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Soybean (*Glycine max* (L.) Merr.) Yield Reduction due to Late Sowing as a Function of Radiation Interception and Use in a Cool Region of Northern Japan

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Abstract: Yields decrease when soybean is sown later than recommended in the cool climate of the Tohoku region of Japan. However, the factors responsible for this decrease are not fully understood. We investigated the effects of late sowing on growth, phenological development, yield, yield components, and radiation interception of three soybean cultivars in two consecutive years and analyzed the relationships of those variables with temperature and soil volumetric moisture content (SMC). Averaged across years and cultivars, yields decreased significantly when plants were sown approximately three weeks late. Yield reductions were partially due to reductions in node number per plant, dry matter production, and capture of cumulative irradiance, resulting from slowed canopy development during vegetative and early reproductive stages. The number of seeds per pod was one of the major determinants of the variation in yield. Owing to the delay in sowing date, the reduction in seeds per pod was likely due to low temperatures during the 20 days after seed filling began. Occasional lower SMC during reproductive stages did not affect yield, yield components, and growth parameters. However, these results were obtained from the two years' experiments. Therefore, further investigations of the relationship of yield with temperature and SMC under different years and sites are needed.

Keywords: developmental phenology; late sowing; northern Japan; soil water content; solar radiation; soybean cultivars; temperature; yield reduction

1. Introduction

Soybean (*Glycine max* [L.] Merr.) is an important crop for food, protein, and oil. In Japan, soybean is used in traditional foods such as tofu, natto, and miso. However, the soybean yield in Japan is much lower than that in the other major producing countries such as the USA, Brazil, and China, and has stagnated over the past 30 years; the average (1982–2012) soybean yield is only 1.65 t ha^{-1} and has increased by less than 0.3 t ha^{-1} since 1982 [1]. Therefore, to increase and stabilize soybean yields, a strategy is needed that includes both practical agronomic management and genetics.

The optimization of sowing dates is the most important and least expensive agronomic practice that affects soybean yield [2]. Several studies show that recent yield increases in the USA were achieved by optimizing the sowing date with the cultivar maturity group (MG) [3,4]. In the Tohoku region of northern Japan, paddy rice monoculture is the dominant crop system, and the optimal transplanting time for paddy rice is mid-May to late May. The currently recommended sowing window for soybeans is late May to early June after the rice transplanting. Recently, the population of farmers in Japan has declined. Consequently, the scale at which farmers must operate has expanded [1]. Therefore, to avoid the increase in competition associated with rice transplanting and soybean sowing for large-scale production, soybean farmers are using a relatively longer period for sowing in the Tohoku region [5]. Reductions in soybean yields with delayed sowing are well documented in the Tohoku region, and the simple explanations for the yield reductions are a shorter period from sowing to maturity and cooler temperatures during the reproductive stage [6].

The effects of sowing date on soybeans depend on the meteorological conditions at the site, particularly the amount and the timing of precipitation [7,8]. Waterlogging is a serious problem in soybean production in converted paddy fields, which sometimes have poor drainage and a high water table in Japan [9]. The rainy season is June to July in the Tohoku region, so waterlogging occurs during the early vegetative stage. Excess soil water during the vegetative stage inhibits root growth of soybean. Plants with poor root systems could not absorb soil water and then seed yield decreased with water deficits in the summer [10]. Recently, at the Tohoku Agricultural Research Center (TARC) in Akita Prefecture, Japan, we used the FAO 56 evapotranspiration model [11] in a long-term continuous performance test and analyzed the correlations between soybean yield and soil volumetric moisture content (SMC) [12]. We found that seed yield over 33 years was significantly negatively correlated with average SMC during August. This result provided evidence that soybean yield decreased in drier years with lower SMC during August.

Yield potential is defined as the maximum yield achieved when a crop is grown in the absence of water and nutrient limitations and of damage by insects and pests [13]. It is parameterized by different efficiencies in the following equation suggested by Monteith [14]:

$$\text{Yield potential} = \text{IR} \times \text{RIE} \times \text{RUE} \times \text{HI}$$

where incident radiation (IR) is the total incident solar radiation during the growing season; radiation interception efficiency (RIE) is determined by the rate of development and the duration of canopy coverage; radiation use efficiency (RUE) is determined by the amount of solar radiation that is transformed into biomass; the harvest index (HI) is determined by the amount of biomass allocated to vegetative versus reproductive organs. This equation can provide insight into how practical agronomic management and genetics determine the yield potential.

