

SOYBEAN DISEASE RESEARCH ON GREEN STEM DISORDER,
EFFECTS OF SEED TREATMENTS ON SCLEROTINIA STEM ROT,
AND RESISTANCE TO CERCOSPORA LEAF BLIGHT

BY

CHELSEA JEAN HARBACH

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Crop Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2015

Urbana, Illinois

Master's Committee:

Professor Glen L. Hartman
Associate Professor Carl A. Bradley
Professor Donald Bullock
Dr. Suzanne Bissonnette

ABSTRACT

Soybean plants often exhibit varying delayed maturity symptoms at the end of the growing season. One delayed maturity malady known as green stem disorder (GSD) is the occurrence of non-senescent, fleshy green stems at harvest maturity with normal, fully mature pods and seeds. Data on GSD incidence were collected for 1090 soybean cultivars from 2009 to 2012 at seven locations throughout Illinois. Data on six agronomic traits were also collected for every GSD observation, including height, lodging, moisture, protein, oil, and yield. Correlations of GSD incidence with agronomic traits were estimated for every year x location x trial (maturity group and herbicide tolerance type) combination to account for the change in cultivars from year to year and the effect of year x location on GSD incidence. To study the effects of location on GSD incidence, pair-wise comparison best linear unbiased predictors (BLUPs) were estimated between all locations within years for all trials. Correlations showed no consistent significant relationship with yield and GSD, but GSD incidence was positively correlated to height, lodging, moisture, and protein, while negatively correlated to oil. Pairwise comparison BLUPs showed an overall trend of locations in northern regions of Illinois having significantly more GSD than regions in the south. There were locations that had significantly more GSD compared to all other locations consistently throughout the analysis. The general significant effect of locations on GSD incidence provides an important consideration when designing future research with GSD in soybean. The significant correlations with agronomic data in this study support the findings that GSD incidence is a quantitatively controlled genetic trait that can be influenced by the environment.

Sclerotinia sclerotiorum is one of the most important pathogens infecting soybean plants. When the fungus is seedborne as mycelia or when it is infested with seeds as sclerotia, the

fungus can kill germinating seedlings. The use of fungicide seed treatments may help to manage this phase of the disease and may provide residual protection to infection beyond the seedling stage. The objectives of my study were to use the data from the University of Illinois Soybean Variety Testing Program (UISVT) to document the increase in deployment of fungicide seed treatments from 2005 to 2014 and to determine if seed treated fungicides would provide control of *S. sclerotiorum* on inoculated germinating seedlings and on plants beyond the seedling stage inoculated with the fungus. The data from the UISVT showed that the deployment of seed treatments with fungicides on cultivars entered into the program increased from 66% in 2005 to 92% in 2014. To test the efficacy of fungicide seed treatments, four fungicide seed treatments, fludioxonil, trifloxystrobin, trifloxystrobin + saponins, and penflufen + prothioconazole, and an untreated control were applied to seeds of four soybean cultivars. The plants were inoculated at various growth stages from seedling germination to flowering. In the seed germination stage, fludioxonil provided complete control, penflufen + prothioconazole provided moderate control while the trifloxystrobin and trifloxystrobin + saponins provided no control. There was little residual activity detected when plants beyond the seed germination stage were inoculated. Although seed treatments included in this study do not offer residual protection against *S. sclerotiorum* infection beyond germination, there is hope that future seed treatment fungicides will have longer residual activity that could provide protection to plants at later growth stages.

Cercospora leaf blight (CLB) and purple seed stain (PSS) caused by *Cercospora kikuchii* are important diseases of soybean worldwide. While there are no commercially available cultivars advertised for resistance to PSS or CLB, sources of resistance have been reported in plant introductions or older public soybean cultivars. In this study, nine public soybean cultivars with varying resistance or susceptibility to CLB, PSS, and frogeye leaf spot (*Cercospora sojina*)

were screened for differences in CLB disease severity. Soybean plants were inoculated with two different *Cercospora* isolates that were isolated from soybean seeds symptomatic of PSS and leaves symptomatic of CLB in Illinois. The intergenic spacer (IGS) region of these isolates were sequenced and compared to previously published IGS sequences of *C. kikuchii* isolates.

Bioassays for differences in disease severity showed no significant differences between leaf and seed *C. kikuchii* isolates though significant differences were observed between cultivars included in the study. Soybean cultivar Mejiro (PI80837) had the highest disease rating overall.

Comparison of the IGS sequences from *Cercospora* isolates showed differences in the sequence that followed the previously published differences defining three haplotypes of the fungus. This indicates that *C. kikuchii* isolates in Illinois vary genetically from those collected in the southern United States.

Acknowledgements

First and foremost, I wish to acknowledge my adviser, Dr. Glen Hartman. Dr. Hartman gave me the chance to prove to myself and the world that I can and will succeed in my dreams of becoming a professional plant pathologist. His guidance has been fundamental in my progress towards my master's degree. I appreciate his expertise and willingness to push me to improve my research, critical thinking, and writing skills as a plant pathologist.

Beyond my adviser, I would also like to thank the rest of my master's committee: Drs. Suzanne Bissonnette, Donald Bullock, and Carl Bradley for all of their help and support throughout my studies and projects.

Second, I would like to thank my mother, Barb, for her undying love and support as I pursue my career goal. Though it may be difficult for her to understand at times, she has been one of my rocks to which I turn for encouragement and inspiration. Along with my mother I would like to acknowledge my little sister, Analiesa, big brother, Shane, and future sister-in-law, Sarah, for playing a similar role as my mother as I continue on my professional journey.

Besides my family I would like to thank all of those I have had the pleasure of working with in Dr. Glen Hartman's lab, both past and present: Ron Warsaw, Joe Scholtes, Shilpi Chawla, Jess Stewart, Michelle Pawlowski, Liwei Wen, Hao-Xun Chang, James Haudenshield, Theresa Herman, Curtis Hill, Doris Lagos, and Lucimara Koga for all of their help. I would especially like to thank Roger Bowen for his immense support and reassurance as I have progressed through my studies at University of Illinois.

I must also thank my corgi, Charles Avocado VanGogh. Though I know he can't tell since he is a dog, he has been an incredible source of emotional support and unyielding love.

Table of Contents

Chapter 1: Agronomic traits associated with green stem disorder incidence in Illinois.....	1
Abstract.....	1
Introduction.....	2
Materials and Methods.....	5
Results.....	9
Discussion.....	11
Literature Cited.....	15
Tables and Figures.....	17
Chapter 2: Fungicide seed treatments in soybean provide differential protection against <i>Sclerotinia sclerotiorum</i> at germination and only slight residual protection beyond germination.....	26
Abstract.....	26
Introduction.....	27
Materials and Methods.....	30
Results.....	35
Discussion.....	37
Literature Cited.....	42
Tables and Figures.....	45
Chapter 3: Leaf and seed isolates of <i>Cercospora kikuchii</i> from soybean differ in intergenic spacer region sequence but not in disease severity on selected soybean genotypes....	55
Abstract.....	55
Introduction.....	56
Materials and Methods.....	57
Results.....	62
Discussion.....	63
Literature Cited.....	66
Tables and Figures.....	68
Appendix A.....	76
Appendix B.....	93
Appendix C.....	94
Appendix D.....	96

CHAPTER 1

Agronomic traits associated with green stem disorder incidence in Illinois

Abstract

Soybean plants often exhibit varying delayed maturity symptoms at the end of the growing season. One delayed maturity malady known as green stem disorder (GSD) is the occurrence of non-senescent, fleshy green stems at harvest maturity with normal, fully mature pods and seeds. Data on GSD incidence were collected for 1090 soybean cultivars from 2009 to 2012 at seven locations throughout Illinois. Data on six agronomic traits were also collected for every GSD observation, including height, lodging, moisture, protein, oil, and yield. Correlations of GSD incidence with agronomic traits were estimated for every year x location x trial (maturity group and herbicide tolerance type) combination to account for the change in cultivars from year to year and the effect of year x location on GSD incidence. To study the effects of location on GSD incidence, pair-wise comparison best linear unbiased predictors (BLUPs) were estimated between all locations within years for all trials. Correlations showed no consistent significant relationship with yield and GSD, but GSD incidence was positively correlated to height, lodging, moisture, and protein, while negatively correlated to oil. Pairwise comparison BLUPs showed an overall trend of locations in northern regions of Illinois having significantly more GSD than regions in the south. There were locations that had significantly more GSD compared to all other locations consistently throughout the analysis. The general significant effect of locations on GSD incidence provides an important consideration when designing future research with GSD in soybean. The significant correlations with agronomic data in this study support the findings that

GSD incidence is a quantitatively controlled genetic trait that can be influenced by the environment.

Introduction

Soybean [*Glycine max* (L.) Merr] plants with non-senescent stems at harvest maturity can result in indirect yield losses as green, fleshy stems are difficult to combine. This can result in harvest delays that may lead to seed weathering and pod shattering (Hill et al., 2006). In turn, many growers, primarily in the south, will resort to the use of harvest aids, such as paraquat, to induce stem senescence and make harvest easier and more-timely (Appendix A.1).

Different terms have been used to describe the varying symptoms that can occur as a result of delayed maturity maladies in soybean. In 1980, green stem syndrome was used to describe the occurrence of thin, small, mature pods on green, nonsenescent soybean stems at harvest maturity associated with *Bean pod mottle virus* (BPMV) (Schwenk and Nickell, 1980). BPMV is known to reduce overall soybean yields (Byamukama et al., 2015). Green stem disorder (GSD) was first described in 2006 as normal, mature pods on green soybean stems without any association with BPMV (Hobbs et al. 2006). These are two different delayed maturity afflictions of soybean that tend to be used interchangeably although their symptoms are somewhat different. Another commonly used term to describe the incidence of green stems in soybean fields at harvest maturity by growers was “green stem” which simply describes the common denominator in symptoms observed in green stem syndrome, GSD, and other delayed maturity maladies (Appendix A.1).

Although the cause of GSD is not fully understood, studies showed that five additional viruses along with BPMV were not associated with GSD incidence (Formento and de Souza,

2009), but the incidence of GSD was associated with soybean cultivars (Hill et al., 2006; Hobbs et al., 2006). Based on a four-year study, significant differences between cultivar GSD incidences were reported in 29 out of 31 field trials (Hill et al., 2006). Furthermore, major quantitative trait loci (QTL) associated with GSD insensitivity were identified by researchers in Japan where GSD is a serious problem in soybean production (Yamada et al., 2014).

Delayed maturity symptoms have positive influences on agronomic traits in other field crops such as maize and sorghum. In maize and sorghum, stay-green is a well-studied trait that has been associated with less diseased stems and increased grain yields (Thomas et al., 2014). While delayed maturity in other major field crops has been extensively studied and understood, GSD in soybean has not been as well studied. Other than yield, information on other agronomic traits associated with GSD is not known. In terms of yield, there has been no well-defined or conclusive study that suggests a positive or negative relationship with GSD, although one study showed that higher GSD incidences were associated with higher yields in 11 out of 28 cultivars (Hill et al., 2013).

GSD incidence has been observed to have environmental relationships. A study on GSD in Japan found that GSD incidence tends to be higher in northern latitudes over more southern latitudes when comparing cultivars grown at 39.7°N over those grown at 35.0°N (Fujii et al., 2015). Another study found that there was no significant effect of temperature on GSD incidence (Mochizuki et al., 2005). To further enhance the understanding of this trait, evaluating the same genotypes in multiple environments would help establish this relationship. Additionally, other than yield, the relationship between GSD and other agronomic traits has yet to be established. By examining the relationship of GSD incidence with agronomic traits, the importance of environment on the expression of this trait can become evident if agronomic traits that are

genetically controlled and influenced by the environment are significantly correlated with GSD incidence.

The University of Illinois Soybean Variety Testing (UISVT) program has been conducting soybean variety trials in Illinois since 1998 (www.vt.cropsci.illinois.edu/soybean.html). The UISVT collects agronomic data including yield, plant height, stand lodging, protein content, oil content, and moisture content for every plot planted in all 13 locations throughout the state. These 13 locations are distributed through five designated “regions” from north to south in order to plant appropriate maturity groups at each location (Fig 1.1). The Varietal Information Program for Soybean (VIPS) collaborated with the UISVT to collect GSD data over four years from 2009 to 2012 at seven locations throughout three regions to evaluate cultivar sensitivity to GSD. A very brief summary of the amount of GSD observed in 2009 to 2011 reported the minimum, average, and maximum GSD percent incidence for each year (Chawla et al., 2013); however, no analysis was conducted to compare agronomic data to GSD or to investigate location effects on GSD incidence.

The first objective of this study was to investigate the relationship between GSD incidence and agronomic traits, including yield, for soybean cultivar observations from Illinois. The second objective was to use a subset of data including observations of public check cultivars only, which have the most number of observations from 2009 to 2012, to determine the effect of year and location on the same cultivar. The third objective was to use all data observations for the same cultivars observed in different locations within the same year to determine the effect that locations have on the GSD incidences of the same cultivars within a single year.

Materials and Methods

Experimental design and data collection. Experimental trials included at UISVT locations are determined by region (Fig. 1.1). Seven locations throughout regions 2, 3, and 5 were used to collect green stem disorder data either before or immediately after the plots were harvested. At each location, the trials were differentiated by maturity groups (MG II to V) and herbicide tolerance types (glyphosate tolerant [R] or conventional [C]). Trials are designated by maturity group Roman numeral and herbicide tolerance type designation; for example, maturity group II glyphosate tolerant will be referred to as trial IIR, etc. All experiments were organized in a randomized complete block design with replications as blocks and three replications. An experimental unit was a plot consisting of one cultivar planted in four 6.4 m rows spaced 76.2 cm apart.

The cultivars included in the program saw a 30 to 40% turnover from year to year, based on nominations by seed companies in Illinois and the surrounding states. From 2009 to 2012, a total of 1090 different cultivars were included in the trials for which GSD data were collected. Several public cultivars were included as “checks” in trials IIC, IIIC, IIIR, and VC. Public check cultivars were selected by the Illinois Soybean Association and had some variability from year to year, though multiple checks were present in nearly every location observed within a year. A subset of data with observations solely from check cultivars was used for analysis of year and location effects on GSD incidence since most check cultivars were grown in multiple years and locations.

GSD incidence data were collected for each plot within a trial based on percent incidence over the entire plot by walking through and visually estimating the amount of plants with GSD symptoms. GSD symptoms observed were consistent with the description used to initially

describe the malady (Hobbs et al., 2006). The UISVT program harvested the center two rows of each plot and collected agronomic data from all locations (<http://vt.cropsci.illinois.edu>). Grain weight was measured for each plot at harvest in pounds with a High Capacity Grain Gauge (Juniper Systems, Logan, Utah). Data were reported as bushels acre⁻¹ and transformed to metric units for analysis. Height notes were taken from R7-R8 in centimeters as an average over the entire plot from the ground to the apical meristem. Lodging was estimated on a 1 to 5 scale as follows: 1 = almost all plants erect, 2 = all plants leaning slightly or a few plants down, 3 = all plants leaning moderately (45°), or 25 to 50% of the plants down, 4 = all plants leaning considerably, or 50 to 80 percent of the plants down, 5 = almost all plants horizontal. Height was measured in centimeters shortly before harvest as the length from the ground to the apical meristem. Seed moisture content was assessed at harvest. Protein and oil composition estimations for each experimental unit were determined post-harvest with the use of a near-infrared spectroscopy machine (Foss Tecator, Infratec 1229, Denmark). Data on agronomic traits were shared for this study as a collaborative effort between UISVT and the Laboratory for Soybean Disease Research at the University of Illinois.

Correlations of GSD with agronomic traits. The dataset including all cultivar observations over all year x location combinations was used to estimate correlations coefficients for GSD with agronomic traits. The percent incidence data for GSD observations were transformed using a natural log transformation so data were normally distributed. A means dataset of all natural log transformed GSD and raw agronomic data observations was generated using Proc Means in SAS 9.3 (Cary, North Carolina) by the average of the three observations for each cultivar in every year x location x trial combination. Pearson correlation coefficients were obtained using Proc Corr in SAS. Correlations for GSD and agronomic traits with each other

were made with all data to observe how and if these traits correlated with each other as expected. Correlations for every year x location x trial combination were made in order to account for the different maturity groups, cultivars, and “environments” which were present in these observations and can influence the measured traits from this study.

Effects of year and location on GSD incidence. The public cultivar data subset was used for analysis of the effects of year and location on GSD incidence of cultivars that occurred in many year x location combinations. Raw percentage GSD incidence data were transformed using a natural log transformation to normalize residuals before analysis using the following model:

$$y_{(ijkl)m} = \mu + E_i + T_j + ET_{ij} + B_{(ij)k} + C_{(j)l} + EC_{i(j)l} + \varepsilon_{(ijkl)m}$$

Where E_i is the random environment (year x location combination), T_j is the fixed trial (based on maturity group and conventional versus glyphosate tolerant), ET_{ij} is the random environment x trial interaction, $B_{(ij)k}$ is the random block within environment x trial combination, $C_{(j)l}$ is the random cultivar within trial, $EC_{i(j)l}$ is the environment x cultivar within trial interaction, and $\varepsilon_{(ijkl)m}$ is the random error (niid 0, σ^2). To estimate the random effect of cultivar within trial and environment x cultivar within trial, best linear unbiased predictors (BLUPs) were used for individual estimates for cultivar and pairwise comparisons between environments to estimate the difference between random environments for the same cultivar. Pairwise comparison BLUPs were compared using Scheffe's S to maintain an experimental alpha = 0.05 and to determine significant pairwise comparison estimates for the differences in GSD incidence for the same cultivar in different environments. Scheffe's S was compared to the absolute value of the difference estimated by SAS and subsequent conclusions were made.

Effects of year and location on overall GSD incidence. The dataset including all cultivar observations was used to assess the effect of year and environment on the overall incidence of GSD. In order to reduce the amount of data in this analysis and account for differences in maturity group, this analysis was conducted by trial. The IIIR dataset was so large that it was split in half (MG 2.9-3.4 and MG 3.5-4.0, designated IIIRa and IIIRb) in order to allow the computer to conduct the analysis. Furthermore, to account for differences in GSD sensitivity between cultivars, cultivars that occurred in all locations observed within the same year were the only cultivars included in these analyses. With the exception of the IVC and IVR trials, the analyses for all trials were conducted on the natural log transformed percentage incidence data of GSD to meet ANOVA assumptions. IVC and IVR trial data were transformed using the square root transformation in order to meet ANOVA assumptions. Data from each trial except the IVC trials were analyzed using Proc Mixed in SAS 9.3 using the following model:

$$y_{ijkl} = Y_i + L_{(i)j} + C_k + YC_{ik} + \varepsilon_{(ijk)l}$$

Where Y_i is the random effect of i^{th} year, $L_{(i)j}$ is the random effect of j^{th} location within i^{th} year, C_k is the random effect of the k^{th} cultivar, YC_{ik} is the random interaction between the i^{th} year and the k^{th} cultivar, and $\varepsilon_{(ijk)l}$ is the random error (niid 0, σ^2). The IVC trials were observed only in 2012, so the model was adjusted to account for only one year of data with multiple locations. BLUPs for the differences between locations observed within same year were estimated in SAS and compared to the calculated Scheffe's S to maintain overall experimental error rate of $\alpha = 0.05$. The absolute value of the estimate for the difference was compared to the critical S value and estimates that were significant were backwards transformed to a percentage. Subsequent conclusions were made with these difference estimates. This analysis was not conducted for the

VC soybean trial since there was only one year and location that data were collected from for this trial.

BLUP estimates of GSD incidence for each year x location x trial combination were calculated based on the natural log or square root transformed data (trial dependent, explained above) that were significantly different from zero ($P \leq 0.05$). Rainfall data were collected by on-site collaborators of all UISVT locations and published with results at the end of each season. Rainfall data were reported for May through September and summed over all months. Data for all year x location x trial combinations were combined and analyzed in SAS Proc Corr.

Results

Correlations of agronomic traits with GSD. GSD incidence ranged from 0 to 100%, yield ranged from 1.1 to 6.4 metric tonnes hectare⁻¹, height ranged from 16 to 72 cm, lodging ranged from 1 to 4.5 on the rating scale, seed moisture ranged from 3.2 to 47%, seed protein content ranged from 29.9 to 43.1%, and oil content ranged from 15.7 to 23%. All correlations were significant ($P < 0.0001$) ($n = 4430$) except for the correlation of protein and yield (Table 1.1). The strongest correlation was negative ($r = -0.75$) ($P \leq 0.0001$) for protein and oil (Table 1.1). GSD was negatively correlated with yield ($r = -0.14$, $P \leq 0.0001$) (Table 1.1).

There were 83 correlation coefficients for year x location x trial combinations as three trials in different year x location combinations had only zeros for GSD observations and were not analyzed. Of the 83 correlation coefficients, 20 were significant ($P \leq 0.05$) for GSD to yield with 15 positive and 5 negative correlation coefficients ranging from -0.89 to 0.66 (Table 1.2). Significant ($P \leq 0.05$) positive correlations of GSD to the agronomic traits were greatest for lodging (32 cases), followed by moisture, height, protein, yield, and oil (Table 1.2). Significant

($P \leq 0.05$) negative correlations of GSD to the agronomic traits were greatest for oil (25 cases), followed by protein, yield, moisture, height, and lodging (Table 1.2).

Year and location effects on GSD incidence. The main effects of trial, block within environment x trial, cultivar within trial, and the interaction between environment x cultivar within trial were significant ($P \leq 0.05$) for GSD incidence (Table 1.3). There were no BLUP estimates for the differences between year and location combinations for the same cultivar that were significantly different from zero compared to Scheffe's S ($\alpha_e = 0.05$). Backwards transformed individual BLUP estimates for cultivars nested within trial over all year and location combinations showed that the overall range of GSD incidences for the cultivars included in the public check cultivar data subset was from 0 to 19% (Fig. 1.2). The cultivars with the greatest number of year x location observations were Dwight and Jack, which had estimated GSD incidences of 1 and 4%, respectively.

