Phytophthora root rot-like symptoms on soybeans containing *Rps* 1k in Wisconsin in 2008.

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Introduction-The heavy rains, flooding, and cool temperatures that started off the 2008 growing season favored soybean diseases like Phytophthora root rot (PRR), caused by *Phytophthora sojae* (*Ps*). However, by mid-July the rains had stopped, fields dried out, and drought-like conditions occurred throughout much of Wisconsin. Despite the drastic change in weather, by early August, reports started to come into county extension office and the University of Wisconsin of PRR-like symptoms in fields throughout the state. Because many of these fields were planted to varieties containing the *Rps* 1k gene, serious concern arose over the breakdown of resistance to PRR conferred by this gene.

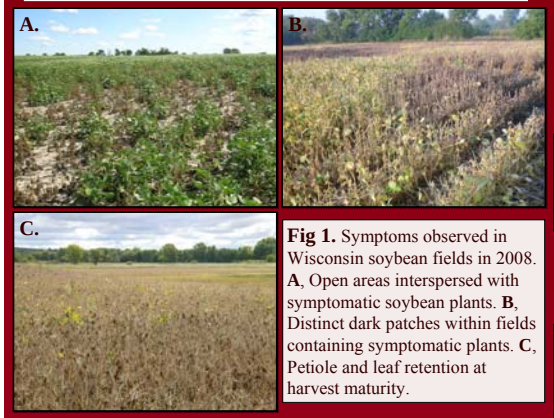

Fig 1. Symptoms observed in Wisconsin soybean fields in 2008. **A**, Open areas interspersed with symptomatic soybean plants. **B**, Distinct dark patches within fields containing symptomatic plants. **C**, Petiole and leaf retention at harvest maturity.

Were symptoms associated with colonization by *Ps*?

• **Field sampling**-From 14 August to 1 October, 22 fields were sampled in 7 counties throughout Wisconsin where soybean varieties containing *Rps* 1k were expressing PRR-like symptoms.

• **Observations**-Symptoms and signs associated with stems and roots of diseased and 'healthy' soybean plants collected from each field were observed and recorded. Observations were made prior to isolation and following incubation in a moist chamber.

•**Isolations**-Isolations were made from both symptomatic and 'healthy' soybean tissues. In addition, small pieces of tissue were suspended in water to favor oomycete sporulation. Methods that favored isolation of *Ps* from plant tissues (A.E. Dorrance and A.E. Robertson, *personal communication*) as well as general isolation techniques were used.

Fig 2. Wisconsin county map. Green dots represent the approximate location of each field where soybean plants expressing PRR-like symptoms were sampled.

Fig 3. **A**, Classical symptoms of PRR. **B**, Symptoms observed in 2008

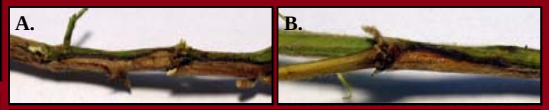

Fig 4. Characteristics of stem lesions observed in 2008. **A**, Reddish brown lesion with a dark brown border surrounded by green tissue. **B**, Lesion with fungal fruiting bodies in the center.

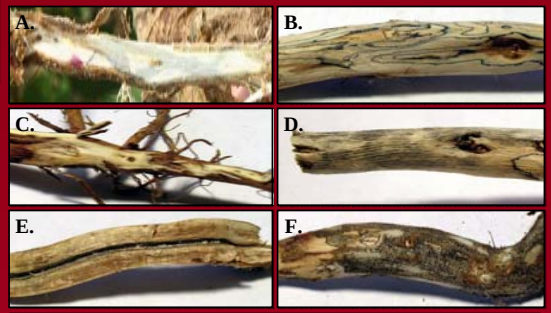

Fig 5. Internal and sub-surface symptoms observed in 2008. **A,** Gray discoloration of cortical tissues. **B,** Black striations in root and stem tissues. **C,** Absence of root rot. **D,** Tattering of stem tissues with microsclerotia underneath. **E,** Brown discoloration of internal tissues and formation of microsclerotia within pith tissues. **F,** Microsclerotia just below the epidermis.

