Chemical hormesis in plant pathogenic fungi and fungus-like oomycetes

Carla D. Garzon and Francisco J. Flores

Department of Entomology and Plant Pathology
Oklahoma State University
Stillwater OK 74078
Hormesis in fungi

• **Schulz 1887-88** - Yeast *(Saccharomyces* spp.) – early chemical hormesis research models. European Journal of Physiology 1:517-541

• **1943** - **Southam and Ehrig** 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 prochloraz - 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
Testing for hormesis

Seedling assay

- Petri dish
- Seedling
- Inoculum
- Filter paper
- Fertilizer solution

Lesion Expansion (AUDPC)

Number of infected seedlings / treatment
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

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

Plant Dis. 95: 1233-1238

Featured Article (October 2012)

• Phytopathology News: Plant Disease Editor’s Choice

• APSnet: Emerging Research
EFFECT OF LOW DOSES OF PESTICIDES ON SOILBORNE PATHOGENS AN APPROACH TO THE HORMETIC RESPONSE

Francisco Flores
M.S. Thesis

Dept. Entomology and Plant Pathology
Oklahoma State University
Stillwater, OK
Evaluating hormesis

• Criteria:
  – Strength of evidence
  – Soundness of data
  – Consistency
  – Biological plausibility

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
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pythium aphanidermatum</em></td>
<td>Ethanol</td>
</tr>
<tr>
<td></td>
<td>Sodium hypochlorite (Clorox) *</td>
</tr>
<tr>
<td></td>
<td>Cyazofamid (Segway)</td>
</tr>
<tr>
<td></td>
<td>Propamocarb (Previcur)</td>
</tr>
<tr>
<td><em>Rhizoctonia zeae</em></td>
<td>Ethanol</td>
</tr>
<tr>
<td></td>
<td>Sodium hypochlorite (Clorox) *</td>
</tr>
<tr>
<td></td>
<td>Propiconazole (ferti-lome) *</td>
</tr>
<tr>
<td><em>Rhizoctonia solani</em></td>
<td>Propiconazole (ferti-lome) *</td>
</tr>
</tbody>
</table>

* 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

P. aphanidermatum

Gordon Beakes © University of Newcastle
Rhizoctonia spp.

- Fungi
  - Basidiomycota
  - Agaricomycetes

- Diseases:
  - Sclerotial diseases
  - Damping off
  - Broad host range
  - Soilborne

- Warm and humid weather
Determining hormetric zone

Benchmark dose (BMD) = EC_{10}

<table>
<thead>
<tr>
<th>Stock solution</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>BMD x 10^{4}</td>
</tr>
<tr>
<td>B</td>
<td>BMD x 10^{3}</td>
</tr>
<tr>
<td>C</td>
<td>BMD x 10^{2}</td>
</tr>
<tr>
<td>D</td>
<td>BMD x 10</td>
</tr>
<tr>
<td>E</td>
<td>BMD x 10^{0.6}</td>
</tr>
<tr>
<td>F</td>
<td>BMD x 10^{0.2}</td>
</tr>
<tr>
<td>G</td>
<td>BMD x 10^{-0.2}</td>
</tr>
<tr>
<td>H</td>
<td>BMD x 10^{-0.6}</td>
</tr>
<tr>
<td>I</td>
<td>BMDL x 10^{-1}</td>
</tr>
<tr>
<td>J</td>
<td>BMD x 10^{-1.4}</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>
Curve Modeling
Schabenberger et al. 1999