In the Tohoku region, late sowing may shorten the growth period from sowing to maturity and thereby may decrease the cumulative solar radiation (CumIR) intercepted. Moreover, it may affect RIE by affecting the development of the canopy and leaf expansion, which depend on climate factors such as temperature, precipitation, and soil water content [15,16]. Although RUE is constant for a given crop species in a given environment, large variability in RUE under optimal conditions has been reported in soybean [17]. The HI also tends to be constant and genotype-dependent; however, some studies show that sowing dates affect biomass partitioning into seed yield [18,19]. Despite many previous investigations of the effect of late sowing on soybean yield in the Tohoku region [6], the importance of radiation interception and use in explaining the lower yields associated with late planting has not been determined. In the USA, yield reductions with late plantings are partially explained by reductions in CumIR around flowering and early pod set [20,21].

In this study, we tested two hypotheses: (1) The yield reduction with late sowing date is explained by the reduction in CumIR during reproductive stages in this region; (2) the yield variations among the combinations of years, sowing dates, and cultivars are explained by variations in SMC. To test the hypotheses, we compared the changes in CumIR and RUE from flowering to late reproductive stages and in HI, yield, and yield components among three leading soybean cultivars between normal (early June) and late (late June) sowing in two consecutive years. Furthermore, we analyzed the effects of temperature and SMC on yields, yield components, and growth parameters of the three cultivars. Our results supported the first hypothesis but did not support the second hypothesis.

2. Materials and Methods

The experiments were conducted at the National Agriculture and Food Research Organization (NARO), TARC in Morioka, Iwate Prefecture, Japan (39°44′ N, 141°7′ E), in 2015 and 2016. In each year, different converted paddy fields were used. The soil characteristics of the two fields are summarized in Table S1. The soil in each field is an Andosol. Each field received 60 g m^{-2} fused phosphate fertilizer and 100 g m^{-2} magnesium lime a month before sowing in each year. In addition, the fields received 3 g m^{-2} N, 12.5 g m^{-2} P (P_2O_5 equivalent), and 5 g m^{-2} K (K_2O equivalent) in the form of a compound fertilizer one day before sowing each year. Fertilizers were applied and incorporated to a depth of approximately 14 cm by using rotary tillers.

We used three determinate soybean cultivars: Ryuhou, Enrei, and Satonohohoemi. Within the Tohoku region, Ryuhou is widely grown in Akita and Iwate prefectures, and Enrei is widely grown in Yamagata Prefecture [22,23]. Satonohohoemi is a new cultivar developed at NARO TARC that was released as a newly recommended cultivar for Yamagata Prefecture in 2009 [22]. Ryuhou is a mid-season maturing cultivar and Enrei and Satonohohoemi are late-season maturing cultivars according to flowering dates at NARO TARC [22,23]. All three cultivars are equivalent to MG IV. Plants were grown to maturity. Seeds were treated with a combined insecticide and fungicide (CruiserMaxx, Syngenta Co., Tokyo, Japan) at the manufacturer's recommended dose and then sown by hand at three seeds per hill on 5 June (normal sowing) and 25 June (late sowing) in 2015 and on 6 June (normal sowing) and 27 June (late sowing) in 2016. The planting density was 28.5 seeds m^{-2} (9.5 hills m^{-2}) with 0.15 m between hills and 0.70 m between rows. This density corresponded to the recommended local planting densities in the Tohoku region (seven to 15 hills m^{-2}). Plants were thinned to one per hill after establishment. A pre-emergence herbicide (Ecotop, Maruwa Biochemical Co., Tokyo, Japan), which contained 1.0% dimethenamide-p and 1.4% linuron, was applied immediately after sowing for weed control at the manufacturer's recommended dose. A month after sowing, the area between the rows was tilled, and ridging was used to control weeds and lodging. In each year, the experimental design was a split-plot arrangement; the main plot was sowing date, and the subplot was cultivar, with three replicates. A subplot consisted of seven rows, each 4.5 m in length.

