

Investigation of Diurnal Rhythms and Circadian Rhythms in *Phytophthora ramorum*

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Fairfield Osborn Preserves

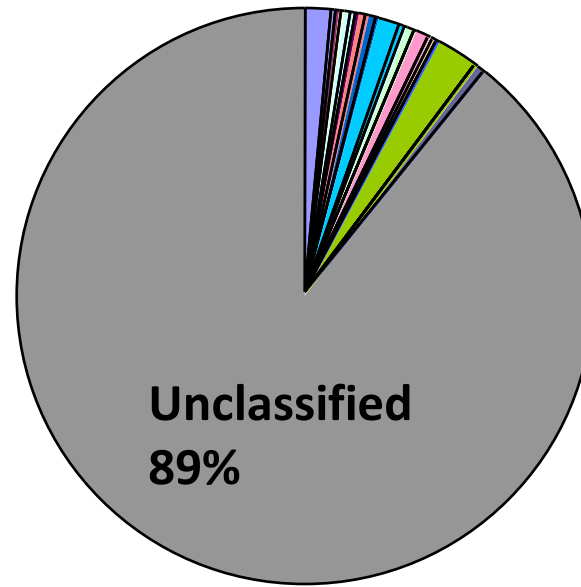
Mission in USDA:

Make the *P. ramorum* genome data more accessible and useful

A large part of *P. ramorum* genome is “Unclassified” according to MIPS Functional Catalogue

Apr. 2004

15,743 gene models



Microarray mRNA profiling

When and where genes are activated.

sexual cycle

sporangia & zoospores

in planta

How genes are regulated to drive the complicated life cycle as a pathogen.

Disturbing fact:

In a model ascomycete *Neurospora crassa*, 20% of genes display circadian rhythmicity (24-hour cycle).

(1) Diurnal rhythm: daily periodicity in e.g. developmental or behavioral process (e.g. breakfast schedule).

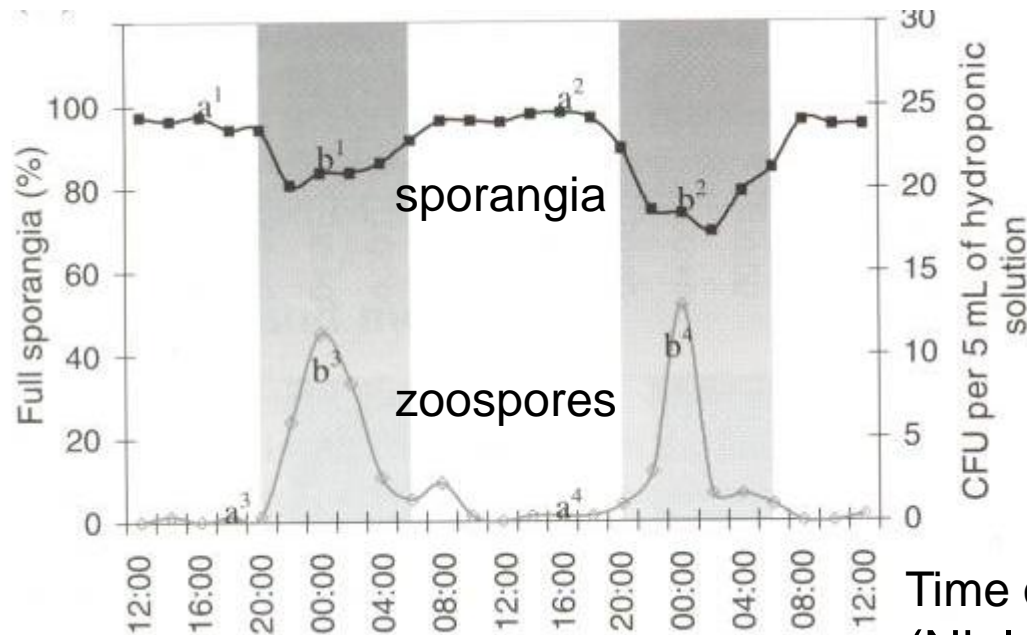
Sporulation of downy mildews (Yarwood, 1937; Rumbolz et al., 2002; Nordskog et al 2007). *P. ramorum?*

(2) Circadian rhythm (body clock): endogenous self sustaining oscillator, which is adjusted to external cues (daylight, temperature).

