

# Fungicide Management Does Not Affect the Rate of Genetic Gain in Soybean

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## ABSTRACT

On-farm U.S. soybean [*Glycine max* (L.) Merr.] yields have increased at an annual rate of 23.3 kg ha<sup>-1</sup> yr<sup>-1</sup> since the 1920s. These gains have come from a variety of sources including genetic, agronomic, and environmental changes. Genetic gains arising from breeding efforts have likely contributed the most to the U.S. soybean yield increase; however, the relative contribution of each source of gain is difficult to estimate. The objectives of this study were to compare yield of soybean varieties with different year of release, understand the effects of fungicide applications on soybean seed yield, and evaluate the composition of soybean cultivars chosen to represent historically significant releases in maturity groups (MGs) II and III released during the last 85 yr. A set of 116 cultivars in these two MGs, released from 1923 to 2008, received a fungicide seed treatment followed by foliar applications at R1, R3, and R5 and were compared to non-treated controls. Seed composition changed over time with protein concentration decreasing 2.1 g kg<sup>-1</sup> for every g kg<sup>-1</sup> increase in oil concentration. The significant interaction between fungicide treatment and MG III cultivar release year for plant stand revealed that such treatments were more beneficial with respect to obsolete cultivars of MG III, though this plant stand interaction did not translate into a significant yield interaction. The rate of genetic yield improvement made by breeders was not influenced by fungicide management and matched the observed rate of on-farm yield improvement that occurred during the same period.

Soybean has been in commercial production in the United States since the early 1920s (USDA-NASS, 2014). In the last 90 yr, soybean production has increased by 29.8 million ha whereas yield increased by more than 2000 kg ha<sup>-1</sup> (USDA-NASS, 2014). Rowntree et al. (2013) and Wilson et al. (2014) provided a summary of the genetic yield gain with respect to its interaction with crop management practices, which in their papers, focused on planting date and N fertilization, respectively. Other factors such as chemical disease management and the introduction of genes for resistance to disease in newer cultivars could also influence the rate of genetic yield gain over time.

Various management practices have been proposed and used to mitigate damage caused by disease. Tillage, crop rotation, use of resistant cultivars, and fungicides are the principal management practices available for soybean disease control (Elmore, 1991; Crookston et al., 1991; Swoboda and Pedersen, 2009). The use of foliar fungicides has been the subject of much current research, and has been shown to have variable effects on soybean seed yield. Yield gains of up to 25% have been reported with the use of pyraclostobin alone (Nelson and Meinhardt, 2011; Nelson et al., 2010; Henry et al., 2011). These authors consistently reported that this fungicide resulted in a reduction in either foliar disease incidence or its severity from the treatments, primarily in Septoria brown spot (*Septoria glycines* Hemmi, SBS) and frogeye leaf spot (*Cercospora sojina* K. Hara, FLS). Kyveryga et al. (2013) found a correlation between above average spring rainfall and an increased likelihood of a response to foliar fungicides. Other authors have not found a positive yield response from pyraclostobin applications (Swoboda and Pedersen, 2009; Dorrance et al., 2010). The primary seed constituents, protein and oil, were not affected by treatment (Swoboda and Pedersen, 2009; Henry et al., 2011). Seed mass increased, but seed number ha<sup>-1</sup> was unchanged (Swoboda and Pedersen, 2009; Henry et al., 2011), and the increase in seed mass accounted for about 80% of the observed yield gain in Henry et al. (2011). Seed mass is heavily influenced by environmental factors such as water availability and disease pressure, but foliar diseases alone can significantly reduce seed mass.

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**Abbreviations:** FLS, frogeye leaf spot; MGs, maturity groups; PRSR, phytophthora root and stem rot; SBS, Septoria brown spot.

Nelson and Meinhardt (2011) found SBS and FLS caused a respective 16 and 29% loss in seed mass. Variation in environments and inoculum levels in the previous studies may account for the inconsistent yield responses to fungicide treatments that have been observed to date in soybean. To our knowledge, there is no published evidence to support the claim that foliar fungicides increase soybean yield from physiological enhancements beyond disease suppression.

Planting disease resistant cultivars is an important management practice. New cultivars with resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe) have been shown to yield an average of 14% more than comparable susceptible cultivars, when the pest is present (De Bruin and Pedersen, 2008). Some diseases such as stem canker [*Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell and var. *meridionalis* Morgan-Jones] are far less common now than they were previously. Stem canker resulted in yield losses of up to 50% in Iowa during the 1950s, but was ranked the 16th most damaging disease of the 21 diseases surveyed in Iowa in 2005, and the least damaging in 2006 and 2007 (Lu et al., 2010). Several *Rps* genes have been identified for resistance to specific races of *Phytophthora sojae* Kaufmann and Gerdeman, and can drastically reduce yield loss in environments suitable for infection (Dorrance et al., 2009). Despite advances in genetic resistance to *P. sojae*, phytophthora root and stem rot (PRSR) was the second most yield-limiting disease between 2001 and 2010 (Wrather and Koenning, 2010). When genetic resistance is lacking, soybean producers have turned to chemical control methods (Swoboda and Pedersen, 2009). Today, more than 50% of soybean land area in the Midwest is planted with a fungicide seed treatment to mitigate seedling damp-off and thus increase seedling emergence (Esler and Conley, 2012).

Genetic improvement in yield potential per se has had a significant role in soybean yield gain over time (Wilcox, 2001). Breeding specifically for disease resistance has provided

appreciable protection against losses in yields arising from soybean diseases. Still, diseases continue to reduce yields, which has led to a greater economic incentive for their control. Understanding the role of management practices for controlling foliar diseases, and identifying interactions between genetics and management will help determine best practices for reducing yield loss, thereby leading to greater realization of cultivar genetic yield potential over time.

We expect both genetic resistance and chemical disease management to effectively limit diseases from reducing yield, with older cultivars likely benefiting more from fungicide treatment than newer cultivars. In experimental terms, our primary hypothesis was that fungicide applications would reduce the apparent rate of genetic gain over time, because yields of obsolete cultivars might be enhanced more by the treatments than the yields of modern cultivars, and the resultant interaction would be testable in our experiment. We also hypothesized that seed mass might be increased by the use of fungicides, but that seed protein and oil were unlikely to be unaffected. The objective of our study was to better understand the impact of fungicide applications on soybean yield, seed protein, seed oil, seed mass, lodging, and plant stands at both establishment and harvest, measured in a historic set of MG II and III cultivars that have been released by breeders during an 85-yr period (1923–2008).

## MATERIALS AND METHODS

This study used the same materials and methods as Wilson et al. (2014) and Rowntree et al. (2013) with an additional location, Waseca, MN, added in 2011, and used the same cultivars as the Wisconsin location. Site coordinates, soil characteristics, soil fertility, and previous crop for these locations are presented in Table 1. A set of 59 MG II soybean cultivars released over eight decades, from 1928 to 2008 were planted, and 57 MG III soybean cultivars released from 1923 to 2007

Table 1. Experimental details with respect to test sites, soils, and soybean cyst nematode (SCN) egg counts in 2010 and 2011.

