Chemical hormesis in plant pathogenic fungi and fungus-like oomycetes

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Hormesis in fungi

- **Schulz** 1887-88 - Yeast (*Saccharomyces* spp.) – early chemical hormesis research models. European Journal of Physiology 1:517-541
- 1943 - **Southam and Ehrlig** coined the term hormesis – red cedar extracts on a wood-decaying fungus (*Fomes officinalis*) in culture. Phytopathology 6:517-524.
- 1949 – **Campbell and Saslaw**, growth enhancement of fungi by streptomycin. Proceedings Of The Society For Experimental Biology And Medicine 3:562-562.
- 1953 – **Hessayon** described biphasic dose responses of *Fusarium oxysporum* to trichothecin. Nature 4284:998-999.
- Recent research: yeast are popular models for caloric restriction and cell aging studies, among others.

Mechanism of fungicide hormesis

2009 - **Ohlsson** et al. imidazole fungicide procloraz - biphasic effects on aldosterone secretion by selective enzymatic inhibition in the steroidogenic pathway.

“A couple of days after routine fungicide applications, we saw **more** disease!”

- Ornamental grower, PA

Question

What effects have subinhibitory doses of fungicides on fungal plant pathogens?

Could they become more aggressive?

Pythium spp.

- Straminipila /Chromista
  - Oomycota
    - Pythiales
      - Pythiaceae
- Sexual and asexual reproduction
- Aggressive plant pathogens
- Broad host range
- Diseases:
  - Damping off
  - Root and stem rot
  - Blight of grasses and fruit
- Soil and water-borne

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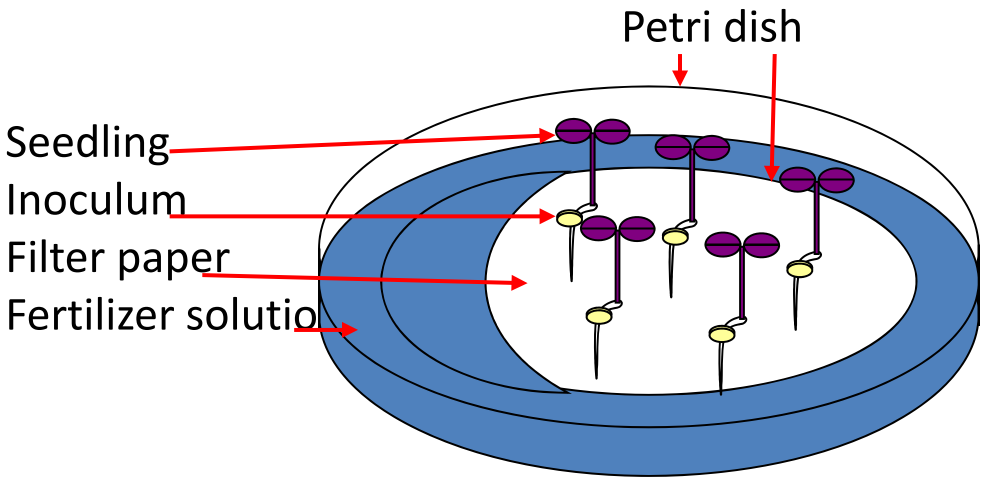
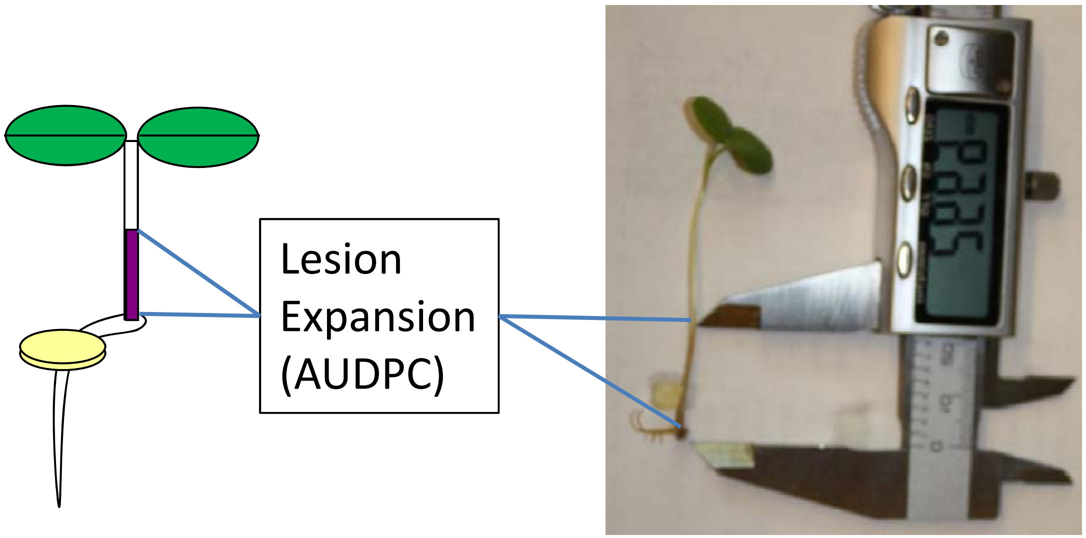
Seedling assay