Animals, Plants, Fungi, & Cyanobacteria
In constant dark, diurnal rhythms are displayed for a week. *Oomycetes?*

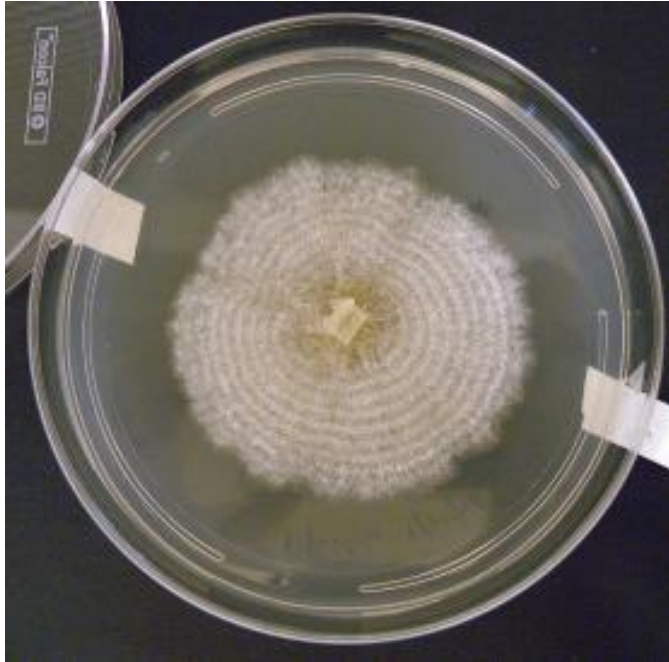
Practical implications of diurnal rhythm:

- Cyclic production of sporangia and zoospores by *P. capsici* on pepper roots in hydroponic system (Nielsen et al., 2006)
- Disease spreads 7x faster if irrigate at night rather than during daytime(Nielsen et al., 2003)



Time of day
(Nielsen et al., 2006)

P. ramorum develops diurnal rings under a photo-period of 12 hrs light: 12 hrs dark



Ring formation occurs around 2-4 PM (time-lapse photography)



Sporangia development on the rings

(Future work) Zoospore release timing

Do oomycetes have circadian systems?

