# Use of oxalic acid to characterize soybean accessions for partial resistance to *Sclerotinia sclerotiorum.*

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## ABSTRACT

Different methods of assessment may be needed to assign the function of soybean QTL putatively linked to physiological resistance to *Sclerotinia sclerotiorum.* Efforts have been made to characterize the genetics of partial resistance through the identification of QTL. Several QTL are linked to phenotypes associated with disease escape mechanisms. Other QTL are linked to phenotypes most likely associated with physiological resistance, but explain only 9.6% of the variability observed among interaction phenotypes. It is not known whether specific combinations of QTL in the same soybean genotype would confer higher degrees, and more environmentally stable forms of resistance. We hypothesize that various QTL are associated with different mechanisms of physiologically-based resistance. Phenotypic data derived from multiple methods of disease assessments would provide guidance as to which QTL could be combined to achieve a more complete and environmentally stable form of resistance. Using multiple assessments to evaluate plants with genetically characterized resistance will help link phenotype to genotype, leading to a greater understanding of resistance. Oxalic acid is a putative virulence factor associated with pathogenesis by *Sclerotinia sclerotiorum*. Researchers have used oxalic acid with varying success to challenge plants in efforts to screen accessions of various host species for resistance to *S. sclerotiorum*. The use of oxalic acid rather than the pathogen has been perceived to reduce variability associated with the plant / *S. sclerotiorum* interaction. In a preliminary experiment we used oxalic acid (20 mM) to challenge trifoliate leaves removed from flowering soybean plants of varying reaction to *S. sclerotiorum.* We assessed tolerance to oxalic acid by measuring the percent mid-rib discoloration of each leaflet. Three resistant accessions, W04.1000, W04.1001, and W04.1002, exhibited a high level of tolerance and differed from NK S19-90, the resistant check, which performed in a similar manner to W01.1305 and BSR-101, the susceptible checks. The results of this preliminary experiment suggest that resistance to *S. sclerotiorum* and tolerance to oxalic acid may be conferred by unique genes or gene combinations. Various aspects of these results were repeated in subsequent experiments. Experiments are currently underway to optimize methodology, incubation environment and assessment variables. We plan to utilize three methods of assessment: field disease severity data, inoculation in controlled light environments, and the oxalic acid assay, to differentiate genotypic and phenotypic differences among soybean accessions reported to express partial resistance to *S. sclerotiorum*.


## INTRODUCTION

Quantitative trait loci (QTL) for resistance to Sclerotinia stem rot have been identified in soybean (1,6). Although work is currently underway to incorporate some of these QTL into commercially viable germplasm, the fact that a maximum of only 9.6% of the variability in disease phenotypes is explained by these QTL indicates a large gap in our understanding (6). Most soybean breeding programs have relied on one method to characterize and select resistant progeny to advance during the breeding process (1,6,7).

Pathogenesis by *S. sclerotiorum* is not completely understood and data to date suggests multiple mechanisms. The working model combines oxalic acid and enzymes capable of degrading various cell wall components (5). We hypothesize that different QTL confer resistance or tolerance to various mechanisms of pathogenesis. Different methods of assessment may be needed to assign the function of these QTL. A comprehensive understanding of partial resistance could lead to stacking the most important forms of resistance resulting in a more complete resistance than we have in the current commercial germplasm.

de Bary was the first to link *S. sclerotiorum*, oxalic acid, and pathogenicity (9). Modern technology has helped to solidify the role of oxalic acid in *S. sclerotiorum* pathogenesis. Oxalic acid has been shown to be an important pathogenicity factor of *S. sclerotiorum* through the development of oxalic acid deficient mutants (4). In addition, a soybean line carrying a transgenic copy of a wheat oxalate oxidase gene in a susceptible genetic background exhibited partial resistance equivalent to commercial cultivars expressing the highest forms of partial resistance (3). In efforts to avoid the variability associated with pathogen delivery, oxalic acid has been used directly to evaluate severed *Phaseolus coccineus* stems (2), *Phaseolus vulgaris* stems (8) and leaves (10), and soybean stems (11).

Here, we report the results of preliminary studies using oxalic acid to challenge detached soybean leaves from accessions of varying reaction to *Sclerotinia sclerotiorum*.

## MATERIALS AND METHODS

Plant Materials. Four partially resistant and two susceptible soybean accessions were included in our studies. The partially resistant accessions were NK S19-90 (industry check) and Wisconsin breeding lines W04.1000, W04.1001, and W04.1002 which are lines derived by selection of single resistant plants from the plant introductions 561.285B, 561.367, and 567.157A, respectively. The susceptible accessions included BSR-101 (industry check) and W01.1305, a progeny line derived from a cross between Dwight and PI 567.323A.