Number of infected seedlings / treatment

Sublethal Doses of Mefenoxam Enhance Pythium Damping-off of Geranium

Carla D. Garzón and **Julio E. Molineros,** Oklahoma State University, Stillwater; **Jennifer M. Yánez,** The Pennsylvania State University, University Park; **Francisco J. Flores,** Oklahoma State University, Stillwater; and **María del Mar Jiménez-Gasco** and **Gary W. Moorman,** The Pennsylvania State University, University Park

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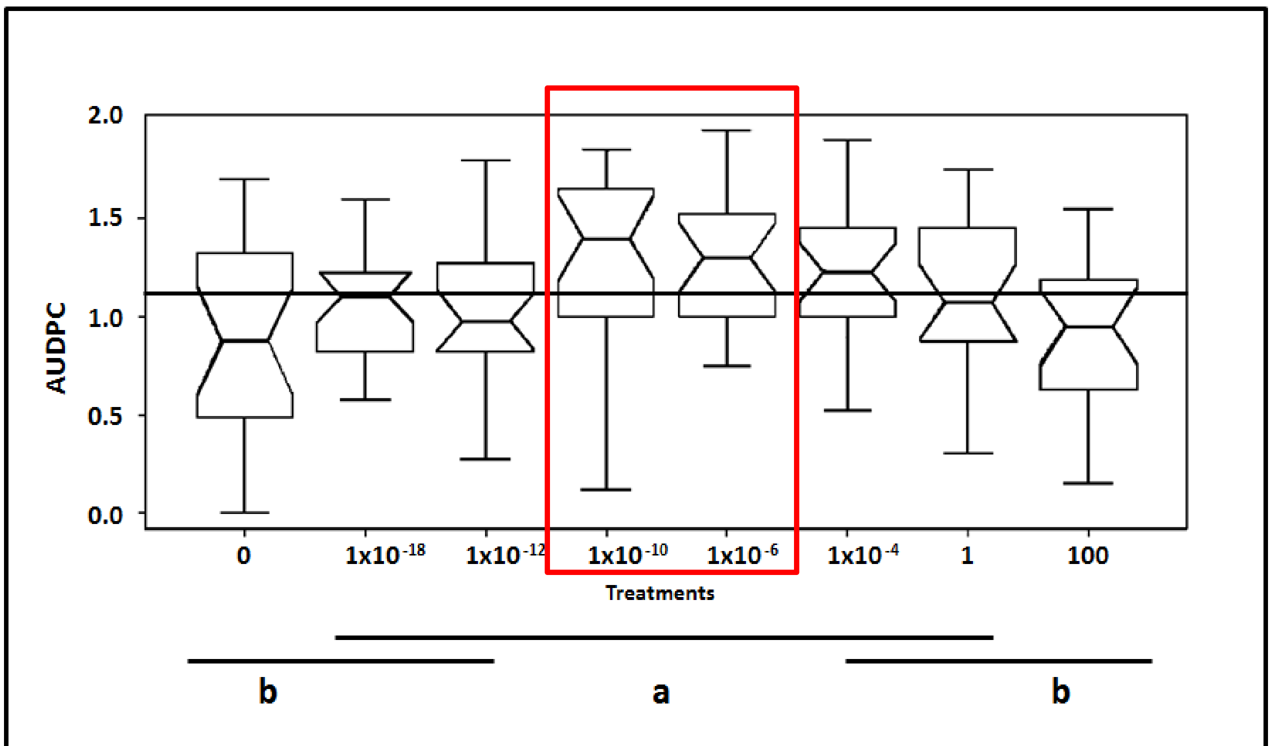
Objectives

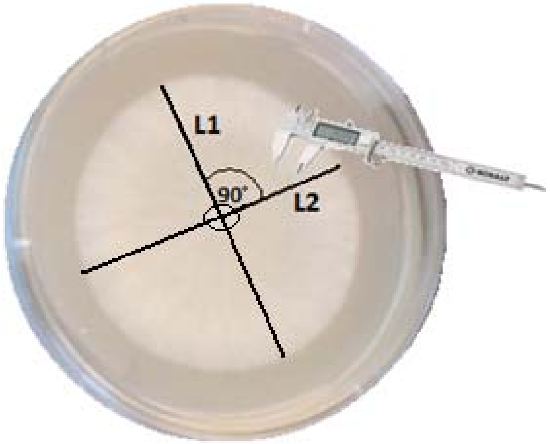
1. Examine the dose effect of mefenoxam on *Pythium* isolates *in vitro*

