# Response of sunflowers to cell-free culture filtrates produced by *Phomopsis*

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# Outline

- ✓ Research Background
- ✓ Rationale
- ✓ Objectives
- ✓ Materials and Methods
- ✓ Results
- ✓ Summary

# Previous Research

- Cytological observations revealed that *P. helianthi* kills the host cells before colonizing tissues

  ✓ Potentially by the toxic metabolites released by fungus (Heller and Gierth, 2001)

- Production of a phytotoxin "phomozin" by *P. helianthi* isolates have been demonstrated during infection on sunflowers (Mazars et al. 1990)

- Purified two phytotoxic metabolites (*cis*- and *trans*-4,6-dihydroxymellein) from cultures of French and Italian of *P. helianthi* isolates with varying degrees of virulence (Avantaggiato et al. 1999)

# Rationale

- ✓ Production of phytotoxic metabolites by isolates of *Phomopsis gulyae* and *P. helianthi* from United States is not investigated

# Research Objectives

1. To evaluate the response of sunflowers to *P. gulyae* and *P. helianthi* isolates, and their cell-free culture filtrates

2. To evaluate the response of ten sunflower accessions to *P. gulyae* and *P. helianthi* and their cell-free culture filtrates

3. To identify putative phytotoxic metabolites produced by the U.S. isolates of *P. gulyae* and *P. helianthi* through untargeted metabolomics approach.

# Objective 1

To evaluate the response of sunflowers to isolates of *P. gulyae* and *P. helianthi,* and their cell-free culture filtrates

# Greenhouse assay

- Two parallel experiments – for each species
  - Fungus inoculation
  - Cell-free culture free filtrate inoculation
- Isolates used:

| Sl. No. | Isolate | State | Species |
|---|---|---|---|
| 1 | DIA40 | SD | *P. gulyae* |
| 2 | DIA66 | NE | *P. gulyae* |
| 3 | DIA73 | ND | *P. gulyae* |
| 4 | DIA233 | ND | *P. gulyae* |
| 5 | DIA234 | ND | *P. gulyae* |
| 6 | DIA235 | ND | *P. gulyae* |

| Sl. No. | Isolate | State | Species |
|---|---|---|---|
| **1** | DIA59 | MN | *P. helianthi* |
| **2** | DIA131 | ND | *P. helianthi* |
| **3** | DIA145 | SD | *P. helianthi* |
| **4** | DIA290 | ND | *P. helianthi* |
| **5** | DIA300 | ND | *P. helianthi* |
| **6** | DIA323 | ND | *P. helianthi* |

- Completely randomized design
- Three week old sunflower plants of a *Phomopsis*-susceptible variety N4HM354 (Nuseed genetics)
- Experiment conducted two times, six plants (replication) for each treatment
- Plants infiltrated with PDB or inoculated with plugs (no fungus) served as controls

# Method of inoculation

- Performed on top most fully opened leaf

Mycelial inoculation – Affixing mycelial plugs with tape

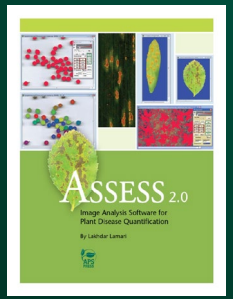
Cell-free culture filtrate inoculation - 200 µl filtrate delivered with a needle less syringe

# Greenhouse conditions

- Temperature regime - 23±2°C
- Light conditions - (16 h photoperiod)
- Relative humidity – 55 - 70%

# Observation

- One week post inoculation
- Disease symptoms were quantified




# Data Analysis

- One-way ANOVA
- Duncan's Multiple Range Test
- Using 'Agricolae' package in R v4.3.0
- Pearson's correlation test

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# Response of sunflower to *P. gulyae*