Effects of year and location on overall GSD incidence. The main effect of location within year was significant in the ANOVA for all trials ($P \leq 0.05$) (Table 1.4) and the main effect of location was significant in the ANOVA for the IVC trial ($P \leq 0.05$) (Table 1.5) indicating the impact that location has on the incidence of GSD for the same cultivars within a single year. Many BLUP estimates for the difference in GSD incidence for cultivars at different locations within the same year were significant when compared to the Scheffe's S calculated for each comparison ($\alpha_e = 0.05$) (Table 1.6). Most locations in Region 2 (Dwight, Goodfield, and Monmouth) having significantly more GSD incidence over locations in Region 3 (New Berlin, Perry, Urbana) in IIC, IIR, IIIC, IIIRa, and IIIRb (Table 1.6). This difference was not estimated for any MGIV trials as cultivars in MGIV were not grown in Region 2. Some of those estimated significant differences were low. For example, Monmouth had significantly more GSD than New

Berlin in 2011 in the IIIC trial with an estimated difference of 1.2%. However, most of the significant differences were estimated to be 4% or greater. In 2010, GSD incidence was estimated to be 12, 17.5, 11.1, 11.7, and 10.1% greater in Goodfield compared to Perry in trials IIC, IIR, IIIC, IIIRa, and IIIRb, respectively (Table 1.6). There was one year (2012) in which there was significantly more GSD in some locations in Region 3 compared to Region 2 where New Berlin and Perry had significantly more GSD (2 and 2.1%, respectively) than Dwight and Goodfield (17.6 and 18.5 percent, respectively) (Table 1.6). In 2012, the only year that GSD data were collected from Elkhville, estimates showed significantly higher GSD in trials IIIC, IIIRb, IVC, and IVR for New Berlin over Elkhville with differences of 2.8, 12.7, 54.4, and 26.6% in each trial, respectively (Table 1.6). Overall, locations in Regions 2 and 3 had significantly more GSD compared to Region 5 in 2012. New Berlin had significantly more GSD compared to other locations in Region 3 in all instances where these locations were compared throughout the BLUP analysis, with differences ranging from a 1.5% difference with Urbana in trial IIIRb in 2011 to an 18.6% difference with Urbana in trial IVR in 2012 (Table 1.6).

The correlation of green stem disorder with seasonal rainfall over all trial observations was significantly ($P \leq 0.01$) negative, -0.28 ($n = 102$). This included 74 year x location x trial BLUP estimates that were not significant from zero. The correlation coefficient for data excluding the BLUP estimates that were not significant from zero was also significantly ($P \leq 0.05$) negative, -0.38 ($n = 28$).

Discussion

The correlations of GSD with agronomic traits showed some overall trends. For yield, there were 20 significant correlation coefficients from 83 comparisons with 15 being positive

and 5 being negative. The lack of a general trend to show that yields increase as GSD incidence increases is unlike the known positive relationship between delayed maturity in maize and sorghum to yield (Thomas et al., 2014). This overall inconsistency of GSD with yield in soybean has been shown before for individual cultivars, with 11 of 28 observed cultivars having a positive relationship with yield (Hill et al., 2013). Some of the most consistently, positively correlated agronomic traits with GSD were lodging, height, moisture, and protein. Oil was most consistently, negatively correlated with GSD. The findings that GSD incidence was most frequently correlated with numerous quantitative agronomic traits that are affected by the environment supports the idea that GSD incidence is a quantitatively conferred trait that can be impacted by environment. While results from this study suggest a negative relationship between rainfall and GSD incidence, these trials were not designed to assess the effect of rainfall on GSD. In order to examine environmental effects on GSD symptom expression, cultivars that are known to be sensitive to GSD should be planted and observed in multiple site-years, and if possible, under different irrigation regimes.

Positive correlations between GSD and seed moisture at harvest are important as seed moisture content can be one of the main factors to consider when it comes to stored grain. This relationship between soybean plants with green stems at harvest maturity and increased seed moisture has been shown in the past (Favero and doCarmo Lana, 2014). Harvested soybean with a moisture content above 13% are recommended to be dried to reduce the likelihood of reducing seed quality due to various factors, including fungal colonization (Agarwal and Sinclair, 1997). Harvested soybean seeds with a moisture content between 13-20% are more susceptible to infection by storage fungi (Tariq et al., 2005). The data from this study support the hypothesis

that soybean harvested from plants with green stems at harvest maturity are positively correlated with seed moisture, yet another problem coinciding with GSD incidence.

When considering GSD incidence of the public cultivars, which had the greatest amount of data over the years, it was unfortunate to find that the majority of these cultivars were not sufficiently sensitive to the development of GSD symptoms. The two cultivars, Dwight and Jack, had the greatest number of observations, but had small estimates of GSD making this data not so useful. Previous studies have provided data that showed differences in GSD sensitivity between cultivars (Hill et al., 2006; Hobbs et al., 2006); however, the cultivars included in this study unknowingly did not include cultivars that were sensitive to GSD symptom development. The cultivars included in this study did not allow for the assessment of how individual environments present in this study influenced GSD incidences.

This study has provided evidence of the effect locations can have on the same soybean cultivars planted within a single year. Perhaps one of the most interesting finds is the overall trend of higher incidences of GSD in region 2 (40.9°N) over 3 (39.78°N) and region 3 over 5 (37.91°N). Researchers in Japan observed that cultivars developed for production in more northern latitudes were more sensitivity to GSD (Fujii et al., 2015), which is not the same as the same cultivars grown in different latitudes having differences in GSD incidences. Further exploration of the effects of latitude and maturity group on GSD incidence is needed. High GSD within specific maturity groups could be attributed to unintentional selection for GSD sensitivity in breeding programs. While comparing maturity groups within this study was not possible, it is also probably not the most sensible approach to make comparisons of GSD sensitivity since research has determined GSD sensitivity to be a genetically controlled trait (Yamada et al., 2014). However, if researchers were to compare cultivars that are sensitive or insensitive to GSD

in different maturity groups, it is possible this inference could be deduced. This was not a reality within the constraints of this study. In addition to attention to cultivar selection, the significant effects of location on GSD are important considerations for future studies with GSD.

Continued research on GSD in soybean is needed to determine what factors in the environment influence the incidence of GSD. Considerations of genotype x environment interaction will be important in future research to identify more QTL for GSD.

Acknowledgements. Thanks to Shilpi Chawla, Tara Slaminko, and Laura Crull for their work in collecting green stem disorder data from 2009 to 2012. I would also like to acknowledge and thank the University of Illinois Soybean Variety Testing program for sharing their agronomic trait data and allowing the Varietal Information Program for Soybean coordinators to collect green stem disorder data from 2009 to 2012, especially Ralph Esgar and Darin Joos and all other on-site collaborators.

Literature Cited

- Agarwal, V.K., Sinclair, J.B. 1997. *Principles of Seed Pathology*. (2nd ed.). Boca Raton, FL: CRC Press Lewis Publishers.
- Byamukama, E., Robertson, A. E., Nutter, F. W., Jr. 2015. Bean pod mottle virus time of infection influences soybean yield, yield components, and quality. *Plant Disease* 99:1026-1032.
- Chawla, S., Bowen, C.R., Slaminko, T.L., Hobbs, H.A., Hartman, G.L. 2013. A public program to evaluate commercial soybean cultivars for pathogen and pest resistance. *Plant Disease* 97:568-578.
- Favero, F., do Carmo Lana, M. 2014. Reduction of green stem and leaf retention in soybean through greater nitrogen availability from seed treatment. *Revista Brasileira de Ciência do Solo* 38:1423-1438.
- Formento, A.N., de Souza J. 2009. Detection of green stem disorder of soybean in Entre Rios, Argentina. *Journal of Plant Pathology* 91:236.
- Fujii, K., Kato, S., Sayama, T., Tanaka, Y., Nakazaki, T., Ishimoto, M., Shiraiwa, T. 2015. Stability verification of the effects of stem determination and earliness of flowering on green stem disorder of soybean against genetic background and environment. *Plant Production Sciences* 18:166-179.
- Hill, C. B., Hartman, G. L., Esgar, R., Hobbs, H. A. 2006. Field evaluation of green stem disorder in soybean cultivars. *Crop Science* 46:879-885.
- Hill, C. B., Bowen, C. R., Hartman, G. L. 2013. Effect of fungicide application and cultivar on soybean green stem disorder. *Plant Disease* 97:1212-1220.
- Hobbs, H. A., Hill, C. B., Grau, C. R., Koval, N. C., Wang, Y., Pedersen, W. L., Domier, L. L., Hartman, G. L. 2006. Green stem disorder of soybean. *Plant Disease* 90:513-518.
- Mochizuki, A., Shiraiwa, T., Nakagawa, H., Horie, T. 2005. The effect of temperature during the reproductive period on development of reproductive organs and the occurrence of delayed stem senescence in soybean. *Japanese Journal of Crop Science* 74:339-343.
- Schwenk, F. W., Nickell, C. D. 1980. Soybean green stem caused by Bean pod mottle virus. *Plant Disease* 64:863-865.
- Tariq, M., Dawar, S., Mehdi, F.S. 2005. Effect of different moisture and storage temperature on seedborne mycoflora of soybean. *International Journal of Biology and Biotechnology* 2:947-950.
- Thomas, H., Ougham, H. 2014. The stay-green trait. *Journal of Experimental Botany* doi:10.1093/jxb/eru037.

Yamada, T., Shimada, S., Makita, H., Hirata, K., Takashashi, K., Nagaya, T., Hamaguchi, H., Maekawa, T., Sayama, T., Hayashi, T., Ishimoto, M., Tanaka, J. 2014. Major QTLs associated with green stem disorder insensitivity of soybean (*Glycine max* (L.) Merr.). *Breeding Science* 64:331-338.

Tables and Figures

Table 1.1. Pearson correlation coefficients between natural log transformed percent incidence data for green stem disorder and all agronomic traits observed over all years, locations, and cultivar observations. Data were collected from 2009 to 2012 from Dwight, Elkhart, Goodfield, Monmouth, New Berlin, Perry, and Urbana, Illinois.

Trait	Trait					
	GSD ^a	Yield ^b	Height ^c	Lodging ^d	Moisture ^e	Protein ^f
Yield	-0.14 ^{h,i}
Height	0.09	0.66
Lodging	0.17	0.42	0.55
Moisture	0.08	-0.06	-0.17	-0.04
Protein	0.39	ns	0.07	0.17	0.10	...
Oil ^g	-0.20	-0.14	-0.13	-0.30	-0.17	-0.75

^a GSD- Green stem disorder, estimated as a percent affected by GSD in the field plot. Natural log transformed data used for correlation estimates.

^b Yield measured in bu acre⁻¹ and transformed to metric units for this study.

^c Plant height measured in centimeters at R8.

^d Lodging estimated on a 1 to 5 scale for no lodging to lodging of the entire stand, respectively.

^e Moisture content measured at harvest with yield and moisture monitor attachment on harvester.

^f Protein content measured post-harvest using a near-infrared spectroscopy machine (Foss Tecator, Infratec 1229, Denmark).

^g Oil content measured using the near-infrared spectroscopy machine post-harvest.

^h Pearson correlation coefficients significant at $P < 0.0001$; ns = not significant.

ⁱ Correlations were conducted using the averages over each cultivar in every trial for every year x location. Cultivars were replicated in three blocks within a trial. Trials were designated by maturity group and herbicide tolerance type (conventional or glyphosate tolerant). The total number of observations for correlation coefficient was $n = 4430$.

Table 1.2. Number of significantly ($P \leq 0.05$) positive or negative Pearson correlation coefficients (r) obtained for each agronomic trait with the natural log transformed percent incidence green stem disorder. Pearson correlation coefficients estimated over the cultivar averages from each trial in every environment yielded 83 correlation coefficients ^a.

	Yield^b	Height^c	Lodging^d	Moisture^e	Protein^f	Oil^g
Positive r	15	26	32	28	20	3
Negative r	5	2	1	2	5	25

^a GSD- Green stem disorder, estimated as a percent affected by GSD in the field plot. Natural log transformed data used for correlation estimates.

^b Yield measured in bu acre⁻¹ and transformed to metric units for this study.

^c Plant height measured in centimeters at R8.

^d Lodging estimated on a 1 to 5 scale for no lodging to lodging of the entire stand, respectively.

^e Moisture content measured at harvest with yield and moisture monitor attachment on harvester.

^f Protein content measured post-harvest using a near-infrared spectroscopy machine (Foss Tecator, Infratec 1229, Denmark).

^g Oil content measured using the near-infrared spectroscopy machine post-harvest.

Table 1.3. Analysis of variance for green stem disorder incidence for public check cultivar subset of data collected from 2009 to 2012 in Dwight, Elkhville, Goodfield, Monmouth, New Berlin, Perry, and Urbana, Illinois with locations observed varying from year-to-year, delineating the different environments (year x location).

Source of variation	df	<i>F</i>^a
Trial (T)	3	3.1*
Environment (E)	18	2.17
E x T	12	1.77
Block(E x T)	68	1.83*
Cultivar (C) (T)	23	5.07*
E x C(T)	69	2.82*
Residual	226	.

^a This analysis was conducted on the natural log transformed percent incidence green stem disorder data in order to meet ANOVA assumptions. Significant ($P \leq 0.05$) model factors are indicated by * next to the *F* statistic.

Table 1.4. Analysis of variance for trials (maturity group x glyphosate tolerance type) to assess the variability of location on the incidence of green stem disorder (GSD) within a year.

Trial ^a	Source of Variation								
	Year		Location(year)		Cultivar		Year x cultivar		Residual
	df ^b	F	df	F	df	F	df	F	df
IIC	3	2.9	13	17.45*	27	3.17*	17	2.15*	553
IIR	3	2.02	15	37*	78	2.16*	20	2.01*	1188
IIIC	3	2.75	14	122.56*	179	4.04*	58	1.98*	2242
IIIRa ^c	3	2	13	177.89*	180	2.01*	65	1.26	2611
IIIRb ^c	3	5.5*	13	57.4*	118	0.84	27	3.06*	1659
IVR	2	1.28	6	75.14*	37	5.06*	11	0.82	369

^a Trials are indicated by maturity group (II-IV) and glyphosate tolerance type (C = conventional, R= glyphosate tolerant). Natural log transformed percent incidence GSD data were used in the analyses of all trials except for the glyphosate tolerant maturity group IV trial in which the square root transformation was used. Data were transformed to meet ANOVA assumptions. Data were collected from 2009-2012 in Dwight, Elkhart, Goodfield, Monmouth, New Berlin, Perry, and Urbana, Illinois with locations observed varying from year-to-year for each trial.

^b The degrees of freedom (df) and calculated F statistic for each factor in the model for each trial are shown. Significant effects ($P \leq 0.05$) are indicated with a * next to the F statistic.

^c Trial IIIR was split into two halves in order to decrease dataset size for analysis. IIIRa includes MG 2.9-3.4 and IIIRb included MG 3.5-4.0.

Table 1.5. Analysis of variance of green stem disorder incidence in the conventional maturity group IV trials which were observed only in 2012 at Elkhville, New Berlin, Perry, and Urbana, Illinois.

Source of Variation	df	<i>F</i>^a
Location	3	50.7*
ID	4	7.55*
Residual	40	.

^a Natural log transformed percent incidence GSD data were used in the analyses of these data to meet ANOVA assumptions. Significant ($P \leq 0.05$) factors are indicated with *.

Table 1.6. Best linear unbiased predictors for pairwise comparisons of green stem disorder (GSD) percent incidences between years and locations for each trial which GSD data were collected. Estimates were made with like cultivars within a year since there is known to be variability in cultivar sensitivity and cultivars in trials varied from year to year.

Year	Location 1 ^a	Location 2	Trial						
			IIC ^b	IIR	IIC	IIRa	IIRb	IVC	IVR
2009	Dwight	Goodfield	- ^c	0	-	-	-	-	-
2009	Dwight	Monmouth	-	-2.7	-	-	-	-	-
2009	Dwight	New Berlin	-	0	-	-	-	-	-
2009	Dwight	Urbana	-	0	-	-	-	-	-
2009	Goodfield	Monmouth	-3.3	-3.8	0	1.9	0	-	-
2009	Goodfield	New Berlin	0	0	-	-	-	-	-
2009	Goodfield	Perry	-	-	7.5	11.1	5.3	-	-
2009	Goodfield	Urbana	-	0.0	-	-	-	-	-
2009	Monmouth	New Berlin	2.6	4.8	-	-	-	-	-
2009	Monmouth	Perry	-	-	10.3	3.8	4.1	-	-
2009	Monmouth	Urbana	-	4.8	-	-	-	-	-
2009	New Berlin	Urbana	-	0	-	-	-	-	-
2010	Dwight	Goodfield	0	0	0	-3.8	0	-	-
2010	Dwight	Monmouth	0	2.9	0	-3.6	0	-	-
2010	Dwight	New Berlin	0	3.0	0	0	0	-	-
2010	Dwight	Perry	3.2	8.8	5.8	2.1	4.4	-	-
2010	Dwight	Urbana	0	0	4.0	2.4	4.2	-	-
2010	Goodfield	Monmouth	3.4	6.2	0	0	0	-	-
2010	Goodfield	New Berlin	4.0	6.5	0	4.5	3.9	-	-
2010	Goodfield	Perry	12.7	17.6	11.1	11.7	10.1	-	-
2010	Goodfield	Urbana	6.1	3.6	7.8	12.9	9.6	-	-
2010	Monmouth	New Berlin	0	0	0	3.1	2.3	-	-
2010	Monmouth	Perry	0	2.0	6.0	8.4	6.4	-	-
2010	Monmouth	Urbana	0	0	4.1	9.3	6.1	-	-
2010	New Berlin	Perry	0	1.9	7.9	1.7	1.7	-	5.4
2010	New Berlin	Urbana	0	0	5.5	2.0	1.6	-	15.5
2010	Perry	Urbana	0	-3.6	0	0	0	-	2.5
2011	Monmouth	New Berlin	0	0	1.2	0	1.3	-	-
2011	Monmouth	Urbana	0	0.9	5.2	1.5	3.5	-	-
2011	New Berlin	Urbana	0	0	2.5	2.1	1.5	-	4.7
2012	Dwight	Elkville	-	-	0.9	-	6.7	-	-
2012	Dwight	Goodfield	0	0	0	6.1	0	-	-
2012	Dwight	New Berlin	0	0	0	-2.0	0	-	-
2012	Dwight	Perry	0	0	0	-2.2	0	-	-

Table 1.6. (cont.)

Year	Location 1	Location 2	Trial						
			IIC	IIR	IIIC	IIIRa	IIIRb	IVC	IVR
2012	Dwight	Urbana	0	0	0	0	0	-	-
2012	Elkville	Goodfield	-	-	0	-	-16.4	-	-
2012	Elkville	New Berlin	-	-	-2.8	-	-12.7	-54.4	-26.6
2012	Elkville	Perry	-	-	0	-	-13.5	-66.3	-7.3
2012	Elkville	Urbana	-	-	0	-	-4.8	-36.5	0
2012	Goodfield	New Berlin	0	0	4.1	-17.6	0	-	-
2012	Goodfield	Perry	0	0	0	-18.5	0	-	-
2012	Goodfield	Urbana	2.3	0	0	-8.9	2.4	-	-
2012	New Berlin	Perry	0	0	0	0	0	0	6.0
2012	New Berlin	Urbana	0	1.6	3.0	1.3	0	1.8	18.4
2012	Perry	Urbana	0	0	0	1.3	0	4.4	3.4

^a Locations are distributed throughout different regions in the University of Illinois Soybean Variety Testing program to account for differences in maturity groups suitable for designated locations.

^b Trials are defined by maturity group (II-IV) and herbicide tolerance type (C – conventional, R – glyphosate tolerant). Trials are distributed throughout the locations based on the region in which the location is found, so not all trials occur in every location.

^c Differences between locations within years are reported as follows: - = no observation was made for this trial, 0 = the best linear unbiased predictor was not significant compared to the calculated Scheffe's *S*, numbers reported are significant compared to Scheffe's *S* when comparing the first locations minus the second location specified within a year. The experimental alpha for pairwise comparisons was maintained at 0.05.



Fig. 1.1. Data were collected from regions 2, 3, and 5 from the plots established by the University of Illinois Soybean Variety Testing Program in the years 2009 to 2012.

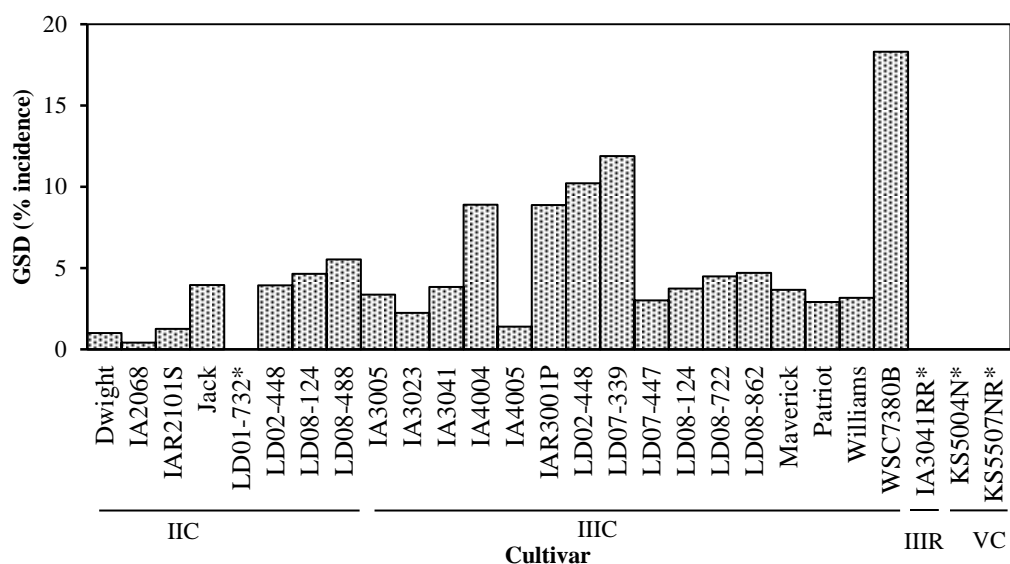


Fig. 1.2. Backward transformed values for narrow best linear unbiased predictor (BLUP) estimates^{a,b} for each within trial^c.

^a Estimates were obtained using the natural log transformed data and were backwards transformed to get a percent incidence estimate.

^b Estimates shown were significantly different from zero ($P \leq 0.05$). Estimates for cultivars with an * were not significantly different from zero.

^c Trial is determined by maturity group (II-V) and herbicide tolerance type (conventional or glyphosate tolerant).

CHAPTER 2

Fungicide seed treatments in soybean provide differential protection against *Sclerotinia sclerotiorum* at germination and only slight residual protection beyond germination

Abstract

Sclerotinia sclerotiorum is one of the most important pathogens infecting soybean plants. When the fungus is seedborne as mycelia or when it is infested with seeds as sclerotia, the fungus can kill germinating seedlings. The use of fungicide seed treatments may help to manage this phase of the disease and may provide residual protection to infection beyond the seedling stage. The objectives of my study were to use the data from the University of Illinois Soybean Variety Testing Program (UISVT) to document the increase in deployment of fungicide seed treatments from 2005 to 2014 and to determine if seed treated fungicides would provide control of *S. sclerotiorum* on inoculated germinating seedlings and on plants beyond the seedling stage inoculated with the fungus. The data from the UISVT showed that the deployment of seed treatments with fungicides on cultivars entered into the program increased from 66% in 2005 to 92% in 2014. To test the efficacy of fungicide seed treatments, four fungicide seed treatments, fludioxonil, trifloxystrobin, trifloxystrobin + saponins, and penflufen + prothioconazole, and an untreated control were applied to seeds of four soybean cultivars. The plants were inoculated at various growth stages from seedling germination to flowering. In the seed germination stage, fludioxonil provided complete control, penflufen + prothioconazole provided moderate control while the trifloxystrobin and trifloxystrobin + saponins provided no control. There was little residual activity detected when plants beyond the seed germination stage were inoculated. Although seed treatments included in this study do not offer residual protection against *S.*

sclerotiorum infection beyond germination, there is hope that future seed treatment fungicides will have longer residual activity that could provide protection to plants at later growth stages.