Growth and Incubation Environments. Plants were grown in the Walnut Street greenhouse facility located at the University of Wisconsin. Sunlight was supplemented with 1000W high pressure sodium lamps to obtain a 16 hour photoperiod. Trifoliate leaves were harvested when plants had reached the R1 growth stage.

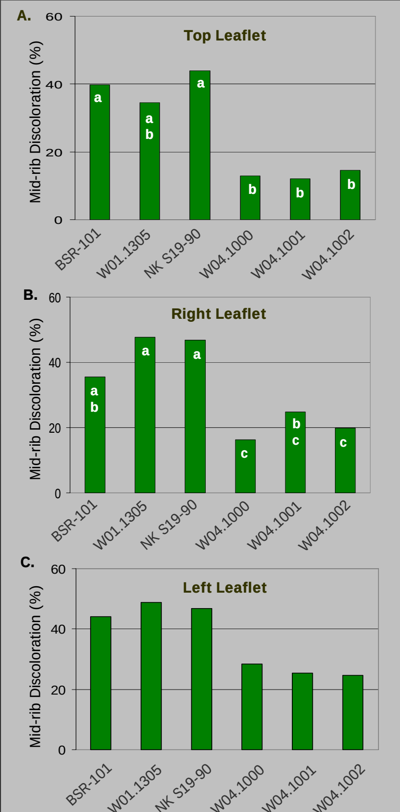


Figure 3. Experiment 1: mid-rib discoloration (%) of detached trifoliate leaves placed in 20 mM oxalic acid, a) Top leaflet, $P = 0.0350$ LSD(0.05) = 25; b) Right leaflet, $P = 0.0007$, LSD(0.05) = 16; c) Left leaflet, $P = 0.1700$, LSD(0.05) = NS.

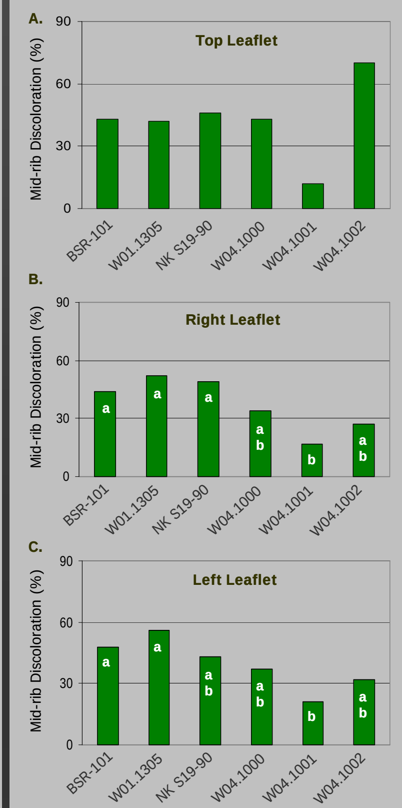


Figure 4. Experiment 2: mid-rib discoloration (%) of detached trifoliate leaves placed in 20 mM oxalic acid, a) Top leaflet, $P = 0.6268$, LSD(0.05) = NS; b) Right leaflet, $P = 0.0551$, LSD(0.05) = 25; c) Left leaflet, $P = 0.0897$, LSD(0.05) = 25.

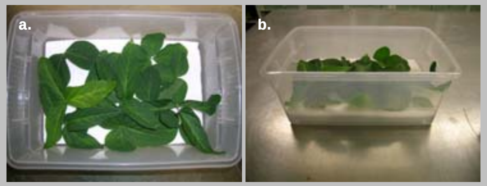


Figure 1. Container used to challenge detached soybean leaves with 20 mM oxalic acid. A piece of 1.3 cm thick Styrofoam was cut to fit a 14.2 L clear plastic storage container. Evenly spaced holes were cut in the Styrofoam using a No. 5 core borer. Petioles from the V-2 trifoliate leaf of flowering plants were cut to 2.5 cm and placed through the holes in the Styrofoam, a) top view, b) side view.

Figure 2. Detached soybean leaves after 24h of treatment in a 20 mM oxalic acid solution, a) W01.1305, a susceptible accession, b) W04.1002, a resistant accession, c) water control.