**Cell-free culture free filtrate infiltration**

**Mycelial inoculation**

DIA234 DIA40 DIA66 DIA233 DIA235 DIA73 Control

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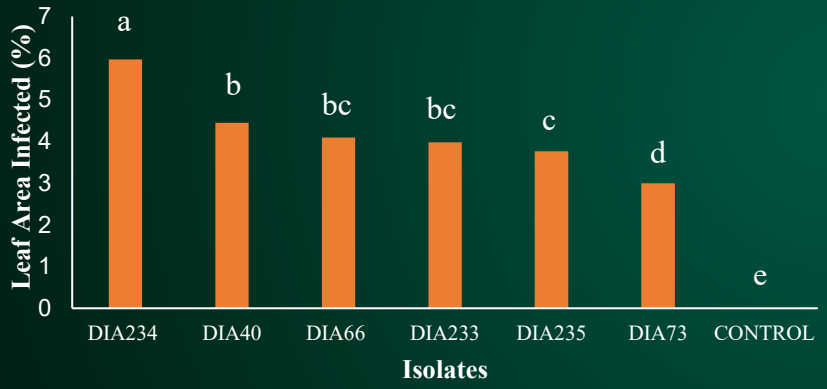
# Sensitivity of sunflower to isolates of *P. gulyae*

**Mycelial Inoculation**

Expressed as Leaf Area Infected (LAI)

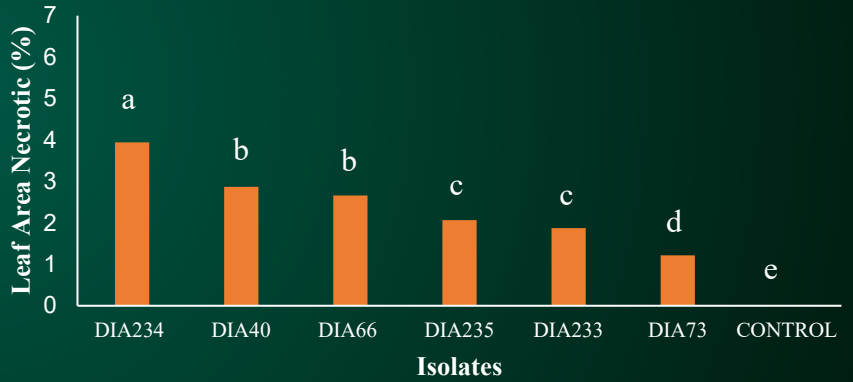
| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Trt | 6 | 246.38 | 41.06 | 82.342 | <0.0001 *** |




**Culture Filtrate Inoculation**

Expressed as Leaf Area Necrotic (LAN)

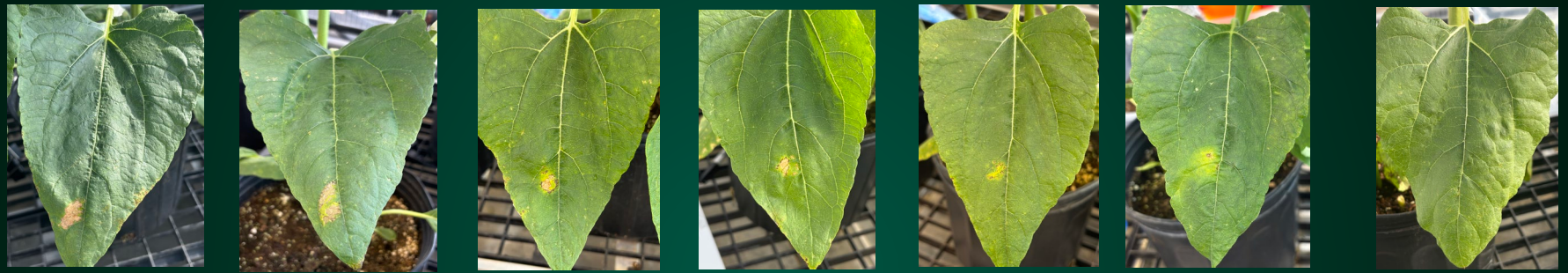
| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Trt | 6 | 117.84 | 19.640 | 75.459 | <0.0001 *** |