2. Determine whether sublethal doses of mefenoxam increased damping-off of geranium seedlings.

Research supported by Fred Gloeckner Foundation

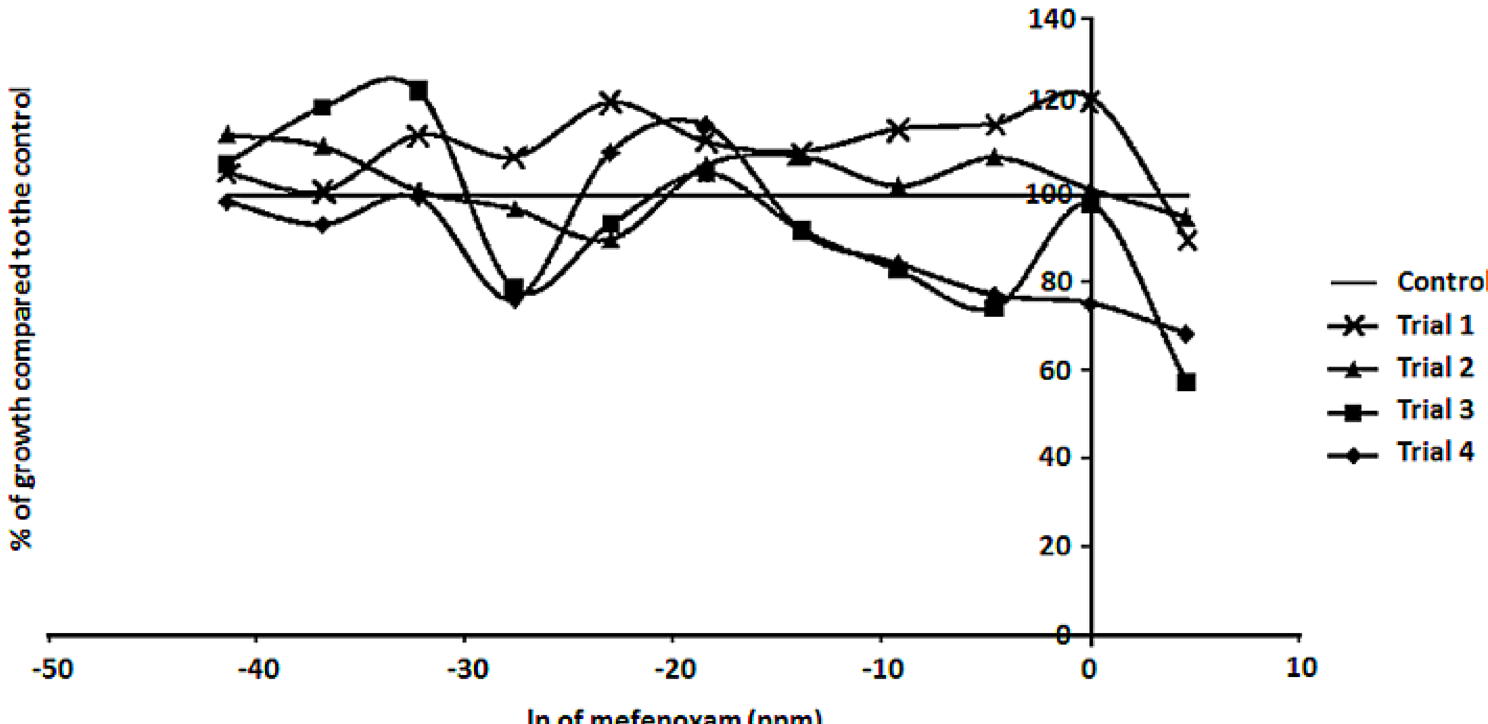
- Disease severity increased 61%
- Reproducible





- Consistent, but not significant, radial growth stimulation (1-22%, aver. 10%)

- Stimulatory dose not reproducible



Creating awareness among Phytopathologists

Sublethal Doses of Mefenoxam Enhance Pythium Damping-off of Geranium

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Plant Dis. 95: 1233-1238

Featured Article (October 2012)

- **Phytopathology News: *Plant Disease* Editor's Choice**
- **APS*net*: Emerging Research**

