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACTGTGACCCCGCTTGAAGTAATTGCGAGCGGA\*

*P. schachtii*/K888585: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACTGTGACCCCGTTTGAAGTAATTGCGAGCGGA

*P. corydalis*/AF528563.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACCGTGAGTCCGTTTGCT-TCATTGCGAGTGGA

*P. variabilis*/AF528556.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACTGTAGTCCGTTTGCT-TCATTGCGAGTGGA

*P. arborescens*/AY695806.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACCGTGAGTCCGTTTGCT-TCATTGCGAGTGGA

*P. sp*/KJ002561: GCACCTACCGATTGAATGCTTCGGTGAAAAATATACGGCTGCAAGGACGCTTGCT-TCATTGCGAGCGGA

*Ps. cubensis*/HQ636505.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACCGTGAGTCCGCTTGCT-TAATTGCGAGTGGA

*Ph. borealis*/JQ626601.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACCGTGAGTCCGTTTGCT-TCATTGCGAGTGGA

*Ph. megasperma*/HM004230.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACCGTGAGTCCGTTTGCT-TTATTGCGAGTGGA

*Ph. nicotianae*/KF147897.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACCGTGAGTCCGTTTGCT-TCATTGCGAGTGGA

*Ph. cactorum*/JN635052.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACCGTGAGTCCGTTTGCT-TCATTGCGAGTGGA

*Hy. sp*/AY211008.1: GCACCTACCGATTGAATGACTCGGTGAAAAAT-TGGGACTGTAGTTTGTGTTGCT-TTATTGCGAGTGGA

Klosterman et al. 2014. *Phytopathology* 104 :1349-1359.

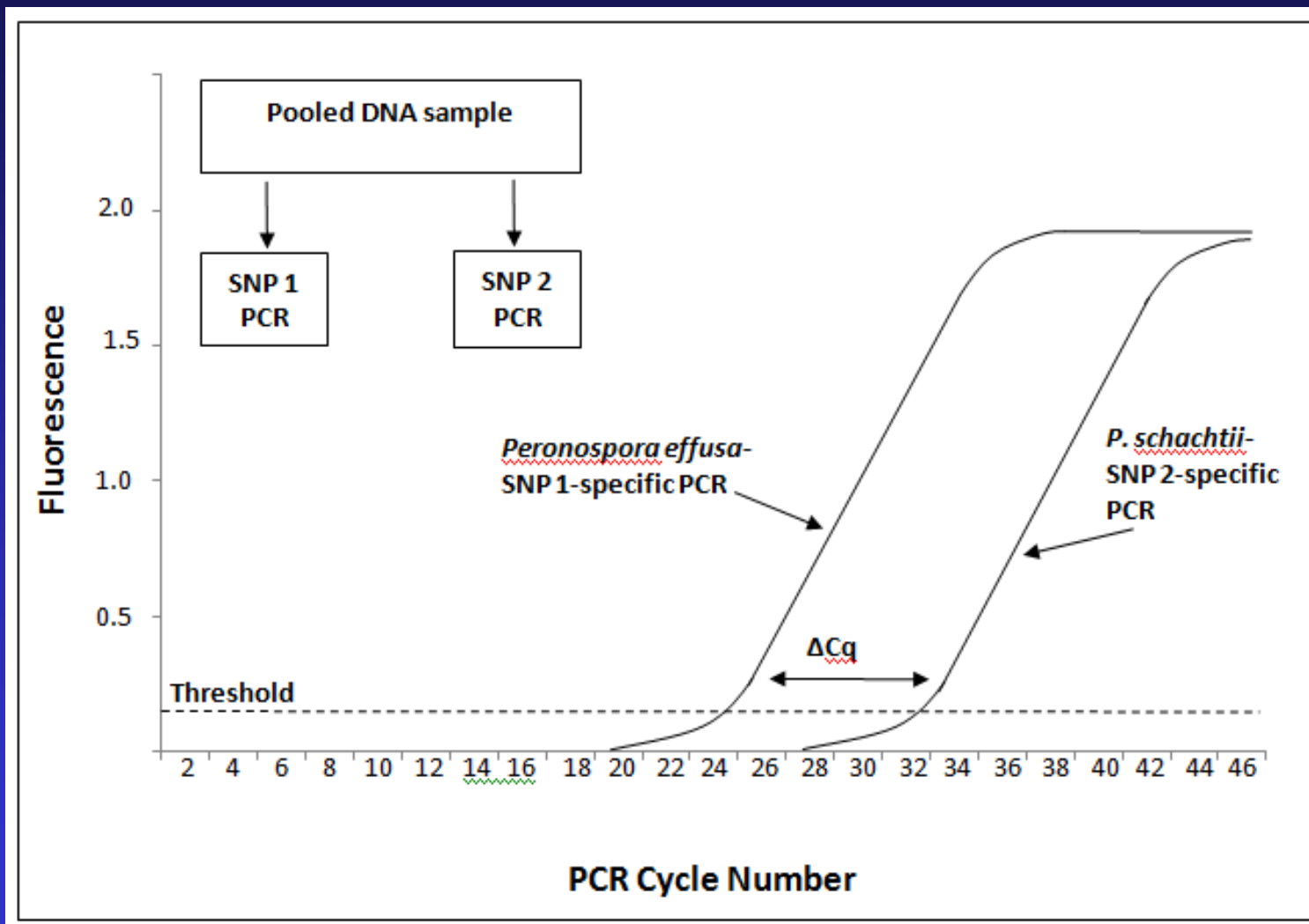
# Test of a TaqMan assay to distinguish *Peronospora effusa* from related species on various plant hosts