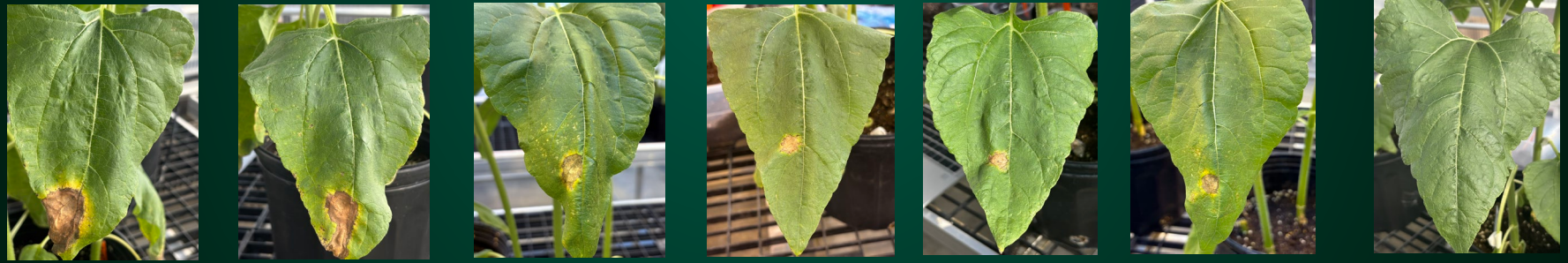

Significant positive correlation ($r = 0.95$, $P = 0.004$) between the LAI (by the isolates) and LAN (by the culture filtrates of the isolates) suggests involvement of metabolites produced by *P. gulyae* isolates from U. S. in symptom development

# Response of sunflower to *P. helianthi*

**Culture free filtrate infiltration**



**Mycelial inoculation**



DIA300 DIA323 DIA290 DIA145 DIA131 DIA59 Control

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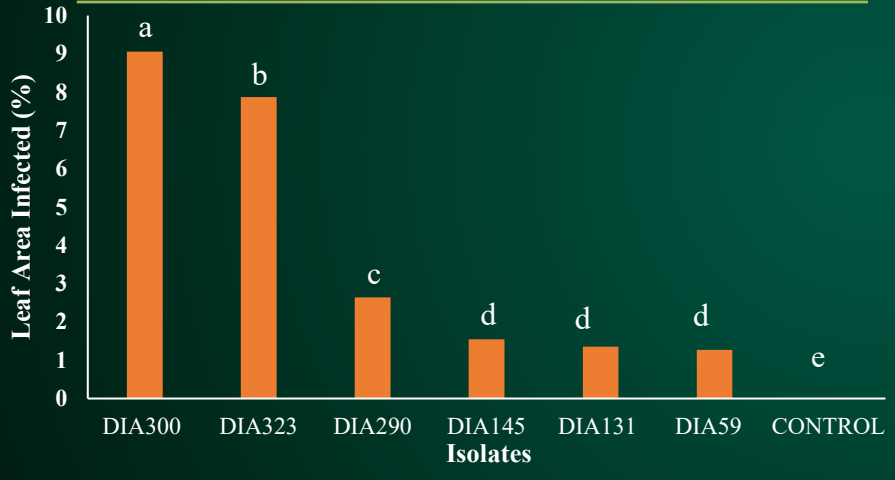
# Sensitivity of sunflower to isolates of *P. helianthi*

**Mycelial Inoculation**

Expressed as Leaf Area Infected (LAI)