Introduction

Soybean [*Glycine max* (L.) Merr.] plants are threatened by a number of pathogens throughout the growing season. In the North Central United States, Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* [(Lib.) de Bary] causes yield reductions particularly when the weather is cooler and wetter than normal. SSR has been ranked as one of the top ten yield-suppressing diseases in the United States and Canada for over a decade (Wrather and Koenning, 2009; Koenning and Wrather, 2010). Yield suppression in Illinois is most common in the northern regions of the state where growing season temperatures are cooler; however, a 1988 survey found *S. sclerotiorum* infected seeds harvested from fields as far south as Madison County (38.3°N) in Illinois, (Hartman et al., 1988). In addition to yield reductions in the field and those associated with the seed-borne phase of the disease, soybean growers can take indirect profit losses if their harvested grain is contaminated with sclerotia, the dark, small, irregularly-shaped, survival structure of *S. sclerotiorum* (Grau and Hartman, 2015). Sclerotia can be confused with soil peds picked up by farm equipment and can be a means of pathogen dispersal (Link and Johnson, 2007). Seeds colonized by *S. sclerotiorum* also contribute to the spread of the disease (Hartman et al., 1998).

S. sclerotiorum, a necrotrophic pathogen, typically infects senescing soybean flowers. In favorable conditions, sclerotia within 5 cm of the soil surface germinate carpogenically to produce apothecia, which release ascospores into the air. The ascospores that land on senescing flower petals colonize that tissue and produce oxalic acid to kill additional tissue that the fungus

will then colonize. If good growth conditions persist, the fungus will continue this process of colonization until the plant dies due to stem girdling at the point usually above the infected flower node. In other cases, the disease can be transferred to healthy plants from neighboring infected plants that are in contact with each other (Grau and Hartman, 2015). In addition to yield reductions of above-ground plant parts, *S. sclerotiorum* can also cause stand reductions and subsequent yield losses by seed-borne mycelia or seeds infested with sclerotia. When seeds are planted that are infested with *S. sclerotiorum*, they can suffer from rot below the soil surface or can be killed off shortly after germination. This reduction in plant stand will reduce yields if significant stand reductions occur.

While host resistance is one of the most economically and ecologically sound methods to manage many soybean pathogens, complete resistance to *S. sclerotiorum* is not found in soybean plants. However, sources of partial resistance and quantitative trait loci (QTL) associated with this partial resistance have been identified and located (Kim and Diers, 2000; Vuong et al., 2008; Zhao et al., 2015). For soybean growers, selection and use of soybean cultivars with partial resistance to SSR is a useful practice to help manage this disease. Some seed companies report the “resistance level” that cultivars have to *S. sclerotiorum* (e.g. www.dairylandseed.com, www.prairiebrand.com) and in some states, commercial cultivar disease screening information is available (Chawla et al., 2013).

In addition to partial resistance, growers that have fields with a history of SSR often use foliar fungicides to aid in disease management. There are different chemistry classes of foliar-applied fungicides that are labeled for control of *S. sclerotiorum* in soybean including the quinone outside inhibitors, demethylation inhibitors, methyl benzimidazole carbamates, and succinate dehydrogenase inhibitors (Mueller et al., 2015). These fungicides are not translocated

throughout the plant systemically, which means the chemical cannot move down the plant where infection is most likely to occur (Peltier et al., 2012).

While foliar fungicides are utilized to protect soybean plants from *S. sclerotiorum*, commercial fungicide seed treatments have the ability to protect seeds from the fungus when it is seed-borne and potentially provide in-season control during flowering. There are a few active ingredients that are used in seed treatments for soybean that are labeled for the control of seed borne *S. sclerotiorum*. Fludioxonil (phenylpyrrole), an active ingredient in the commercial soybean seed treatment Fludioxonil (www.syngenta.com), is labeled for the control of seed-borne *S. sclerotiorum*. This active ingredient was an effective method to reduce sclerotia formation from infected seeds (Mueller et al., 1999), which in turn can suppress inoculum sources for mid-season infections. The use of commercial seed treatments in soybean has increased over the years; an estimated 8 and 30% of all soybean planted in 1996 and 2008, respectively, had a seed treatment applied (Munkvold, 2009), with an industry estimate of 60-70% in 2014 (United Soybean Board, 2014). New seed treatments released in 2014 and 2015 are now labeled for protection against soybean cyst nematode (*Heterodera glycines* Ichinohe) and sudden death syndrome (*Fusarium viguliforme* O'Donnell and T. Aoki) (www.bayer.com; www.syngenta.com). With continued development and deployment of seed treatments by seed companies, my objectives were to (i) document the deployment of fungicides seed treatments by commercial soybean companies from 2005 to 2014 based on cultivars entered into the University of Illinois Soybean Variety Testing Program (UISVT) and (ii) determine if soybean fungicide seed treatments will provide control of *S. sclerotiorum* from seedling germination to reproductive growth stages of the plant.

Materials and Methods

Seed treatment deployment by commercial seed companies. Information on soybean cultivars entered into the UISVT was collected from 2005 through 2014 from the UISVT website (<http://vt.cropsci.illinois.edu/soybean.html>). New cultivars entered into the UISVT generally range from 30 to 40% from year to year as soybean seed companies choose to enter cultivars into the program for various reasons from evaluating performance of advanced breeding lines to newly released cultivars. Soybean entries in the UISVT are planted at several of 13 locations throughout Illinois in different trials based on maturity group and herbicide tolerance type (glyphosate tolerant or conventional). Agronomic trait information is published for each cultivar in each year along with information specific to each cultivar tested (<http://vt.cropsci.illinois.edu/soybean.html>). Published information included seed treatments that were deployed on each entry. The seed treatments were classified by UISVT project managers as untreated, fungicide only, or fungicide + insecticide. The percentage of each type of seed treatment was calculated for each year, from 2005-2014.

Plant material for seed treatment tests. Four cultivars were selected for these experiments based on previous experiments and previous information. These were DSR2400 (Dairyland Seed), IP2991 (Prairie Hybrids), Resnik (Public), and Fairbault (Public). DSR2400 was used as a partially resistant check for SSR greenhouse assays by the Varietal Information Program for Soybean (VIPS) program (Chawla et al., 2013). Resnik and Fairbault were used for susceptible checks for the same assays. IP2991 was a cultivar that had contradictory results from one year of assays to the next, with seed treatment being the only difference in those years (data not shown).

Fungicides materials used for seed coating. Four different soybean seed fungicide treatments were used including fludioxonil (CruiserMaxx, Syngenta), trifloxystrobin (Trilex6000, Bayer Crop Science), trifloxystrobin + saponins (Heads Up, Plant Protectants Inc.), and penflufen + prothioconazole (EverGol Energy, Bayer Crop Science). Most of these seed treatments included an insecticide component listed in Table 2.1. In addition, there was an untreated control included in this study. The active fungicide ingredients in seed treatments varied from treatment to treatment with fludioxonil being the only treatment labeled for control of *S. sclerotiorum* (seed-borne) (Table 2.1). Fludioxonil and trifloxystrobin seed treatments included an insecticide component (thiamethoxam and imidacloprid, respectively) (Table 2.1). Trifloxystrobin treatment included a biological component (*Bacillus pumulis*, a biological agent for activating an induced systemic resistance) (Table 2.1). Penflufen + prothioconazole was the most recently released seed treatment for soybean at the time of this study, released in 2013 with the new active ingredient penflufen labeled for the control of *Rhizoctonia* spp. and *Fusarium* spp. (Table 2.1). Saponins, a derivative of quinoa (*Chenopodium quinoa*), were included in this study only as a combination with trifloxystrobin. Saponins labeled to induce a systemic acquired resistance in the plant (Table 2.1).

For each cultivar, 120 grams of seed were added to 324 grams of sorghum to provide the weight needed (444 g) for proper use of the Gustafson Bowl Treater (Model #529288, Bayer Crop Science, Research Triangle Park, North Carolina). Active ingredient rates are outlined in Table 2.1. After treating, sorghum was separated using a mechanical screen (SeedBuro Equipment Company, Des Plaines, Illinois). Seed were stored in individual envelopes in a cold room (4°C) until used in the experiments.

Experimental design. There were five experiments; all organized as complete factorials arranged in an RCBD with replication as the block unless otherwise noted. Seeds were planted in LC1 professional growing mix (Sun Gro Horticulture, Agawam, Massachusetts). After seeds were planted, plants were grown in a warm greenhouse room with a 13-hour photoperiod with a daytime temperature of 23-27°C and a nighttime temperature of 18.5-22.5 °C. The *S. sclerotiorum* isolate “Rudd” (Isolate U2; Koga et al., 2014) was used for all inoculations in this study. For all experiments inoculated post-emergence, plants were moved to an air-conditioned greenhouse room without supplemental lighting and a maintained temperature between 20.5-22.5°C for a 48-hour incubation period in a mist chamber. Following the incubation period, plants were removed from the mist chamber and kept in the air-conditioned greenhouse room until the experiment was completed. Models for each experiment are included below. Models include abbreviations for any of the following terms, depending on the factors of the experiment: D = day after planting, C = cultivar, T = seed treatment, I = inoculum, B = block, and R = replication (within block).

Effect of fungicide seed treatments on seeds inoculated with *S. sclerotiorum*. Two seeds of each cultivar x seed treatment combination were planted about 2 cm deep in each of 32-cell inserts filled with LC1 soilless mix. The 32-cell inserts were placed into 26.5 x 51.5 x 6 cm flats. There was one inoculum type used, potato dextrose agar (PDA) plug with *S. sclerotiorum* mycelia, and two controls, a sterile agar plug and nothing. Inoculum was added to the growth media before seeds were covered with coarse vermiculite after planting and placed in the warm greenhouse room.

The experiment was a three-factor factorial with a total of 60 experimental units per replication consisting of inoculum type (three) x fungicide treated or not treated (five) x cultivar

(four) treatment combinations. This experiment was replicated in three blocks with one replication per block. Germination notes were taken at 5, 7 and 10 days after planting. Emergence count data collected 10 days after planting were analyzed using a chi-square test of homogeneity in Excel to test for the effect of seed treatment x inoculum. Means and standard deviations were also estimated for each seed treatment in the positive inoculum treatment.

Effect of seed treated fungicides on 7 and 14 day old seedling inoculated with *S. sclerotiorum*. Four seeds of each of the 20 fungicide seed treatment x cultivar combinations were planted one week apart in 18-cell inserts filled with LC1 placed into 26.5 x 51.5 x 6 cm flats. The plants were grown to 7 and 14 days old or at V1 and V2, respectively (Fehr et al., 1971), before inoculation. Plants were inoculated using 1.5-2 ml of a mycelial slurry made from three to four day-old colonies of Rudd grown on PDA that were homogenized in a 60 ml plastic syringe. The inoculum was applied on the cotyledons of the seven day old plants and onto the internode between the unifoliate and the first trifoliate of 14 day old plants. After inoculation, plants were placed into aforementioned greenhouse conditions.

This experiment was planted in blocks consisting of two replications within each block which served as one trial. This was repeated four times. Count survival data were recorded 7 and 10 days after inoculation (DAI). Since data from these trials did not meet the homogeneity of variance and normality of residual requirements simultaneously, they were analyzed using the Kruskal-Wallis test in SAS using Proc npar1way with percent data that were transformed using the square root transformation to meet homogeneity of variance assumption. This was conducted for each day after planting and focused on the main effect of seed treatment.

Effect of seed treated fungicides on 18, 25, and 32-day old plants inoculated with *S. sclerotiorum*. Six seeds of each cultivar x seed treatment combination were planted in 15 cm

pots filled with LC1 and fertilized with 5 ml of 13-13-13 slow release osmocote fertilizer. Pots were thinned to five plants per pot two weeks after planting. Plantings occurred in one-week successions for three weeks and plants grew until they were 18, 25, and 32 days old, approximately at V1-V2, V2-V3, and V3-V4, respectively (Fehr et al. 1971). PDA plates with *S. sclerotiorum* were prepared three to four days before inoculation. For inoculation, stems were cut 2.54 cm above the most mature leaf axil and inoculated by applying a 200 µl pipette tip filled with a plug of PDA with *S. sclerotiorum* to the cut end of the stem. After the incubation period, pots were placed into 26.5 x 51.5 x 2.5 cm flats and bottom-watered with drip irrigation.

This was conducted as a three-factor factorial consisting of DAP (three) x fungicide treated or not treated (five) x cultivar (four) with two trials (one repeat) and with two replications in the first trial and three replications in the second trial. Lesion length (cm) measurements were taken 10 DAI. Plants with no lesion expansion were counted as escapes and were not included in the data analysis. Data were analyzed using the following mixed model:

$$Y_{(ijklm)} = \mu + D_i + T_j + DT_{ij} + C_k + DC_{ik} + TC_{jk} + DTC_{ijk} + B_l + \epsilon_{(ijklm)}$$

Effect of seed treated fungicides on 32, 39, and 46-day old plants inoculated with *S. sclerotiorum*. Resnik and Fairbault were included in this experiment with all seed treatment combinations for both cultivars. Seeds were planted using the same method described above but allowed to grow until all of the plants in each block were 32, 39, and 46 days old at inoculation at estimated growth stages V4-V5, R1, and R2, respectively (Fehr et al. 1971), and fertilized as needed with 13-13-13 slow release fertilizer. Plants were then inoculated using the same cut-stem method as described above.

The experiment was planted with two replications within each trial and was repeated two times. Stem lesion lengths were measured at 7, 14, and 21 DAI the area under disease progress

curve (AUDPC) was calculated in Excel (Shaner and Finney, 1977). AUDPC values were analyzed using the following mixed model:

$$Y_{ijkl} = \mu + C_i + T_k + CT_{ik} + B_j + R_{(j)l} \epsilon_{ijkl}$$

Further analyses were conducted with the 21 DAI measurement dataset using the same model as was used in the 18, 25, and 32 DAP analyses with the added random effect of replication within block.

Data analyses. Data collected from experimental repeats were combined and checked for homogeneity of variances in Proc Glimmix and the normality of residuals was verified in Proc Univariate to meet ANOVA assumptions. Data collected from all experiments were subject to analyses using Proc Mixed in SAS 9.4 unless otherwise noted. Least square means of significant terms of interest were separated using the pdmix800 macro (Saxton, 1998).

Results

Seed treatment deployment by commercial seed companies. In 2005, 34% of the cultivars entered into the UISVT were non-treated, 33% treated with a fungicide only, and 33% treated with a mixture of a fungicide and insecticide (Fig. 2.1). By 2014, less than 8% of the cultivars were non-treated while most of the cultivars were treated with a combination of a fungicide and insecticide. The use of fungicides in both treatments with solely fungicide components and treatments with fungicide and insecticide components increased from 66% to 92% from 2005 to 2014. The percent of seed treatments used in UISVT cultivars with only fungicide components went from 33% in 2005 to 1% in 2014 (Fig 2.1).

Effect of seed treated fungicides on seed inoculated with *S. sclerotiorum*. The chi-square test of homogeneity revealed that fludioxonil and penflufen + prothioconazole were

significantly different ($P \leq 0.05$) from the other treatments. The mean estimates for percent germination of seeds treated with fludioxonil and penflufen + prothioconazole were 100 and 36.3%, respectively (Table 2.2). Trifloxystrobin + saponins, trifloxystrobin, and the untreated check had 10% germination or lower when planted in the positive SSR inoculum treatment (Table 2.2).

Effect of seed treated fungicides on 7 and 14 day old seedling inoculated with *S. sclerotiorum*. There were no significant ($P \leq 0.05$) differences in the medians detected by the Kruskal-Wallis test for the 7 or 14-day old plants (Table 2.3). The associated standard deviation with each backwards transformed means from DAP x seed treatment combinations were relatively large indicating the overall variability of this assay (Table 2.4)

Effect of fungicide seed treatments on 18, 25, and 32-day old plants inoculated with *S. sclerotiorum*. The three-way interaction of DAP x cultivar x seed treatment was not significant in the analysis of variance ($P < 0.05$) (Table 2.5). Factors that were significant included the main effects of DAP and cultivar and the interactions between seed treatment x cultivar, DAP x cultivar, and DAP x seed treatment ($P < 0.05$) (Table 2.5). Least square mean separation for the DAP x seed treatment interaction showed that at 18 DAP, plants grown from untreated seed, fludioxonil, and trifloxystrobin + saponins treated seed have significantly shorter stem lesions compared to plants grown from penflufen + prothioconazole treated seed ($P < 0.05$) (Fig. 2.2). At the same date, plants grown from trifloxystrobin + saponins treated seed have significantly shorter stem lesions compared to plants grown from trifloxystrobin and penflufen + prothioconazole treated seed ($P < 0.05$) (Fig. 2.2). At 25 and 32 DAP, no treatments were significantly different from the control or other treatments ($P < 0.05$) (Fig. 2.2).

The ranking of the least square means for the estimate of lesion length for the significant main effect of cultivar ranked IP2991, DSR2400, Fairbault, and Resnik from smallest overall lesion length to the largest lesion length, respectively.

Effect of fungicide seed treatments on 32, 39, and 46-day old plants inoculated with *S. sclerotiorum*. Analysis of variance for the AUDPC calculation of each treatment combination showed a significant interaction between cultivar and seed treatment ($P < 0.05$) (Table 2.6). The least square mean separation of the AUDPC estimates for seed treatment x cultivar treatment combinations showed that the only two treatment combinations that were significantly different were Resnik plants grown from untreated seed, with the smallest AUDPC, and Fairbault grown from penflufen + prothioconazole treated seed, with the largest AUDPC ($P < 0.05$) (Fig. 2.3).

ANOVA of the stem lesion length measurements at 21 DAI showed that the three-way interaction of DAP x seed treatment x cultivar was significant along with the two-way interactions of seed treatment x cultivar and DAP x seed treatment ($P < 0.05$) (Table 2.7). The main effects of DAP, seed treatment, and cultivar were also significant ($P < 0.05$). Least square mean separation ($P < 0.05$) of estimates for 21 DAI lesion length for all cultivar x DAP x seed treatment combinations show an overall trend of a decrease in lesion length as plants become age (Appendix B). Contrasts showed that the overall stem lesion length for plants that were 46 days old was significantly less than plants that were 32 and 39 days old ($P < 0.05$), while there was no significant difference between 32 and 39 days old ($P < 0.05$).

Discussion

The estimated amount of seed treatments employed on commercial soybean cultivars subsequently grown by soybean farmers has increased greatly from when it was at 8% in 1996 to

30% in 2008 (Munkvold, 2009). Based on soybean cultivars entered into the UISVT from 2005-2014, the percentage of soybean cultivars entered into the UISVT with fungicide seed treatments increased from 66% to 92% from 2005 to 2014. This exemplifies not only the overall increase in use of soybean seed treatments but also the importance of seed treatments in commercial soybean companies. The likely reason for the differences between the 30% estimate for 2008 (Munkvold, 2009) and the 66% estimate for 2005 in the present study is the survey population. Since this study gathered data from a very specific niche of soybean cultivars, the estimate could be relatively skewed as commercial soybean cultivars most likely benefit from seed treatments when entered into the UISVT program. The global seed treatment market saw a doubling in value between 2002 and 2008 (Munkvold, 2009) and was estimated to be worth more than \$2.43 billion dollars in 2011 and estimated to be \$4.45 billion by 2018 (Transparency Market Research, 2013). This increase is largely due to the increased value of seed as an agricultural input, heightening the need for growers to protect their investments (Munkvold, 2009).

While fungicide seed treatments had a significant effect on emergence of soybean seeds infested with *S. sclerotiorum*, results from my study do not support the hypothesis that soybean seed treatments with fungicide components have a lasting effect on plant tissue colonization by *S. sclerotiorum* after emergence. Results from a previous study showed that fungicide seed treatments can increase germination of soybean seeds infected by *S. sclerotiorum* as treatments that included fludioxonil and/or one that included captan + PCNB + thiabendazole + metalaxyl completely inhibited mycelial growth on infected seeds (Manandhar et al., 1999). In my study, fludioxonil provided the best control against *S. sclerotiorum*, which is labeled for control of the pathogen. Penflufen + prothioconazole provided moderate control of *S. sclerotiorum* but still had <50% seed germination. The active fungicide ingredient in a soybean seed treatment will

determine how effectively seed-borne *S. sclerotiorum* is controlled or how well soybean seeds grown in *S. sclerotiorum* infested fields germinate.

The residual activity of fungicide components of seed treatments has not been extensively studied with regards to protection against *S. sclerotiorum* though the longevity of residual activity of insecticides has been shown in soybean. Research shows that an insecticide component of treated soybean seed provided protection against soybean aphids (*Aphis glycines*) for up to 60 days after planting (Pedersen and Lang, 2006). The active ingredients of these insecticides are systemic, meaning they are absorbed by and translocated throughout the plant. Fungicide components of seed treatments can be either systemic or non-systemic (contact). Of the active ingredients used in this study, fludioxonil, is the only contact fungicide with some translaminar movement. Metalaxyl (Oomycota), penflufen and prothioconazole are all systemic fungicides (Giesler and Ziem, 2008). Trifloxystrobin has low translaminar movement and is not systemic (Bartlett et al., 2002). The residual activity of fungicide active ingredients can vary depending on the environment, seedling development, and the target pathogen. From the experiment conducted in the present study with 18, 25, and 32 day old plants, separation between seed treatments occurred at 18 DAP and there were no significant differences between fungicide seed treatments at 25 and 32 DAP.

The results from the 7 and 14 DAP experiment did not show any variation between fungicide treatments or cultivars in the survival of *S. sclerotiorum* inoculated seedlings. A previous study showed that soybean genotypes could be evaluated for resistance to *S. sclerotiorum* by inoculating 10-14 days old seedlings (Manandhar et al., 1999). While there were no differences in fungicide treatments detected in this experiment, it is possible that other

inoculation methods could have proven more consistent such as a modification of the cotton pad method (Bastien et al., 2012), although that was not used in my experiment.