We surveyed the phenology of 10 plants in the center row of each plot at intervals of two or three days and recorded the dates of growth stages based on the staging system of Fehr and Caviness [24]: VE, emergence; R2, full flowering; R5, the beginning of seed filling; R7, the beginning of maturity. We determined the dry weight of aboveground parts per m^2 by periodic sampling at R2, R5, and 20 days after R5. The aboveground parts of 0.84 m^2 of the center four rows were harvested on each date. Their dry weights were measured after oven drying at 80 °C for three days. The differences in the dry weights of aboveground parts (increase in aboveground biomass—ΔAGB) between R2 and R5 and between R5 and R5 + 20 days were calculated. We estimated the intercepted solar radiation by using digital imaging techniques (GACS1; Kimura Ouyou-Kougei Co., Ltd., Saitama, Japan) according to Kumagai [8]: The fractional canopy cover was determined from digital images taken above the canopy at one-week intervals from the early vegetative to late reproductive stages. In each year, total daily solar radiation (MJ m^{-2} day^{-1}) was measured by a pyranometer (CMP11, Campbell Scientific Inc., Logan, UT, USA) and recorded by a data logger (CR1000, Campbell Scientific) at a weather station near the study field, and daily incident radiation above the canopy was computed as the product of daily solar radiation and the fractional canopy cover. The CumIR from R2 to R5 and from R5 to R5 + 20 days was computed by summing the values of daily incident radiation above the canopy. The mean radiation use efficiency (RUE) for biomass production was determined by dividing the ΔAGB by the CumIR.

At maturity, we manually harvested the aboveground parts from 4.62 m^2 of the center four rows of each plot. After the plants were completely air-dried, we removed the few remaining leaves and petioles and then weighed the remaining aboveground parts. The aboveground biomass equaled the sum of the weights of the stems, pod shells, and seeds. The seed yield per m^2 was adjusted to 15% moisture content, and the harvest index (HI) was determined as the seed yield divided by the aboveground biomass. The yield components (numbers of nodes, pods, seeds per pod, pods per node, and 100-seed weight) were estimated from five representative plants in each plot. The 100-seed weight was also adjusted to 15% moisture.

In addition to solar radiation, daily means of temperature and precipitation during the growing season were recorded at the nearby weather station. The soil volumetric moisture content (SMC, mm^3 mm^{-3}) was monitored with time-domain transmission (TDR) sensors (CS625; Campbell Scientific) at approximately seven cm below the soil surface (the midpoint depth of the plow layer) in a plot with the normal sowing date at the two sites. SMC was measured and recorded every 30 min on a data logger (CR200X; Campbell Scientific).

At the end of June (approximately three weeks after sowing in the plot with the normal sowing date), we sampled the soil of the plow layer in 100 mL sampling tubes. The SMC of the samples at field capacity (θ_{FC}, pF = 1.5) was determined by the sand column method (DIK-3521; Daiki Rika Kogyo Co., Ltd., Saitama, Japan) and that at a permanent wilting point (θ_{WP}, pF = 4.2) by a dew point potentiometer (WP4C; Meter Group, Inc., Pullman, WA, USA). Because θ_{FC} and θ_{WP} differed somewhat between the two years (Table S1), the fraction of available soil water (FASW) was calculated as $(SMC - \theta_{WP})/(\theta_{FC} - \theta_{WP})$ in each year.

We first conducted ANOVA with a split-split-plot design, where treating year as the main factor, sowing date as the split factor, and cultivar as the split-split factor. Year, sowing date, cultivar, and their interaction were each considered as fixed effects, and replicate (block) was considered as a random effect. When the ANOVA produced a significant result ($p < 0.05$), Fisher's LSD test was used to detect significant differences between means. We used multiple regression analysis to examine the relative importance of yield components in the two years of yield data. We used the natural logarithm transformation because the relationships between yield and its components were multiplicative. We also examined the relations between yield and yield components and between temperature and FASW during the reproductive stage by simple correlation (Pearson's) and partial correlation analyses. All procedures were performed in SPSS v. 23.0 software (IBM, Tokyo, Japan).