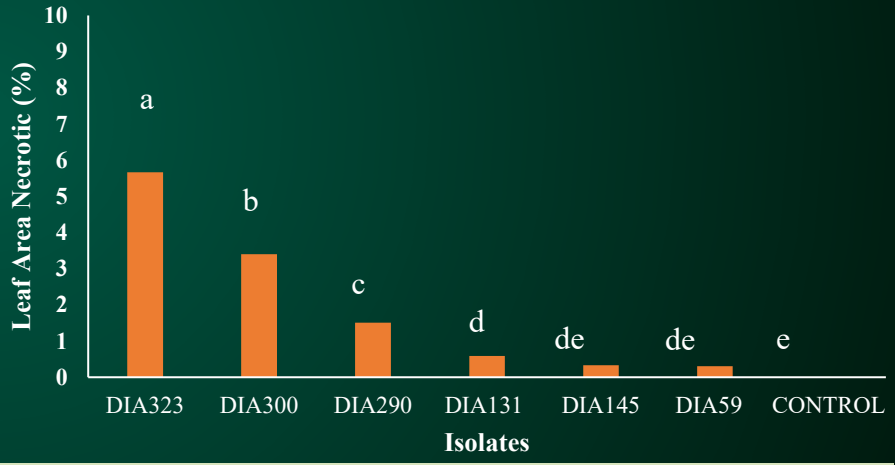
| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| **Trt** | 6 | 915.4 | 152.57 | 156.468 | <0.0001 *** |




**Culture Filtrate Inoculation**

Expressed as Leaf Area Necrotic (LAN)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| **Trt** | 6 | 319.8 | 53.31 | 237.585 | <0.0001 *** |




Significant positive correlation ($r = 0.90$, $P = 0.015$) between the LAI (by the isolates) and LAN (by the culture filtrates of the isolates) suggests involvement of metabolites produced by *P. helianthi* isolates from U.S. in symptom development

2. To evaluate the response of ten sunflower accessions to *P. gulyae* and *P. helianthi* and their cell-free culture filtrates

# Methodology

| Sl. No | Name of accessions | Line name | Class | Country of origin |
|---|---|---|---|---|
| 1 | PI 603991 | HA410 | HA-Oil | USA |
| 2 | PI 509064 | RHA 354 | RHA-Non-oil | USA |
| 3 | PI 552934 | HA 288 | HA-Non-oil | USA |
| 4 | PI 561918 | HA 378 | HA-Oil | USA |
| 5 | PI 599984 | HA 821 | HA-Oil | USA |
| 6 | PI 618725 | HA 421 | HA-Oil | USA |
| 7 | PI 650657 | Ames 10101 | Open-pollinated variety | China |
| 8 | PI 650675 | CO-PB 39 | Open-pollinated variety | Spain |
| 9 | PI 650755 | HA-R4 | HA-Oil | USA |
| 10 | PI 650839 | Taiyo | Open-pollinated variety | Netherlands |

- Ten accessions, including susceptible check – HA410 (Underwood and Misar 2024)

# Methodology

- Two parallel experiments - Fungal inoculation and cell-free culture filtrates infiltration
- Isolates
    - *P. gulyae* (DIA234)
    - *P. helianthi* (DIA323)
- Experiments repeated twice
- For both inoculation and infiltration - six plants as replication per experiment
- Inoculation/Infiltration performed as mentioned before

# Response of sunflower accessions

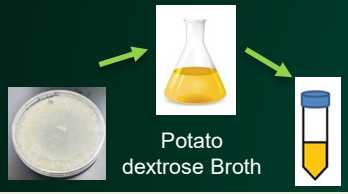
| Sl. No | Name of accessions | *P. gulyae* | | *P. helianthi* | |
|---|---|---|---|---|---|
| | | Reaction to fungus | Reaction to cell-free culture filtrates | Reaction to fungus | Reaction to cell-free culture filtrates |
| 1 | PI 603991 | Susceptible | + | Susceptible | + |
| 2 | PI 509064 | Less susceptible | + | Less susceptible | + |
| 3 | PI 552934 | Less susceptible | + | Less susceptible | + |
| 4 | PI 561918 | Less susceptible | - | Less susceptible | + |
| 5 | PI 599984 | Susceptible | + | Less susceptible | + |
| 6 | PI 618725 | Less susceptible | + | Less susceptible | + |
| 7 | PI 650657 | Less susceptible | + | Less susceptible | + |
| 8 | PI 650675 | Susceptible | + | Less susceptible | + |
| 9 | PI 650755 | Less susceptible | + | Less susceptible | + |
| 10 | PI 650839 | Susceptible | + | Less susceptible | + |

Reaction to fungus: + (sensitive), - (insensitive)

3. To identify putative phytotoxic metabolites produced by the U.S. isolates of *P. gulyae* and *P. helianthi* through an untargeted metabolomics approach.