While the experiments conducted on soybean 32, 39, and 46 days old did not yield any consistent differences in lesion length or AUDPC for seed treatment, it is interesting to note the decrease in lesion length in plants that were 46 days old. This decrease in disease severity caused by *S. sclerotiorum* with plant age has been documented before in soybean. Chun et al. (1987) found that soybean plants that were 6 and 7 week old had overall decreased lesion lengths compared to soybean that were 5 weeks old or younger. A later study found the opposite to be true when comparing soybean plants inoculated with *S. sclerotiorum* that were 5, 6, and 7 weeks old (Vuong et al., 2004), who found that soybean that were 6 and 7 weeks old had overall longer lesion lengths compared to plants which were 5 weeks old (Vuong et al., 2004). The current study found a decrease in lesion length in soybean plants that were 46 days old (almost 7 weeks) over both groups of younger soybean.

The use of fungicide seed treatments in soybean cultivars entered into the UISVT has increased considerably over the years. Furthermore, the inclusion of insecticide and fungicides in seed treatments has increased since 2005. Research has found that drift of seed treatment powder onto neighboring flowering plants can harm pollinators, including the honeybee (*Aphis mellifera*) (Alburaki et al., 2015). The utility of seed treatments from an integrated pest management standpoint is questioned as well. Regardless, the industry in the United States increasingly markets the benefits of seed treatments and includes them on cultivars included in the UISVT. The overwhelming deployment of seed treatments by farmers is an extra insurance to ensure and maintain soybean stands (United Soybean Board, 2014). Fungicide chemistry continues to improve, keeping the fungicides included in seed treatments ever-evolving. While

the results from this study were unable to discern seed treatment effects on seedlings, there were differences in fungicide seed treatments when plants were 18 days old. There is hope that new active ingredients for seed treatments will provide more systemic protection against pests and pathogens. These kinds of improvements in seed treatments could alter management options for early, mid, and late-season diseases.

Acknowledgements. Thanks to Nathan Karplus at Bayer in White Heath, Illinois for helping me to treat my soybean seeds for my *Sclerotinia* stem rot experiments.

Literature Cited

- Alburaki, M., Boutin, S., Mercier, P.-L., Loublier, Y., Chagnon, M., Derome, N. 2015. Neonicotinoid-coated *Zea mays* seeds indirectly affect honeybee performance and pathogen susceptibility in field trials. PLoS ONE 10: e0125790. doi:10.1371/ journal.pone.0125790.
- Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., Parr-Dobrzanski, B. 2002. The strobilurin fungicides. Pest Management Science 58:649-662.
- Bastien, M., Thanh-Huynh, T., Giroux, G., Iqura, E., Rioux, S., Belzile, F. 2012. A reproducible assay for measuring partial resistance to *Sclerotinia sclerotiorum* in soybean. Canadian Journal of Plant Science 92:279-288.
- Chawla, S., Bowen, C.R., Slaminko, T.L., Hobbs, H.A., Hartman, G.L. 2013. A public program to evaluate commercial soybean cultivars for pathogen and pest resistance. Plant Disease 97:568-578.
- Chun, D., Kao, L.B., Lockwood, J.L., Isleib, T.G. 1987. Laboratory and field assessment of resistance in soybean to stem rot caused by *Sclerotinia sclerotiorum*. Plant Disease 71:811-815.
- Fehr, W.R., Caviness, C.F., Burmood, D.T., Pennington, J.S., 1971. Stage of development descriptions for soybean, *Glycine max* (L.) Merrill. Crop Science 11:929-931.
- Giesler, L.J., Ziem, A.D. 2008. Seed treatment fungicides for soybean. University of Nebraska-Lincoln Extension. <http://www.ianrpubs.unl.edu/live/g1852/build/g1852.pdf>.
- Grau, C.R., G.L. Hartman. 2015. Sclerotinia stem rot, pp. 59 – 62. In: *Compendium of Soybean Diseases and Pests*. Hartman, G.L., Rupe, J.C., Sikora, E.F., Domier, L.L., Davis, J.A. and Steffey, K.L. (eds.). St. Paul: American Phytopathological Society.
- Hartman, G.L., Kull, L., Huang, Y.H. 1998. Occurrence of *Sclerotinia sclerotiorum* in soybean fields in east-central Illinois and enumeration of inocula in soybean seed lots. Plant Disease 82:560-564.
- Kim, H.S., Diers, B.W. 2000. Inheritance of partial resistance to Sclerotinia stem rot in soybean. Crop Science 40:55-61.
- Koenning, S., J. Wrather. 2010. Suppression of soybean yield potential in the continental United States by plant disease from 2006 to 2009. Plant Health Progress. doi:10.1094/PHP-2010-1122-01-RS.
- Koga, L., Bowen, C., Godoy, C., de Oliveira, M., and Hartman, G. 2014. Mycelial compatibility and aggressiveness of *Sclerotinia sclerotiorum* isolates from Brazil and the United States. Pesquisa Agropecuaria Brasileira 49:265-272.

Link, H.V., Johnson, K.B. 2007. White Mold. The Plant Health Instructor DOI: 10.1094/PHI-I-2007-0809-01.

Manandhar, J.B., Kull, L.S., Mueller, D.S., Hartman, G.L., Pedersen, W.L. 1999. *Sclerotinia sclerotiorum* in soybean: pathogenic variability, host resistance, and seed infection. Nelson, B.D. and Gulya, T.J. (eds.) Proceedings of the 1998 International Sclerotinia Workshop, Fargo, ND 9-12 Sept. 1998. pp. 36-37.

Mueller, D.S., Hartman, G.L., and Pedersen, W.L. 1999. Development of sclerotia and apothecia of *Sclerotinia sclerotiorum* from infected soybean seed and its control by fungicide seed treatment. Plant Disease 83:1113-1115.

Mueller, D.S., Wise, K.A., Tylka, G.L., Dufault, N. 2015. Management through pesticides. pp. 171-1723. In: *Compendium of Soybean Diseases and Pests*. Hartman, G.L., Rupe, J.C., Sikora, E.F., Domier, L.L., Davis, J.A. and Steffey, K.L. (eds.). St. Paul: American Phytopathological Society.

Munkvold, G. 2009. Seed pathology progress in academia and industry. Annual Review of Phytopathology 47:285-311.

Pederson, P., Lang, B. 2006. Use of insecticide seed treatments for managing soybean aphids. Iowa State Extension Integrated Crop Management Newsletter-496.
<http://www.ipm.iastate.edu/ipm/icm/2006/1-23/seedtreat.html>.

Peltier, A.J., Bradley, C.A., Chilvers, M.I., Malvick, D.K., Mueller, D.S., Wise, K.A., Esker, P.D. 2012. Biology, yield loss, and control of *Sclerotinia* stem rot of soybean. Journal of Integrated Pest Management doi: <http://dx.doi.org/10.1603/IPM11033>.

Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Proc. 23rd SAS Users Group Intl., SAS Institute, Cary, NC, pp 1243-1246.

Shaner, G., and R. Finney. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.

Transparency Market Research. 2013. "Seed treatments (insecticides, fungicides, other chemical and nonchemical treatment) market for corn, soybean, wheat, canola, cotton, and others- global industry analysis, size, share, growth forecast, 2012-2018".
<http://www.transparencymarketresearch.com/seed-treatment-market.html>.

United Soybean Board. 2014. Six things farmers should know about seed treatments.
<http://unitedsoybean.org/article/six-things-farmers-should-know-about-seed-treatments/>.

Vuong, T.D., Hoffman, D.D., Diers, B.W., Miller, J.F., Steadman, J.R., Hartman, G.L. 2004. Evaluation of soybean, dry bean, and sunflower for resistance to *Sclerotinia sclerotiorum*. Crop Science 44:777-783.

Vuong, T.D., Diers, B.W., Hartman, G.L. 2008. Identification of QTL for resistance to *Sclerotinia* stem rot in soybean plant introduction 194639. *Crop Science* 48:2209-2214.

Wrather, J.A., Koenning, S.R. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. Online. *Plant Health Progress* doi:10.1094/PHP-2009-0401-01-RS.

Zhao, X., Han, Y., Li, Y., Liu, D., Sun, M., Zhao, Y., Lv, C., Li, D., Yang, C.Z., Huang, L., Teng, W., Qiu, L., Zheng, H., Li, W. 2015. Loci and candidate gene identification for resistance to *Sclerotinia sclerotiorum* in soybean (*Glycine max* L. Merr.) via association and linkage maps. *The Plant Journal* 82:245-255.

Tables and Figures

Table 2.1. Active ingredients and activity of four fungicide seed treatments included in this study to determine residual effects on soybean colonization of *Sclerotinia sclerotiorum* from germination to R2.

Active Ingredient	Active Against/Activity	Rate (mg ai / g seed)
Fludioxonil ^a	<i>Fusarium</i> , <i>Rhizoctonia</i> and seed-borne <i>Phomopsis</i> and <i>S. sclerotiorum</i>	0.025
Mefoxonam-M and S isomer ^a	<i>Pythium</i> spp. and <i>Phytophthora sojae</i>	0.0375
Thiamethoxam ^a	Insecticide	0.5
Trifloxystrobin ^b	<i>Rhizoctonia solani</i> , <i>Fusarium</i> spp., <i>Aspergillus</i> spp., <i>Penicillium</i> spp., <i>Alternaria</i> spp. and <i>Phomopsis</i> spp.	0.0445
<i>Bacillus pumilis</i> ^b	Biological agent activated Induced Systemic Resistance	0.000175
Metalaxyl ^b	<i>Pythium</i> spp. and <i>Phytophthora sojae</i>	0.1331
Imidacloprid ^b	Insecticide	0.4870
Saponins ^c	Induce Systemic Acquired Resistance (SAR)	0.001
Prothioconazole ^d	<i>Fusarium</i> , <i>Phomopsis</i>	0.0449
Penflufen ^d	<i>Rhizoctonia</i> , <i>Fusarium</i>	0.0224
Metalaxyl ^d	<i>Pythium</i> , <i>Phytophthora</i>	0.0359

^a Active ingredient in formulation for Cruiser Maxx (Syngenta).

^b Active ingredient in formulation for Trilex 6000 (Bayer Crop Science).

^c Active ingredient in Heads Up (Plant Protectants Inc.).

^d Active ingredient in formulation for EverGol Energy (Bayer Crop Science).

Table 2.2. Percent emergence of soybean seedlings that were either not treated or treated with seed applied fungicides and then inoculated with a potato dextrose agar plug infested with *Sclerotinia sclerotiorum*, and a chi-square test to compare the germination rate from the control.

Seed treatment	Germination (%)	Standard deviation	Chi-Square calculated ^a
Fludioxonil	100	0	138.67*
Penflufen + prothioconazole	36.4	0.323	46.22*
Trifloxystrobin + saponins	8.3	0.29	11.56
Trifloxystrobin	0	0	0
Control	0	0	0

^a Chi-square values significant ($P \leq 0.05$) from Chi-square critical value (15.51) indicated by “*”.

Table 2.3. Kruskal-Wallis test for differences in the median of the survival of soybean grown from fungicide treated or non-treated seed inoculated at 7 or 14 days after planting (DAP).

Kruskal-Wallis	14 DAP	7 DAP
Chi-Square	3.20	1.80
Df ^a	4	4
<i>P</i> ^b	0.53	0.77

^a Included a non-treated control, Cruiser Maxx (Syngenta), Trilex 6000 (Bayer Crop Science), Trilex 6000 with Heads Up (Plant Protectants Inc.), and EverGol Energy (Bayer Crop Science). This analysis was conducted over all cultivars included in the study: DSR2400 (Dairyland Seed), IP2991 (Prairie Hybrids), Resnik (Public), and Fairbault (public).

^b Analysis was conducted with the square root transformed percent survival data that were collected 10 days after inoculation. ($P \leq 0.05$)

Table 2.4. The mean survival percentage and standard deviation of soybean grown from fungicide treated or untreated seed that were inoculated with *Sclerotinia sclerotiorum* at 7 or 14 days after planting.

Seed treatment ^a	Day after planting			
	14		7	
	\bar{x}^b	<i>s</i>	\bar{x}	<i>s</i>
Fludioxonil	55.0	0.53	48.7	0.41
Penflufen + prothioconazole	41.6	0.46	47.3	0.45
Trifloxystrobin + saponins	50.6	0.47	58.9	0.37
Trifloxystrobin	62.6	0.46	57.5	0.42
Control	55.0	0.53	48.7	0.41

^a Percent survival data taken ten days after inoculation were analyzed over all cultivars included (DSR2400, IP2991, Resnik, and Fairbault) using a chi-square test of homogeneity which showed that fludioxonil and penflufen + prothioconazole were significantly ($P \leq 0.05$) different from the other seed treatments.

^b Means were estimated in SAS using Proc Means using arcsine transformed data which were used in the ANOVA for this experiment. The means shown are the backwards transformed means with the associated standard deviation for each seed treatment x day after planting combination.

Table 2.5. Analysis of variance for the effects of soybean seed treatment on lesion length of four soybean cultivars at different growth stages grown from seeds with different seed treatments. Lesion lengths were measured 10 days after inoculation with *Sclerotinia sclerotiorum*. This data was used analyzed in the ANOVA.

Source of variation	df	F ^a
Day after planting (DAP) ^b	2	34.38*
Fungicide seed treatment (T) ^c	4	1.3
DAP x T	8	2.59*
Cultivar (C) ^d	3	89.57*
DAP x C	6	4.63*
T x C	12	1.81*
DAP x T x C	24	0.95
Replication	4	14.3*
Error	1114	.

^a The experiment was carried out as a three-factor factorial in an RCBD blocked by replication with six replications. Significant model factors are indicated by a * ($P \leq 0.05$).

^b There were three levels of days after planting in this study including 18, 25, and 32 DAP.

^c The five levels of seed treatment included in this study were a non-treated control, fludioxonil, trifloxystrobin, trifloxystrobin + saponins, and penflufen + prothioconazole.

^d The four levels of cultivar included in these experiments were DSR2400 (Dairyland Seed), IP2991 (Prairie Hybrids), Resnik (Public), and Fairbault (public).

Table 2.6. Analysis of variance for the effects of fungicide seed treatments on the area under disease progress curve (AUDPC) in soybean inoculated at 32, 39, and 46 days after planting with *Sclerotinia sclerotiorum*. Stem lesion lengths were measured at 7, 14, and 21 days to generate an AUDPC.

Source of variation	df	F ^a
Day after planting (DAP) ^b	2	11.87*
Seed treatment (T) ^c	4	1.07
DAP x T	8	0.98
Cultivar (C) ^d	1	4.01*
DAP * C	2	3.67*
C x T	4	2.76*
DAP x C x T	8	1.85
Block (B)	2	10.6*
Rep (B)	3	1.25
Residual	145	.

^a The experiment was carried out as a three-factor factorial in an RCBD with two replications per block and three blocks total. Significant model factors are indicated by a * ($P \leq 0.05$).

^b Days after planting included in this study were 32, 39, and 46.

^c The five levels of seed treatment included in this study were a non-treated control, fludioxonil, trifloxystrobin, trifloxystrobin + saponins, and penflufen + prothioconazole.

^d The two levels of cultivar included in these experiments were Resnik (Public), and Fairbault (public).

Table 2.7. Analysis of variance for the effects of fungicide seed treatments on stem lesion length of plants inoculated with *Sclerotinia sclerotiorum* at 32, 39, and 46 days after planting (DAP). Stem lesion lengths were measured at 21 days after planting.

Source of variation	df	F ^a
Day after planting (DAP) ^b	2	26.11*
Seed treatment (T) ^c	4	1.97
DAP x T	8	2.5*
Cultivar (C) ^d	1	19.48*
DAP x C	2	1.12
T x C	4	5.45*
DAP x T x C	8	3.32*
Block	2	3.77
Replication(Block)	3	3.15*
Residual	781	.

^a The experiment was carried out as a three-factor factorial in an RCBD with two replications per block and three blocks total. Significant model factors are indicated by a * ($P \leq 0.05$).

^b The three levels of day after planting included in this experiment were 32, 39, and 46.

^c The five levels of seed treatment included in this study were a non-treated control, fludioxonil, trifloxystrobin, trifloxystrobin + saponins, and penflufen + prothioconazole.

^d The two levels of cultivar included in these experiments were Resnik (Public), and Fairbault (public).

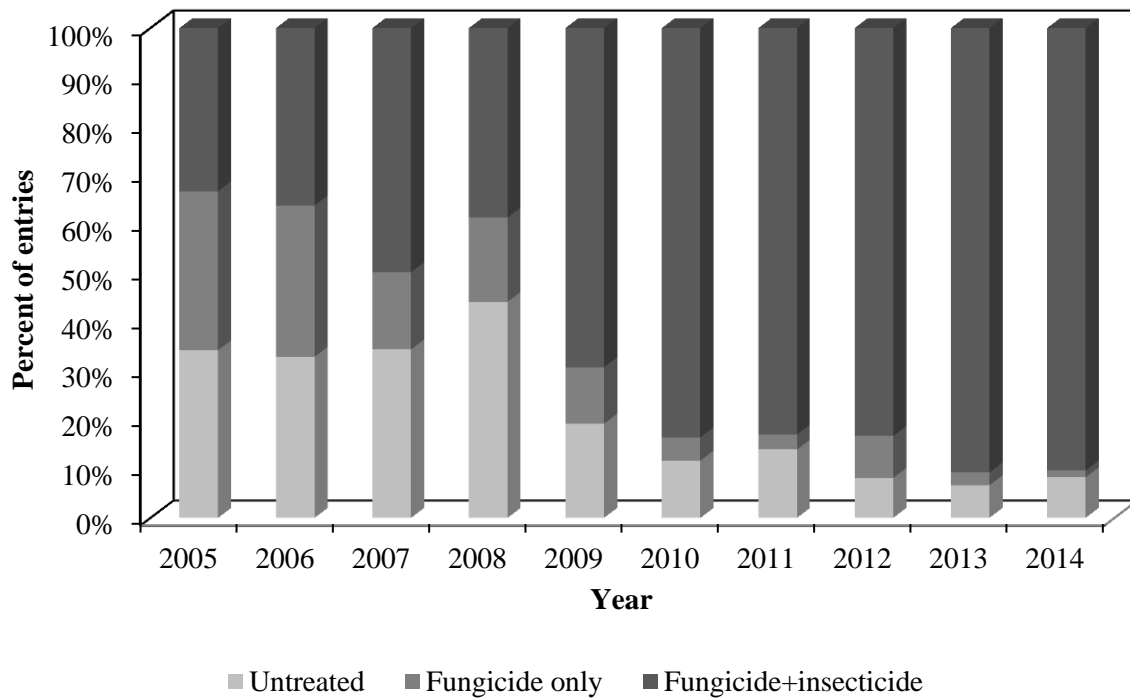


Fig. 2.1. The percent of soybean seed treatments with fungicide components in commercial cultivars based on soybean cultivars entered into the University of Illinois Soybean Variety Testing Program (UISVT) from 2005-2013. For each year from 2005 to 2014, $n = 767, 686, 601, 617, 649, 588, 515, 514, 345,$ and 289 , respectively.

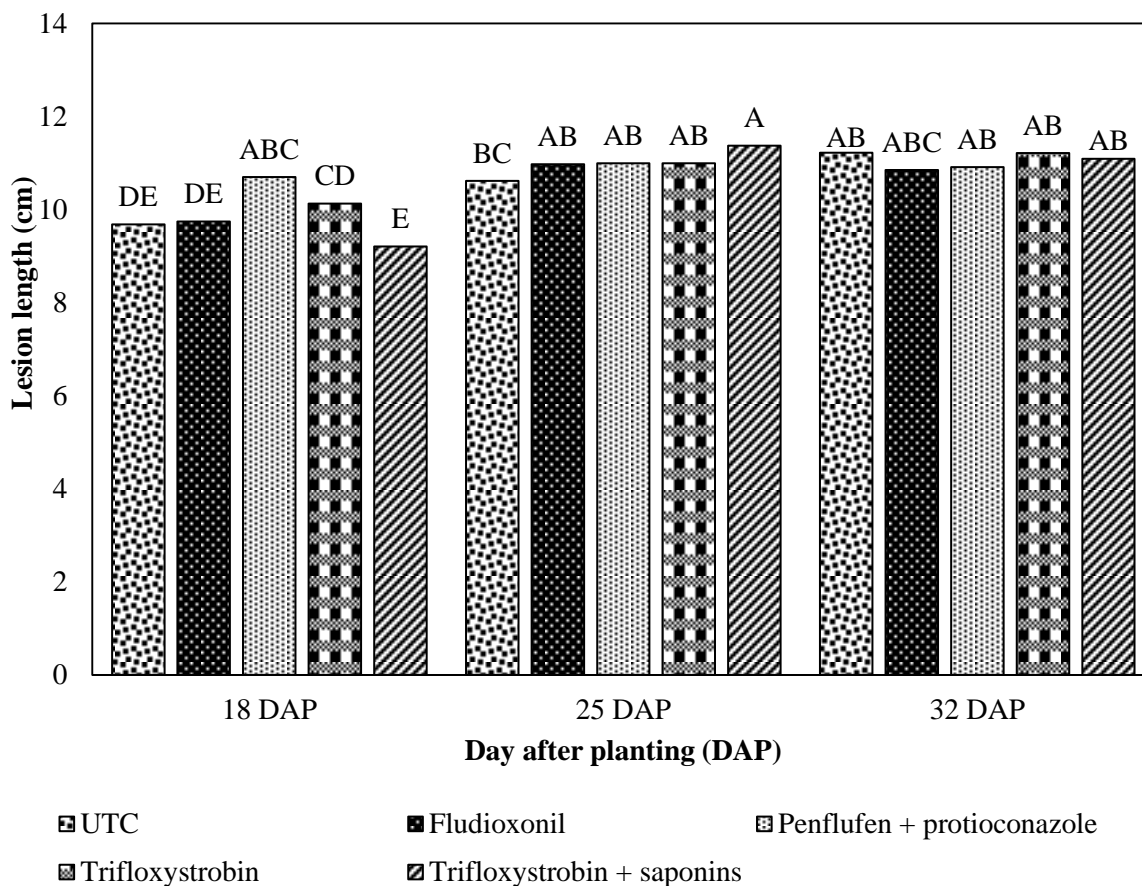


Fig. 2.2. Least square mean estimates^a for lesion lengths between seed treatments and day after planting (DAP) when stems were inoculated with *Sclerotinia sclerotiorum*. Least square mean estimates that are significantly different from one another are indicated by letter groupings ($P \leq 0.05$).

^a Means were separated using the pdmix800 macro in SAS (Saxton, 1998) ($P \leq 0.05$). This experiment was conducted as a factorial in an RCBD with three factors, DAP, seed treatment, and cultivar. Soybean were inoculated using the cut-stem method and the stem lesion lengths were measure at 10 days after inoculation.