Research site	Arlington Agricultural Research Station		Southern Research & Outreach Center		Crop Sciences Research & Education Center		Throckmorton Purdue Agricultural Center	
Location	Arlington, WI 43°18' N, 89°20' W		Waseca, MN 44°4' N, 93°31' W		Urbana, IL 40°3' N, 88°14' W		Lafayette, IN 40°17' N, 86°54' W	
2010 Previous crop	Corn harvested for silage		Corn harvested for grain		Corn harvested for grain		Corn harvested for grain	
2011 Previous crop	Corn harvested for silage		Corn harvested for grain		Corn harvested for grain		Corn harvested for grain	
Soil Series	Plano silt loam		Webster & Nicollet clay loam		Flanagan silt loam & Drummer silty clay loam		Throckmorton silt loam	
Soil Family	Fine-silty, mixed, mesic Typic Argiudoll		Fine-loamy, mixed, mesic Typic Endoaquolls & fine-loamy, mixed, mesic Aquic Hapludolls		Fine-silty, mixed, mesic Typic Endoaquolls & fine, smectitic, mesic Aquic Argiudolls		Fine-silty, mixed, mesic mollic Oxyaquic Hapludalf	
Tillage	1 pass fall chisel + 1 pass spring field cultivation + 1 pass spring soil finisher		1 pass fall chisel + 1 pass spring field cultivation		1 pass fall chisel + 2 pass spring mulch till		1 pass fall chisel + 2 pass spring field cultivation	
Soil fertility	2010	2011	2010	2011	2010	2011	2010	2011
Phosphorous Bray, mg kg <sup>-1</sup>	43.5	55.5	32.0	37.0	23.4	33.5	66.1	38.6
Potassium, mg kg <sup>-1</sup>	172.5	165.5	184.0	165.0	121.6	122.0	146.3	137.5
pH	7.1	6.9	7.1	5.9	6.1	5.8	6.1	6.0
Organic matter, g kg <sup>-1</sup>	32	32	63	54	41	36	30	29
CEC, cmol <sub>c</sub> kg <sup>-1</sup> †	18.4		19.4–21.5		22.1–22.4		13.6	
SCN Egg Counts (per 100 cc soil)	0	n/a	25	n/a	40	n/a	n/a	n/a

† Data from NRCS Web Soil Survey, <http://websoilsurvey.nrcs.usda.gov> (accessed 17 Feb. 2014) (USDA-NRCS, 2014).

were planted. The list of cultivars, chosen to represent a set of historically significant releases, along with available information on pedigree and genetic disease resistance are presented in Table 2. To provide an estimate of experimental error, 13 MG II cultivars and 15 MG III cultivars were replicated twice within each planting date, for a total of 72 plots per treatment in each maturity group. A limited number of cultivars were

chosen for replication due to limited seed supply and field space constraints. Replicated cultivars within each maturity group were evenly distributed across years of release. The experiment was replicated by environment, defined as location within year, for each maturity group. Plots were mechanically seeded in four rows, spaced 76 cm apart, at a rate of 370,650 seeds ha<sup>-1</sup>. Planted plot dimensions at all locations were 3.1 m wide by

Table 2. Cultivars, year of release, maturity group, plant introduction (PI) number, and disease resistance of the entries used in this study.

Cultivar	Year of release	Maturity Group	PI no.†	<i>P. sojae</i> ‡§	HG type 2.5.7¶	HG type 0
Dunfield#	1923	III	PI548318	n/a	mr††	nr
Illini#	1927	III	PI548348	n/a	nr	nr
Korean#	1928	II	PI548360	n/a	nr	–
AK (Harrow) #	1928	III	PI548298	n/a	nr	mr
Mukden#	1932	II	PI548391	n/a	nr	nr
Mandell	1934	III	PI548381	n/a	nr	nr
Richland#	1938	II	PI548406	n/a	mr	nr
Mingo	1940	III	PI548388	n/a	nr	nr
Lincoln#	1943	III	PI548362	n/a	nr	nr
Hawkeye#	1947	II	PI548577	n/a	nr	nr
Adams	1948	III	PI548502	n/a	nr	–
Harosoy#	1951	II	PI548573	nr	nr	nr
Lindarin	1958	II	PI548589	nr	nr	mr
Shelby	1958	III	PI548574	n/a	nr	mr
Ford	1958	III	PI548562	n/a	mr	mr
Ross	1960	III	PI548612	n/a	nr	nr
Harosoy 63	1963	II	PI548575	R	nr	nr
Hawkeye 63	1963	II	PI548578	R	nr	nr
Wayne#	1964	III	PI548628	mr	nr	nr
Adelphia	1964	III	PI548503	nr	nr	nr
Amsoy	1965	II	PI548506	nr	nr	nr
Corsoy#	1967	II	PI548540	nr	nr	nr
Beeson	1968	II	PI548510	r	nr	nr
Calland#	1968	III	PI548527	r	nr	nr
Amsoy 71#	1970	II	PI548507	r	nr	nr
Williams#	1971	III	PI548631	ms	nr	nr
Wells	1972	II	PI548630	n/a	nr	nr
Woodworth#	1974	III	PI548632	ms	nr	nr
Harcor	1975	II	PI548570	<i>Rps I</i>	nr	nr
Private 2-7	1977	II	n/a	n/a	nr	mr
Private 2-8	1977	II	n/a	n/a	nr	nr
Wells II	1978	II	PI548513	<i>Rps I c</i>	nr	nr
Vickery	1978	II	PI548617	<i>Rps I c</i>	nr	nr
Private 3-1#	1978	III	n/a	n/a	nr	nr
Cumberland	1978	III	PI548542	ms	nr	nr
Oakland	1978	III	PI548543	r	nr	nr
Corsoy 79	1979	II	PI518669	<i>Rps I c</i>	nr	nr
Beeson 80	1979	II	PI548511	<i>Rps I c</i>	nr	nr
Century#	1979	II	PI548512	r	nr	–
Amcor	1979	II	PI548505	r	nr	mr
Pella	1979	III	PI548523	r	nr	nr
Williams 82#	1981	III	PI518671	<i>Rps I k</i>	nr	nr
Private 2-11	1982	II	n/a	n/a	nr	–
Private 3-15	1983	III	n/a	n/a	nr	nr
Century 84	1984	II	PI548529	<i>Rps I k</i>	nr	nr
Elgin	1984	II	PI548557	nr	nr	nr
Zane	1984	III	PI548634	nr	nr	nr
Harper	1984	III	PI548558	mr	nr	nr
Preston	1985	II	PI548520	nr	nr	nr

Continued next page.

Table 2. (continued).