3. Results and Discussion

3.1. Meteorological Conditions and Fraction of Available Soil Water

The two years had summer with higher temperatures and abundant solar radiation. The amount and pattern of precipitation during summer differed between years. Overall, 2015 was drier and 2016 was wetter than the long-term averages. In 2015 and 2016, the five-month mean temperature (from June to October) was 0.4 to 0.7 °C higher than the 30-year mean (Table 1). The largest departure of 2.1 °C above the 30-year mean was observed in July 2015 and September 2016. In addition, mean temperatures in August 2016 were 1.2 °C higher than the 30-year mean. The five-month mean daily solar radiation in 2015 and 2016 was 1.6 and 1.3 MJ m^{-2} day^{-1} respectively, higher than the 30-year mean. In 2015, the solar radiation in June and July was higher than normal, and in 2016, that in August was 3.1 MJ m^{-2} day^{-1} higher than the 30-year mean. The five-month mean precipitation in 2015 was lower than the 30-year mean (121.2 vs. 147.0 mm, respectively), whereas that in 2016 was higher (179.0 mm). In 2015, the monthly total precipitation was lower than the 30-year mean by 87.9 mm in July, 36.4 mm in August, and 51.4 mm in September. In 2016, the monthly total precipitation in June, August, and October was higher than the 30-year mean but that in July was lower.

The changes in the FASW during the two growing seasons were different as shown in Figure 1. In 2015, the FASW decreased to low levels at the end of July (day of the year—DOY, 203) and in the middle of August (DOY 223), which corresponded to the vegetative stages and the early reproductive stage (around R2), respectively of the normal sowing. However, it was high and relatively stable afterward. In 2016, the FASW fluctuated and decreased to relatively low levels several times from late July to early September (DOY 206, 211, 218, 226, and 247). DOY 218 and 226 corresponded to the early reproductive stage (around R2) in normal and late sowing, respectively. DOY 247 corresponded to the late-reproductive stage (after R5) in both sowing treatments. According to Allen et al. [11], soybean transpiration is inhibited when the FASW is depleted below 0.5. Therefore, drought stress might have sometimes limited soybean growth during the two growing seasons.

Table 1. Monthly and five-month (June–October) mean air temperature (°C), solar radiation (MJ m^{-2} day^{-1}), and total precipitation (mm) in soybean fields during the 2015 and 2016 growing seasons compared with the 30-year means in the Tohoku region of northern Japan.

		June	**July**	**August**	**September**	**October**	**Five-Month Mean**
Temperature (°C)	2015	18.5	23.4	22.7	17.9	10.8	18.7
	2016	18.1	21.6	24.1	20.3	11.2	19.0
	30-year mean	17.8	21.3	22.9	18.2	11.5	18.3
Solar radiation (MJ m^{-2} day^{-1})	2015	19.2	17.8	15.6	13.2	11.1	15.4
	2016	16.4	16.9	18.2	12.5	11.6	15.1
	30-year mean	17.1	15.1	15.1	11.9	9.9	13.8
Precipitation (mm)A	2015	123.5	108.5	145.5	104.5	124.0	121.2
	2016	160.0	131.0	258.5	160.5	185.0	179.0
	30-year mean	108.1	196.4	181.9	155.9	92.5	147.0

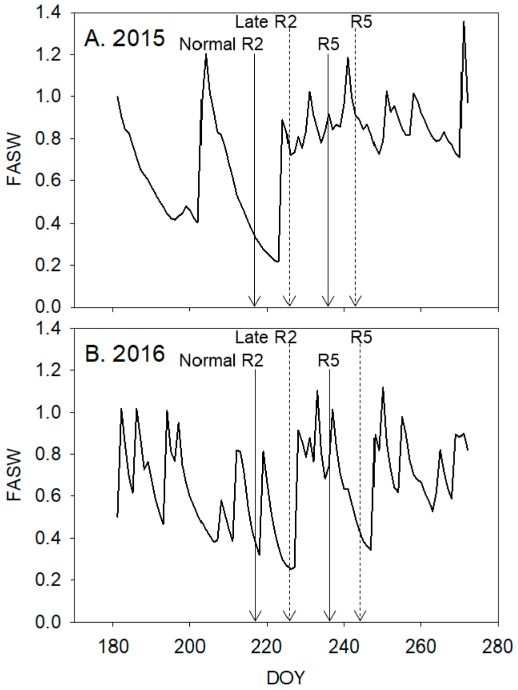

Figure 1. Changes in the fraction of available soil water (FASW) from July to September during the soybean growing season in (**A**) 2015 and (**B**) 2016 in the Tohoku region of northern Japan. DOY, day of the year. R2, beginning of flowering; R5, beginning of seed filling stage; | normal sowing date; ⁝ late sowing date; arrows indicate means of the three cultivars.

