**Resistance to multiple SDHI fungicides in *Stemphylium vesicarium.***

Emily McFaul¹, Bruce D. Gossen² and Mary Ruth McDonald¹

¹Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada and ²Agriculture and Agri-Food Canada, Saskatoon, SK, Canada

**Introduction**

*Stemphylium vesicarium*, the causal agent of Stemphylium leaf blight (SLB), is an important foliar pathogen of onion in the Holland Marsh, Ontario, Canada. SLB causes premature defoliation of onion (Fig. 1), which can reduce yield, and also sprout inhibitor absorption, resulting in post-harvest losses. All onion cultivars are susceptible. Fungicides are sprayed to manage the disease, but are often not effective, likely because of fungicide resistance. Most of the fungicides used contain FRAC 7 active ingredients. Previous studies have identified resistance to FRAC 2, 7, 9 and 11 fungicides. Isolates collected from Ontario in 2021–2023 were assessed for resistance to two relatively new FRAC 7 active ingredients, fluxapyroxad and penflufen. Penflufen seed treatment (Evergil Prime) may provide early season protection against *S. vesicarium*. Fluxapyroxad is a component of several foliar fungicides, such as Merivon.

**Objective:** Evaluate sensitivity of *S. vesicarium* populations to penflufen and fluxapyroxad.

**Materials and Methods**

- **Isolates of *S. vesicarium* were grown on V8 agar under UV light to stimulate sporulation (Fig. 2A). All isolate responses were compared to a baseline isolate from 1995 to identify shifts in resistance overtime.**
- **Media was amended with 50 μg/mL penflufen (FRAC 7), 50 μg/mL or 100 μg/mL fluxapyroxad (FRAC 7). S. vesicarium isolates from 2021–2023, were evaluated using the mycelial growth assay to fluxapyroxad (n = 68) and penflufen (n = 76).**
- **In conidial germination assays, 76 isolates and 84 isolates from 2021–2023 were exposed to fluxapyroxad or penflufen, respectively.**

**Conidial germination assay**

- Conidia were collected and pipetted onto fungicide-amended media (Fig. 2B).
- Germination was examined using a light microscope after 24 hours (Fig. 2C).

**Mycelial growth assay**

- Mycelial plugs from the margins of actively-growing cultures were placed mycelium side down in the center of each of the five amended-media laneways.
- Growth was evaluated after 48 and 96 hours (Fig. 2D).

**Results**

**Fig. 2.** Graphic of sensitivity assays; (A) growth of isolates on agar medium and assessment of (B, C) conidial germination and (D) radial growth.

- Isolates of *S. vesicarium* were grown on V8 agar under UV light to stimulate sporulation (Fig. 2A). All isolate responses were compared to a baseline isolate from 1995 to identify shifts in resistance overtime.
- Media was amended with 50 μg/mL penflufen (FRAC 7), 50 μg/mL or 100 μg/mL fluxapyroxad (FRAC 7). S. vesicarium isolates from 2021–2023, were evaluated using the mycelial growth assay to fluxapyroxad (n = 68) and penflufen (n = 76).
- In conidial germination assays, 76 isolates and 84 isolates from 2021–2023 were exposed to fluxapyroxad or penflufen, respectively.

**Fig. 3.** Mycelium and conidial resistance of *S. vesicarium* isolates collected from 2021–2023 to fluxapyroxad at 50 μg/mL and 100 μg/mL.

**Fig. 4.** Fungicide resistance to penflufen at 50 μg/mL of mycelium and conidia of *S. vesicarium* isolates collected from 2021–2023.

- A baseline isolate from 1995 was sensitive to both active ingredients based on mycelial growth but was resistant to both based on conidial germination.
- In the mycelial growth assay 28% (n=68) of isolates from 2021–2023 were resistant to fluxapyroxad (Fig. 3) and 43% (n=76) were resistant to penflufen (Fig. 4).
- The conidial germination assay showed 88% (n=76) of isolates were resistant to fluxapyroxad (Fig. 3) and 84% (n=84) were resistant to penflufen (Fig. 4).
- Most isolates from 2023 were resistant to fluxapyroxad (94%, n=32) and penflufen (95%, n=35) in the conidial assay, as expected but resistance was lower when the mycelium was assessed (fluxapyroxad: 44%, n=36, penflufen: 63%, n=35) (Figs. 3 & 4).

**Conclusions**

- Fluxapyroxad and penflufen do not inhibit conidial germination but may be effective against mycelial growth.
- Resistance to fluxapyroxad and penflufen in the *S. vesicarium* population in the Holland Marsh has increased over time compared to the baseline, possibly because of cross-resistance with other FRAC 7 active ingredients.