Cultivar	Year of release	Maturity Group	PI no.†	<i>P. sojae</i> ‡§	HG type 2.5.7¶	HG type 0
Private 2-15	1985	II	n/a	n/a	nr	nr
Chamberlain#	1986	III	PI548635	<i>Rps 1</i>	nr	nr
Private 3-2	1986	III	n/a	n/a	nr	nr
Resnik	1987	III	PI534645	<i>Rps 1k</i>	nr	nr
Pella 86	1987	III	PI509044	<i>Rps 1k</i>	nr	nr
Burlison	1988	II	PI533655	<i>Rps 1b &amp; 3</i>	nr	nr
Private 2-9	1988	II	n/a	n/a	nr	–
Elgin 87	1988	II	PI518666	<i>Rps 1k</i>	nr	nr
Conrad#	1988	II	PI525453	nr	nr	mr
Jack#	1989	II	PI540556	nr	nr	hr
Kenwood	1989	II	PI537094	<i>Rps 1</i>	nr	–
Private 2-1	1989	II	n/a	n/a	nr	nr
Private 3-9	1989	III	n/a	n/a	nr	mr
Private 2-2	1990	II	n/a	n/a	nr	nr
Private 3-10	1990	III	n/a	n/a	nr	mr
RCAT Angora	1991	II	PI572242	<i>Rps 1c</i>	nr	nr
Private 2-6	1991	II	n/a	n/a	nr	–
Private 3-16	1991	III	n/a	n/a	nr	hr
Dunbar	1992	III	PI552538	r	nr	nr
Thorne	1992	III	PI564718	<i>Rps 1k</i>	nr	nr
Private 3-17	1992	III	n/a	n/a	nr	nr
Private 2-5	1993	II	n/a	n/a	nr	nr
Private 3-18	1993	III	n/a	n/a	nr	nr
Private 2-10	1994	II	n/a	n/a	nr	nr
Private 2-16	1994	II	n/a	n/a	mr	hr
Private 3-19	1994	III	n/a	n/a	nr	nr
IA 2021	1995	II	n/a	<i>Rps 1k</i>	nr	nr
Macon#	1995	III	PI593258	nr	nr	nr
IA 3004	1995	III	n/a	n/a	nr	nr
Savoy	1996	II	PI597381	<i>Rps 1b &amp; 3</i>	nr	nr
Private 2-12	1996	II	n/a	n/a	nr	nr
Maverick	1996	III	PI598124	<i>Rps 1k</i>	nr	r
Private 3-4	1996	III	n/a	n/a	nr	nr
Private 3-11	1996	III	n/a	n/a	nr	nr
Dwight#	1997	II	PI597386	nr	nr	hr
Private 218	1997	II	n/a	n/a	nr	nr
Pana	1997	III	PI597387	nr	nr	hr
Private 3-5	1997	III	n/a	n/a	nr	r
Private 3-12	1997	III	n/a	n/a	nr	nr
IA 2038	1998	II	n/a	n/a	nr	nr
Private 3-6	1998	III	n/a	n/a	mr	hr
IA 3010	1998	III	n/a	nr	nr	nr
Private 3-7#	1999	III	n/a	n/a	nr	nr
IA 2050	2000	II	n/a	n/a	nr	nr
IA 2052	2000	II	n/a	nr	nr	nr
Private 3-20	2000	III	n/a	n/a	nr	nr
Loda#	2001	II	PI614088	nr	mr	–
Private 2-4	2001	II	n/a	<i>Rps 1k</i>	nr	nr
Private 2-17	2001	II	n/a	n/a	nr	hr
U98-311442	2001	III	n/a	n/a	mr	hr
IA 3014	2001	III	n/a	r	nr	r
Private 3-8#	2002	III	n/a	n/a	nr	r
IA 2068	2003	II	n/a	n/a	mr	hr
IA 3023	2003	III	n/a	nr	mr	nr
Private 2-3	2004	II	n/a	<i>Rps 1k</i>	nr	r
NE3001	2004	III	n/a	n/a	nr	nr
Private 3-13#	2004	III	n/a	n/a	nr	nr

Continued next page.

Table 2. (continued).

Cultivar	Year of release	Maturity Group	PI no.†	<i>P. sojae</i> ‡§	HG type 2.5.7¶	HG type 0
IA 3024	2004	III	n/a	nr	nr	nr
IA 2065	2005	II	n/a	n/a	nr	nr
Private 2-19	2005	II	n/a	n/a	nr	hr
Private 2-20	2005	II	n/a	n/a	nr	nr
IA 2094	2006	II	n/a	nr	nr	nr
Private 3-22	2006	III	n/a	n/a	nr	nr
Private 3-23	2006	III	n/a	n/a	nr	nr
Private 3-14	2007	III	n/a	n/a	mr	r
Private 2-13	2008	II	n/a	<i>Rps 1k</i>	nr	r
Private 2-14#	2008	II	n/a	<i>Rps 1k</i>	nr	hr

† n/a, not applicable.

‡ n/a, not available.

§ Resistance gene listed if available.

¶ *Heterodera glycines* population type, Type 2.5.7 can reproduce on PI88788, PI209332, and PI548316 sources of resistance. Type 0 does not reproduce on known sources of resistance.

# Cultivars replicated within location.

†† mr, moderately resistant; nr, not resistant; ms, moderately susceptible; r, resistant; hr, highly resistant.

4.6 m long. Plant populations were recorded for all plots at the V1 (first trifoliolate) and R8 (95% pod maturity) growth stages, as defined by Fehr and Caviness (1977). The center two rows of each plot were mechanically harvested a few days after R8. Grain weight and moisture data were collected simultaneously at harvest so that seed yield could be expressed on a 130 g kg<sup>-1</sup> seed moisture content basis.

At each location in both 2010 and 2011, two blocks were established, each with the same completely randomized set of cultivars. One block was intensively managed with disease control measures and the other remained non-treated. The cultivars planted in the treated block received fungicidal seed treatment in combination with three applications of foliar fungicides occurring at the R1, R3, and R5 growth stages. See Table 3 for additional details regarding the fungicide active ingredients and application timing. Both the seed treatment and foliar fungicides were applied according to company recommended label rates and growth stages. The three foliar fungicide applications were both prophylactic and more frequent than standard grower practices to assure maximum disease protection. In Minnesota, Indiana, and Illinois, foliar treatments were applied with CO<sub>2</sub>-pressurized hand sprayers. In Wisconsin, the R1 application in 2010 was applied with a CO<sub>2</sub>-pressurized hand sprayer, and all subsequent applications in 2010 and 2011 used a tractor-mounted sprayer. Sprayers were equipped with XR TeeJet 8002 nozzles (Spraying Systems Co., Wheaton, IL) and calibrated to deliver 140 L ha<sup>-1</sup> in WI, IN, and IL, and 150 L ha<sup>-1</sup> in MN.

In 2011, a planting error in MN resulted in the experiment being planted at half the target rate. Despite low plant

populations, data from MN were still collected and retained for the analysis, given that the magnitude of the treatment effect was similar to that of the other locations. The MN results, as expected, exhibited greater variation in yield and loss of precision in determining treatment effects. Additionally, 13 contiguous plots from MN in 2011 were removed from the analysis due to severe water damage. Early season ponding resulted in uneven stands and yield. Plant populations were recorded for all plots and locations at V1 and R8 growth stages. Lodging scores were recorded for each plot before harvest using a 1 to 5 scale, with 1 being fully erect and 5 being fully prostrate. Foliar disease severity ratings in the center two rows of a 4.6 m plot were accomplished by visual estimation of the percentage (0–100%) of foliar area exhibiting disease symptoms. Ratings were performed only in 2011, but repeated at the R1, R3, R5, and R7 stages for Arlington, Waseca, and Lafayette, and at the R3, R5, and R7 stages in Urbana.

Yield, seed mass, seed protein and oil, lodging, and plant stands at establishment and harvest were subjected to the same analysis as Rowntree et al. (2013). The main effects were fungicide treatment, cultivar year of release, and fungicide treatment × year of release interaction. Variables were regressed over year of release to evaluate the change over time with the two treatments. The change in rate per year is estimated by the slope of the regression line plus or minus the standard error. The interaction of fungicide by release year was examined to determine if differences existed in the rate of change for each variable. Final models were chosen using appropriate statistics (AIC, BIC, -2 Res Log Likelihood), as well as biological interpretation.

Table 3. Fungicide treatments and applications used for all locations.

Trade name	Active ingredient	Percent composition	Application rate	Application timing†	FRAC Code‡
Apron Maxx	Fludioxonil	2.31	2.5 g 100 kg seed <sup>-1</sup>	SA§	12
	Mefenoxam	3.46	3.75 g 100 kg seed <sup>-1</sup>		
Endura	Boscalid	70	538 g ha <sup>-1</sup>	R1 & R3	7
Headline	Pyraclostrobin	23.6	220 g ha <sup>-1</sup>	R3	11
Stratego	Propiconazole	11.4	91 g ha <sup>-1</sup>	R5	3
	Trifloxystrobin	11.4	91 g ha <sup>-1</sup>		

† Soybean reproductive stages (Fehr and Caviness, 1977).