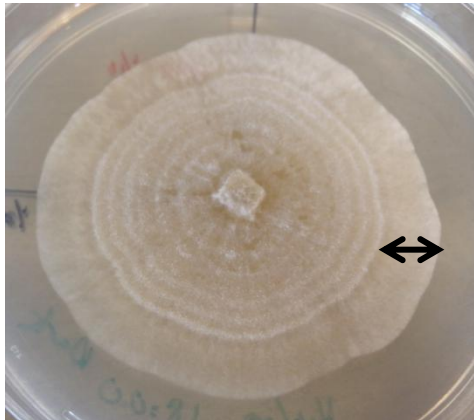
12 hours light / 12 hours dark



24 hours dark

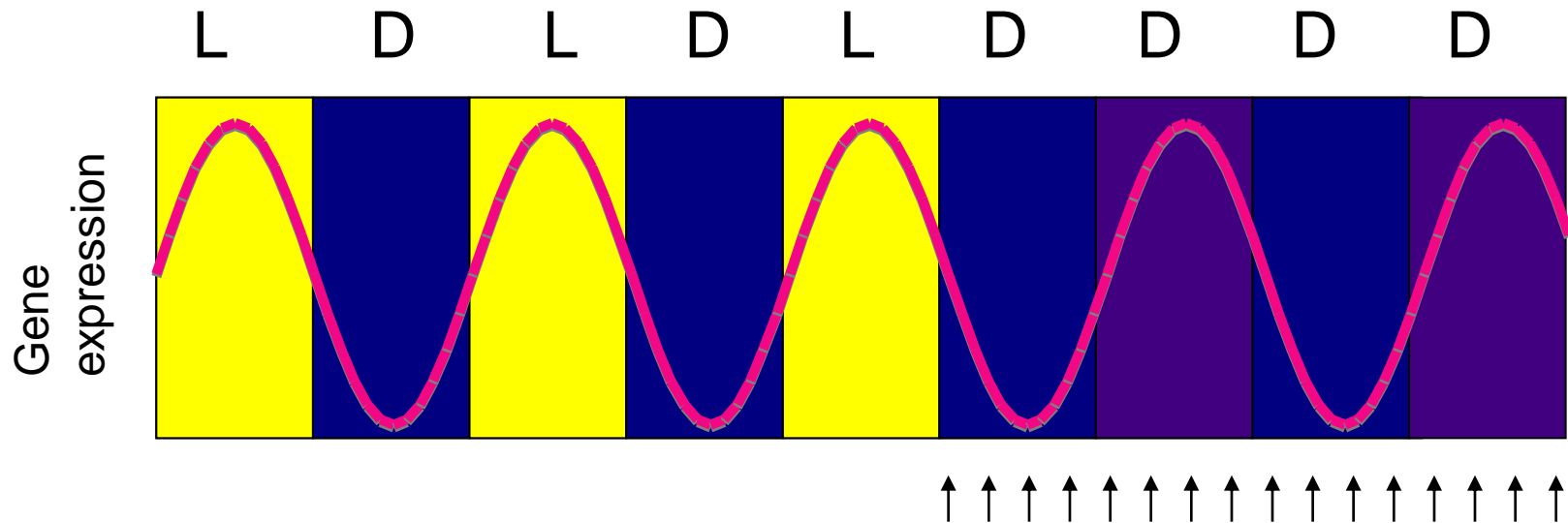


Cease of diurnal production of sporangia



3 days in
dark

Clock-controlled genes are regulated at the transcriptional level



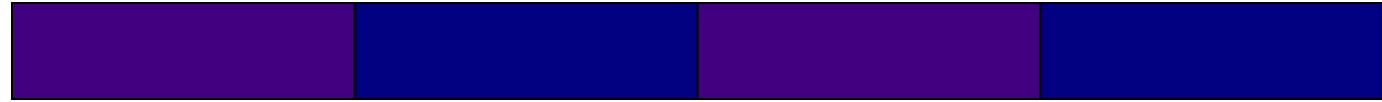
- (1) *P. ramorum* grown under 12 hrs light: 12 hrs dark for 6 days
- (2) Switched to constant darkness, and harvest every 3 hours (16 samples in 48 hours)
- (3) Microarray mRNA profiling