# Methodology

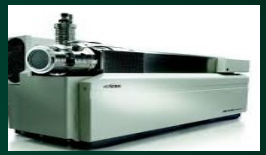
- Novogene America Inc., Sacramento, CA




1. Sample preparation


acetonitrile: methanol (1:4, v/v)

2. Metabolite extraction



LC/MS instrument

3. Mass spectrometry

ProteoWizard (v3.x)
(Kessner et al. 2008)

4. Data acquisition


(Dührkop et al. 2020)

5. Feature selection

HMDB (Wishart et al. 2022)
MassBank (Horai et al. 2010)
Novogene's spectral library

6. Identification of compounds

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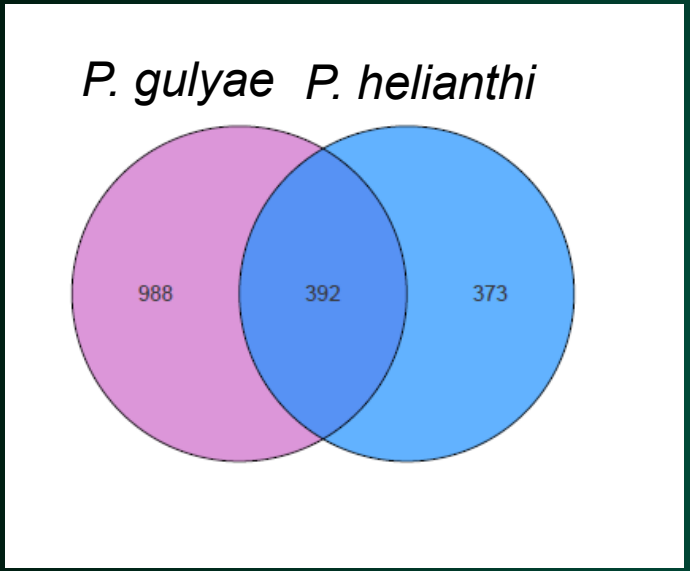
# Untargeted LC–MS analysis of cell-free culture filtrates




Putative phytotoxic metabolites

| ***P. gulyae*** | ***P. helianthi*** |
|---|---|
| 3-nitropropionic acid (*P*=0.013)<br>Cercosporin(*P* = 0.0024)<br>Fumonisin $B_2$ (*P* = 0.001)<br>3,4-dehydro-6-hydroxymellein (*P* = 0.0) | Gliotoxin (*P* = 0.017) |

- No common phytotoxic metabolites between *P. gulyae* and *P. helianthi*
- Phytotoxic metabolites previously reported in *P. helianthi*, such as phomozin and 4,6-dihydroxymellein (Mazars et al. 1990; Avantaggiato et al. 1999) were not detected

# Summary

- Significant correlation between LAI and LAN for isolates of *P. gulyae* ($r = 0.95$, $P = 0.004$) and *P. helianthi* ($r = 0.90$, $P = 0.015$)
  - ✓ Suggest potential role of fungal metabolites in symptom development

- PI 561918 was insensitive to cell-free culture filtrates of *P. gulyae*

# Summary

- Untargeted metabolomics identified several metabolites putatively annotated to previously identified metabolites

- Previously reported metabolites in *P. helianthi* (phomozin and 4,6-dihydroxymellein) were not detected
  - ✓ Due to variations in culture media

- Further research is required to confirm the role of these metabolites in pathogenicity

# Acknowledgement

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United States Department of Agriculture
National Institute of Food and Agriculture

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THANK YOU