‡ Fungicide Resistance Action Committee Code List 2011.

§ Seed applied.

## RESULTS AND DISCUSSION

Climatic conditions during the study were reported in Rowntree et al. (2013). Additionally, the Waseca, MN, location had above average early-season rainfall and was affected by a slight frost on 15 Sept. 2011, damaging the upper canopy (Table 4).

Soybean yield increased at a rate of  $20.9 \pm 2.20 \text{ kg ha}^{-1} \text{ yr}^{-1}$  with respect to MG II cultivars released over the 85-yr time frame, and  $23.4 \pm 1.85 \text{ kg ha}^{-1} \text{ yr}^{-1}$  for the group III cultivar releases (Fig. 1). These estimated rates of genetic gain are very close to the realized rate of U.S. on-farm yield gain of  $23.3 \text{ kg ha}^{-1} \text{ yr}^{-1}$  from 1924 to 2013 (USDA-NASS, 2014). The on-farm USDA rate represents what the average U.S. soybean producer achieves each year by adopting both genetic technology and improved or novel agronomic practices. Our estimates are dependent on the agronomic yield potential of the field sites where the historic cultivar sets were grown. While our estimated rates of genetic yield gain are about equal to the rate of on-farm yield gain achieved by the average U.S. producer, these genetic rates of gain may not account for the entire rate of on-farm yield gain achieved in the most highly productive environments.

The genetic gain estimated here and in Rowntree et al. (2013) and Wilson et al. (2014) is similar to previous estimates by Luedders (1977), Boerma (1979), Wilcox et al. (1979), Voldeng et al. (1997), and Morrison et al. (2000). The linear fit suggests the rate of gain has remained relatively constant over the past 85 yr. However, there is evidence that the rate of gain has been increasing in recent years (Voldeng et al., 1997; Specht et al., 1999).

Seed protein concentrations modestly decreased by  $0.22 \pm 0.07 \text{ g kg}^{-1} \text{ yr}^{-1}$  in MG II, and  $0.28 \pm 0.06 \text{ g kg}^{-1} \text{ yr}^{-1}$  for the group III cultivar releases (Fig. 2). Over the 85 yr of cultivar releases examined in this study, there was a total reduction in protein of 18.7 and  $23.6 \text{ g kg}^{-1}$  for MG II and III, respectively. Rowntree et al. (2013) similarly found that protein decreased  $0.191 \text{ g} \pm 0.07 \text{ kg}^{-1} \text{ yr}^{-1}$  in MG II, and MG III decreased by  $0.242 \pm 0.06 \text{ g kg}^{-1} \text{ yr}^{-1}$ . Morrison et al. (2000) estimated

a greater decrease in protein of  $0.537 \text{ g kg}^{-1} \text{ yr}^{-1}$  in MG 0 and 00. Protein may have been indirectly selected against as new varieties were selected for higher yield. The decline in seed protein concentration coincided with an increase in seed oil concentrations, as is often observed (Hartwig and Kilen, 1991). Oil concentration gained  $0.11 \pm 0.03 \text{ g kg}^{-1} \text{ yr}^{-1}$  and  $0.13 \pm 0.04 \text{ g kg}^{-1} \text{ yr}^{-1}$  for MG II and III, respectively (Fig. 3). Over the 85 yr, there was a total gain of 9.1 and  $10.9 \text{ g kg}^{-1}$ , respectively. Wilson et al. (2014) similarly found that oil increased  $0.13 \pm 0.03 \text{ g kg}^{-1} \text{ yr}^{-1}$  in MG II and  $0.12 \pm 0.03 \text{ g kg}^{-1} \text{ yr}^{-1}$  in MG III. This is less than the  $0.449 \text{ g kg}^{-1} \text{ yr}^{-1}$  for oil estimated by Morrison et al. (2000). The sum of protein and oil concentrations decreased by  $0.11 \pm 0.05 \text{ g kg}^{-1} \text{ yr}^{-1}$  for MG II and  $0.15 \pm 0.05 \text{ g kg}^{-1} \text{ yr}^{-1}$  in MG III. Over the 85 yr, there was a combined loss of 10.7 and  $13.7 \text{ g kg}^{-1}$  for MG II and III, respectively. This result suggests that selection for greater yield has diverted photoassimilate from these metabolically costly constituents into carbohydrates to obtain greater seed production. This loss in total seed quality shows the inverse relationship is not 1:1 (Wilcox and Guodong, 1997).

Fungicide treatment altered seed protein and oil contents for MG II but not MG III (Table 5). The protein in treated MG II cultivars increased  $6.8 \text{ g kg}^{-1}$  and the oil decreased  $4.0 \text{ g kg}^{-1}$ . Similarly, Nelson et al. (2010) reported a change in seed constituents from the application of a fungicide and insecticide. They found an increase in grain oil concentration and a decrease in protein with an R4 application of azoxystrobin plus lambda-cyhalothrin. Our MG III results are in agreement with those of Swoboda and Pedersen (2009) who observed no effect of fungicides on seed protein or oil.

Seed mass was not affected by the cultivar release year or by fungicide treatment in the historic MG II cultivar set. Release year in MG III cultivars had an effect (Table 5), with an increase in seed mass per year of  $0.015 \pm 0.008 \text{ g } 100 \text{ seeds}^{-1}$  (Fig. 4). The change in seed mass is consistent with previous reports that seed mass has shown little (Specht and Williams, 1984) or no (Morrison et al., 2000) change over time. There was no fungicide treatment effect on seed mass in our

Table 4. Mean monthly air temperature and total monthly precipitation at the four locations during the 2010 and 2011 growing seasons, and 30-yr average.†

Month	Arlington, WI			Waseca, MN		Urbana, IL			Lafayette, IN		
	2010	2011	30 yr	2011	30 yr	2010	2011	30 yr	2010	2011	30 yr
°C											
Air temperature											
April	10.4	6.2	7.1	6.3	7.7	15.1	11.9	11.1	14.9	11.6	10.7
May	15.3	13.4	13.2	13.9	14.6	18.3	16.9	16.9	18.1	17.1	16.6
June	19.7	19.6	18.7	20.1	20.1	23.8	22.8	22.3	23.3	22.6	21.8
July	22.9	24.0	20.8	24.6	22.1	25.2	26.8	23.8	24.4	26.0	23.4
August	22.2	21.0	19.6	21.1	14.1	25.1	24.1	23.0	24.3	22.7	22.4
September	15.6	14.5	15.2	15.5	16.1	19.7	17.5	19.0	19.4	17.1	18.8
mm											
Precipitation											
April	107.5	106.4	88.9	168.1	82.9	48.5	214.6	93.5	72.9	192.6	86.6
May	88.9	55.4	93.7	118.6	100.1	78.5	121.9	124.2	72.6	113.4	117.9
June	169.4	98.8	118.9	250.4	119.4	198.6	106.7	110.2	95.0	92.8	115.6
July	222.8	64.3	105.7	183.1	115.5	90.7	39.9	119.4	66.3	45.5	103.6
August	114.0	39.9	99.1	23.4	99.8	40.1	44.7	99.8	42.2	26.3	100.1
September	50.5	96.5	89.9	45.2	90.8	76.7	70.9	79.5	24.1	82.8	71.2

† 30-yr average from 1971 to 2000.