Downy mildew infected host plant	qPCR detection
<i>Spinacia oleracea</i> (spinach)	+
<i>Beta vulgaris</i> (beet/Swiss chard)	+/-
<i>Chenopodium album</i> (lambsquarters)	-
<i>Atriplex patula</i> (spear saltbush)	-
<i>Spergula arvensis</i> (corn spurry)	-
<i>Bassia scoparia</i> (burningbush)	-
<i>Chenopodium polyspermum</i> (manyseed goosefoot)	-
<i>Chenopodium bonus-henricus</i> (good King Henry)	-
<i>Rumex acetosa</i> (garden sorrel)	-
<i>Dysphania ambrosiodes</i> (epazote)	-

DNA template integrity tested by SYBR green assays prior to specificity tests.

# Single nucleotide polymorphism (SNP)-specific PCRs for determining frequencies of target alleles

Freq. SNP<sub>1</sub> =  $1/(2^{\Delta Cq} + 1)$  where  $\Delta Cq = (Cq \text{ of SNP}_1\text{-specific PCR}) - (Cq \text{ of SNP}_2\text{-specific PCR})$



Klosterman et al. 2014. *Phytopathology*. 104 :1349-1359.  
Adapted from Germer et al. 2000. *Genome Research* 10:258–266.



# Spore trap system to collect airborne downy mildew spores

Spore traps from Dr. Walt Mahaffee, USDA ARS

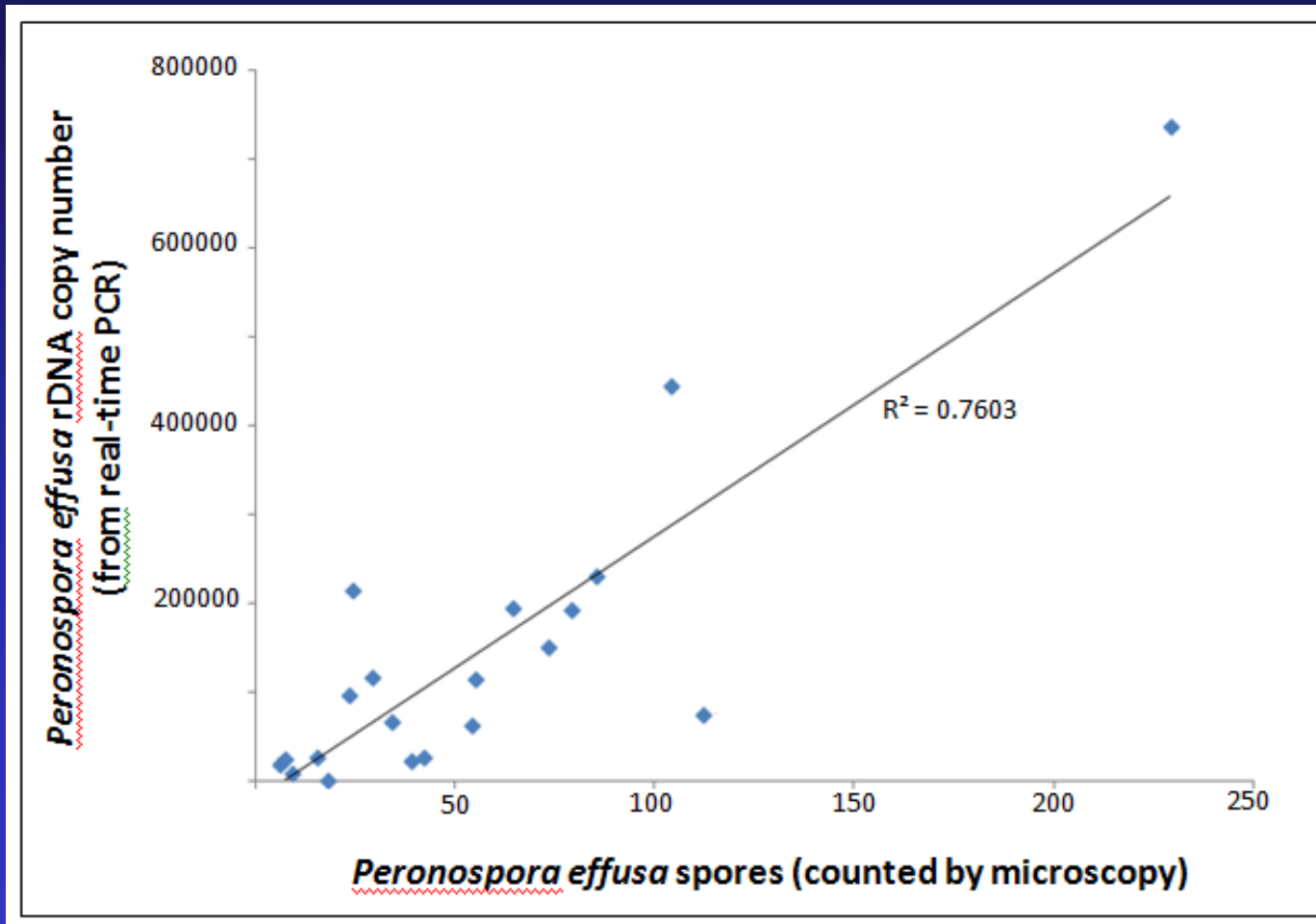


## Relative frequency of rDNA amplification from *Peronospora effusa* and *P. schachtii* at two spore trap sites

Sample location	Date	<i>P. effusa</i> assay Cq±SD	<i>P. schachtii</i> assay Cq±SD	Frequency ( <i>P. effusa</i> )	Frequency ( <i>P. schachtii</i> )	DNA copy # qPCR assay ( <i>P. effusa</i> )