EFFECT OF LOW DOSES OF PESTICIDES ON SOILBORNE PATHOGENS AN APPROACH TO THE HORMETIC RESPONSE

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Evaluating hormesis

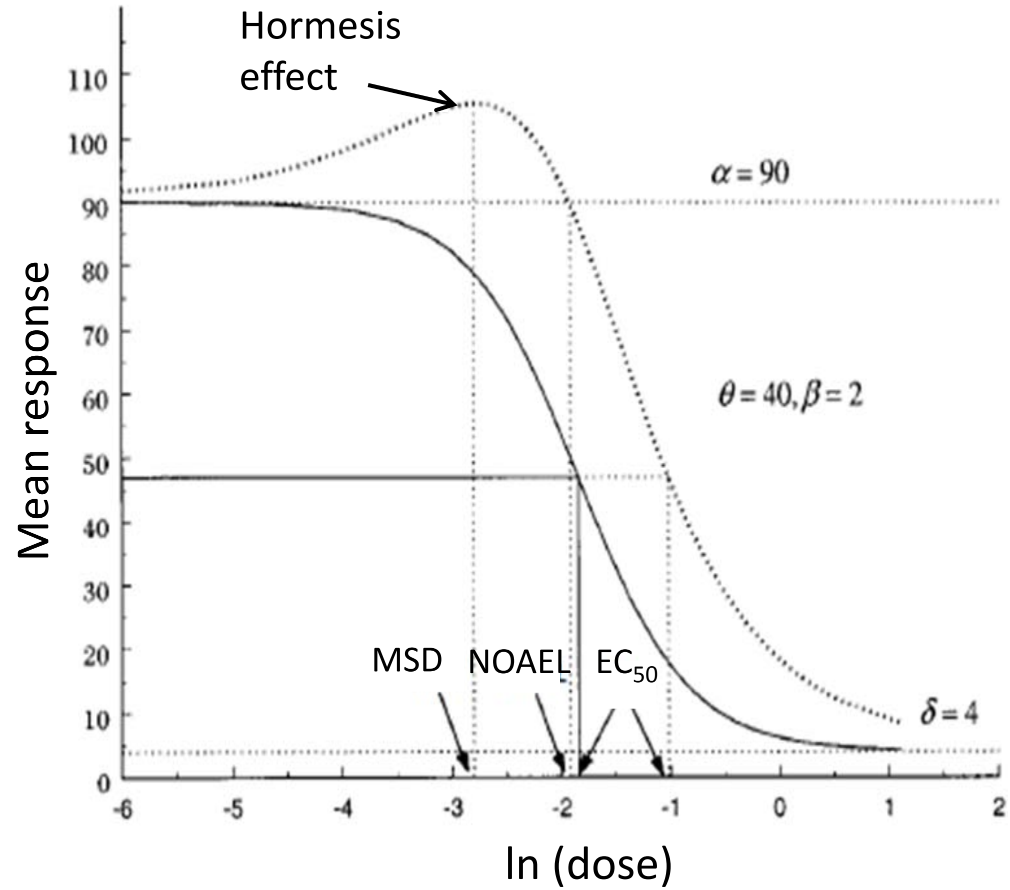
- Criteria:
  - Strength of evidence
  - Soundness of data
  - Consistency
  - Biological plausibility

Crump, K. 2001. Human & Ecological Risk Assessment (4):781.

Objectives

1. Establish an experimental design for the correct assessment of hormetic responses in fungal plant pathogens
2. Assess growth responses *in vitro* of soilborne fungal plant pathogens exposed to subinhibitory doses of disinfectants and pesticides

- Determine no observed adverse effect level (NOAEL)
- Test five equally spaced doses below the NOAEL
- Separation between doses smaller than one order of magnitude
- Background incidence in the control



Schabenberger et al. 1999

Pathogen	Compound
Pythium aphanidermatum	**Ethanol**
	Sodium hypochlorite (Clorox) *
	Cyazofamid (Segway)
	Propamocarb (Previcur)
Rhizoctonia zeae	**Ethanol**
	Sodium hypochlorite (Clorox) *
	Propiconazole (ferti-lome) *
Rhizoctonia solani	Propiconazole (ferti-lome) *

* Threshold model dose responses

Pythium spp.

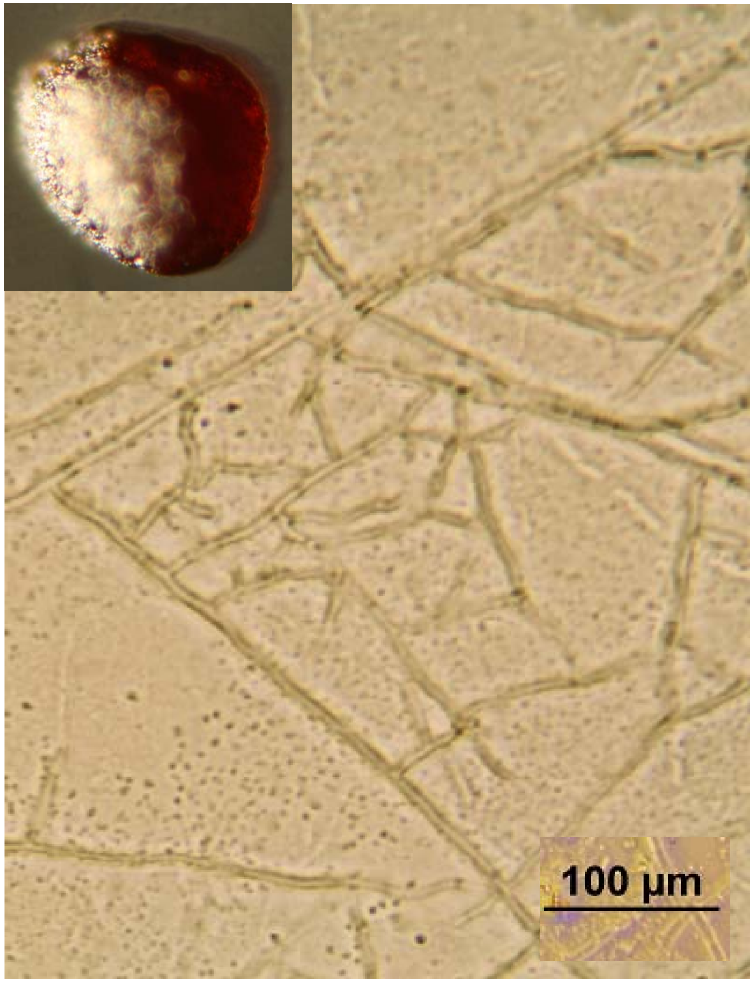
- Straminipila /Chromista
  - Oomycota
    - Pythiales
      - Pythiaceae
- Sexual and asexual reproduction
- Aggressive plant pathogens
- Broad host range
- Diseases:
  - Damping off
  - Root and stem rot
  - Blight of grasses and fruit
- Soil and water-borne