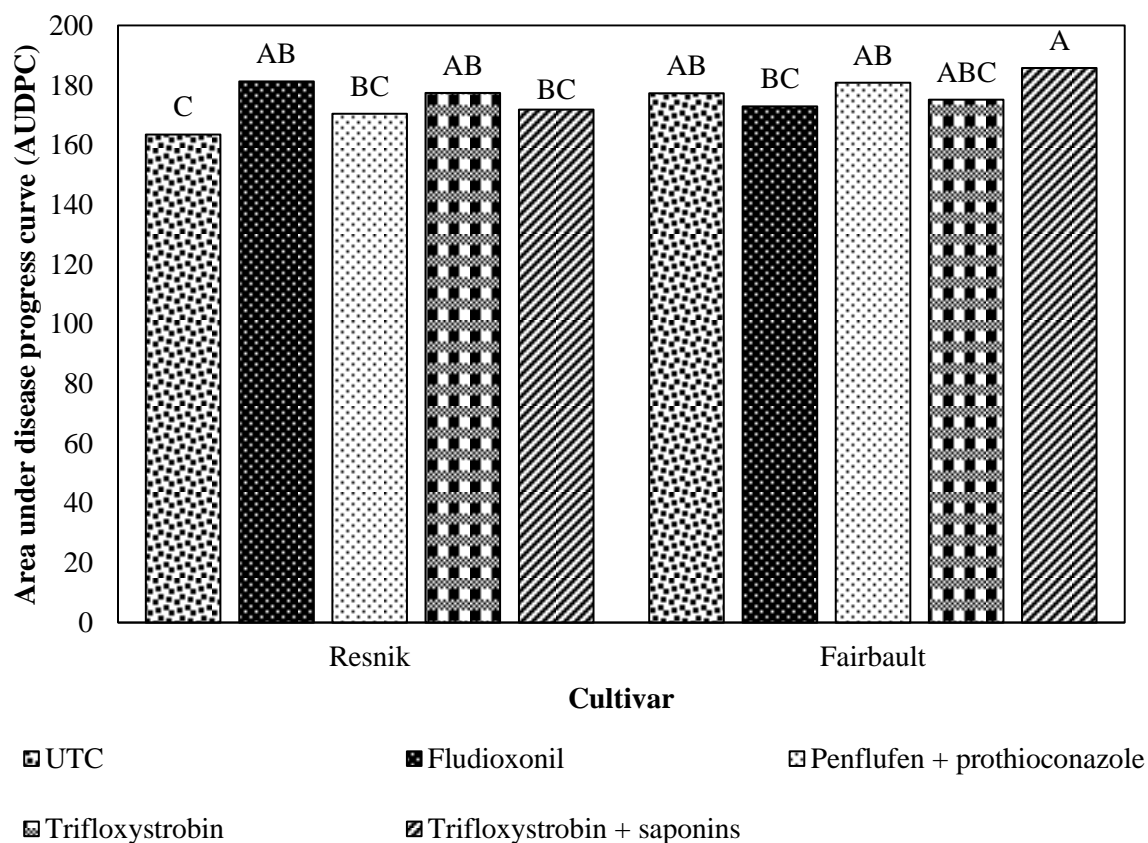


Fig. 2.3. Least square means estimates^a of area under disease progress curve (AUDPC) from two soybean cultivars that were either treated or not treated with a seed fungicide and inoculated with *Sclerotinia sclerotiorum* 32, 39, and 46 days after planting with lesions measured at 7, 14, and 21 days after inoculation. Different letters indicate least square means that differ ($P \leq 0.05$).

^a Means were separated using the pdmix800 macro in SAS (Saxton, 1998) ($P \leq 0.05$). This experiment was conducted as a factorial in an RCBD with three factors, DAP, seed treatment, and cultivar.

CHAPTER 3

Leaf and seed isolates of *Cercospora kikuchii* from soybean differ in intergenic spacer region sequence but not in disease severity on selected soybean genotypes

Abstract

Cercospora leaf blight (CLB) and purple seed stain (PSS) caused by *Cercospora kikuchii* are important diseases of soybean worldwide. While there are no commercially available cultivars advertised for resistance to PSS or CLB, sources of resistance have been reported in plant introductions or older public soybean cultivars. In this study, nine public soybean cultivars with varying resistance or susceptibility to CLB, PSS, and frogeye leaf spot (*Cercospora sojina*) were screened for differences in CLB disease severity. Soybean plants were inoculated with two different *Cercospora* isolates that were isolated from soybean seeds symptomatic of PSS and leaves symptomatic of CLB in Illinois. The intergenic spacer (IGS) region of these isolates were sequenced and compared to previously published IGS sequences of *C. kikuchii* isolates. Bioassays for differences in disease severity showed no significant differences between leaf and seed *C. kikuchii* isolates though significant differences were observed between cultivars included in the study. Soybean cultivar Mejiro (PI80837) had the highest disease rating overall. Comparison of the IGS sequences from *Cercospora* isolates showed differences in the sequence that followed the previously published differences defining three haplotypes of the fungus. This indicates that *C. kikuchii* isolates in Illinois vary genetically from those collected in the southern United States.

Introduction

Cercospora leaf blight (CLB) and purple seed stain (PSS) of soybean [*Glycine max* (L.) Merr.] are caused by *Cercospora kikuchii* (Tak. Matsumoto & Tomsoy.) M.W. Gardner. Both diseases are important worldwide. Yield losses of up to 30 to 50% have been attributed to CLB. CLB is also known as the most important disease the Gulf South United States in terms of yield losses and management costs (Ward-Gauthier et al., 2015). CLB symptoms often are first observed on petioles in the early reproductive stages with foliar symptoms becoming apparent after growth stage R5. In severe cases, soybean foliage will appear as purple to bronze on the upper leaves, while small and discrete blotches will occur in less severe cases (Ward-Gauthier et al., 2015). PSS symptoms appear as purple blotches on the seed coat that range in size from small, irregular spots to entire coverage of the seed coat, which can reduce seed quality and plant stands (Ward-Gauthier et al., 2015).

Both diseases may be difficult to manage although fungicides and host resistance have been investigated (Price et al., 2015). The fungus is known to infect plants during the early vegetative stages and remains latent for a period of time while the plants are asymptomatic until the reproductive growth stages (Chanda et al., 2014). To investigate this early infection, a qPCR assay specific to *C. kikuchii* has been used to detect the presence and quantity of the pathogen in field studies prior to fungicide application (Chanda et al., 2014). Recently identified resistance to quinone outside inhibitor (QoI) fungicides in *C. kikuchii* isolates from Louisiana presents another CLB management obstacle (Price et al., 2015).

Although no sexual stage has been characterized for *C. kikuchii*, a recent study found genetic variation among *C. kikuchii* isolates, and sequencing results of the inter genetic spacer (IGS) region showed random mating occurred in different haplotypes, suggesting that sexual

recombination may occur in some *C. kikuchii* isolates or alternatively, some form of parasexual reproduction functions in this species (Cai and Schneider 2008).

Although sources of resistance including plant introduction and older cultivars have been reported to have resistance to one or the other disease, there are no private cultivars that show or have been advertised with resistance to *C. kikuchii* (Gould, 2014). In the past, separate studies screened for resistance to CLB and PSS, though each study used only one *C. kikuchii* isolate isolated from a soybean seed that was symptomatic of PSS (Orth and Schuh, 1994) or did not state the source of the isolate used in disease screening (Walters, 1980). Out of 17 soybean cultivars evaluated for resistance to CLB, the cultivar Hobbit was found to be the most resistant and the cultivar Hack was the most susceptible (Orth and Schuh, 1994). In addition, the cultivar Tracy was found to be resistant to both CLB and PSS (Walters, 1980).

The objectives of this study were to (i) examine the genetic relationship of the isolated fungi compared to previously published findings (Cai and Schneider, 2008) and (ii) determine if fungal isolates causing CLB and PSS differentially infected soybean cultivars shown to have resistance or susceptibility to CLB, PSS, and/or frog-eye leaf spot.

Materials and Methods

Isolation and molecular identification of pathogens. Symptomatic seeds of Williams 82 harvested in 2012 were surface disinfested in a 20% bleach solution for 1 minute with two subsequent sterile water rinses and placed onto acidified potato dextrose agar (PDA). Once the fungus began to colonize the agar, it was subcultured and maintained on PDA at 24°C in ambient light. This isolate was named PSS1 (Fig. 3.1a).

One leaf isolate of CLB was collected in September of 2014. Symptomatic soybean leaves obtained from field experiments in Urbana, Illinois were rubbed onto water agar to release spores which were observed through a compound microscope (100x). Single spores were transferred to acidified PDA plates using a hypodermic needle. One spore produced a colony which was identified as CLB1 (Fig. 3.1b). This colony was transferred to and maintained on PDA in the same conditions described above.

DNAs were extracted from 7 mm diameter punches collected at the edge of actively growing colonies of each isolate using the FastDNA Spin Kit (MP Biomedicals, Santa Ana, California). These DNAs were included in a quantitative polymerase chain reaction (qPCR) experiment using previously reported primers (Chanda et al., 2014) designed to amplify the NADPH-dependent oxidoreductase gene, a gene in the cercosporin biosynthetic pathway (*CTB6*). A total of 10 qPCR reactions were set up with two duplicates for each of the following: PSS1, CLB1, no template control, and a high (1 ng) and low (10 pg) concentration standard of *C. kikuchii* DNA. Each reaction had a total volume of 25 µl including 5 µl of template DNA Platinum SYBR Green qPCR SuperMix (1x) (Invitrogen), “CkCTBY-2F” and “CkCTB6-2R” (forward and reverse primers) (666 nM each), ROX passive Reference Dye (50 nM), bovine serum albumin (BSA) (400 ng/µl), and ultrapure water. SYBR Green qPCR SuperMix was used in this assay instead of the FAM probe “CkCTB6-PbFAM” (Chanda et al., 2014) to allow for better amplification. The cycle for the qPCR assay was as follows: hold 120 s at 90°C, 40 cycles of 15 s at 95°C and 45 s at 60°C, 60 s at 95°C followed by a dissociation curve from 55°C to 95°C. This assay was conducted using a Stratogene Mx3005p qPCR machine (Aligent Technologies, Santa Clara, California).

Sequencing of IGS region. The IGS region of the *Cercospora* isolates were selected for sequencing. Primers were designed to aid with amplification and sequencing specific accessions listed in GeneBank (Cai and Schneider, 2008) and fabricated by Invitrogen (Life Technologies, Thermo Fischer Scientific Brand, Waltham, Massachusetts)(Table 3.1). One forward primer, two internal primers for sequencing (one forward and one reverse), and two reverse primers were designed. Two reverse primers accounted for single nucleotide polymorphisms which were present at the end of published IGS sequences (Cai and Schneider, 2008). For PCR amplification, 2 µl of extracted DNAs were used for each reaction which included 0.5 µM of the forward primer, 0.25 µM of each reverse primer, 1x High-Fidelity Phusion enzyme (New England Biolabs, Inc., Ipswich, Massachusetts), and super pure water to make each reaction 23µl. The PCR reaction was conducted in PTC-100 programmable thermal controller thermocycler (MJ Research, Inc., Quebec, Canada) with the following cycle: hold 120 s at 98°C, 35 cycles of 15 s at 98°C and 60 s at 72°C, with a final extension of 10 minutes at 72°C and a final hold with an indefinite time period at 6°C. The final hold ended whenever the PCR product was removed from the machine.

DNA fragments were confirmed before sequencing using gel electrophoresis. PCR products were loaded into designated lanes of a 1% agarose gel with loading buffer. To identify fragment length, DNA ladder F-303SD was loaded in one lane. The gel ran for one hour at 80 volts. Bands were stained using GelRed (Biotium, Hayward, California) and visualized using ultraviolet light.

Raw PCR products were purified using the QIA Quick PCR Purification Kit (Qiagen, Valencia, California). After DNA was purified, it was quantified using a NanoDrop (Thermo Scientific, Waltham, Massachusetts) spectrophotometer to ensure an adequate quantity of PCR

product before sequencing. DNAs and primers were submitted to the Roy J. Carver Biotechnology Center at the University of Illinois for sequencing. Before analysis, sequences were trimmed and aligned using Clustal 2.1 multiple sequence alignment tool (Bioinformatics, Oxford, England) and analyzed. Sequences from isolates in this study were also aligned with the IGS sequences published in GenBank for *C. kikuchii* (Cai and Schneider, 2008) (GenBank accession numbers DQ178942 to DQ178957) for overall comparison and in attempt to determine haplotypes of the isolates.

Assay of reaction to infection by different isolates of CLB and PSS. Nine soybean cultivars were selected based on reactions to inoculation with *C. kikuchii* isolates or *Rcs* genes for resistance to frogeye leaf spot (Table 3.2) (Walters, 1980; Walters, 1984; Orth and Schuh, 1994; Mengistu et al, 2012). Two seeds of each cultivar were planted in 18-cell inserts filled with LC1 Sunshine Mix. Soybean were grown for three weeks prior to initial inoculation in a greenhouse room with a 13-hour photoperiod maintained at daytime and nighttime temperatures of 23-27°C and 18.5-22.5°C, respectively. Inoculum preparation began one week before inoculation by adding 10-12 plugs of fungus-infested agar to 250 ml flasks containing 100 ml of potato dextrose broth. Liquid cultures were then placed in a shaker at room temperature with ambient lighting. Mycelial fragments were used because of the difficulty in producing conidia.

Liquid mycelial grown cultures of each isolate were blended with 100 ml of sterile distilled water in a Waring blender at a low speed for 30 seconds twice with a 15 second rest between 15 seconds of rest and then 30 more seconds at low for each isolate. Hyphal fragment suspensions were transferred to spray bottles before colony forming unit (CFU) quantification. CFUs were quantified by placing 10 µl of the hyphal fragment suspension into a drop of lactophenol cotton blue dye in a 60 x 15 mm polystyrene petri dish and subsequently counted.

Hyphal fragments suspensions were adjusted to 100,000 CFUs per ml by adding sterile distilled water. The inoculum suspensions and water control were amended with Tween80 to a 0.01% concentration. After inoculum preparation was complete, all parts of the soybean plants were sprayed with inoculum until runoff with corresponding inoculum sources for each treatment. After the inoculum was applied, plants were incubated in a warm (26-28°C) and high humidity (>90%) environment for 48 hours in the dark. After incubation was complete, plants were moved to the greenhouse bench at the same settings as previously noted. Plants were inoculated twice, one week apart at three and four weeks after planting using the same inoculum prep and application methods.

Symptoms were assessed at 14 and 21 days after the initial inoculation using the following rating scale from Chanda et al. (2014): 1 = no symptoms, 2 = few symptoms on petioles with up to 10% of the petioles showing symptoms, 3 = up to 10% of petioles and 5% of leaf area showing symptoms, including leaf veins, 4 = up to 10% of petioles and leaf area affected, including leaf veins, 5 = approximately 50% of petioles and 30% of leaves with symptoms, including leaf veins, 6 = up to 100% of petioles affected but leaves do not have a reddish cast and are not chlorotic, 7 = up to 20% of upper leaves becoming reddened or chlorotic, 8 = moderate chlorosis, reddening, and necrosis on up to 50% of upper leaves, 9 = moderate chlorosis, reddening, and necrosis on up to 50% of upper leaves, symptoms are severe with some defoliation.

The experiment was planted in a split-plot design with isolate as the whole plot factor and replication as the subplot factor, with two replicate plantings of each of the nine selected cultivars (Table 3.2) planted per trial. This experiment was conducted in four trials with three repeats and the homogeneity of the variance of the trials was checked in Proc Glimmix before

combining data for analysis. The normality of residuals was verified using Proc Univariate. The three isolate treatments were water-sprayed control, PSS1, and CLB1. Data analysis was conducted using the data collected 21 days after the initial inoculation using Proc Mixed in SAS 9.4 with the following model:

$$Y_{ijkl} = \mu + B_i + I_j + e1_{(ij)} / R_{(i)k} + e2_{(ijk)} / C_l + CI_{jl} + e3_{(ijkl)}$$

Where B_i was the random effect of the i^{th} block, I_j was the fixed effect of the j^{th} inoculum, $e1_{(ij)}$ was the random error term used to test the effects of block and inoculum, $R_{(i)k}$ was the random effect of the k^{th} replication nested within block, $e2_{(ijk)}$ was the random error term to test the main effect of replication within block, C_l was the fixed effect of the l^{th} cultivar, CI_{jl} was the fixed effect of the interaction between the j^{th} inoculum with the l^{th} cultivar and $e3_{(ijkl)}$ was the random error to test the significance of the main effect of cultivar and interaction between cultivar and inoculum. Mean separations were conducted using the least square means and the pdmix800 macro (Saxton, 1998).

Results

Molecular identification of isolated *Cercospora* isolates. The dissociation curve generated by the qPCR experiment (Fig. 3.2) shows the seed isolate (PSS1) followed the same trend as the 10 ng *C. kikuchii* standard, each producing one amplicon with the same melting temperature of 84.6°C. CLB1 produced two amplicons with different melting temperatures (83.0 and 86.8°C, respectively).

IGS sequence results from isolated *Cercospora* isolates. The IGS region sequences from the two *Cercospora* isolates were between 520-650 bp per isolate. Aligning the sequences showed that there were several sequence differences between CLB1 and PSS1 (Appendix D).

Isolates PSS1 and CLB1 differed from the published IGS sequences (Cai and Schneider, 2008) in only 7 positions. The 7 positions where PSS1 and CLB1 differed are shown in Table 3.3, along with the outlined haplotypes from Louisiana (Cai and Schneider, 2008).

Assay of reaction to infection by different isolates of CLB and PSS. The disease severity analysis from the fungus-inoculated plant assays had a significant ($P \leq 0.05$) main effect of cultivar (Table 3.4). The main effect of isolate and interaction of cultivar with isolate were not significant (Table 3.4) ($P \leq 0.05$). Least square mean estimates for all of the cultivars averaged over all of the isolates are shown in Fig. 3.3. Mejiro was estimated to have the highest disease severity overall (3.3) (Fig. 3.4a), which was significantly greater than Davis, Hack, Tracy, and Hood 75 (Fig. 3.4b), which had the lowest disease severity (2.1) ($P \leq 0.05$) (Fig. 3.3). There were no significant differences in resistance or susceptibility between the cultivars included with varying resistance genes for frog-eye leaf spot ($P \leq 0.05$) (Fig. 3.3).

Discussion

The differences in the IGS sequences found in the *Cercospora* isolates used in this study did not align with previously defined haplotypes for *C. kikuchii* (Cai and Schneider, 2008). This suggests that there are more haplotypes than those outlined by Cai and Schneider (2008) and that the random events of sexual recombination are not exclusive to *C. kikuchii* populations in Louisiana (Cai and Schneider, 2008). Additionally, the results from the qPCR assay showed different amplicons for PSS1 and CLB1. Since the qPCR assay was meant to amplify the *CTB6* gene, which is part of the cercosporin biosynthesis pathway (Chanda et al., 2014), it would be interesting to examine the differences in cercosporin production between these two isolates since

cultural morphology varied between the two; PSS1 turns the growth media purplish-red and CLB1 turns the media yellow (Fig 3.1).

There were no significant differences in disease severity when comparing inoculations with different *Cercospora* isolates as the data shows that there was no difference between the seed isolate and the leaf isolate. This could be an indication that *C. kikuchii* isolated from seeds will not cause different foliar symptoms even if the cultivar being assayed has resistance to the seed disease. Previous research has provided evidence of variability in aggressiveness of *Cercospora kikuchii* related to cercosporin production differences in isolates (Almeida et al., 2005; Cai et al., 2009). I attribute the lack of variability in severity of disease symptoms between isolates to the low number of isolates that were used in my study.

Interestingly, the results from my study conflict with some of the results published about cultivar resistance or susceptibility to CLB. In my study, Mejiro showed the most severe leaf and petiole symptoms of *Cercospora* leaf blight indicating that even though it has a single, dominant gene for resistance to purple seed stain (Jackson et al., 2006; Jackson et al., 2008), it does not confer resistance to CLB. The cultivar Hood 75 had the lowest estimated rating for *Cercospora* leaf blight in my study, but it was listed as a susceptible cultivar in an older study (Walters, 1980). The cultivar Hack was also reported to be susceptible to CLB (Orth and Schuh, 1994), but had significantly less severe symptoms compared to Mejiro and Williams 82 in my study. With no separation in disease severity between cultivars that had resistance genes for frog-eye leaf spot, resistance to *C. sojina* does not appear have an effect on *Cercospora* leaf blight disease severity.

Overall, results from this study supported and expanded the genetic variability that was previously described for *C. kikuchii* (Cai and Schneider, 2008). The increased genetic variation

with the addition of haplotypes shows that genetic recombination in a seemingly asexual fungus occurs in Illinois in addition to the observations from Louisiana. Additionally, my data shows that there are no differences in disease severity between cultivars that are known to be susceptible or resistant Cercospora leaf blight, PSS, or frogeye leaf spot when inoculated with different sources of the causal pathogen. What could be one of the most interesting areas to continue to investigate is where the genetic diversity of this fungus stems from since a sexual stage has never been observed. The potential for this fungus to recombine genes and change in breeding stock are most likely largely to blame for the increase in Cercospora leaf blight disease severity in the United States (Cai and Schneider, 2008).

Acknowledgements. A special thanks to Dr. James Haudenshield for his patience and help in guiding me through the molecular experiments and analyses involved in this chapter.

Literature Cited

- Almeida, A.M.R., Piuga, F.F., Marin, S.R.R., Binneck, E., Sartori, F., Costamilan, L.M., Teixeira, M.R.O., Lopes, M. 2005. Pathogenicity, molecular characterization, and cercosporin content of Brazilian isolates of *C. kikuchii*. *Fitopatologia Brasileira* 30:594-602.
- Cai, G., Schneider, R. W. 2008. Population structure of *Cercospora kikuchii*, the causal agent of *Cercospora* leaf blight and purple seed stain in soybean. *Phytopathology* 98:823-829.
- Cai, G., Schneider, R.W., Padgett, G.B. 2009. Assessment of Lineages of *Cercospora kikuchii* in Louisiana for Aggressiveness and Screening Soybean Cultivars for Resistance to *Cercospora* Leaf Blight. *Plant Disease* 93:868-874.
- Chanda, A.K., Ward, N.A., Robertson, C.L., Chen, Z.-Y., and Schneider, R.W. 2014. Development of a quantitative polymerase chain reaction detection protocol for *Cercospora kikuchii* in soybean leaves and its use for documenting latent infection as affected by fungicide application. *Phytopathology* 104:1118-1124.
- Clustal W and Clustal X version 2.0. 2007. *Bioinformatics* (Oxford, England) 23:2947-8.
- Gould, F. 2014. Researchers seeking *Cercospora*-resistant soybean. Louisiana University Agriculture Center. <http://www.lsuagcenter.com/en/crops_livestock/crops/soybean/Soybean+Grain+Promotion+Board+Reports/Researchers-seeking-Cercosporaresistant-soybean.htm>.
- Jackson, E.W., Fenn, P., and Chen, P. 2006. Inheritance of resistance to purple seed stain caused by *Cercospora kikuchii* in PI 80837 soybean. *Crop Science* 46:1462-1466.
- Jackson, E.W., Feng, C., Fenn, P., Chen, P. 2008. Genetic mapping of resistance to purple seed stain in PI80837 soybean. *The Journal of Heredity* 99:319-322.
- Mengistu, A., Bond, J., Mian, M.A.R., Nelson, R., Shannon, G., Wrather, A. 2012. Resistance to leaf spot in selected soybean accessions in MG I through MG VI. Online. *Plant Health Progress* doi:10.1094/PHP-2012-0521-02-RS.
- Orth, C.E., Schuh, W. 1994. Resistance of 17 soybean cultivars to foliar, latent, and seed infection by *Cercospora kikuchii*. *Plant Disease* 78:661-664.
- Price, P.P., Purvis, M.A., Padgett, G.B., Robertson, C.L., Schneider, R.W., Albu, S. 2015. Fungicide resistance in *Cercospora kikuchii*, a soybean pathogen. *Plant Disease* <http://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS-07-14-0782-RE>.
- Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Proc. 23rd SAS Users Group Intl., SAS Institute, Cary, NC, pp 1243-1246.