Subjective
day

Subjective
night

Subjective
day

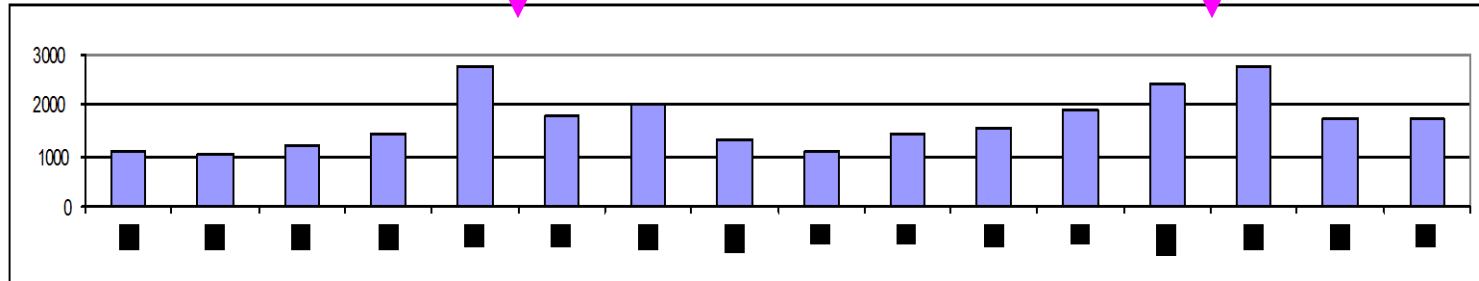
Subjective
night



S/T Kinase PR_79668



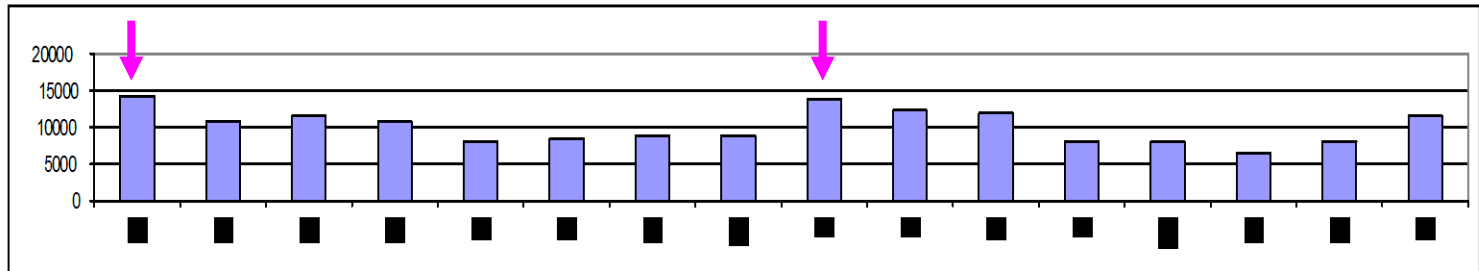
mRNA
expression



Aquaporin PR_72419

9 12 15 18 21 24 3 6 9 12 15 18 21 24 3 6

Time of day



Summary of circadian controlled genes

P. ramorum: 112 genes (0.9% of transcripts) displayed 24 hour periodicity.

(diverse functions: metabolic enzymes, effector proteins etc.)

Drosophila: 1.0% (McDonald & Rosbash, 2001)

Arabidopsis: 5.5% (Harmer et al, 2000)

Neurospora: 20% (Correa et al, 2003)

Conclusion

(1) Sporangia formation of *P. ramorum* was diurnal on Petri dish.

(2) 1% of genes with various functions were circadian-controlled.

Implications for nursery practice

Both host plants and pathogens are under circadian control.

Timings of e.g. irrigation and fungicide application may be optimizable.

Acknowledgements:

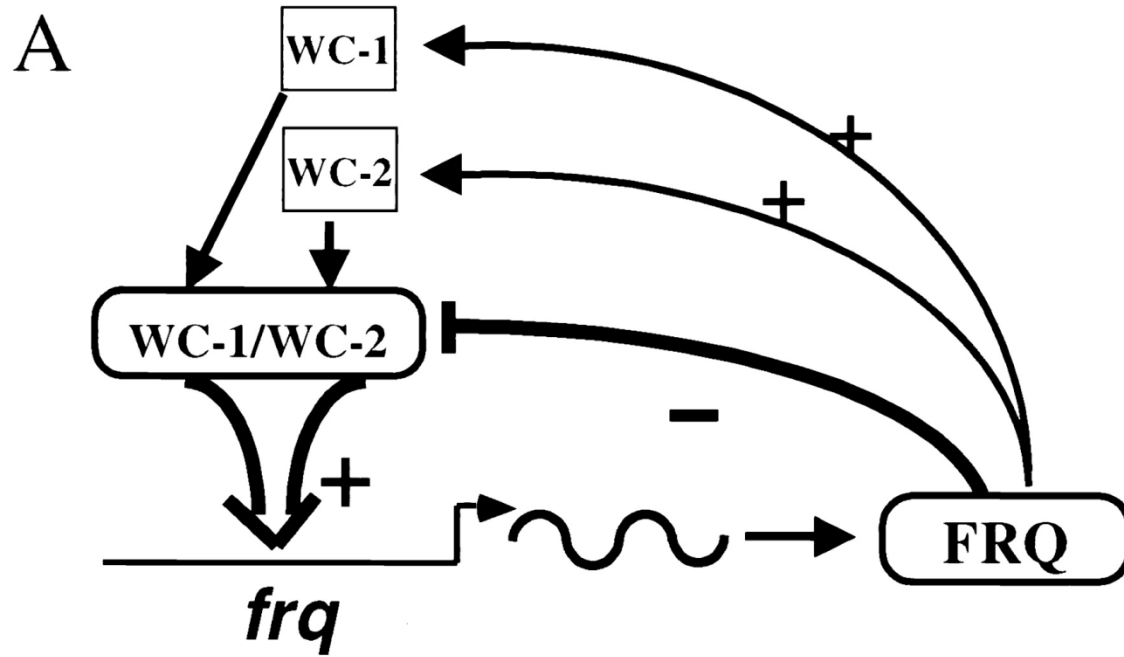
David Rizzo + Rizzo Lab members

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Cheng, Yang and Liu, 2001