| Model                         | Parameter | Defining relationship | $\omega =$ | Expression $E[Y|x]=$ |
|------------------------------|-----------|-----------------------|------------|---------------------|
| Log-logistic, Eq. [1]        | $EC_{50}$ | $0 = \omega \exp[-\beta \ln(EC_{50})]$ | 1          | $\delta + \frac{\alpha - \delta}{1 + \exp[\beta \ln(x/EC_{50})]}$ |
| Log-logistic, Eq. [1]        | $EC_{x}$  | $0 = \omega \exp[-\beta \ln(EC_{x})]$ | $\frac{K}{100 - K}$ | $\delta + \frac{\alpha - \delta}{1 + \omega \exp[\beta \ln(x/EC_{x})]}$ |
| Brain–Cousens, Eq. [3]       | $EC_{50}$ | $0 = \omega \exp[-\beta \ln(NOAE)]$ | 1 + $\frac{\alpha - \delta}{\alpha - \gamma}$ | $\delta + \frac{\alpha - \delta + \gamma x}{1 + \omega \exp[\beta \ln(x/EC_{50})]}$ |
| Brain–Cousens, Eq. [3]       | $EC_{x}$  | $0 = \omega \exp[-\beta \ln(EC_{x})]$ | $\frac{K}{100 - K} + \frac{100}{\alpha - \delta}$ | $\delta + \frac{\alpha - \delta + \gamma x}{1 + \omega \exp[\beta \ln(x/EC_{x})]}$ |
| Brain–Cousens, Eq. [3]       | NOAEL     | $0 = \omega \exp[-\beta \ln(NOAE)]$ | $\gamma \frac{NOAE}{(\alpha - \delta)}$ | $\delta + \frac{\alpha - \delta + \gamma x}{1 + \omega \exp[\beta \ln(x/NOAE)]}$ |
| Brain–Cousens, Eq. [3]       | MSD$\dagger$ | $0 = \omega \exp[-\beta \ln(MSD)]$ | $\frac{MSD}{(\alpha - \delta)}$ | $\delta + \frac{\alpha - \delta + \gamma x}{1 + \omega \exp[\beta \ln(x/MSD)]}$ |

$\dagger$ The parameter to be incorporated into the model.
$\ddagger$ The highest response in the presence of hormesis.

---

```plaintext
/* ______________ */
/* Test the dose response data for a hormesis effect */
/* This is the Brain–Cousens model parameterized in terms of EC50 */
/* ______________ */

proc nlin data = pythsegw nostprint 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 resp >= 0;
run;
```
Results

**P. aphanidermatum** vs. ethanol

![Graph showing growth compared to control vs. ethanol concentration](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated values</th>
<th>Approximate 95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta )</td>
<td>1.90</td>
<td>Lower bound: 1.67 Upper bound: 2.1</td>
</tr>
<tr>
<td>( EC_{50} )</td>
<td>7863 ppm</td>
<td>6701 ppm - 9024 ppm</td>
</tr>
<tr>
<td>( Y )</td>
<td>0.032</td>
<td>0.014 - 0.049</td>
</tr>
<tr>
<td>NOAEL</td>
<td>2966 ppm</td>
<td>2394 ppm - 3537 ppm</td>
</tr>
<tr>
<td>MSD</td>
<td>1296 ppm</td>
<td>897.6 ppm - 1514.3 ppm</td>
</tr>
</tbody>
</table>
Rhizoctonia zeae vs. ethanol

% of growth compared to the control vs. In Ethanol concentration (ppm)

Repetition 1
Repetition 2
Repetition 3
Repetition 4
Modeled curve

Images:
- 11887 ppm: Inhibition: 38%
- 0 ppm: Control
- 1880 ppm: Stimulation: 15%
P. aphanidermatum vs. cyazofamid

![Graph showing growth comparison](image)

- % of growth compared to the control vs. In Cyazofamid concentration (ppb)

- Repetition 1
- Repetition 2
- Repetition 3
- Repetition 4
- Repetition 5

- Modeled curve

17 % radial growth increase at 0.1ppb
P. aphanidermatum vs. propamocarb

% of growth compared to the control

In Propamocarb concentration (ppm)

-8 -6 -4 -2 0 2 4 6 8 10 12 14 16

Repetition 1
Repetition 2
Repetition 3
Repetition 4
Repetition 5

modeled curve

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 hormetric 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 hormetric 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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