Walters, H.J. 1980. Soybean leaf blight caused by *Cercospora kikuchii*. Plant Disease 64:961-962.

Walters, H.J. 1984. Purple seed stain and *Cercospora* leaf blight. World Soybean Research Conference III. pp. 503-506.

Ward-Gautheier, N. A., Schneider, R.W., Chanda, A., Silva, E. C., Price, P. P., III, and Cai, G. 2015. *Cercospora* leaf blight and purple seed stain. pp. 37-40. In: *Compendium of Soybean Diseases and Pests*. (Hartman, G.L., Rupe, J.C., Sikora, E.F., Domier, L.L., Davis, J.A. and Steffey, K.L., eds.). St. Paul: American Phytopathological Society.

Tables and Figures

Table 3.1. Primers designed and fabricated for amplification of IGS region of *Cercospora kikuchii* isolates^a which were sequenced.

Primer name	Tm(°C)	Primer sequence (5'-3')
Ck IGS Fwd	77	TAATTGGTTTTTTGCGGCTGTCCGACCGG
Ck IGS Int1	67	AGCTGGGACATCGCCACGA
Ck IGS Int2	67	TCGTGGCCATGTCCCAGCT
Ck IGS Rev1	75	CACTGGACCCAACACTGGCGGGG
Ck IGS Rev2	76	CACCCGACCCAACACTCCCGGG

^a Primers were designed based on IGS sequences of *Cercospora kikuchii* published by Cai and Schneider (2008).

Table 3.2. Soybean cultivars selected for *Cercospora* leaf blight (CLB) evaluation through infection by different fungal isolates obtained from symptomatic *Cercospora* leaf blight leaves and a purple seed stain (PSS).

Name	Germplasm accession	Release year	MG	Traits selected for	Inoculum used in study	Sources
Hobbit 87	PI546373	1992	III	CLB resistant	Seed isolate of <i>C. kikuchii</i> .	Orth and Schuh, 1994
Hack	PI548569	1986	II	CLB susceptible	Seed isolate of <i>C. kikuchii</i> .	Orth and Schuh, 1994
Davis	PI553039	1966	IV	CLB moderate susceptibility	No note of isolate source	Walters, 1980 ^a
				<i>Rcs3</i> (leaf spot resistance)	<i>C. sojae</i>	Mengistu et al., 2012
Hood 75	PI559371	1976	VI	CLB susceptible, PSS resistant	No note of isolate source	Walters, 1980 ^a
Tracy	PI548983	1975	VI	PSS resistant, CLB resistant	No note of isolate source	Walters, 1980 ^a Walters, 1984
Mejiro	PI80837	1929	IV	PSS resistant	Without supplemental inoculum ^b	Jackson et al., 2006
Lincoln	PI358362	1991	III	<i>Rcs1</i> (leaf spot resistance)	<i>C. sojae</i>	Mengistu et al., 2012
Kent	PI548586		IV	<i>Rcs2</i> (leaf spot resistance)	<i>C. sojae</i>	Mengistu et al., 2012
Williams 82	PI518671	1988	III	overall susceptibility to diseases		

^a Author noted that isolates were gathered from seeds, leaves, stems, and petioles though no note was made of the isolate used in the evaluation of plant materials.

^b Researchers inoculated field experiments with *Phomopsis longicolla* but did not include any supplemental inoculum for *C. kikuchii*, relying on naturally occurring disease pressure.

Table 3.3. Comparison of the 12 differences in the intergenic spacer region (IGS) sequences of *Cercospora kikuchii* haplotypes.

Isolate/ haplotype	1	2	3	4	5	6	7	8	9	10	11	12
PSS1 ^a	..A ^d	CCCCTCGGCTTCACTG					
CLB1 ^b	..GGGGG			
A ^c	..GGGTGCTGCA
B ^c	..GGGCGCGTCA
C ^c	..AAAAGCCCCTCAGCTTGACTACCCTTGG

^a PSS1 was isolated from a soybean seed symptomatic of purple seed stain from seeds grown in Urbana, IL.

^b CLB1 was is a single-spore isolate from soybean leaves symptomatic of Cercospora leaf blight collected in Urbana, IL.

^c These haplotypes were outlined by Cai and Schneider (2008) using IGS sequences from 16 *C. kikuchii* isolates collected from Louisiana.

^d Regions of the sequences that were similar are indicated by and gaps are indicated by -. Sequences from this study were not as long as the sequences used to determine haplotype differences in the previous study, resulting in fewer differences included for PSS1 and CLB1.

Table 3.4. Analysis of variance for disease severity ratings in an experiment that compared nine cultivar reactions to three isolates of *Cercospora kikuchii*. The analysis was conducted on the disease severity data rated 21 days after inoculation on a 1-9 scale^a.

Source of variation	df	F ^b
Block (B)	3	2.41
Isolate (I) ^c	1	0.52
Error 1	3	4.73
Replication within block	4	0.37
Error 2	4	1.42
Cultivar (C) ^d	8	3.98*
I x C	8	0.51
Error 3	217	.

^a Symptoms were assessed at 14 and 21 days after the initial inoculation using the following qualitative rating scale adapted from Chanda et al. (2014): 1 = no symptoms, 2 = few symptoms on petioles with up to 10% of the petioles showing symptoms, 3 = up to 10% of petioles and 5% of leaf area showing symptoms, including leaf veins, 4 = up to 10% of petioles and leaf area affected, including leaf veins, 5 = approximately 50% of petioles and 30% of leaves with symptoms, including leaf veins, 6 = up to 100% of petioles affected but leaves do not have a reddish cast and are not chlorotic, 7 = up to 20% of upper leaves becoming reddened or chlorotic, 8 = moderate chlorosis, reddening, and necrosis on up to 50% of upper leaves, 9 = moderate chlorosis, reddening, and necrosis on up to 50% of upper leaves, symptoms are severe with some defoliation.

^b Significant terms indicated by a "*" next to the *F* statistic.

^c PSS1- isolated from soybean seed symptomatic of purple seed stain, CLB1- single-spore isolate from soybean leaves with *Cercospora* leaf blight symptoms.

^d Cultivars assayed in this study were PI358362 (Lincoln), PI518671 (Williams 82), PI546373 (Hobbit 87), PI548569 (Hack), PI548983 (Tracy), PI548586 (Kent), PI553039 (Davis), PI559371 (Hood 75), and PI80837 (Mejiro). These cultivars were selected based on results for purple seed stain, *Cercospora* leaf blight, or frog-eye leaf spot susceptibility or resistance published by previous studies (Walters, 1980; Walters, 1984; Orth and Schuh, 1994; Mengistu et al., 2012).

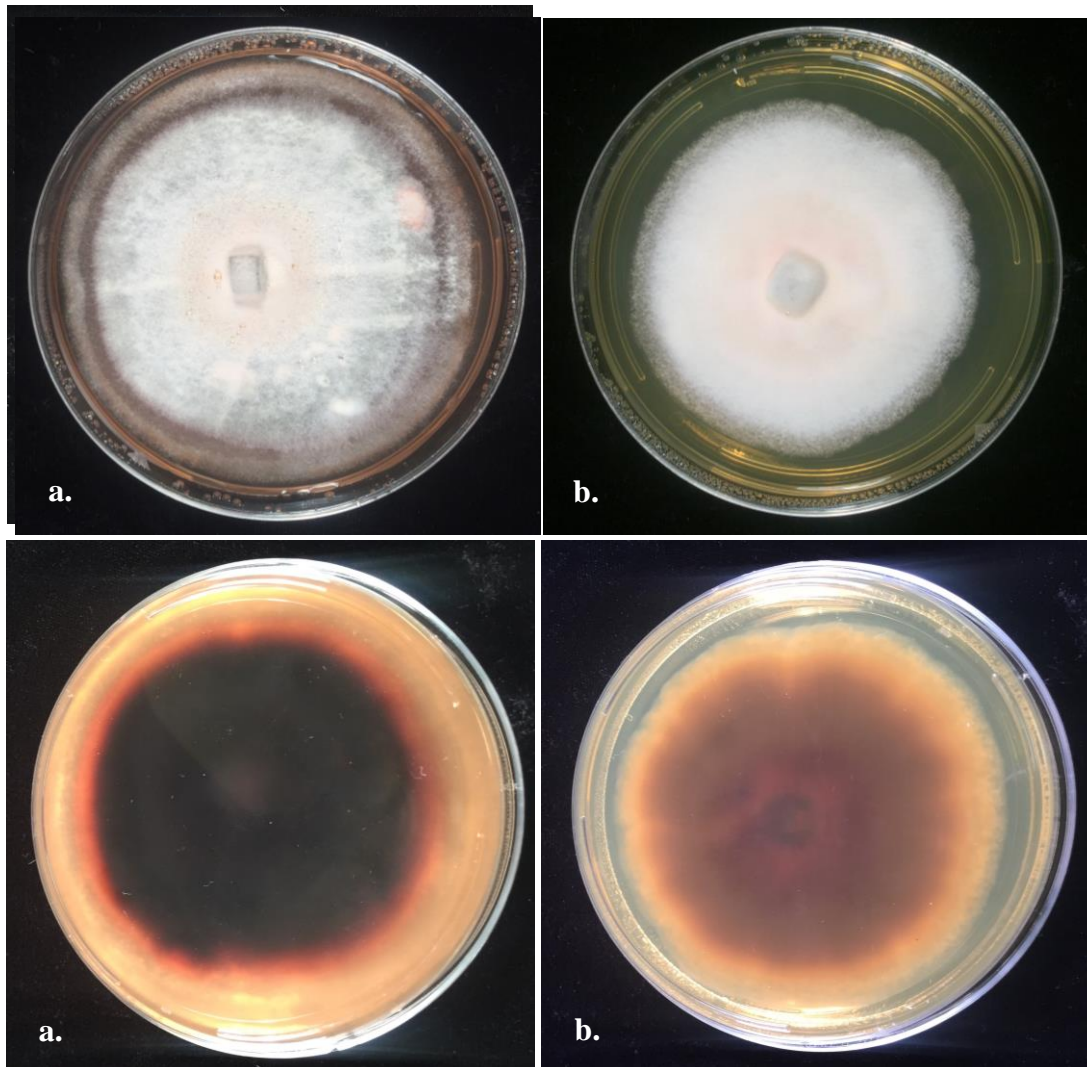


Fig. 3.1. *Cercospora* isolates used in this study grown on potato dextrose agar for four weeks. a. PSS1 culture, which turns medium reddish-purple (a. lower), was isolated from a soybean seed symptomatic of purple seed stain. b. CLB1 isolate, which turns medium yellowish (b. lower), was isolated from soybean leaves symptomatic of *Cercospora* leaf blight.

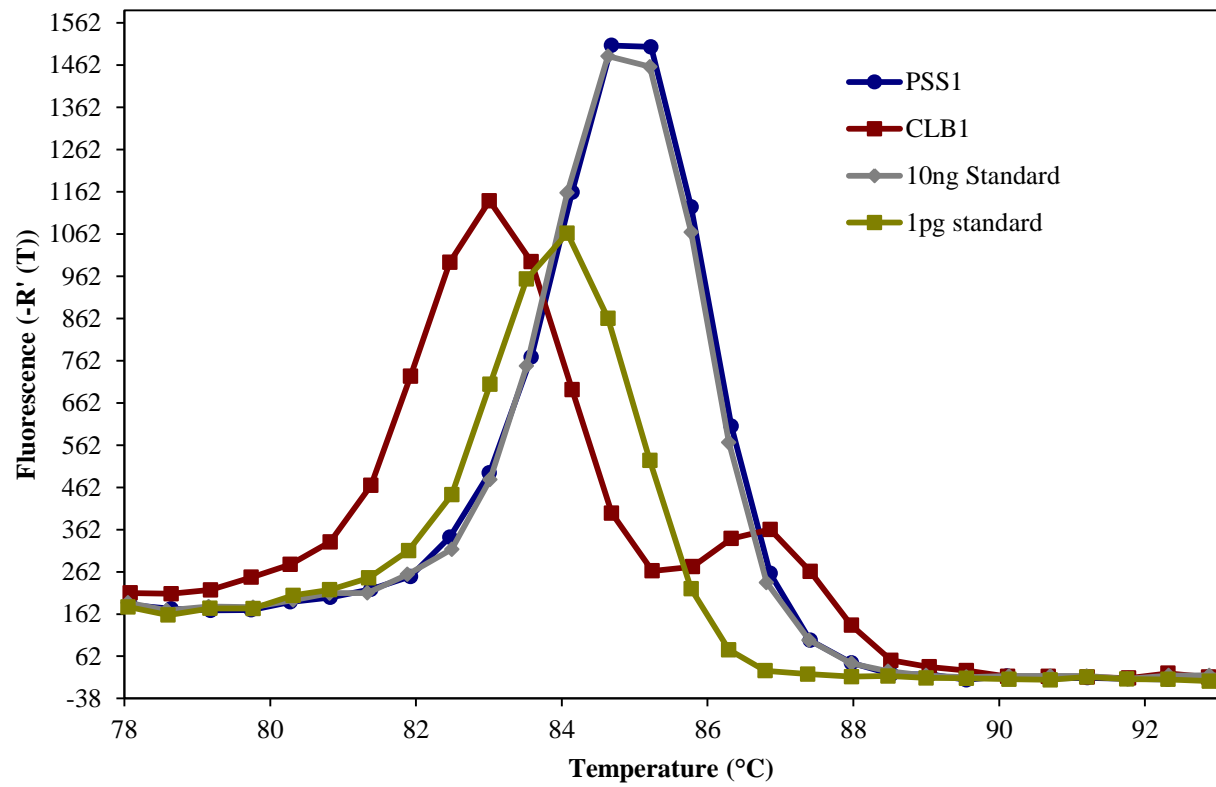


Fig. 3.2. The dissociation curves generated by the qPCR experiment to detect the *CTB6* gene in *Cercospora kikuchii* shows the differences in amplicons produced by this assay using two different *Cercospora* isolates from Illinois^a

^a Isolates included one isolated from a soybean seed exhibiting purple seed stain symptoms (PSS1) and one single-spore isolate from soybean leaves with *Cercospora* leaf blight collected in Urbana, Illinois.

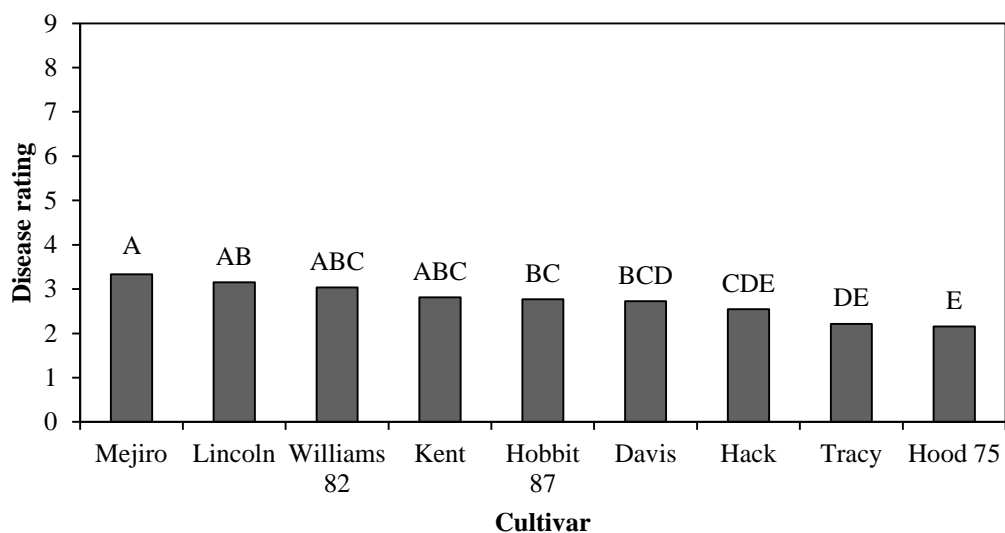


Fig. 3.3. Mean separation of least square mean estimates of disease severity averaged over both seed and leaf isolates of *C. kikuchii* on nine soybean cultivars. While these cultivars were inoculated with different sources of *Cercospora*, PSS1 and CLB1, the interaction between cultivar x isolate was not significant ($P \leq 0.05$).

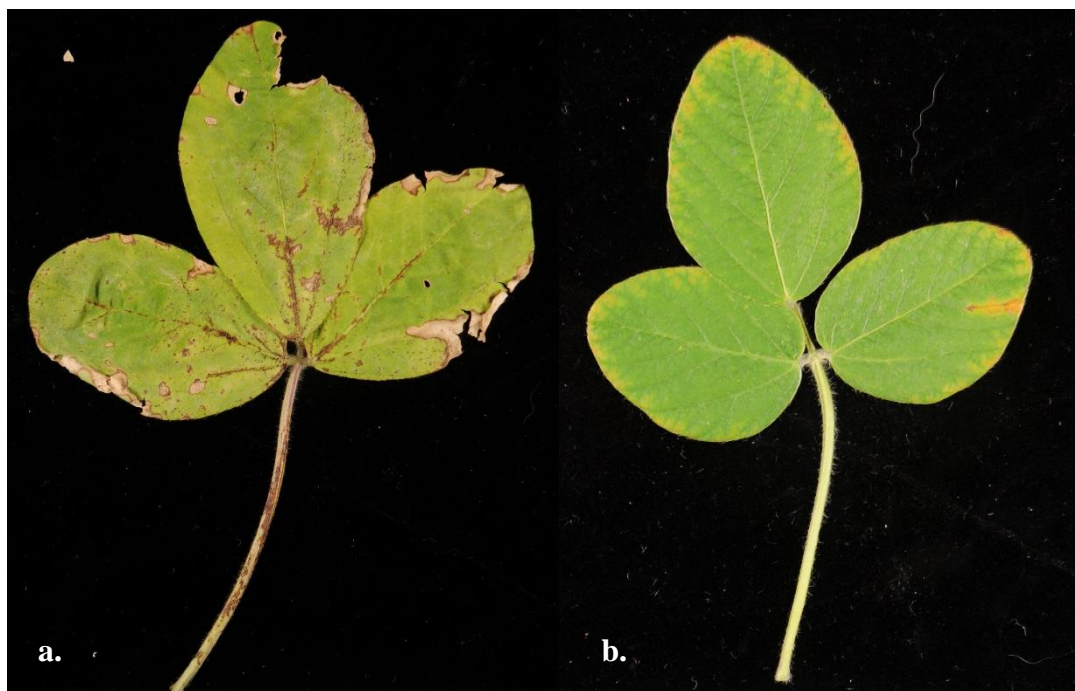


Fig 3.4. *Cercospora* leaf blight foliar and petiole symptoms observed Mejiro (a) and Hood 75 (b) with different *Cercospora* isolates^a. Mejiro was estimated to have the highest disease severity over both *Cercospora* isolates and Hood 75 was estimated to have the lowest disease severity.

^a Soybean were inoculated with either isolate PSS1, a *Cercospora kikuchii* isolate isolated from a soybean seed with purple seed stain, or CLB1, a *C. kikuchii* isolate isolated from soybean leaves with CLB in Urbana, Illinois.

APPENDIX A

Delayed Senescence In Soybean: What Is It?

Abstract

The terms used to describe delayed senescence symptoms in soybean often are used inconsistently or interchangeably and do not adequately distinguish the observed symptoms related to the delayed senescence. Soybean plants with delayed senescence symptoms have been referred to as delayed stem senescence, green bean syndrome, green stem, green stem disorder, and green stem syndrome. Various causes have been put forth to explain the delayed senescence symptoms in soybean. Only a few studies have used specific treatments to study delayed senescence symptoms. In this article, we review published reports on delayed senescence symptoms in soybean, provide an example of what terms relate to what symptoms, and provide an overview of the results of a survey of soybean grower's views on delayed senescence.

Introduction

Over the years, many terms have been used to describe symptoms associated with delayed maturity of some tissue or tissues of the soybean plant. Some of these names include delayed stem senescence, green bean syndrome, green stem, green stem disorder, and green stem syndrome.

The terms used for delayed senescence symptoms in soybean often do not adequately describe the specific malady at hand. As a result, articles often refer to various terms inconsistently or interchangeably to describe a set of particular delayed senescence symptoms in soybean. There have only been a few studies that have used specific treatments to study delayed

senescence. The purpose of this article is to briefly review published reports, provide an example of what terms relate to what symptoms, and provide an overview of the results of a survey of soybean grower's views on delayed senescence.

Soybean with delayed senescence symptoms are probably not new, but there is no clear documentation on when it was first described, or if the incidence of delayed senescence has increased over time. One of the first images of delayed senescence symptoms may have been from the 1950s where plants with green stems at pod/seed maturity were photographed (Fig. A.1) (Craig Grau, pers. comm.). The range of delayed senescence symptoms includes mature pods on green stems with and without petioles or leaves attached, few to no pods on green stems, and immature green pods at the top of the plants with green stems. Several factors have been put forward as possible causes of delayed plant senescence, including abiotic factors such as environmental stresses, pesticide applications, plant genetics and biotic causes including bean leaf beetle, *Bean pod mottle virus* (BPMV), phytoplasmas, *Soybean mosaic virus* or SMV, and stinkbugs.