Gordon Beakes © University of Newcastle

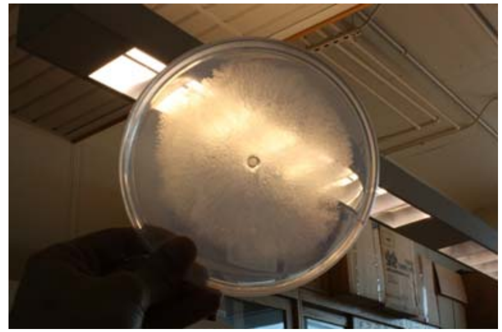
Rhizoctonia spp.

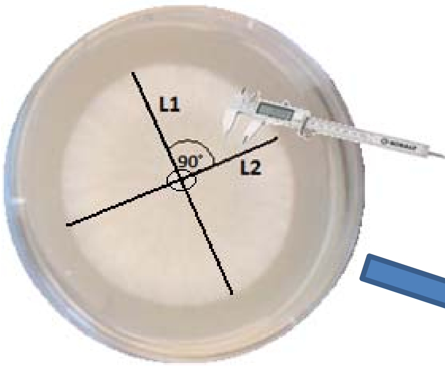
- Fungi
  - Basidiomycota
    - Agaricomycetes

- Diseases:
  - Sclerotial diseases
  - Damping off
  - Broad host range
  - Soilborne
- Warm and humid weather