Results-*Ps* was neither isolated nor observed in any sample. Instead, numerous isolates of *Diaporthe phaseolorum* var. *sojae* (*Dps*), *D. phaseolorum* var. *caulivora* (*Dpc*), and *Macrophomina phaseolina* (*Mp*) were obtained from both roots and stems of symptomatic plants. *Dpc* and *Dps* are the causal agents of Northern stem canker and pod and stem blight, respectively, while charcoal rot is caused by *Mp.*

• Isolates of *Dpc* and *Dps* were obtained from plants collected in each of the seven counties whereas *Mp* was obtained from plants collected in each county except the northern counties of Brown and Kewaunee.

• Soybean plants grown in soil with a high percentage of sand expressed more symptoms characteristic of charcoal rot than stem canker and pod and stem blight and yielded more isolates of *Mp* than *Dpc* or *Dps*.

• Of the soybean varieties sampled in 2008, those with *Rps* 1k and a high field tolerance rating or varieties with multiple *Rps* genes (i.e. *Rps* 1k, *Rps* 6) expressed minimal symptom severity.

• Data suggest symptoms were the result of infection by *Dpc*, *Dps*, and/or *Mp* and that *Rps* 1k is still effective against *Ps* in Wisconsin.

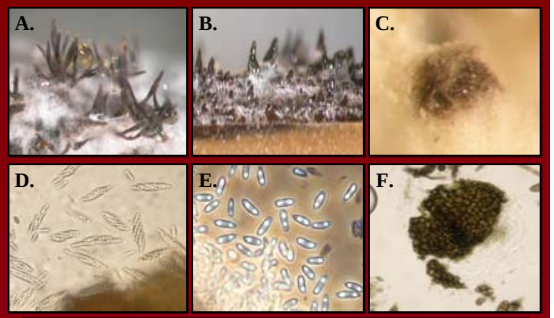

Fig 6. Fungi associated with PRR-like symptoms. Fungal structures and fruiting bodies were induced by using sterile wheat straw on 1% water agar. **A & D,** Perithecia and asci containing ascospores of *Dpc*. **B & E** Pycnidia and α-condia of *Dps* (β-condia were also observed). **C & F**, Microsclerotia of *Mp*.

Koch's postulates

• **Inoculation and growth conditions**-Seeds of Pioneer 92M32 or Pioneer 92Y20 (both containing *Rps* 1k) were planted directly into pasteurized sandy soil infested with sterile sorghum seed or seed colonized by *Dps* or *Mp*. Pots were watered to capacity daily to maintain saturated soils conditions until R1when the soil was dried-down and the plants were water-stressed. At R7, plants were assessed for symptom development and colonization by *Dps* or *Mp*.

• **Results**-External symptoms were not observed for either variety grown in the presence of *Dps* or *Mp*. However, internal stem and root discoloration were observed and both *Dps* and *Mp* were recovered from 92M32 and 92Y20. Control plants remained asymptomatic and did not yield isolates of *Dps* or *Mp*.

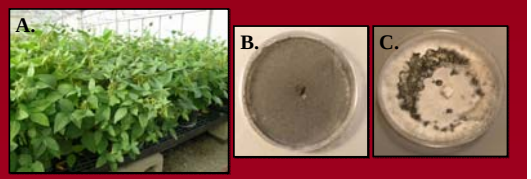

Fig 7. Experimental design for Koch's postulates. **A,** Plants were grown in 5 gal pots at the Walnut St. greenhouses. **B,** Isolate of *Mp* recovered from a plant grown in soil infested with *Mp*. **D,** Isolates of *Dps* recovered from a plant grown in soil infested with *Dps*.

Future direction

• Determine if the PRR-like symptoms observed in 2008 were the result of an interaction between *Ps* and *Dps*, *Dpc* and/or *Mp.*

• Determine if resistance to *Ps* conferred by the *Rps* 1k gene increased susceptibility to *Dps*, *Dpc*, and/or *Mp*.

• Assess the role of biotic and abiotic stress in soybean production.

Additional information

1. University of Wisconsin Soybean Health Website at www.plantpath.wisc.edu
2. Esker, P.D., and Conley, S.P. 2009. Early season assessments for soybean stress. Wisconsin Crop Manager. Vol. 16, No. 15, Pages 59-60.
3. Hughes, T.J., Esker, P.D., and Conley, S.P. 2009. Taking advantage of a stressful situation: stem canker and charcoal rot in soybeans. Wisconsin Crop Manager. Vol. 16, No. 2, Pages 9-11.
4. Esker, P.D., Conley, S.P. Gaska, J., and Hughes, T.J. 2008. Charcoal rot-A disease of drought stressed environments. Wisconsin Soy Sentinel, Vol. 5, Page 16.

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