Besides soybean, delayed senescence has been reported in other crops. For example, a genetic trait called stay-green in maize and sorghum, which causes leaves and stalks to remain green during and after the grain filling stage has completed, is thought to result in healthier stems and higher crop yields (Thomas, 2014). In maize and cowpea, quantitative trait loci (QTL) that control the stay-green trait have been mapped and marker-assisted selection has been used in breeding programs to select plants for stay-green genes (Wang, 2012). Stay-green was also reported to increase drought tolerance in cowpea (Gwathmey, 1992) and sorghum (Borrell, 2014). In soybean, of the various terms used to explain delayed senescence, the main

characteristic that is common with all the definitions is the occurrence of green stems. The green stems may be accompanied by delayed maturity of other plant parts, from the entire plant (leaves, pods, and seeds) remaining immature to the plant being at harvest maturity except for the green stems. The following sections review, in alphabetic order, the names or terms and their definitions and use based on published literature to describe delayed senescence in soybean.

Green Bean Syndrome

Green bean syndrome has been described primarily in the southern USA for the condition in which the maturity of the whole plant is delayed, including the pods (Greene, 2015). This condition is often associated with stink bug feeding, but that has not been firmly established as the only cause (deltafarmpress.com/what-causes-green-bean-syndrome). Additional anecdotes have implicated *Rhizoctonia* aerial blight and mycoplasmas transmitted by leaf hoppers as causes of green bean syndrome, possibly through inhibition of auxin movement in affected plants.

Green Stem Disorder

This term was first used in 2006 in a study conducted in Illinois and Wisconsin and was defined as plants with non-senescent stems with normal, mature pods and seeds at harvest maturity (Figure A.2 and A.4; Hobbs, 2006). In addition, this study showed that green stem disorder was not associated with BPMV infection or feeding from bean leaf beetles, leaf hoppers, or stink bugs (Hobbs, 2006). In a study from Argentina, green stem disorder was shown to be independent of *Alfalfa mosaic virus* (AMV), *Bean common mosaic virus* (BCMV), BPMV, *Soybean mosaic virus* (SMV), *Tobacco ringspot virus* (TRSV), and *Tobacco streak virus* (TSV) (Formento, 2009). In addition, soybean stems with green stem disorder symptoms were shown to have fewer soybean fungal pathogens than stems without green stem disorder symptoms (Fig. A.3; Hill, 2013).

Variability in sensitivity to green stem disorder among soybean cultivars exists (Hill, 2006). In addition, fungicide applications increased the incidence of green stem disorder, especially in green stem disorder-sensitive soybean genotypes (Hill, 2013). Regarding soybean yields, analyses of data collected from 1090 soybean genotypes at seven different locations in Illinois from 2009-2012 indicated no consistent positive or negative correlation with the incidence of green stem disorder and yield (Table A.1). Green stem disorder in Japan, also called delayed stem senescence (Isobe, 2014; Mochizuki, 2005; Sato, 2007; Yamada, 2014), was also found to be dependent of soybean genotype and independent of yield (Isobe, 2014). Researchers in Japan have identified major QTLs associated with green stem disorder insensitivity (Yamada, 2014). The discovery of these QTL may aid soybean breeders through the use of marker-selected breeding, which can lead to cultivars that are less sensitive to green stem disorder. Selection of insensitive soybean cultivars may be the best practice soybean growers can currently use to manage the problem.

Green Stem Syndrome

The term green stem syndrome in soybean was used in a report published in 1980 to describe plants with green stems at maturity in Kansas (Schwenk and Nickell, 1980). The symptoms of green stem syndrome described in that report included delayed maturity of stems, with non-senescent petioles in some cases, and pods of plants with green stems were thinner than normal, brown or mature, and dried with small seeds and overall fewer pods per node. In this report, BPMV was implicated as the main cause of green stem syndrome (Schwenk, 1980). The term green stem syndrome was also used in Virginia and referred to plants that had green stems and often had reduced yields associated with plant stress during pod and seed development (Holshouser, 2009).

In Kentucky, researchers found that by removing soybean pods at growth stage R6, soybean stems remained green longer than the stems of the control plants, and this difference was observed independent of cultivar tested (Egli, 2006). This was likely due to the plants continuing to reproduce to replace the lost pods. After finding that green stems had higher concentrations of soluble sugars, starch, and nitrogen, the researchers speculated that the green stems became a metabolic sink for moisture from the roots and photosynthate from the leaves produced to feed the new developing replacement pods. In Brazil, they showed that by applying cobalt, molybdenum, and a seed inoculant, that leaf nitrogen increased which corresponded to a decrease in green stem syndrome (Favero, 2014).

2014-15 Grower Survey

In a survey supported by the United Soybean Board, growers and crop consultants were asked a series of questions relating to delayed senescence in soybean. Survey results from two North-Central states (Illinois, Indiana, and Wisconsin) and six Southern states (Alabama, Kentucky, Mississippi, North Carolina, South Carolina, and Tennessee) were collected in 2015. The total number of people responding to the survey was 68. Of these, 79% reported observing soybean plants or plant parts that remained green beyond a normal harvest date in their fields (Table A.2). Of those 79%, 98% reported that soybean fields with a range of plants affected from 5% to more than 100% with delayed senescence symptoms caused delays in harvesting the crop (Table A.2). In order to combat the harvest delays or problems with combining, 31% of those with delayed senescence problems resorted to the use of harvest aids (Fig. A.4), the most frequently employed practice. All responses confirming the use of harvest aids came from Southern soybean growing states. Other practices employed included cultivar selection (10%), fungicide (9%), insecticides (11%), planting date management (5%), and stress prevention (3%)

(Fig. A.4). The term most frequently used to describe delayed maturity symptoms in soybean was “green stem,” with 74% of those surveyed using this term. Green stem syndrome and green bean syndrome were less frequently reported (15% and 9%, respectively) followed by green stem disorder (2%) (Fig. A.5). Most surveyors attributed these delayed senescence symptoms to changes in breeding and cultivar selection (36%) (Fig. A.6). The next most frequent responses with regards to cause of delayed maturity symptoms in soybean were insect damage, use of strobilurin fungicides, and changes in weather patterns, with 16% of the responses for each (Fig. A.6).

Further Considerations

Delayed stem senescence of soybean results in persistence of green stems at growth stage R8, which makes the crop more difficult to harvest as the green stems are not as easily cut and processed by the combine. To amend this, fields with high incidences of delayed stem senescence may require combines to reduce the ground speed and increase the engine power, which reduces fuel efficiency and takes more time. Perhaps the most time-consuming issue when it comes to harvesting fields with green stems is when farmers must continuously get out of the combine to manually unclog the combine head. Furthermore, many farmers in the south resort to harvest aids to increase the rate of senescence of the green stems and allow for quicker harvests, or wait until frost or time to pass so more stems mature. A delay in harvest often increases the vulnerability to factors that can reduce grain yield and quality and include such factors as lodging, shattering, and seed decay.

It is important to make a distinction between these delayed senescence symptoms, because the management options may vary depending upon the type of delayed senescence symptoms. For example, growers that encounter green stem disorder, which does not appear to

be yield limiting, may consider varietal selection as a criteria for management. Whereas other delayed senescence symptoms that clearly have yield implications, other management options may need to consider including the use of insecticides. Based on the farmer survey results, farmers do not make a distinction between these maladies, and mostly refer to any delayed senescence symptoms as “green stem”, which indicates that more outreach is required to educate growers on current scientific knowledge of the problem with less reliance on anecdotal evidence.

Literature Cited

- Borrell, A. K., Mullet, J. E., George-Jaeggli, B., Oosterom, E. J. v., Hammer, G. L., Klein, P. E., and Jordan, D. R. 2014. Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. *Journal of Experimental Botany* doi:10.1093/jxb/eru232.
- Egli, D. B., and Bruening, W. P. 2006. Depodding causes green-stem syndrome in soybean. *Crop Management* doi:10.1094/CM-2006-0104-01-RS.
- Favero, F., and Lana, M. C. 2014. Reduction of green stem and leaf retention in soybean through greater nitrogen availability from seed treatment. *Revista Brasileira de Ciência do Solo (R. Bras. Ci. Solo)* [Brazilian Journal of Soil Science] 38:1432-1438.
- Formento, A.N. and de Souza, J. 2009. Detection of Green Stem Disorder in Entre Rios, Argentina. *Journal of Plant Pathology* 91:231-240.
- Greene, J., and Davis, J. 2015. Stink bugs. Page (in press) in: *Compendium of Soybean Diseases and Pests*, G. L. Hartman, J. C. Rupe, E. F. Sikora, L. L. Domier, J. A. Davis and K. L. Steffey, eds. American Phytopathological Society, St. Paul.
- Gwathmey, C. O., and Hall, A. E. 1992. Adaptation of midseason drought of cowpea genotypes with contrasting senescence traits. *Crop Science* 32:773-778.
- Hill, C. B., Bowen, C. R., and Hartman, G. L. 2013. Effect of fungicide application and cultivar on soybean green stem disorder. *Plant Disease* 97:1212-1220.
- Hill, C. B., Hartman, G. L., Esgar, R., and Hobbs, H. A. 2006. Field evaluation of green stem disorder in soybean cultivars. *Crop Science* 46:879-885.
- Hobbs, H. A., Hill, C. B., Grau, C. R., Koval, N. C., Wang, Y., Pedersen, W. L., Domier, L. L., and Hartman, G. L. 2006. Green stem disorder of soybean. *Plant Disease* 90:513-518.
- Holshouser, D. 2009. Green Stem Syndrome in Soybean. Virginia Cooperative Extension. <https://pubs.ext.vt.edu/2912/2912-1430/2912-1430.html>
- Isobe, K., Kurose, T., Sasaki, Y., Someya, T., Terasawa, A., Higo, M., and Torigoe, Y. 2014. Effects of early sowing cultivation on yield and occurrence of delayed stem senescence in several soybean cultivars in south Kanto. *Japanese Journal of Crop Science* 83:195-202.
- Mochizuki, A., Shiraiwa, T., Nakagawa, H., and Horie, T. 2005. The effect of temperature during the reproductive period on development of reproductive organs and the occurrence of delayed stem senescence in soybean. *Japanese Journal of Crop Science* 74:339-343.

Sato, J., Shiraiwa, T., Sakashita, M., Tsujimoto, Y., and Yoshida, R. 2007. The occurrence of delayed stem senescence in relation to *trans*-zeatin riboside level in the xylem exudate in soybean grown under excess-wet and drought soil conditions. *Plant Production Science* 10:460-467.

Schwenk, F. W., and Nickell, C. D. 1980. Soybean green stem caused by bean pod mottle virus. *Plant Disease* 64:863-865.

Thomas, H., and Ougham, H. 2014. The stay-green trait. *Journal of Experimental Botany* doi:10.1093/jxb/eru037.

Wang, A., Li, Y., and Zhang, C. 2012. QTL mapping for stay-green in maize (*Zea mays*). *Canadian Journal of Plant Science* 92:249-256.

Yamada, T., Shimada, S., Hajika, M., Hirata, K., Takahashi, K., Nagaya, T., Hamaguchi, H., Maekawa, T., Sayama, T., Hayashi, T., Ishimoto, M., and Tanaka, J. 2014. Major QTLs associated with green stem disorder insensitivity of soybean (*Glycine max* (L.) Merr.). *Breeding Science* 64:331-338.

Fig. A.1. A soybean field bordering an alfalfa/red clover field in 1950, which has plants with green stems. Photo shared by Craig Grau (University of Wisconsin- Madison).



Fig. A.2. Soybean exhibiting green stem disorder symptoms: green, fleshy, non-senescent stems with normal, mature pods at harvest maturity.



Fig. A.3. Close-up of a soybean plant exhibiting green stem disorder symptoms next to a plant with a normally senescing stem.



Photo credit: Herman, T.

Table A.1. Pearson correlation coefficients for green stem disorder incidence with yield over all cultivars observed in 19 year x location combinations for Variety Testing trials in Illinois. Significant correlations are underlined ($P \leq 0.05$), with six significantly positive, seven significantly negative, and six holding no significance.

Year	Location	n	r
2009	Goodfield	808	0.08
2009	Monmouth	857	<u>0.28</u>
2009	New Berlin	138	-0.05
2009	Perry	684	<u>0.11</u>
2010	Dwight	677	<u>0.20</u>
2010	Goodfield	676	<u>0.21</u>
2010	Monmouth	678	<u>0.34</u>
2010	New Berlin	753	<u>-0.11</u>
2010	Perry	745	<u>0.28</u>
2010	Urbana	945	<u>-0.09</u>
2011	Monmouth	678	<u>-0.09</u>
2011	New Berlin	711	<u>-0.13</u>
2011	Urbana	711	0.00
2012	Dwight	726	<u>-0.09</u>
2012	Elkville	402	<u>-0.28</u>
2012	Goodfield	726	<u>-0.12</u>
2012	New Berlin	753	-0.02
2012	Perry	753	<u>0.23</u>
2012	Urbana	753	0.00

Table A.2. Summary of responses to several questions from survey conducted by United Soybean Board about delayed maturity symptoms in soybean presented by region (North Central and South) and in total. There were 17 responses from the North Central region and 51 responses from the Southern region, giving a total of 68 responses.

	Region		Total
	North Central	South	
Delayed maturity symptoms observed	100	73	79
Subsequent delays in harvest	100	97	98
Fields affected (low-(mean)-high)	10-(57)-100	5-(29)-100	5-(43)-100
Applied production practices to alleviate delayed maturity symptoms	12	76	74

^a States surveys collected from in North Central Region: Illinois, Indiana, Wisconsin.

^b States surveys collected from in South Region: Alabama, Kentucky, Mississippi, North Carolina, South Carolina, and Tennessee.

Fig. A.4. Frequency of types production practices employed to help prevent or alleviate delayed maturity symptoms by surveyors that responded positively when asked if they apply such practices, based on survey responses from soybean consultants and farmers from Alabama, Indiana, Kentucky, Mississippi, South Carolina, Tennessee, and Wisconsin.

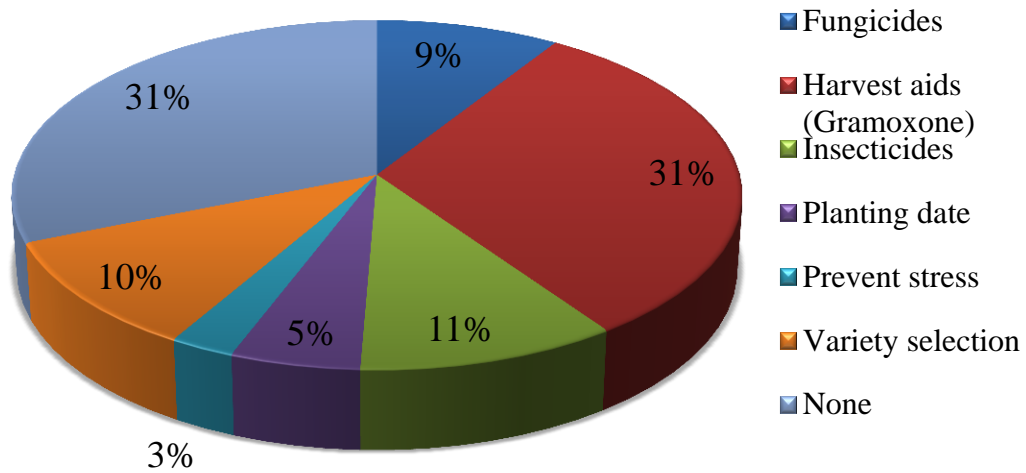


Fig. A.5. Frequency of terms used by soybean growers and consultants to describe delayed maturity symptoms in soybean obtained from a United Soybean Board survey (Responses from soybean consultants and farmers from Alabama, Illinois, Indiana, Kentucky, Mississippi, South Carolina, Tennessee, and Wisconsin).

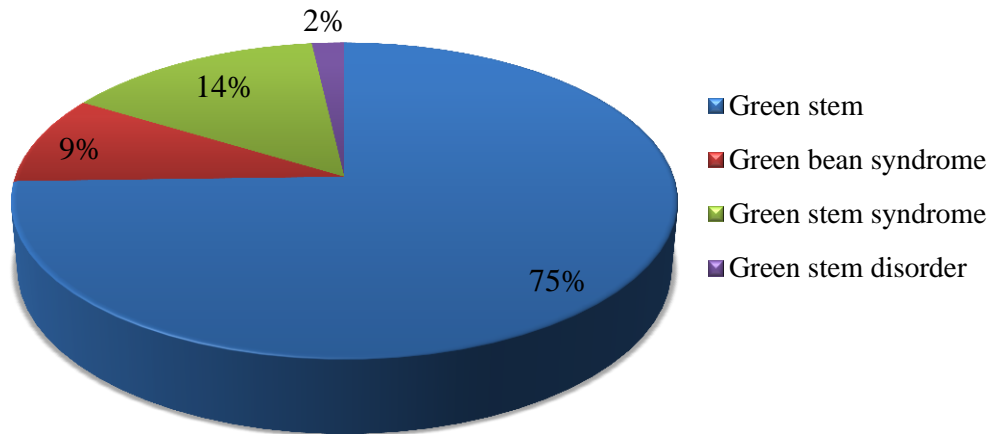
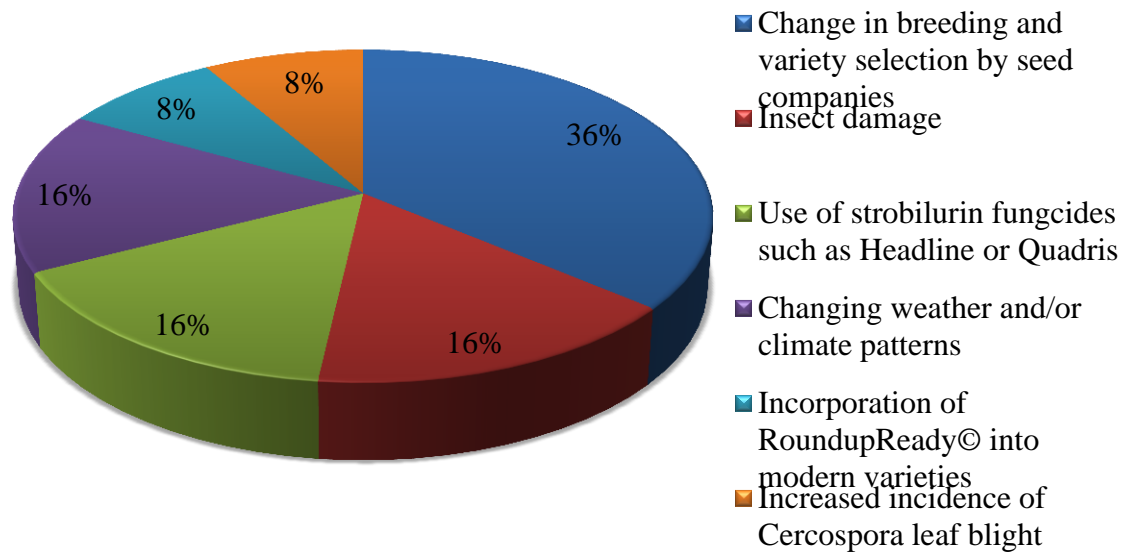
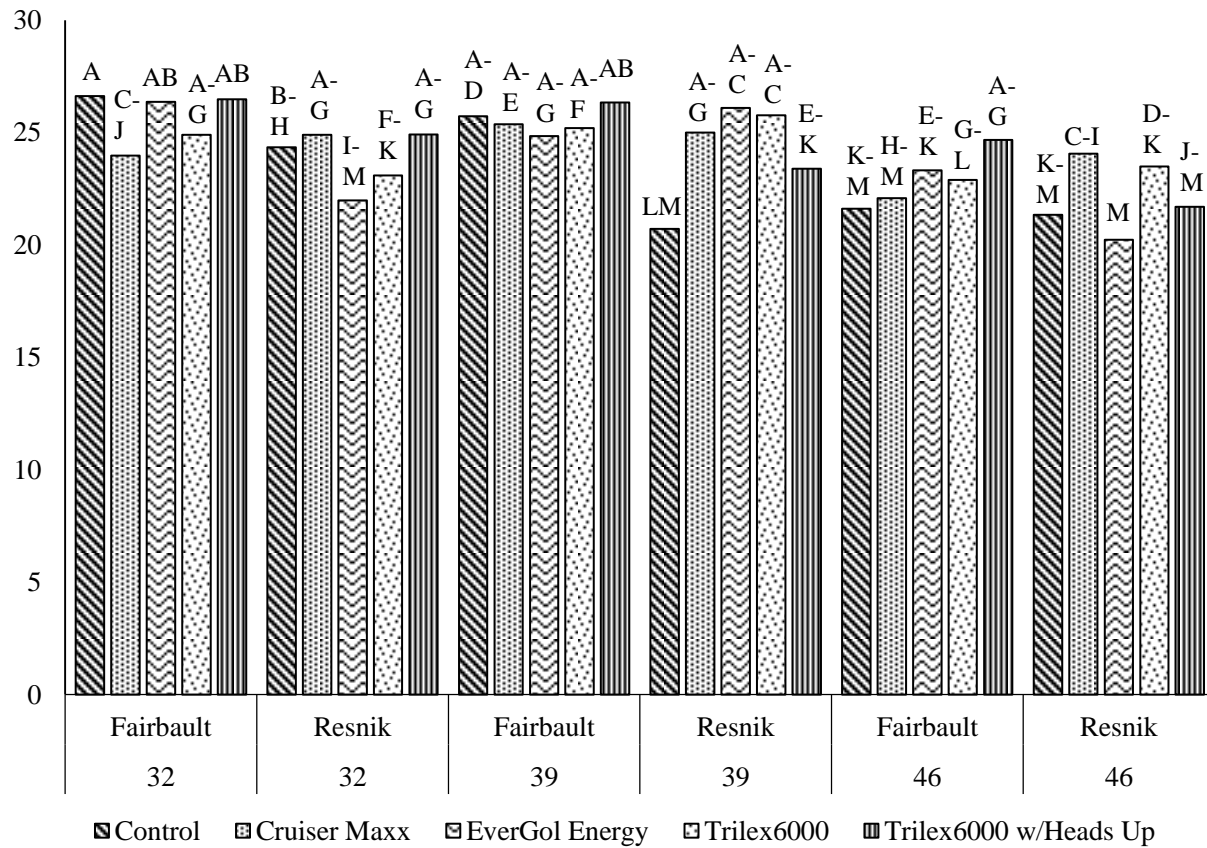


Fig. A.6. Responses from soybean consultants and farmers from Alabama, Illinois, Indiana, Kentucky, Mississippi, South Carolina, Tennessee, and Wisconsin when asked what they think is the leading cause in increased incidence of delayed maturity in soybean. Majority surveyors attribute an increase in delayed senescence symptoms in soybean to changes in breeding and variety selection by seed companies.



Appendix B

Fig. B.1. Least square mean separation of cultivar x day after planting x seed treatment combinations for soybean grown from non-treated or fungicide-treated seed, inoculated with *Sclerotinia sclerotiorum* at 32, 39, and 46 days after planting. Mean separation is indicated with letter grouping significant at $P \leq 0.05$.



