Glyphosate Herbicides
How Do They Kill Plants?

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Acknowledgements

Guri Johal
PhD, 1988
Pioneer documentation of GSI

Andre Levesque
PhD, 1990
LD 50’s, Molecular diagnostics for Pythium

Rolando Descalzo
PhD, 1996
Ecology of GSP

Lixing Liu
PhD, 1995
Physiology of GSI
Outline of presentation

I: Contribution of root rot fungi to herbicidal activity of glyphosate

II: Ecology of Pythium spp. as glyphosate synergistic fungi

III: How glyphosate predisposes roots of bean seedlings to colonization by root fungi
Glyphosate herbicides - Roundup, et al.

- Non-selective
- Absorbed and translocated
- Negligible residual activity in most soils
- Water soluble
- Low mammalian toxicity
Glyphosate herbicides - uses

- Directed sprays
- Preplant cleanup
- Chemfallow
- Crop desiccation
- Injection (forestry)
- Glyphosate-tolerant GMP’s
Glyphosate herbicides - behavior in plants

- Foliar absorbed
- Slow to kill
- Activity mainly in roots
- Shikimic acid pathway targeted
- Long term effects of sublethal doses
Shikimic acid pathway and some of its main products

Pentose phosphate pathway:
- D-erythrose-4-P
- 3-deoxy-3-arabino-heptulosonic acid-7-P
- Phosphoenolpyruvate

TCA cycle

Shikimic acid:
- 5-P-shikimate
- Glyphosate

Chorismic acid
- Prephenic acid
- Phenylalanine

Tyrosine

Tryptophan
Some questions about the herbicidal effect of glyphosate

If death of treated plants is directly due to inhibition of the shikimic acid pathway . . .

Why does glyphosate have to be translocated to roots to be effective?

Why do effects of sublethal doses on perennial plants sometimes appear a year after exposure, and persist for two or more years?
Effect of soil sterilization on herbicidal activity of glyphosate

Symptoms on seedlings killed by glyphosate in untreated soil appear similar to symptoms of damping off caused by Pythium spp. (dose per plant)
Fungal colonization of roots of glyphosate treated bean seedlings growing in untreated soil
Particulate, Heat-Sensitive Factors From Untreated Soil Restore Herbicidal Activity of Glyphosate to Seedlings Growing in Sterilized Soil

% Mortality in Bean Seedlings Grown in Autoclaved Soil Amended with Extracts from Untreated Soil

<table>
<thead>
<tr>
<th>Extract:</th>
<th>Raw</th>
<th>Filtered</th>
<th>Autoclaved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Rates of Colonization of Roots of Bean Seedlings Growing In Untreated Soil by Pythium and Fusarium Following Foliar Treatment With Glyphosate
Fusarium and Pythium restore herbicidal activity of glyphosate to bean seedlings growing in sterilized soil

* FUNGUS APPLIED BY
C = CONTROL
S = SEED TREATMENT
R = ROOT DIP
W = WOUNDING

DAYS AFTER TREATMENT

% MORTALITY

PYTHIUM-
STERILIZED SOIL
Effect of Ridomil on % Mortality on Bean Seedlings Growing in Different Media, 12 Days After Treatment with Glyphosate

<table>
<thead>
<tr>
<th></th>
<th>Pythium - no</th>
<th>Pythium - yes</th>
<th>Pythium - yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ridomil - no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterilized soil</td>
<td>0*</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Vermiculite</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Untreated Soil</td>
<td>100</td>
<td>100</td>
<td>88</td>
</tr>
</tbody>
</table>

Conclusion: Ridomil, a systemic fungicide specific for Oomycete fungi, negates the ability of Pythium to restore herbicidal activity of glyphosate to seedlings growing in sterilized soil or soilless media (vermiculite). Ridomil does not protect seedlings growing in untreated soil where Fusarium and other non-Oomycete root rot fungi are present.
Summary of Evidence that Pythium and Fusarium Contribute to Herbicidal Activity of Glyphosate in Bean Seedlings

Herbicidal activity is reduced in sterilized soils, and in soilless plant growth media

Pythium and Fusarium spp. rapidly colonize roots of glyphosate treated plants growing in non sterile soil

Adding Pythium or Fusarium restores herbicidal activity of glyphosate to seedlings growing in sterilized soil or soilless media

Metalaxyl blocks restoration of activity when Pythium is used to amend sterilized soil
II: Ecology of Pythium spp. as glyphosate synergistic fungi

- Glyphosate treated bean and wheat seedlings were used to bait Pythium from diverse agricultural soils.