Salinas	March 13	37.33±1.52	34.24±0.15	0.01	0.99	2
Salinas	--	35.34±0.41	29.67±0.36	0.00	1.00	0
Soledad	--	30.04±0.15	NA	1.00	0.00	18,129
Soledad	--	29.15±0.26	34.40±4.48	0.98	0.02	31,683
Salinas	March 15	36.42±1.17	38.60±0.00	0.99	0.01	282
Salinas	--	37.90±1.05	NA	1.00	0.00	109
Soledad	--	24.91±0.14	36.85±0.44	1.00	0.00	508,689
Soledad	--	23.39±0.43	35.44±0.41	1.00	0.00	1,368,163
Salinas	March 18	NA	36.06±0.32	0.00	1.00	0
Salinas	--	NA	NA	0.00	0.00	0
Soledad	--	28.45±0.28	35.69±0.08	0.99	0.01	50,359
Soledad	--	23.01±0.14	28.57±1.15	0.98	0.02	1,711,320
Salinas	March 20	31.48±0.33	30.49±0.75	0.33	0.67	2,339
Salinas	--	35.37±0.91	31.72±0.92	0.07	0.93	39
Soledad	--	21.36±0.49	30.97±0.53	1.00	0.00	5,131,043
Soledad	--	21.71±0.61	30.53±0.00	1.00	0.00	4,073,110

Klosterman et al. Phytopathology 104 :1349-1359.

# Correlation between DNA copy number (qPCR) and spore counts



Klosterman et al. 2014. *Phytopathology*. 104 :1349-1359.

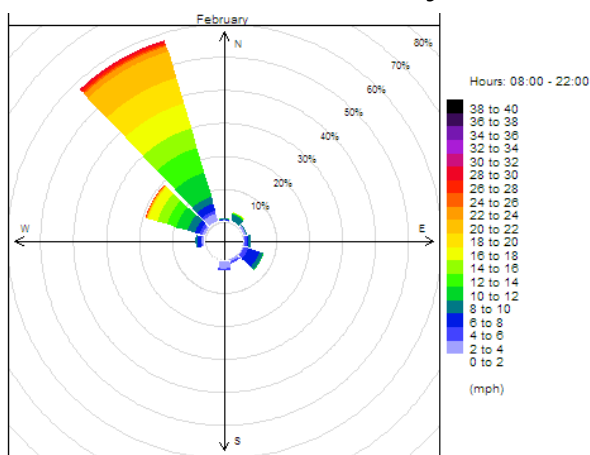


# Assessments of inoculum levels of *Peronospora effusa* at the onset of disease development (scouting and qPCR)

## USDA spinach plot, Salinas, California



Wind rose, February 2013



Frequency of counts by wind direction (%)

Fox weather

Planted Viroflay Nov. – Feb. in both 2013-2014 and 2014-2015

Spore traps on each side/monitored 3 times/weekly

Downy mildew observed mid-Jan.