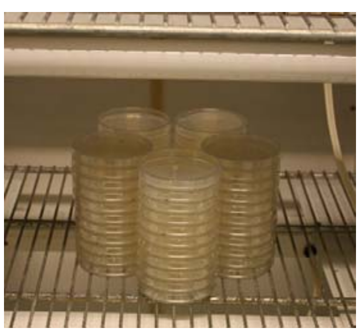


Laboratory methods

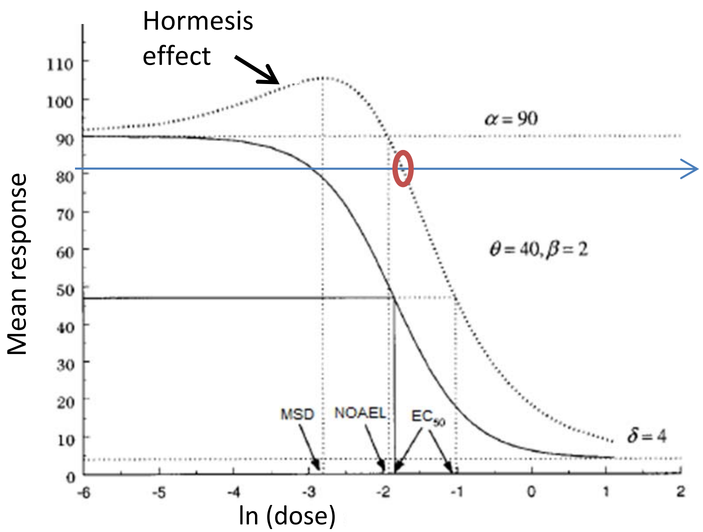




Statistical Data Analysis

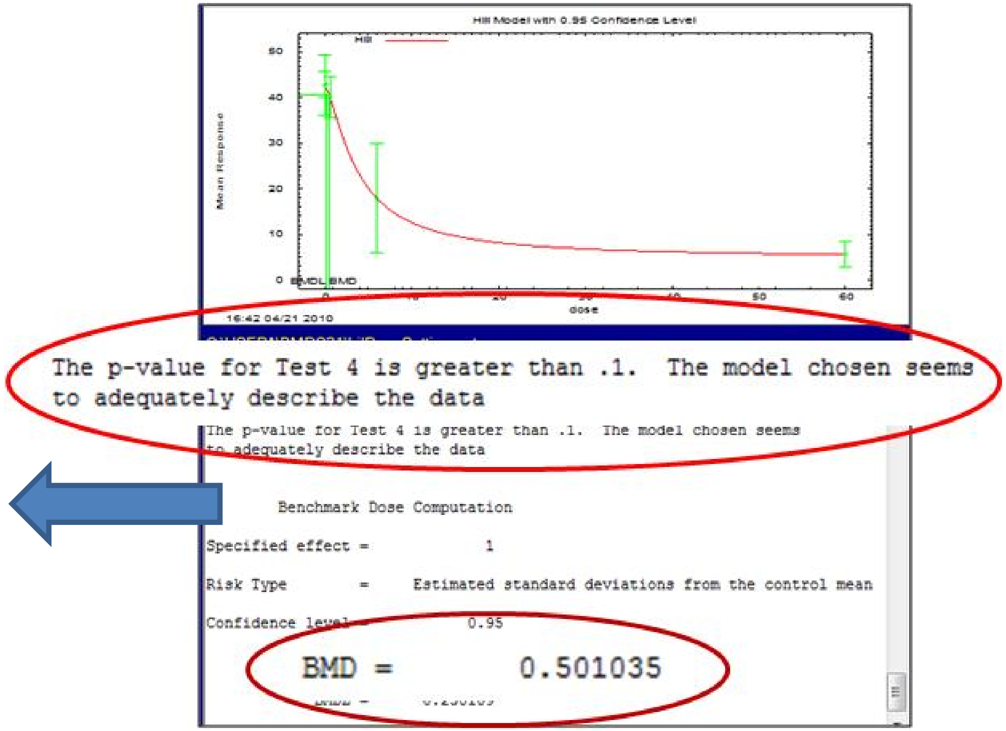


Determining hormetic zone



Benchmark dose (BMD) = EC_{10}

BMDS 2.1.2



Stock solution	Concentration
A	BMD x 10^{4}
B	BMD x 10^{3}
C	BMD x 10^{2}
D	BMD x 10
E	BMD x $10^{0.6}$
F	BMD x $10^{0.2}$
G	BMD x $10^{-0.2}$
H	BMD x $10^{-0.6}$
I	BMDL x 10^{-1}
J	BMD x $10^{-1.4}$
Control	0

Curve Modeling

Schabenberger et al. 1999

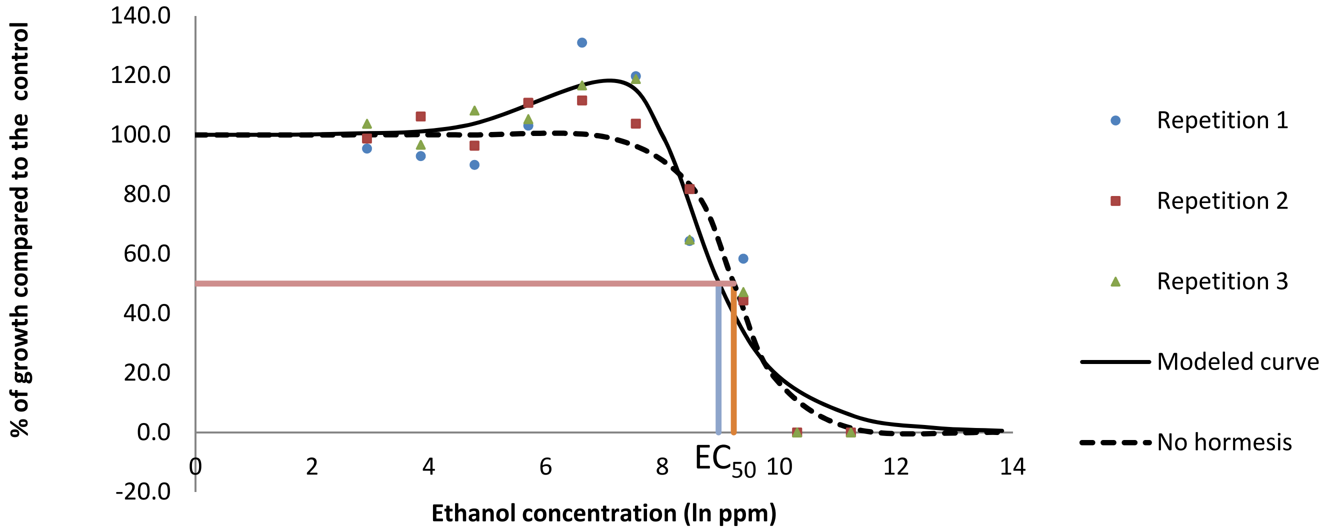
Model	Parameter†	Defining relationship	$\omega =$	Expression $E[Y\|x] =$
Log–logistic, Eq. [1]	EC_{50}	$\theta = \omega \exp[-\beta \ln(EC_{50})]$	1	$\delta + \dfrac{\alpha - \delta}{1 + \exp[\beta \ln(x/EC_{50})]}$
Log–logistic, Eq. [1]	EC_K	$\theta = \omega \exp[-\beta \ln(EC_K)]$	$\dfrac{K}{100 - K}$	$\delta + \dfrac{\alpha - \delta}{1 + \omega \exp[\beta \ln(x/EC_K)]}$
Brain–Cousens, Eq. [3]	EC_{50}	$\theta = \omega \exp[-\beta \ln(EC_{50})]$	$1 + \dfrac{2\gamma EC_{50}}{\alpha - \delta}$	$\delta + \dfrac{\alpha - \delta + \gamma x}{1 + \omega \exp[\beta \ln(x/EC_{50})]}$
Brain–Cousens, Eq. [3]	EC_K	$\theta = \omega \exp[-\beta \ln(EC_K)]$	$\dfrac{K}{100 - K} + \left(\dfrac{100}{100 - K}\right)\dfrac{\gamma EC_K}{\alpha - \delta}$	$\delta + \dfrac{\alpha - \delta + \gamma x}{1 + \omega \exp[\beta \ln(x/EC_K)]}$
Brain–Cousens, Eq. [3]	NOAEL	$\theta = \omega \exp[-\beta \ln(NOAEL)]$	$\gamma\, NOAEL/(\alpha - \delta)$	$\delta + \dfrac{\alpha - \delta + \gamma x}{1 + \omega \exp[\beta \ln(x/NOAEL)]}$
Brain–Cousens, Eq. [3]	MSD‡	$\theta = \omega \exp[-\beta \ln(MSD)]$	$\dfrac{MSD\gamma}{(\alpha - \delta)\beta - MSD\gamma(1 - \beta)}$	$\delta + \dfrac{\alpha - \delta + \gamma x}{1 + \omega \exp[\beta \ln(x/MSD)]}$