Appendix C

Fig. C.1. Boxplots to show the range of green stem disorder (GSD) percent incidence observations collected in 2009 and 2010. Plots include the overall GSD incidence for each year x location combination as well as the range of the public check cultivars observed in the same year x location combination, indicated by “*”.

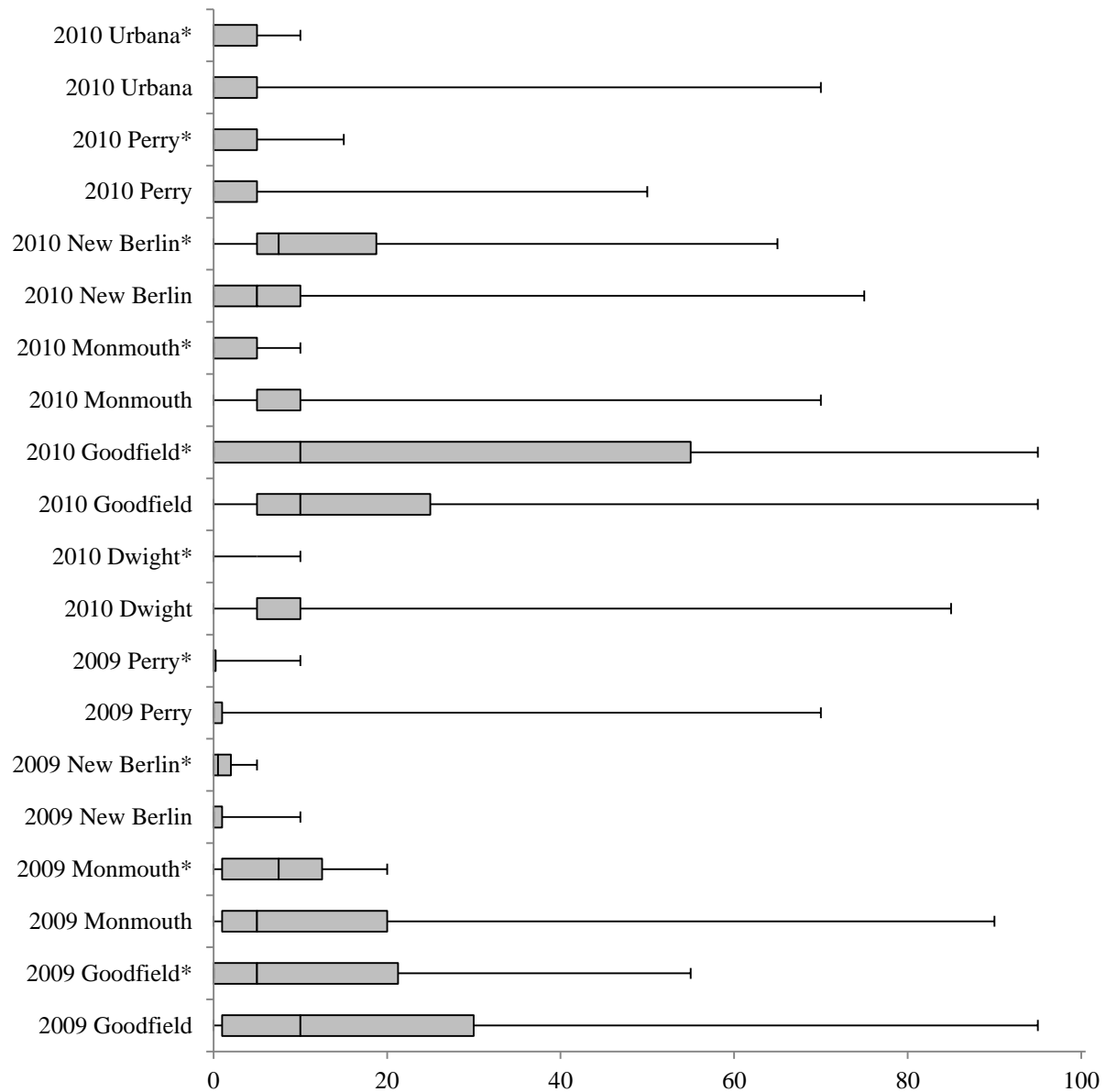
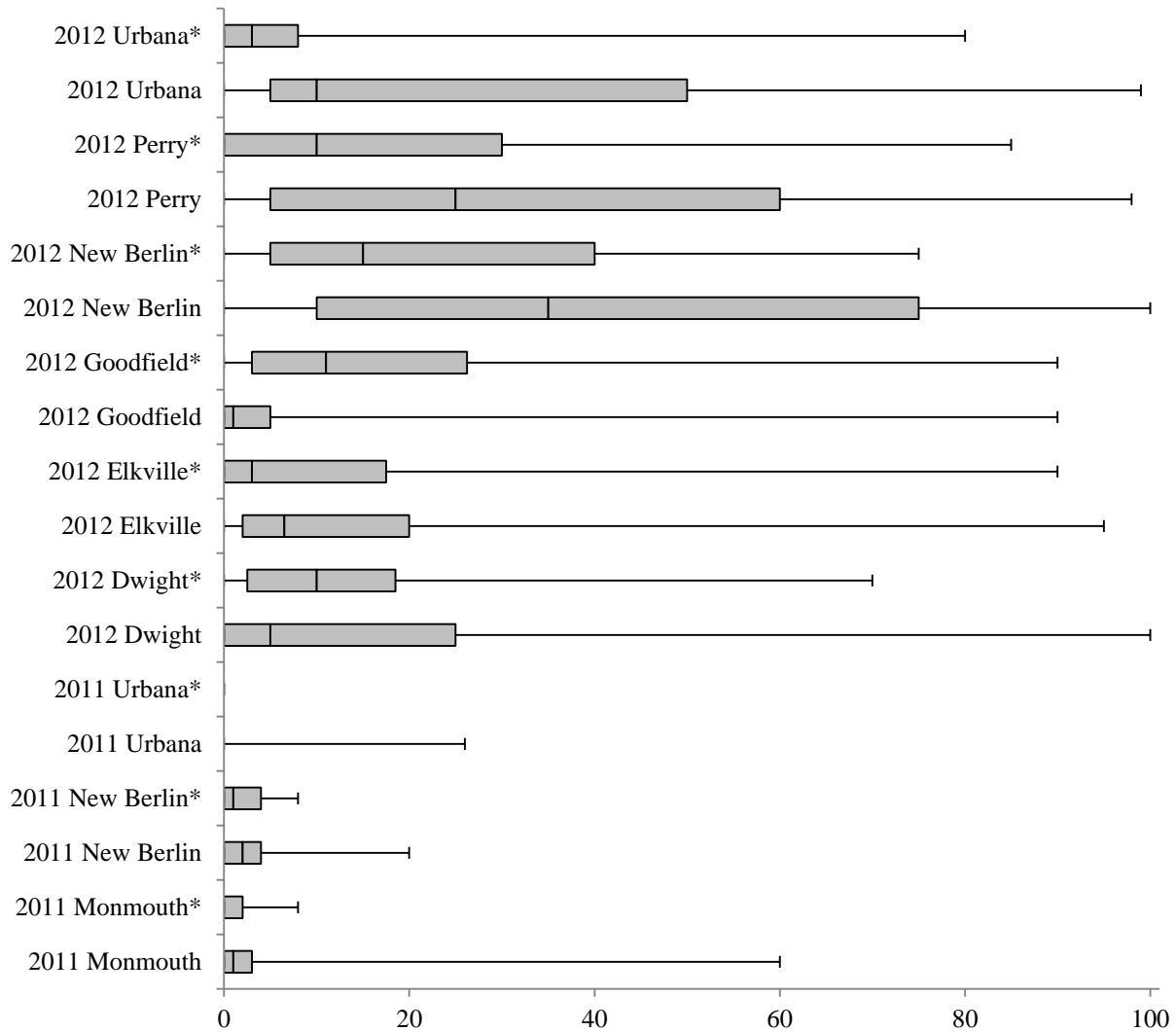


Fig. C.2. Boxplots to show the range of green stem disorder (GSD) percent incidence observations collected in 2011 and 2012. Plots include the overall GSD incidence for each year x location combination as well as the range of the public check cultivars observed in the same year x location combination, indicated by “*”.



Appendix D.

Table D.1. Multiple sequence alignment of IGS sequences from Ck2(PSS1) and CLB1 isolates used in *Cercospora* study with IGS sequences from Cai and Schneider, 2008.

MRL6020-4A	-----	
MRS5098-2B	-----	
MRL5070-3A	-----	
MRL6020-1A	-----	
MRL5070-1B	-----	
DLL6013-1B	-----	
DLS5012-1A	-----	
DLS5070-2A	-----	
DLS5070-2B	-----	
DLS5070-3A	-----	
CLB1	-----	
CKBR1	-----	
DLS5098-1B	TAATTGGTTTTTGC	60
MRS5012-1A	-----	
MRL6020-2B	-----	
DLS6020-4B	-----	
DLS5012-4A	-----	
PSS1	-----	
MRL6020-4A	TAGCTGAACGCCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	60
MRS5098-2B	TAGCTGAACGCCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	60
MRL5070-3A	TAGCTGAACGCCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	60
MRL6020-1A	TAGCTGAACGCCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	60
MRL5070-1B	-----CCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	50
DLL6013-1B	-----CCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	50
DLS5012-1A	-----CCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	50
DLS5070-2A	-----CCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	50
DLS5070-2B	-----CCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	50
DLS5070-3A	-----CCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	50
CLB1	-----	
CKBR1	-----CCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	50
DLS5098-1B	TAGCTGAACGCCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	120
MRS5012-1A	TAGCTGAACGCCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	60
MRL6020-2B	TAGCTGAACGCCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	60
DLS6020-4B	-----CCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	50
DLS5012-4A	-----CCTCTAAGTTAGAATCCATGCCAGAACGGGACGATCCTCTCCAGCACGCC	50
PSS1	-----	
MRL6020-4A	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	120
MRS5098-2B	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	120
MRL5070-3A	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	120
MRL6020-1A	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	120
MRL5070-1B	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	110
DLL6013-1B	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	110
DLS5012-1A	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	110
DLS5070-2A	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	110
DLS5070-2B	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	110
DLS5070-3A	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	110
CLB1	-----CCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	36
CKBR1	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	110
DLS5098-1B	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	180
MRS5012-1A	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	120
MRL6020-2B	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	120
DLS6020-4B	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	110
DLS5012-4A	TTAGGCGGATAAGAATAGGCACTGCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	110
PSS1	-----GCCAGTACCTGGGACCCTCTCATCCCTCGCAAGACAC	37

Table D.1. (cont.)

MRL6020-4A	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	180
MRS5098-2B	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	180
MRL5070-3A	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	180
MRL6020-1A	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	180
MRL5070-1B	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	170
DLL6013-1B	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	170
DLS5012-1A	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	170
DLS5070-2A	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	170
DLS5070-2B	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	170
DLS5070-3A	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	170
CLB1	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	96
CKBR1	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	170
DLS5098-1B	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	240
MRS5012-1A	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	180
MRL6020-2B	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	180
DLS6020-4B	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	170
DLS5012-4A	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	170
PSS1	GCGGGGGCGAAGGGCGTATCATAATTTTATAGCGCGCTGGGATGAATCCCTTGCAGACGA	97

MRL6020-4A	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	240
MRS5098-2B	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	240
MRL5070-3A	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	240
MRL6020-1A	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	240
MRL5070-1B	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	230
DLL6013-1B	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	230
DLS5012-1A	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	230
DLS5070-2A	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	230
DLS5070-2B	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	230
DLS5070-3A	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	230
CLB1	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	156
CKBR1	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	230
DLS5098-1B	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	300
MRS5012-1A	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	240
MRL6020-2B	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	240
DLS6020-4B	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	230
DLS5012-4A	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	230
PSS1	CTTGGACGTCGGATCGGGTCGTGTAAGCAGTCGAGTAGCCTTGTTGTTACGAGCTGCTGA	157

MRL6020-4A	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	300
MRS5098-2B	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	300
MRL5070-3A	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	300
MRL6020-1A	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	300
MRL5070-1B	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	290
DLL6013-1B	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	290
DLS5012-1A	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	290
DLS5070-2A	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	290
DLS5070-2B	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	290
DLS5070-3A	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	290
CLB1	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	216
CKBR1	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	290
DLS5098-1B	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	360
MRS5012-1A	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	300
MRL6020-2B	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	300
DLS6020-4B	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	290
DLS5012-4A	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	290
PSS1	GCGTAAGCCC GTTATCCGCTCGATTTGTTGAATACCTCCCCATTAGTTGAAGGGCTAATC	217

Table D.1. (cont.)

MRL6020-4A	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	360
MRS5098-2B	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	360
MRL5070-3A	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	360
MRL6020-1A	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	360
MRL5070-1B	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	350
DLL6013-1B	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	350
DLS5012-1A	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	350
DLS5070-2A	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	350
DLS5070-2B	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	350
DLS5070-3A	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	350
CLB1	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	276
CKBR1	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGGATGGATTTGATGGATTTC	350
DLS5098-1B	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGAATGGATTTGATGGATTTC	420
MRS5012-1A	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGAATGGATTTGATGGATTTC	360
MRL6020-2B	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGAATGGATTTGATGGATTTC	360
DLS6020-4B	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGAATGGATTTGATGGATTTC	350
DLS5012-4A	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGAATGGATTTGATGGATTTC	350
PSS1	AAATGTGATGAGCCCGCACCTGGAGGCGAGTGTATTGGACGAATGGATTTGATGGATTTC	277

MRL6020-4A	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	420
MRS5098-2B	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	420
MRL5070-3A	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	420
MRL6020-1A	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	420
MRL5070-1B	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	410
DLL6013-1B	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	410
DLS5012-1A	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	410
DLS5070-2A	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	410
DLS5070-2B	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	410
DLS5070-3A	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	410
CLB1	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	336
CKBR1	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	410
DLS5098-1B	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	480
MRS5012-1A	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	420
MRL6020-2B	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	420
DLS6020-4B	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	410
DLS5012-4A	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	410
PSS1	TGCATGTCCGCGGAAGACGGCAGGGTCGCCCCCGGAAGCTGGGACATGGCCACGAGCTGC	337

MRL6020-4A	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	470
MRS5098-2B	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	470
MRL5070-3A	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	470
MRL6020-1A	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	470
MRL5070-1B	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	460
DLL6013-1B	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	460
DLS5012-1A	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	460
DLS5070-2A	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	460
DLS5070-2B	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	460
DLS5070-3A	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	460
CLB1	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	386
CKBR1	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATT-----TGGCACTGGG	460
DLS5098-1B	TGAGCGTAAAGAAAGGCATAGGCGCTGATCTCCGGCGGGAGATTCCCTCTGGCACTAGG	540
MRS5012-1A	TGAGCGTAAAGAAAGGCATAGGCGCTGATCTCCGGCGGGAGATTCCCTCTGGCACTAGG	480
MRL6020-2B	TGAGCGTAAAGAAAGGCATAGGCGCTGATCTCCGGCGGGAGATTCCCTCTGGCACTAGG	480
DLS6020-4B	TGAGCGTAAAGAAAGGCATAGGCGCTGATCTCCGGCGGGAGATTCCCTCTGGCACTAGG	470
DLS5012-4A	TGAGCGTAAAGAAAGGCATAGGCGCTGATCTCCGGCGGGAGATTCCCTCTGGCACTAGG	470
PSS1	TGAGCGTAAAG----GCATAGGCGCTGATCTCCGGCGGGAGATTCCCTCTGGCACTGGG	393

Table D.1. (cont.)

MRL6020-4A	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	530
MRS5098-2B	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	530
MRL5070-3A	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	530
MRL6020-1A	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	530
MRL5070-1B	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	520
DLL6013-1B	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	520
DLS5012-1A	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	520
DLS5070-2A	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	520
DLS5070-2B	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	520
DLS5070-3A	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	520
CLB1	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	446
CKBR1	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	520
DLS5098-1B	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	600
MRS5012-1A	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	540
MRL6020-2B	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	540
DLS6020-4B	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	530
DLS5012-4A	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	530
PSS1	CCAGCGAGGCCTCTGGCGATGCACGTCCGCAGTGGACGGTAGGGTCGCCCCCGGAAGCTG	453

MRL6020-4A	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	582
MRS5098-2B	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	582
MRL5070-3A	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	582
MRL6020-1A	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	582
MRL5070-1B	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	572
DLL6013-1B	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	572
DLS5012-1A	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	572
DLS5070-2A	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	572
DLS5070-2B	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	572
DLS5070-3A	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	572
CLB1	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	498
CKBR1	GGACATGG-----CGGCTTGAGGCGGCCGTGTGCGGTGGGGGGTGGAGGTATATGAG	572
DLS5098-1B	GGACATGGGCTTGACTCGGCTTGAGGCGGCCGTGTGCGGTAGGGGGTGGAGGTATATGAG	660
MRS5012-1A	GGACATGGGCTTGACTCGGCTTGAGGCGGCCGTGTGCGGTAGGGGGTGGAGGTATATGAG	600
MRL6020-2B	GGACATGGGCTTGACTCGGCTTGAGGCGGCCGTGTGCGGTAGGGGGTGGAGGTATATGAG	600
DLS6020-4B	GGACATGGGCTTGACTCGGCTTGAGGCGGCCGTGTGCGGTAGGGGGTGGAGGTATATGAG	590
DLS5012-4A	GGACATGGGCTTGACTCGGCTTGAGGCGGCCGTGTGCGGTAGGGGGTGGAGGTATATGAG	590
PSS1	GGACATGGGCTTGACTCGGCTTGAGGCGGCCGTGTGCGGTAGGGGGTGNAGGTATATGAN	513

MRL6020-4A	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	642
MRS5098-2B	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	642
MRL5070-3A	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	642
MRL6020-1A	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	642
MRL5070-1B	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	632
DLL6013-1B	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	632
DLS5012-1A	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	632
DLS5070-2A	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	632
DLS5070-2B	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	632
DLS5070-3A	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	632
CLB1	TTGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	558
CKBR1	TCGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	632
DLS5098-1B	TCGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	720
MRS5012-1A	TCGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	660
MRL6020-2B	TCGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	660
DLS6020-4B	TCGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	650
DLS5012-4A	TCGGGAAACATGGAGATCTAGCACGAACGACCACAGATACGTGAAAGCTAGGCATCCCGT	650
PSS1	TCNGNAA-----	520
* * *		

Table D.1. (cont.)

MRL6020-4A	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	702
MRS5098-2B	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	702
MRL5070-3A	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	702
MRL6020-1A	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	702
MRL5070-1B	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	692
DLL6013-1B	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	692
DLS5012-1A	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	692
DLS5070-2A	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	692
DLS5070-2B	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	692
DLS5070-3A	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	692
CLB1	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	618
CKBR1	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	692
DLS5098-1B	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	780
MRS5012-1A	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	720
MRL6020-2B	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	720
DLS6020-4B	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	710
DLS5012-4A	CCGCTCTGCCATATATAAGCACGTAATCGCTGGATTAGTACTACGGTGGGTGACCACGTG	710
PSS1	-----	
MRL6020-4A	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	762
MRS5098-2B	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	762
MRL5070-3A	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	762
MRL6020-1A	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	762
MRL5070-1B	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	752
DLL6013-1B	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	752
DLS5012-1A	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	752
DLS5070-2A	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	752
DLS5070-2B	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	752
DLS5070-3A	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	752
CLB1	GGAATCCCCANTGTTGTTTCGTTTTGCTTTTTGCCCG--	655
CKBR1	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTGGACGGCGGAGTC	752
DLS5098-1B	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTCGACGGCGGAGTC	840
MRS5012-1A	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTCGACGGCGGAGTC	780
MRL6020-2B	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTCGACGGCGGAGTC	780
DLS6020-4B	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTCGACGGCGGAGTC	770
DLS5012-4A	GGAATCCCCAGTGTTGTTTCGTTTTGCTTTTTGCCCGGGAGGGGCGCTCGACGGCGGAGTC	770
PSS1	-----	
MRL6020-4A	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	822
MRS5098-2B	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	822
MRL5070-3A	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	822
MRL6020-1A	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	822
MRL5070-1B	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	812
DLL6013-1B	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	812
DLS5012-1A	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	812
DLS5070-2A	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	812
DLS5070-2B	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	812
DLS5070-3A	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	812
CLB1	-----	
CKBR1	CCCTCGTGCGCGGCGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	812
DLS5098-1B	CCCTTGTCGCGGCGGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	900
MRS5012-1A	CCCTTGTCGCGGCGGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	840
MRL6020-2B	CCCTTGTCGCGGCGGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	840
DLS6020-4B	CCCTTGTCGCGGCGGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	830
DLS5012-4A	CCCTTGTCGCGGCGGGAATCCCTCCTTTTTGCACGCCGGGAGACGTCTCCGGCCATTTT	830
PSS1	-----	

Table D.1. (cont.)

MRL6020-4A	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	882
MRS5098-2B	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	882
MRL5070-3A	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	882
MRL6020-1A	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	882
MRL5070-1B	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	872
DLL6013-1B	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	872
DLS5012-1A	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	872
DLS5070-2A	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	872
DLS5070-2B	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	872
DLS5070-3A	TATTTACCAGCGCGCGTGCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	872
CLB1	-----	
CKBR1	TATGTACCAGCGCGCGTTCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	872
DLS5098-1B	TATTTACCAGCGCGCGTTCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	960
MRS5012-1A	TATTTACCAGCGCGCGTTCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	900
MRL6020-2B	TATTTACCAGCGCGCGTTCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	900
DLS6020-4B	TATTTACCAGCGCGCGTTCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	890
DLS5012-4A	TATTTACCAGCGCGCGTTCCACGGCCGTGGGATCAGAAAACGGTAGGGTCGCCCCCGGGA	890
PSS1	-----	
MRL6020-4A	GTGTTGGGTCCAGTG	897
MRS5098-2B	GTGTTGGGTCCAGTG	897
MRL5070-3A	GTGTTGGGTCCAGTG	897
MRL6020-1A	GTGTTGGGTCCAGTG	897
MRL5070-1B	GTGTTGGGTCCAGTG	887
DLL6013-1B	GTGTTGGGTCCAGTG	887
DLS5012-1A	GTGTTGGGTCCAGTG	887
DLS5070-2A	GTGTTGGGTCCAGTG	887
DLS5070-2B	GTGTTGGGTCCAGTG	887
DLS5070-3A	GTGTTGGGTCCAGTG	887
CLB1	-----	
CKBR1	GTGTTGGGTCCAGTG	887
DLS5098-1B	GTGTTGGGTTCGGGTG	975
MRS5012-1A	GTGTTGGGTTCGGGTG	915
MRL6020-2B	GTGTTGGGTTCGGGTG	915
DLS6020-4B	GTGTTGGGTTCGGGTG	905
DLS5012-4A	GTGTTGGGTTCGGGTG	905
PSS1	-----	