# Observed *P. effusa* rDNA copy number for four spore traps at a USDA, Salinas plot, Jan 1 to Feb 11



Spores first observed on leaves, Jan 21

Spores first observed on leaves, Jan 21

# Placement of spore traps for collection of spinach downy mildew spores

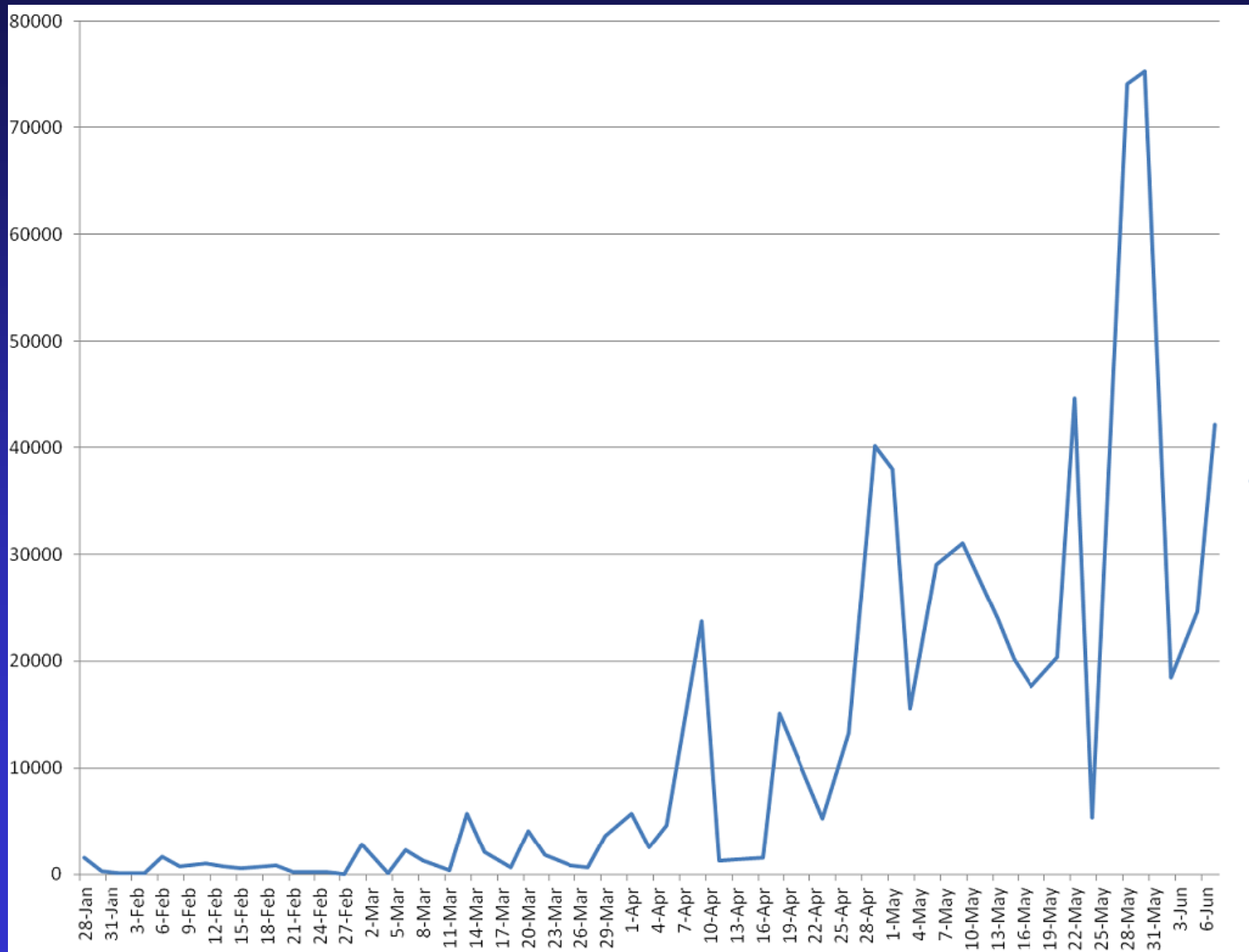
Feb-Jun 2013  
Feb-Jun 2014





# Average values for *Peronospora effusa* (copy number) at all four spore trap sites in the Salinas Valley

DNA Copy number (from qPCR)



## Conclusions:

1. Developed an assay (qPCR) for detection and quantification of DNA from airborne *Peronospora effusa*.
2. Validated the assay in the field.
3. Assessed levels of DNA from *Peronospora effusa* associated with disease development in a field plot.



Long term goal:

Application of detection tools in disease management:

- 1) disease forecasting (for timing fungicide applications)
- 2) reduction of primary inoculum sources of the pathogen

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