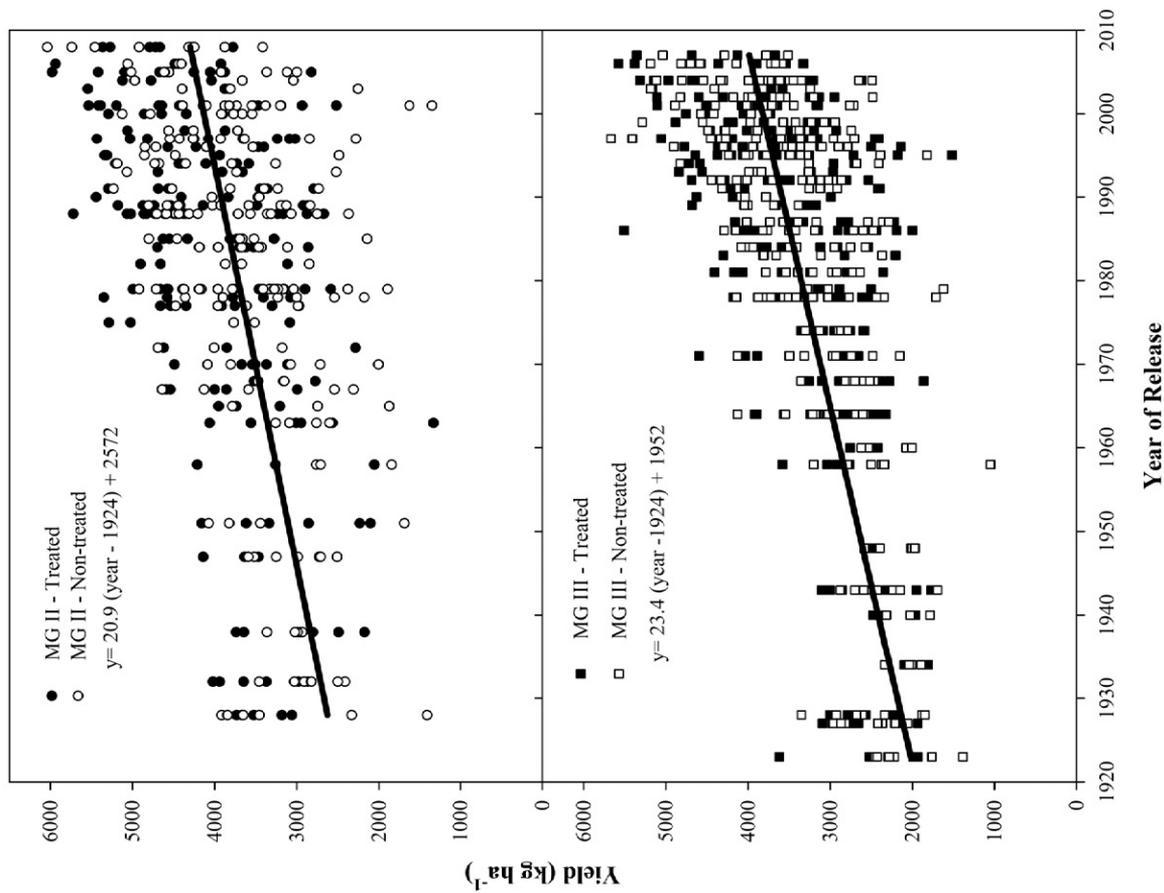


Fig. 1. Seed yield of maturity group II (top) and III (bottom) cultivars vs. year of release, with and without a fungicide treatment applied on the seed and during reproductive development. Data points are 2010–2011 means. Best-fit linear regression was used to derive the trend lines, with the equations formatted to display a 1924 y intercept value.

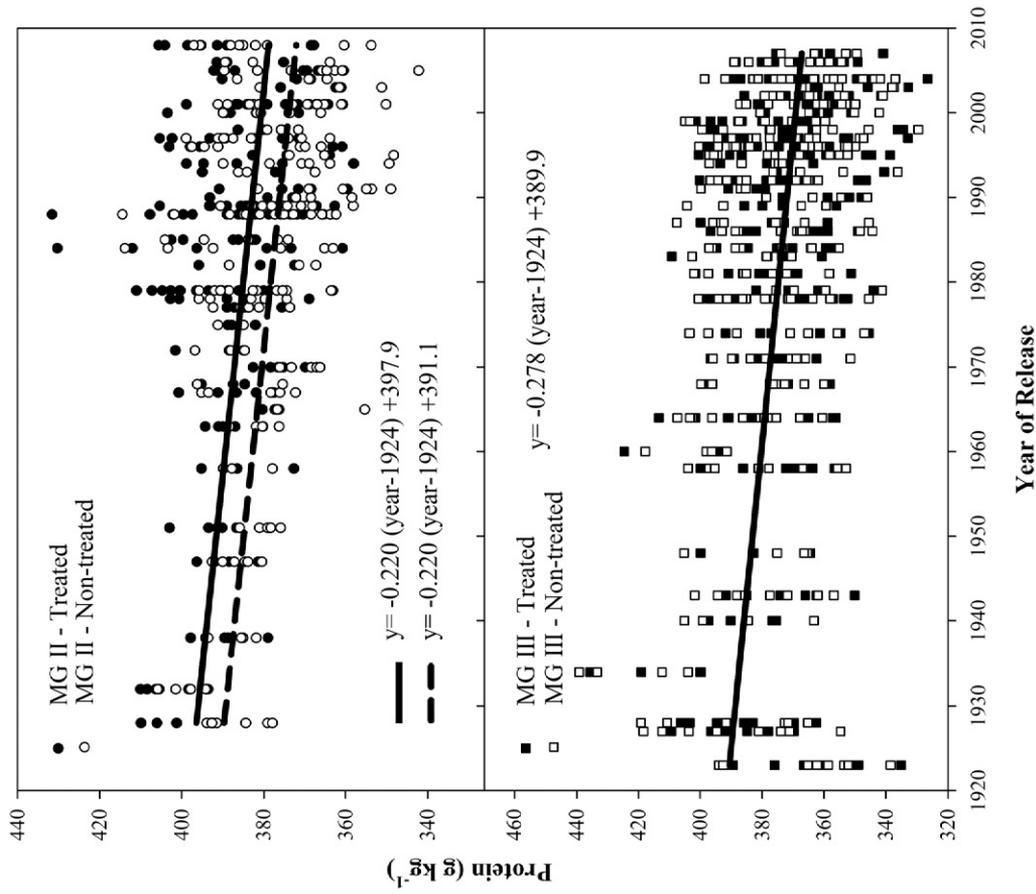


Fig. 2. Seed protein of maturity group II (top) and III (bottom) cultivars vs. year of release, with and without a fungicide treatment applied on the seed and during reproductive development. Data points are 2010–2011 means. Best-fit linear regression was used to derive the trend lines (solid and dashed, respectively), with the equations formatted to display a 1924 y intercept value.