3.2. *Aboveground Biomass, Seed Yield, Harvest Index, and Yield Components*

The effects of year, sowing date, and cultivar on aboveground biomass, seed yield, harvest index, and yield components determined by ANOVA are shown in Table 2. The main finding of this study was the negative effects of late sowing date on aboveground biomass, seed yield, HI, number of pods, seeds per pod, and number of nodes as discussed below.

Table 2. Aboveground biomass (g m^{-2}), seed yield (g m^{-2}), harvest index (HI), and yield components (numbers of pods per plant, seeds per pod, nodes per plant, and pods per node, and 100-seed weight, g) of three soybean cultivars (Ryuhou, Enrei, Satonohohoemi) with normal and late sowing dates in 2015 and 2016 in the Tohoku region of northern Japan.

		Above-Ground Biomass (g m^{-2})	Seed Yield (g m^{-2})	HI	Number of Pods (Per Plant)	Seeds Per Pod	Number of Nodes (Per Plant)	Number of Pods Per Node	100-Seed Weight (g)
Year (Y)	2015	447	237	0.53	53.4	1.42	40.0	1.34	31.0
	2016	512	277	0.54	76.9	1.23	49.2	1.57	30.8
Sowing date (S)	Normal	542	300	0.55	69.0	1.44	48.3	1.43	32.3
	Late	416	214	0.51	61.3	1.20	40.9	1.48	29.4
Cultivar (Cv)	Ryuhou	459	274	0.59a	64.9	1.51a	41.7b	1.54a	30.0b
	Enrei	460	246	0.52b	61.9	1.41b	45.6a	1.34b	28.6b
	Satonohohoemi	519	252	0.47c	68.6	1.04c	46.3a	1.48a	34.0a
ANOVA	Y	ns	ns	ns	**	**	**	**	ns
	S	**	***	**	*	***	**	ns	**
	Cv	ns	ns	***	ns	***	*	***	***
	Y × S	ns	ns	*	ns	**	ns	ns	ns
	Y × Cv	ns	ns	***	ns	ns	ns	**	ns
	S × Cv	ns	ns	**	ns	ns	ns	***	ns
	Y × S × Cv	ns	ns	***	ns	*	ns	ns	ns

Values of a parameter followed by the same letter did not differ significantly between cultivars (ANOVA followed by Fisher's LSD test, $p < 0.05$). ANOVA results: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant.

Although the effect of sowing date on aboveground biomass and seed yield was significant ($p < 0.01$), these variables were not affected by year or cultivar. Aboveground biomass and seed yield were not significantly affected by the interaction of year × sowing date × cultivar. When averaged across years and cultivars, late sowing led to significant decreases in aboveground biomass (23%, $p < 0.01$) and seed yield (29%) ($p < 0.001$). Cultivar and sowing date significantly affected the HI ($p < 0.01$). Averaged across years and sowing dates, Ryuhou had the highest HI, followed by Enrei and Satonohohoemi. Averaged across years and cultivars, late sowing decreased HI significantly from 0.55 to 0.51. However, the decrease in the HI with late sowing differed between years and among cultivars, as indicated by the significant interactions between year and sowing date ($p < 0.05$), between sowing date and cultivar ($p < 0.01$), and among year, sowing date, and cultivar ($p < 0.001$). The largest decrease in HI occurred in Enrei in 2015 and in Satonohohoemi in 2016 (Table S2).