- Pythium isolates from beans were assigned to 15 RFLP groups; isolates from wheat were assigned to 14 RFLP groups.

- Representatives of each RFLP group were tested for efficacy as glyphosate synergists on various monocot and dicot plant species.
Number of isolates of different species and groups of Pythium obtained from roots of glyphosate treated bean seedlings grown in five different soils

<table>
<thead>
<tr>
<th>Pythium spp. and groups</th>
<th>Arid climate</th>
<th>Humid climate</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>virgin replant crown rot loam muck</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P. ultimum</strong></td>
<td>0 4 7</td>
<td>9 6</td>
<td>26</td>
</tr>
<tr>
<td><strong>P. irregulare</strong></td>
<td>0 7 0</td>
<td>1 0</td>
<td>8</td>
</tr>
<tr>
<td><strong>P. sylvaticum</strong></td>
<td>2 0 6</td>
<td>1 5</td>
<td>14</td>
</tr>
<tr>
<td><strong>P. coloratum</strong></td>
<td>0 0 0</td>
<td>0 3</td>
<td>3</td>
</tr>
<tr>
<td><strong>P. ‘G’ group</strong></td>
<td>0 1 3</td>
<td>3 4</td>
<td>11</td>
</tr>
<tr>
<td><strong>P. ‘HS’ group</strong></td>
<td>0 0 1</td>
<td>0 2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Σ</strong></td>
<td>2 12 17</td>
<td>14 20</td>
<td>65</td>
</tr>
</tbody>
</table>
GSI in Virgin and Orchard Soil Compared

**Graph:**

- **LD<sub>50</sub> (µg of Glyphosate/Plant):
  - Virgin Soil: 20 µg
  - Orchard Soil: 100 µg
  - Raw Apple Seedlings: 140 µg
  - Autoclaved Apple Seedlings: 160 µg
Pythium isolates and GSI efficacy of 15 isolates from bean, tested on wheat and corn seedlings
Pythium isolates and GSI efficacy of 14 isolates from wheat, tested on wheat and bean seedlings.
Pythium isolates and GSI efficacy of 15 isolates from bean, tested on sunflower and pepper seedlings
Pythium isolates and GSI
efficacy of isolates from type cultures, tested on bean seedlings
Pythiaceous fungi as glyphosate synergists - Summary

- GSP were present in and isolated from diverse soil types.
- All isolates and species tested were able to enhance herbicidal efficacy of glyphosate on at least some of the plant species used for testing.
- Individual isolates were typically active on several different plant species.
- Pythium GSI was generally strong (10X-50X) on dicot species, and weak (2X-3X) on monocot species.
III: How Does Glyphosate Predispose Plant Roots to Colonization by Soil Fungi?

Lixing Liu

Studies of Mechanisms of Predisposition by Glyphosate of Bean Roots (Phaseolus vulgaris L.) to Colonization by Pythium spp.

How Does Glyphosate Predispose Plant Roots to Colonization by Soil Fungi?

Four hypotheses

- glyphosate directly stimulates growth of Pythium
- glyphosate enhances root exudation
- glyphosate blocks phytoalexin production
- glyphosate interferes with induced lignification
Shikimic acid pathway and some of its secondary metabolites

- Shikimic acid pathway
  - 5-P-shikimate
  - Chorismic acid
  - Glyphosate
  - IAA
  - Tryptophan
  - Prephenic acid
  - Phenylalanine, Tyrosine
  - Isoflavanoid phytoalexins
  - Lignin
Effect of formulations of glyphosate herbicides with and without surfactant on percent germination of sporangia of *Pythium ultimum*

<table>
<thead>
<tr>
<th>ppm glyphosate</th>
<th>0.0</th>
<th>0.1</th>
<th>1.0</th>
<th>10</th>
<th>100</th>
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</thead>
<tbody>
<tr>
<td>Formulation*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accord</td>
<td>30</td>
<td>35</td>
<td>41</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>Roundup</td>
<td>31</td>
<td>34</td>
<td>39</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>

*Accord contains no surfactant; Roundup contains proprietary surfactant

Conclusions:
1. Glyphosate @ 1 ppm stimulates germination of sporangia
2. Inhibition @ 10, 100 ppm by Roundup formulation probably caused by surfactant
How does 1 ppm glyphosate relate to concentrations of glyphosate in soil solution?