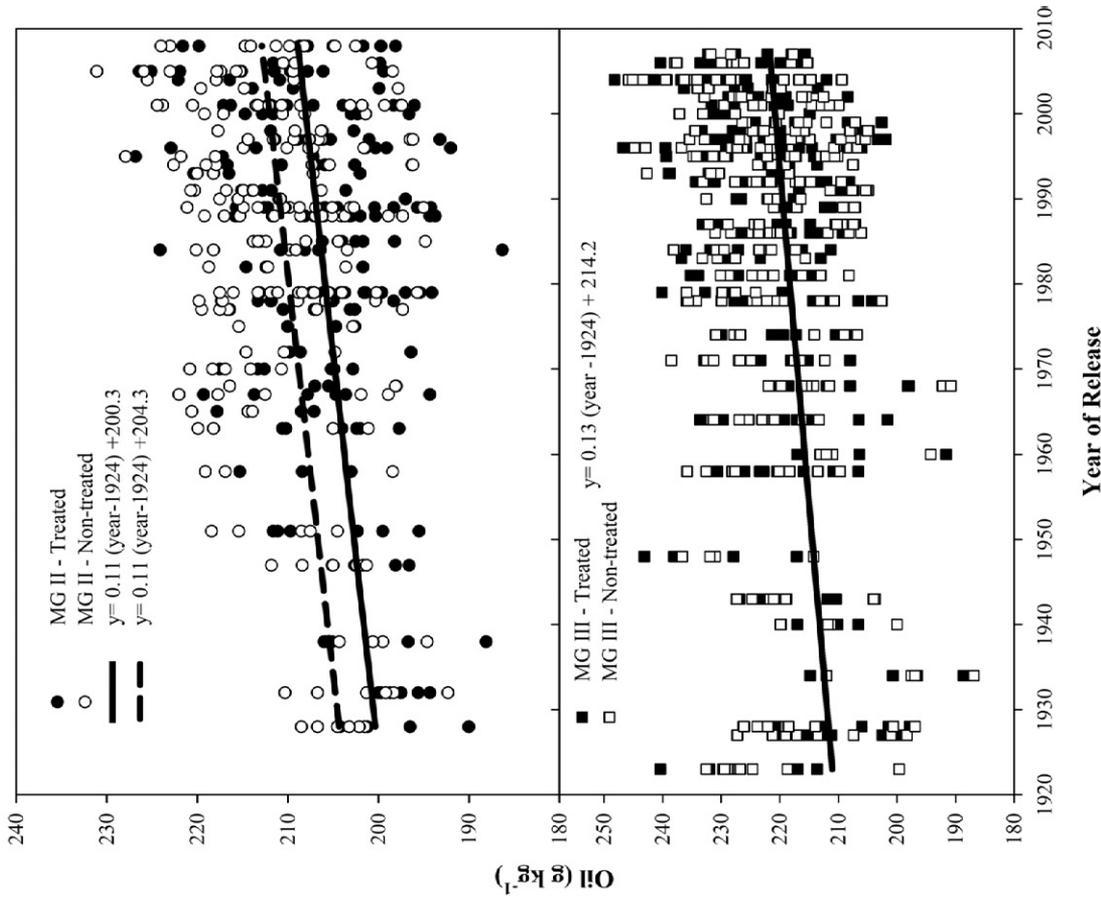


Fig. 3. Seed oil of maturity group II (top) and III (bottom) cultivars vs. year of release, with and without a fungicide treatment applied on the seed and during reproductive development. Data points are 2010–2011 means. Best-fit linear regression was used to derive the trend lines (solid and dashed, respectively), with the equations formatted to display a 1924 *y* intercept value.

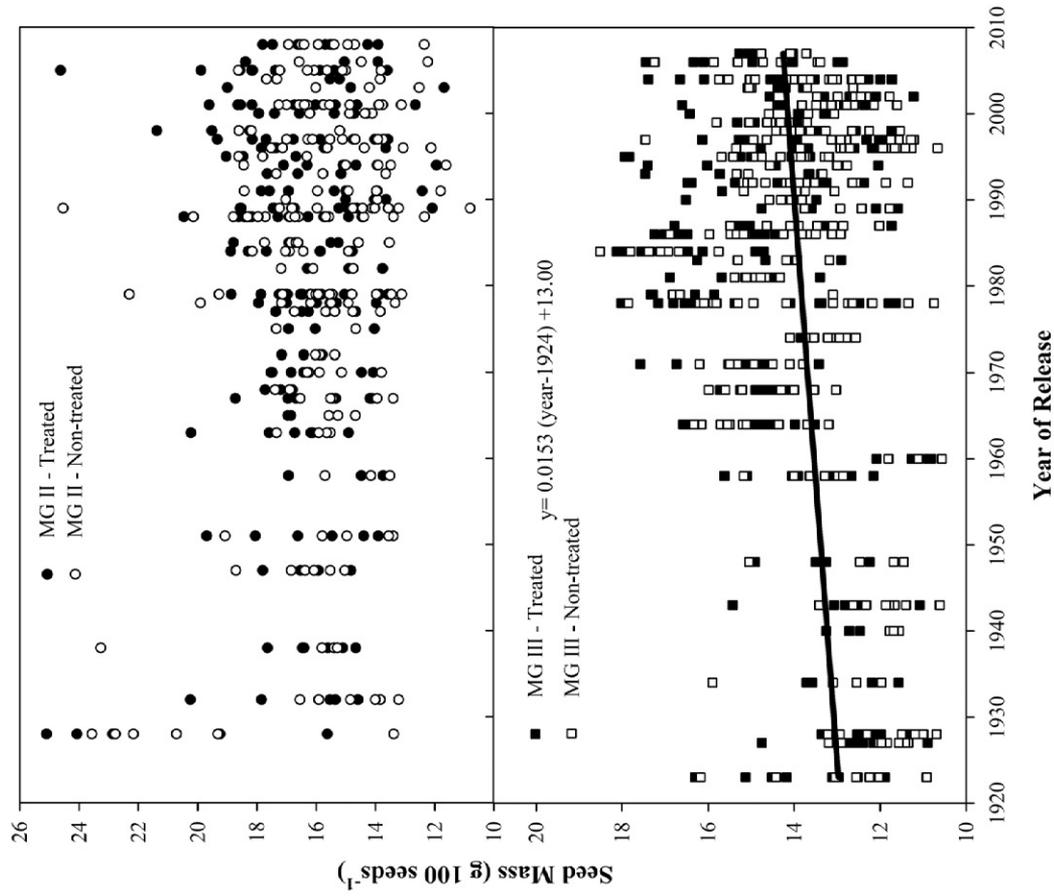


Fig. 4. Seed mass of maturity group II (top) and III (bottom) cultivars vs. year of release, with and without a fungicide treatment applied on the seed and during reproductive development. Data points are 2010–2011 means. Best-fit linear regression was used to derive the trend lines, with the equations formatted to display a 1924 *y* intercept value.

Table 5. Statistical significance of cultivar release year, fungicide treatment, and their interaction on the measured traits.

Factor	Yield	Protein concentration	Oil concentration	Seed mass	Lodging	VI † pop	R8 † pop
<u>Maturity Group II</u>							
Release year (RY)	***	***	***	ns ‡	***	ns	**
Fungicide treatment (F)	ns	***	***	ns	ns	ns	ns
RY × F	ns	ns	ns	ns	ns	ns	ns
<u>Maturity Group III</u>							
RY	***	***	**	*	***	ns	**
F	ns	ns	ns	ns	ns	**	*
RY × F	ns	ns	ns	ns	ns	**	*

\* Significant at  $P \leq 0.05$ .

\*\* Significant at  $P \leq 0.01$ .

\*\*\* Significant at  $P \leq 0.001$ .

† Populations recorded after emergence and before harvest at the VI and R8 stages described by Fehr and Caviness (1977).

‡ ns, not significant.