† The parameter to be incorporated into the model.
‡ The highest response in the presence of hormesis.

doseresp

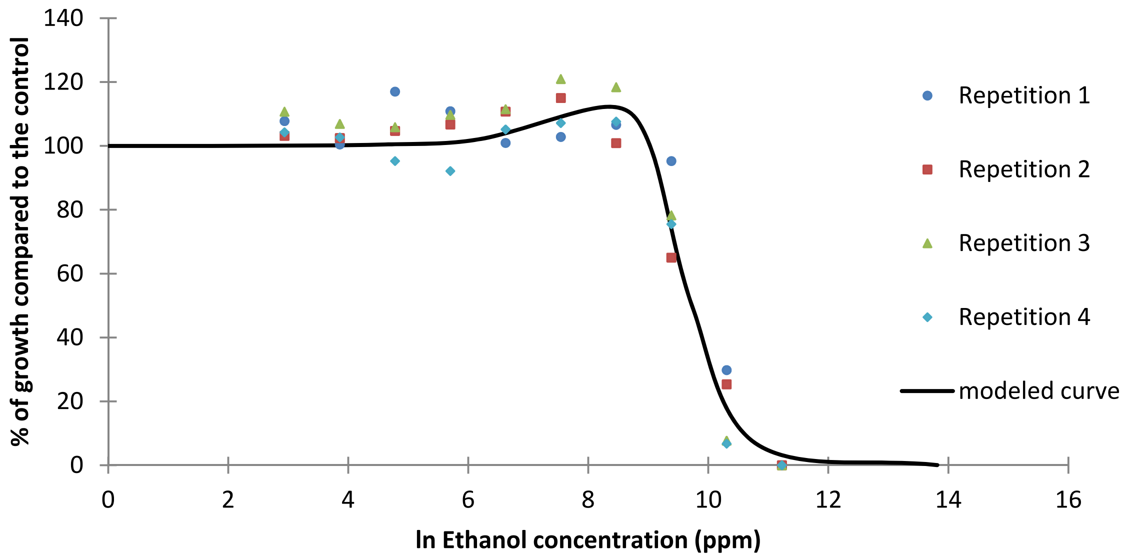
```
/* ——————————————————————————————————————— */
/* Test the dose response data for a hormesis effect */
/* This is the Brain-Cousens model parameterized in terms of EC50 */
 /* ——————————————————————————————————————— */
proc nlin data = pythsegw noitprint method = marquardt;
parameters beta 2.0 EC50 0.10 gamma 2 ;
alpha =100; delta=0;
omega= 1 + 2*gamma*EC50/(alpha-delta);
term=1 + omega * exp(beta*log(rate/EC50));
model resp= (delta +( alpha-delta + gamma*rate) / term);
bounds gamma >= 0;
run;
```