Rates of application; adsorption to soil colloids; losses over time due to microbial degradation and leaching

How much water in 1 ha plow depth?

1 ha = 1 x 10^4 m^2; = 1 x 10^8 cm^2
1 x 10^8 cm^2 x 17.5 cm = 1.75 x 10^9 cc/ha plow depth
@ bulk density = 1.4, ha plow depth = 2.5 x 10^9 g
Water content of different soils typically 30% - 50% w/w
@ -1bar matric potential
Therefore, water content of ha plow depth
= 0.7-1.2 x 10^6 L (for simplicity, let’s assume 1 x 10^6 L/ha)

Usual application rates 0.9-2.7 L a.i.(glyphosate) /ha.
Therefore, ~1 ppm in soil water IF no adsorption to soil colloids; if adsorption is 99.9%, ~1 ppb at time of application
Growth (colony diameters, mm @ 24 h) of *Pythium ultimum* in water agar and corn meal agar amended with Roundup and Accord

<table>
<thead>
<tr>
<th>Formulation</th>
<th>ppm glyphosate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>Accord</td>
<td></td>
</tr>
<tr>
<td>water agar</td>
<td>43</td>
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<tr>
<td>corn meal agar</td>
<td>44</td>
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<tr>
<td>Roundup</td>
<td></td>
</tr>
<tr>
<td>water agar</td>
<td>45</td>
</tr>
<tr>
<td>corn meal agar</td>
<td>48</td>
</tr>
</tbody>
</table>
Effect of root exudates from glyphosate-treated and untreated bean seedlings on fungal germination and germ tube growth

<table>
<thead>
<tr>
<th></th>
<th>Glyphosate</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Germination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pythium ultimum</em></td>
<td>50.5%</td>
<td>36.0%</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>54.0%</td>
<td>43.1%</td>
</tr>
<tr>
<td><strong>Germ tube length</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pythium ultimum</em></td>
<td>60.5μm</td>
<td>44.6μm</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>19.4μm</td>
<td>15.6μm</td>
</tr>
</tbody>
</table>
Effect of root exudates from bean seedlings treated with glyphosate or water on growth of germ tubes of *Pythium ultimum* and FORL*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glyphosate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. ultimum</em></td>
<td>44.6</td>
<td>60.5</td>
</tr>
<tr>
<td>FORL*</td>
<td>15.6</td>
<td>19.4</td>
</tr>
</tbody>
</table>

* Fusarium oxysporum f.sp. radicis lycopersici*
Effect of root exudates collected at different times after treatment of bean seedlings with glyphosate on sporangial germination and germ tube growth by *Pythium ultimum*

<table>
<thead>
<tr>
<th>Exudate collected X hours after Treatment</th>
<th>Germination (%)</th>
<th>Germ tube length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>treatment</td>
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<tr>
<td>0</td>
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<tr>
<td>6</td>
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<td>42</td>
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<tr>
<td>12</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>24</td>
<td>34</td>
<td>36</td>
</tr>
</tbody>
</table>
Phytoalexin content of roots of bean seedlings grown in different media

![Graph showing phytoalexin content in various growth media.](image)
Effect of glyphosate on phytoalexin production by roots of bean seedlings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phaseollin (μg/g fresh wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.5</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>3.4</td>
</tr>
<tr>
<td><em>Pythium ultimum</em></td>
<td>13.7</td>
</tr>
<tr>
<td><em>P. ultimum</em> + glyphosate</td>
<td>10.0</td>
</tr>
</tbody>
</table>
Effect of glyphosate on induced lignification in roots of bean seedlings

- a: glyphosate at time of inoculation
- b: glyphosate 2 days prior to inoculation
Research by Johal, Levesque, Descalzo, Liu, Rahe provides conclusive evidence that glyphosate predisposes plant roots to colonization by root rot fungi such as Pythium and Fusarium within 2-3 days of treatment.

These fungi, ubiquitous in agricultural soils, contribute significantly to the herbicidal efficacy of glyphosate on dicot seedlings.
Predisposition of roots to colonization by low doses of glyphosate may explain why

- glyphosate has to be translocated to roots to be effective.

- effects of sublethal doses on perennial plants sometimes appear a year after exposure, and persist for two or more years.
Whole Root Plating to Determine # of CFU Per Plant