## MATERIALS AND METHODS

Oxalic Acid Assay. The oxalic acid experiments were arranged as randomized complete block designs with leaves from two plants of each accession in each container and the containers as blocks. A piece of 1.3 cm thick Styrofoam was cut to fit a 14.2 L clear plastic storage container. Holes were cut with a No. 5 core borer and were equally spaced from the container walls and other holes, for a total of 20 holes. Treatments consisted of 2 L of either 20 mM oxalic acid or distilled water (control) placed into a container and the Styrofoam was floated on top of the solution. Petioles from the V-2 trifoliate leaf of flowering plants were cut to 2.5 cm and placed through the holes in the Styrofoam (Figure 1). Containers were incubated for 16 hours in the dark. Experiment 1 was incubated on a lab bench at room temperature and Experiment 2 at 24 °C. Leaves were rated after 16-24 hours incubation for the oxalic acid assays depending upon variation in symptom progression. Measurements were taken of the length of each leaflet (cm) and the length of the discoloration (cm) along the mid-rib (Figures 2a and b). Ratings for each leaflet were expressed as percents of symptomatic tissue.

Data Analysis. Percent leaflet mid-rib discoloration from the oxalic acid assay were analyzed as a randomized complete block design using PROC GLM in SAS. If significance was achieved for the main effect, Fisher's protected least significant differences (LSD) were calculated for pair-wise comparisons among accessions.

## RESULTS

Experiment 1:

* differences among accessions were detected: top leaflet {$P = 0.0350$, LSD(0.05) = 25}, right leaflet {$P = 0.0007$, LSD(0.05) = 16}, left leaflet {$P = 0.1700$} (Fig. 3)

* the resistant W04.1000, W04.1001, and W04.1002 were more tolerant than NK S19-90 (Fig 3a and 3b)

* NK S19-90 was indistinguishable from W01.1305 and BSR-101 (Fig. 3)

Experiment 2

* differences among accessions were detected: right leaflet {$P = 0.0551$, LSD(0.05) = 25}, left leaflet {$P = 0.0897$, LSD(0.05) = 25}, top leaflet {$P = 0.6268$} (Fig. 4)

* trend: W04.1000, W04.1001, and W04.1002 had greater tolerance than R and S checks (Fig. 4b and 4c)

* W04.1001 performed better than BSR-101, W01.1305 and NK S19-90 in the right and left leaflets (Fig. 4b and 4c)

* although it remains untested it is likely that differences in incubation conditions may be responsible for variability observed between experiments

## DISCUSSION

Others have attempted to use oxalic acid to indirectly characterize *Phaseolus vulgaris*, *P. coccineus*, and soybean for partial resistance to *S. sclerotiorum.* Tu (10) conducted oxalic acid experiments with severed *P. vulgaris* leaves and differences were observed between resistant and susceptible varieties. Chipps, et al. (2) tested 20 d old *P. coccineus* seedlings and found that lines differed in their tolerance to oxalic acid.

Kolkman and Kelly (8) used a foam board floated over an oxalic acid bath to test the tolerance of 27 varieties of *P. vulgaris* seedlings (20 d old) severed at the soil line. Pearson's correlations of oxalic acid ratings and field data yielded coefficients of 0.580, 0.571 and -0.505 for disease severity indices (DSI), disease incidence and yield, respectively. Wegulo, et al (11) used oxalic acid to test stems of flowering soybean plants (12 varieties). Our data, in addition to that of others, supports the concept that although oxalic acid may be an appropriate way to test varieties for resistance to *S. sclerotiorum* it should not be the exclusive method used for assessment. For example, in our studies, although NK S19-90 expresses a partially resistant interaction phenotype in field and controlled environments (data not shown), it is not tolerant to oxalic acid (Figure 3). This suggests that field reactions to *S. sclerotiorum*, oxalic acid tolerance and environmental sensitivity are conferred by different genes or gene combinations. It also implies that the genetics of resistance in NK S19-90 differs from that in W04.1000, W04.1001, and W04.1002.

A working model is that pathogenesis by *S. sclerotiorum* is a step-wise process involving oxalic acid and cell wall degrading enzymes (5). We hypothesize that different QTL confer resistance or tolerance to various aspects of pathogenesis and appropriate QTL can be combined into a single soybean accession to achieve complete resistance. The coordination of methods to assess various aspects of the soybean- *S. sclerotiorum* and/or pathogenicity factor interaction, will facilitate a greater understanding of the function of QTL for optimal deployment.

Experiments are currently underway to optimize the methodology, incubation environment and assessment variables of the oxalic acid assay.


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