P. aphanidermatum vs. ethanol

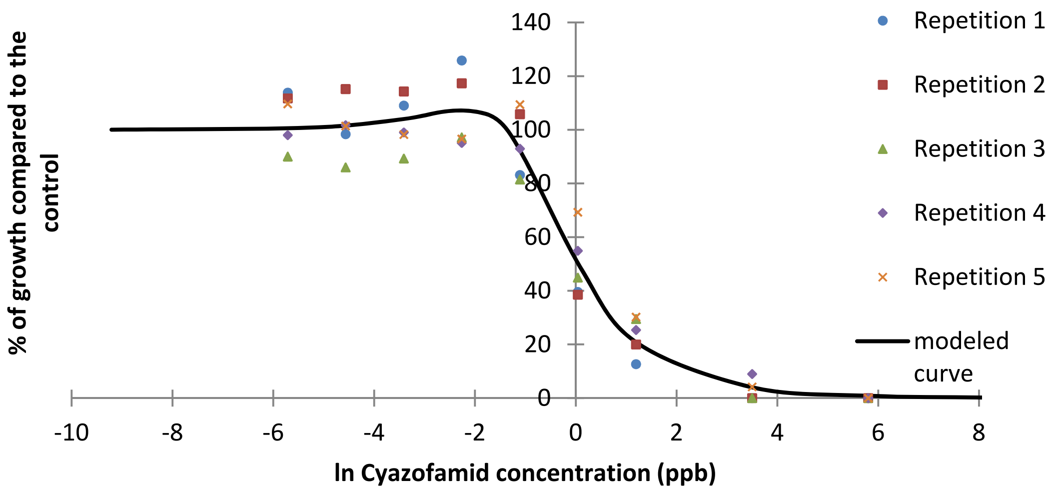


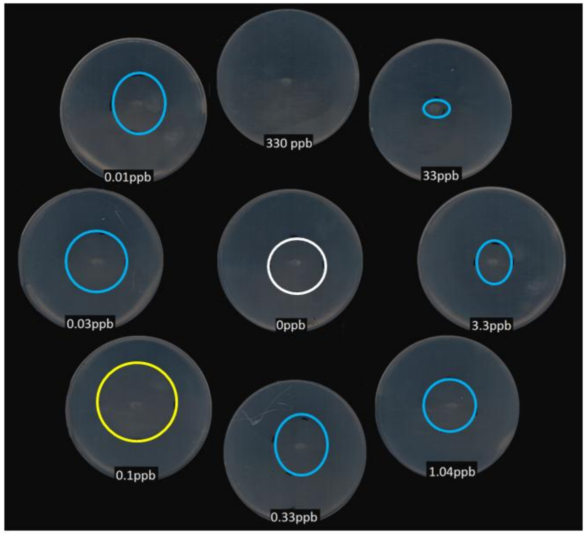
Parameter	Estimated values	Approximate 95% confidence limits	
		Lower bound	Upper bound
β	1.90	1.67	2.1
EC_{50}	7863 ppm	6701 ppm	9024 ppm
γ	0.032	**0.014**	**0.049**
NOAEL	2966 ppm	2394 ppm	3537 ppm
MSD	1206 ppm	897.6 ppm	1514.3 ppm

Rhizoctonia zeae vs. ethanol



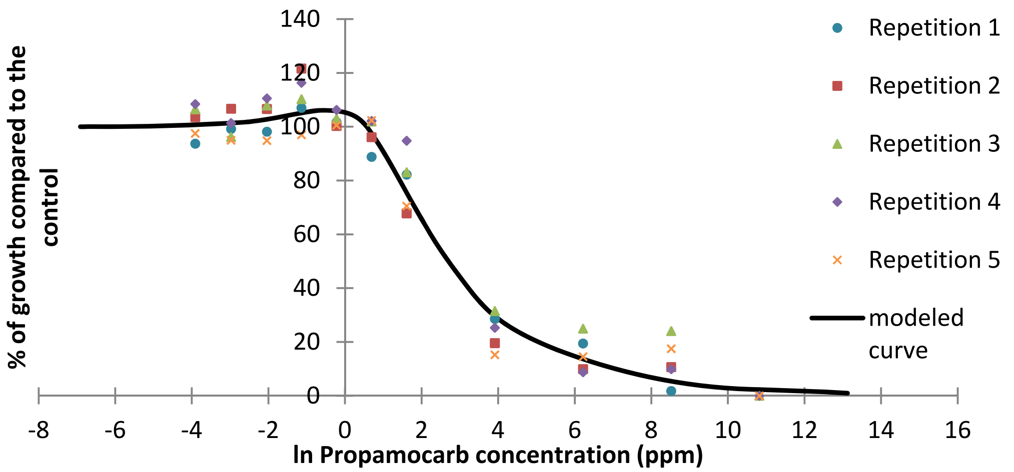
P. aphanidermatum vs. cyazofamid

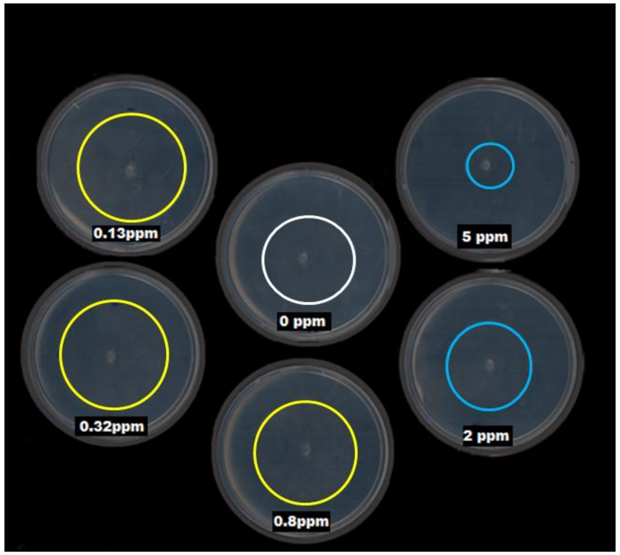




17 % radial growth increase at 0.1ppb

P. aphanidermatum vs. propamocarb





16 % radial growth increase at 0.32 ppm

Conclusions

1. Laboratory methods standardized can be used for true fungi as well as oomycetes for assessment of dose responses in fungal plant pathogens to fungicides at subinhibitory levels
2. Curve modeling is necessary for statistical detection of hormesis. Schabenberger et al. 1999 hormesis test fit our data best
3. The hormetic responses displayed by Pythium aphanidermatum to ethanol, cyazofamid, and propamocarb may be related to the particular plasticity of the studied strain
4. *Rhizoctonia zeae* only displayed hormetic responses to ethanol. Other endpoints should be tested
5. Hormesis should be considered in fungicide EC50 estimation

Final remarks

- Potential impact of fungicide hormesis is great
- More awareness among phytopathologists is needed to prevent crop losses due to accidental stimulation of fungal pathogens
- Until a broader acceptance and understanding of hormetic processes is achieved access to funding will be limited, particularly in agricultural research

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