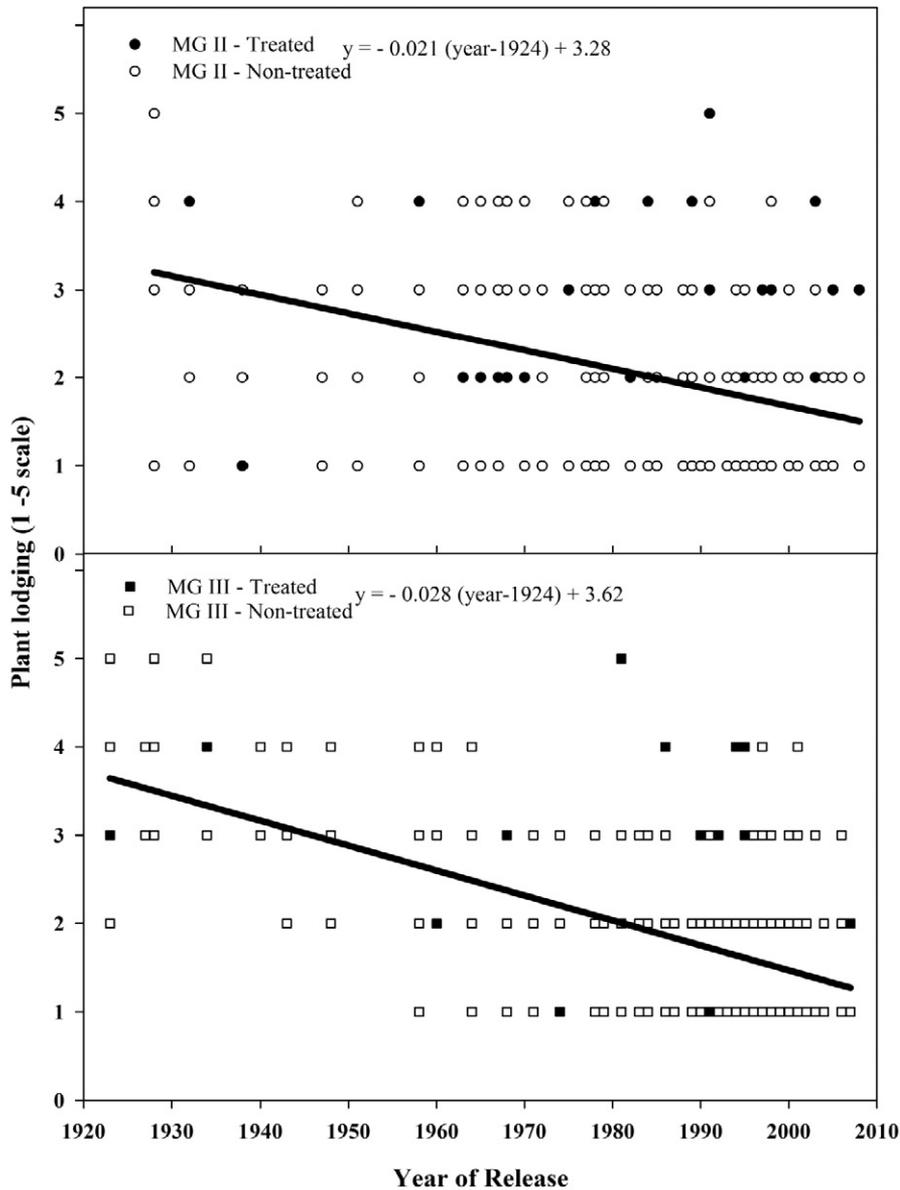


Fig. 5. Lodging score of maturity group II (top) and III (bottom) cultivars vs. year of release, with and without a fungicide treatment applied on the seed and during reproductive development. Data points are 2010–2011 means. Best-fit linear regression was used to derive the trend lines, with the equations formatted to display a 1924 y intercept value.

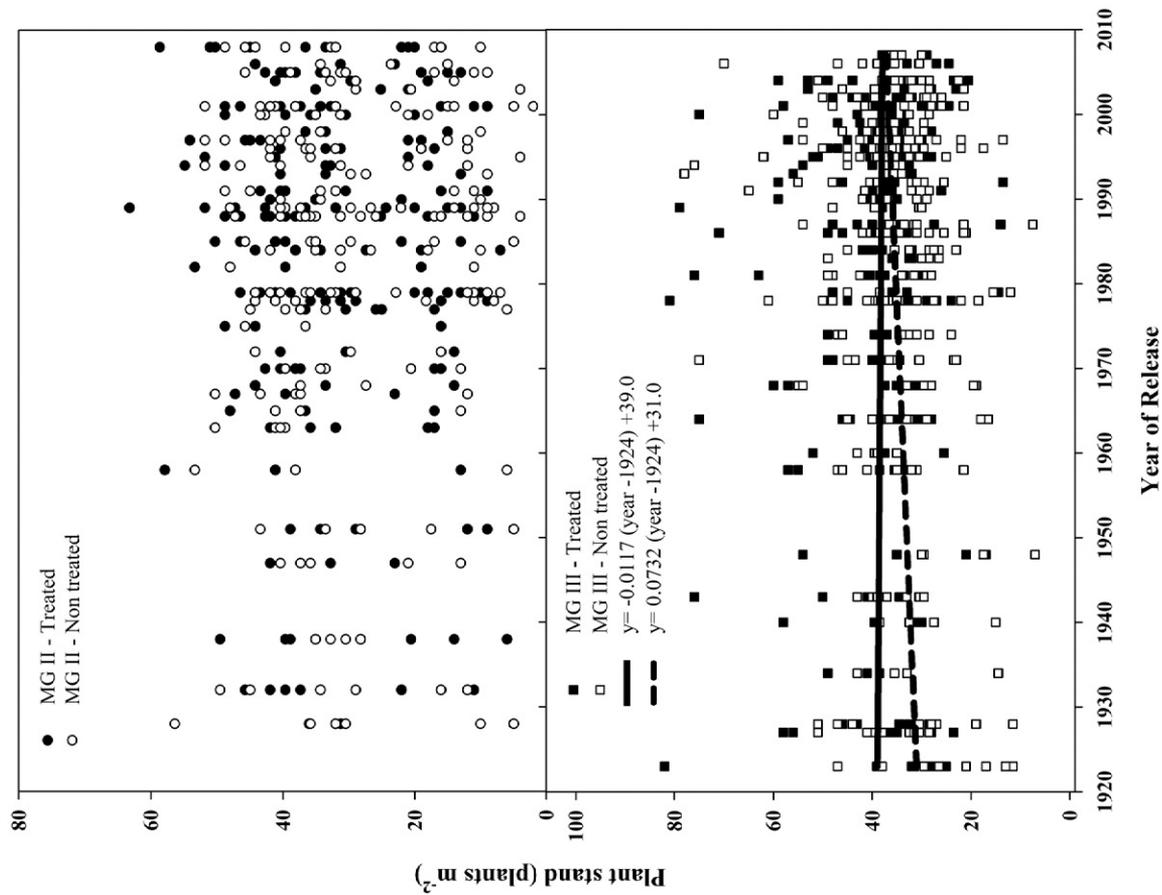


Fig. 6. Plant population recorded after emergence (at the VI stage) for maturity group II (top) and III (bottom) cultivars vs. year of release, with and without a fungicide seed treatment. Best-fit linear regression was used to derive the trend lines (solid and dashed, respectively), with the equations formatted to display a 1924 y intercept value. Data points are 2010–2011 means.

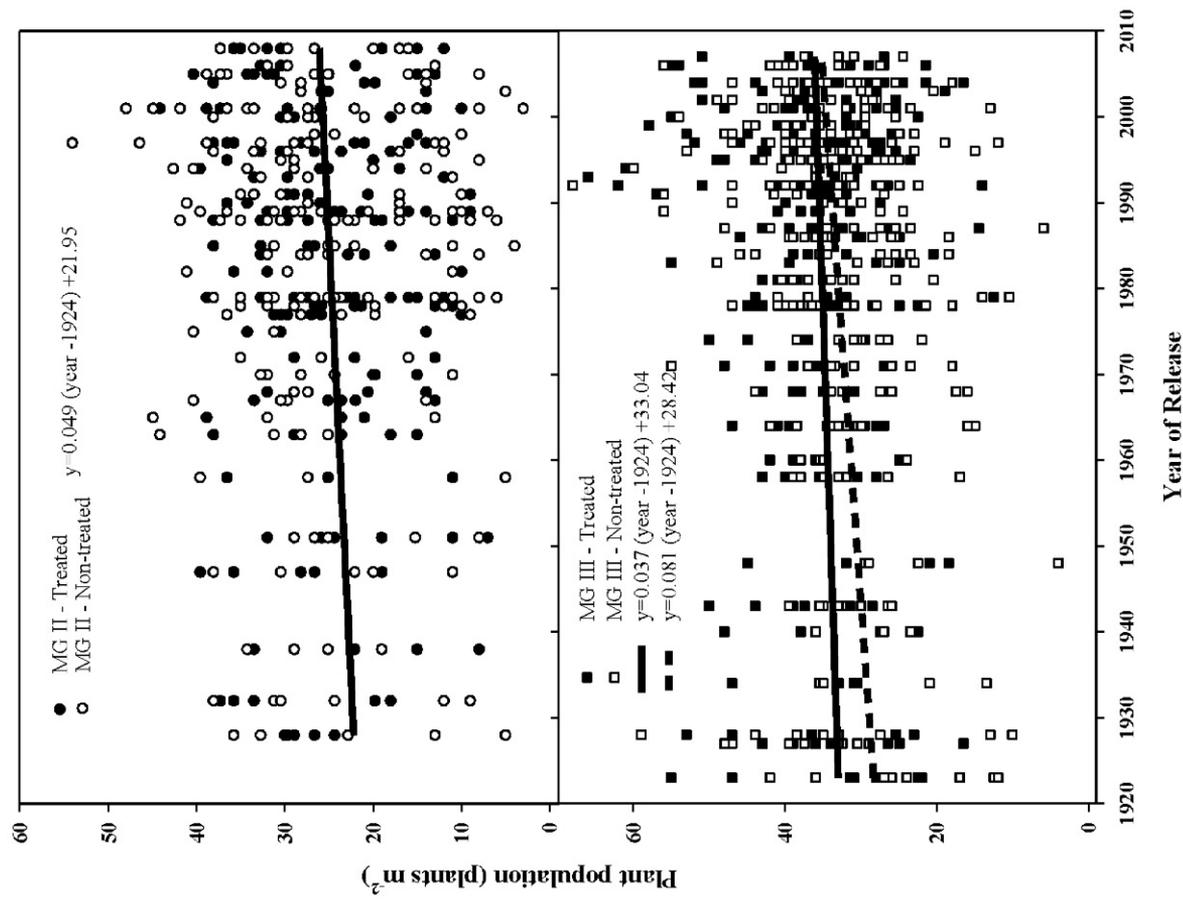


Fig. 7. Plant population recorded before harvest (at the R8 stage) for maturity group II (top) and III (bottom) cultivars vs. year of release, with and without a fungicide seed treatment and foliar applications at reproductive stages. Best-fit linear regression was used to derive the trend lines (solid and dashed, respectively), with the equations formatted to display a 1924 y intercept value. Data points are 2010–2011 means.

experiment, inconsistent with previous reports by Swoboda and Pedersen (2009), and Henry et al. (2011). The late season frost and below average rainfall in 2011 may have contributed to reduced seed mass and could have mitigated a putative positive effect of the fungicide treatment that year.

Recently released cultivars exhibited less lodging than older cultivars when averaged over maturity groups (Table 5). This trend of less lodging in newer, improved cultivars has been well-documented in previous genetic gain research (Luedders, 1977; Wilcox et al., 1979; Specht and Williams, 1984; Voldeng et al., 1997). An upright stand helps to control disease by reducing both the relative humidity under the canopy, and free water required for infection, effectively allowing escape from disease development (Kim and Diers, 2000). We detected different rates of lodging reduction over the 85 yr between the two maturity groups; in MG II the total decrease was 1.8 score units, whereas in MG III, the total decrease was 2.4 units (Fig. 5). These lodging reduction rates were not affected by fungicide treatment. The rates of lodging reduction we noted were greater than the  $-0.014$  units  $\text{yr}^{-1}$  previously reported by Voldeng et al. (1997).

Plant populations at establishment (V1) and at harvest (R8) were different in both MGs. In MG II, there was no difference between modern and obsolete cultivars or between treated and non-treated plots relative to stand establishment (Fig. 6), although the low seeding rate in Minnesota decreased the precision in detecting treatment effects. A different response was observed in MG III. Initial plant stands in the seed-treated plots remained constant across year of release. However, in non-treated plots, initial plant stands were lower than treated stands in obsolete cultivars, but equal to treated stands in modern cultivars. The increase in plant stand establishment over time for MG III cultivars may be due to greater resistance to damping-off diseases, though this was not directly measured. This is further supported by the gradual loss of seed treatment effect as ever improved cultivars replaced their predecessors over release years. It appears breeders have successfully selected for traits leading to greater stand establishment in MG III. The plant stands at harvest showed a slightly different response than those at establishment. Both MGs had greater harvest populations in newer cultivars than the older cultivars. Maturity group II averaged 4.2 more plants  $\text{m}^{-2}$  for cultivars released in 2008 than for those released in 1924 (Fig. 7). Maturity group III cultivars averaged 3.1 and 6.9 more plants  $\text{m}^{-2}$  in treated and non-treated plots, respectively. The increased slope of the harvest population compared to the establishment population indicates that older cultivars had greater plant mortality during the season. This mortality could be the result of disease or from the obsolete cultivars being unable to support the population due to interplant competition.

At every location in 2011, SBS and bacterial blight (BB) were the most prevalent diseases observed and averaged a respective 70 to 100% and 0 to 80% incidence in plots. Study cooperators in West Lafayette, IN, in 2010 observed 38% incidence of Sudden Death Syndrome (caused by the soilborne *Fusarium virguliforme* [Aoki et al, 2003], SDS). At the Urbana site in 2011, FLS was observed in 24% of plots. At the Waseca site in 2011, PRSR was observed in 23% of plots, and was more severe on cultivars without genetic resistance (data not shown). At the

Arlington site in 2011, stem canker incidence was observed in 45% of plots. In both years, foliar disease severity ranged between 0 and 10% at all locations, and was below economic threshold.

## CONCLUSIONS

We found that fungicides were equally effective at reducing yield losses from disease in obsolete and modern cultivars. Across Minnesota, Wisconsin, Illinois, and Indiana, we estimated genetic yield improvement of  $20.9 \pm 2.20$   $\text{kg ha}^{-1} \text{yr}^{-1}$  for MG II and  $23.4 \pm 1.85$   $\text{kg ha}^{-1} \text{yr}^{-1}$  for MG III over the years 1923 to 2008. It appears that our observed rate of genetic improvement made by breeders matches the rate of on-farm yield improvement that occurred in the same time frame. Selection for higher yield has likely resulted in decreased protein and decreased combined protein and oil concentrations. Additionally, obsolete cultivars had greater plant mortality during the season than modern cultivars, and the fungicide treatment reduced the magnitude of this effect. Average soybean seed mass increased slightly over time for MG III and remained constant for MG II. Increasing seed mass while maintaining seed number could explain how fungicides have contributed to grain yield in some environments but not others. Further research is needed to understand what mechanisms could lead to greater seed mass with a fungicide treatment.

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