Year significantly affected numbers of pods, seeds per pod, nodes, and pods per node ($p < 0.01$) but had no effect on 100-seed weight (Table 2). Averaged across sowing dates and cultivars, numbers of pods, nodes, and pods per node were higher and the number of seeds per pod was lower in 2016 than in 2015. Although we detected no difference in the number of pods, the numbers of seeds per pod, nodes, and pods per node and 100-seed weight differed significantly among the cultivars ($p < 0.05$). The number of seeds per pod was highest in Ryuhou. The number of nodes was higher in Enrei and Satonohohoemi. The number of pods per node was higher in Ryuhou and Satonohohoemi, and the 100-seed weight was highest in Satonohohoemi. Averaged across years and cultivars, late sowing significantly decreased the number of pods by 11% ($p < 0.05$), the number of nodes by 15% ($p < 0.01$), and 100-seed weight by 9% ($p < 0.01$). Late sowing significantly decreased the number of seeds per pod ($p < 0.001$), for which the interaction year × sowing date × cultivar was significant ($p < 0.05$). The decrease in seeds per pod differed among the combinations of years and cultivars (Table S2); that of Satonohohoemi in 2016 was the greatest among all combinations. The main effect of sowing date on pods per node was not significant, but the effect on pods per node differed among cultivars, as indicated by a significant interaction between sowing date and cultivar ($p < 0.001$). The number of pods per node in Enrei and Satonohohoemi decreased with late sowing, whereas that in Ryuhou did not change.

A complex relationship among yield components determined the seed yield. Therefore, we used multiple regression to determine the relative contributions of yield components (numbers of nodes, pods per node, and seeds per node, and 100-seed weight) to seed yield. All four components significantly positively affected yield (Figure 2). The number of seeds per pod contributed the most to yield (standardized multiple regression coefficient $\beta = 0.661$, $p < 0.001$), followed by the number of nodes (β = 0.598, $p < 0.001$). The value of β was significant ($p < 0.01$) but low for pods per node and 100-seed weight. This result indicates that the yield reduction due to late sowing was explained primarily by decreases in numbers of seeds per pod and nodes in the Tohoku region. In a review of previous experiments conducted in the Midwestern USA, which has the same latitude and a similar climate as the Tohoku region, soybean yield declined rapidly when seeds were planted after late May [7]. The yield decline following late sowing in north-central USA was due to fewer nodes [25]. De Bruin and Pedersen [26] show that reductions in the number of seeds per m^{-2} accounted for the yield decline associated with delayed planting. They also show that 100-seed weight is not associated with the yield decline due to late planting. The linear decline in yield per day of planting delay they observed was similar to what we observed (1.7–4.3 g m^{-2} d^{-1} vs. 4.2 g m^{-2} d^{-1}).

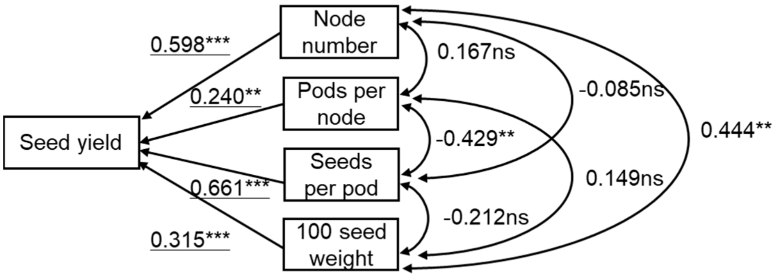

Figure 2. Diagram showing the regression coefficients of yield components in the multiple regression on seed yield and the coefficients of correlations between variables obtained across two years and three cultivars in the Tohoku region of northern Japan. Because the nature of the relationship between yield and yield components was multiplicative, the data of all cultivars in each block ($n = 36$) were natural-log transformed. Values with the single-headed arrows are the standardized multiple regression coefficients, and those with double-headed arrows are Pearson's correlation coefficients. *** $p < 0.001$; ** $p < 0.01$; ns, not significant.

3.3. Phenological Development

The other finding of this study was the negative effects of late sowing on the phenology from emergence (VE) to full flowering (R2), from R2 to the beginning of seed filling (R5), from R5 to the beginning of maturity (R7), and from VE to R7 (total growth, Table 3). The main factors of year, sowing date, and cultivar significantly affected the phenology from VE to R2 ($p < 0.01$), from R2 to R5 ($p < 0.01$), from R5 to R7 ($p < 0.001$), and from VE to R7 ($p < 0.001$), with the exception of the effect of sowing date on R5 to R7. Compared with 2015, the periods from VE to R2, from R5 to R7, and from VE to R7 in 2016 were longer but that from R2 to R5 was shorter. When averaged across years and cultivars, late sowing significantly decreased the time from VE to R2 by 12 days ($p < 0.001$), from R2 to R5 by one day ($p < 0.05$), and from VE to R7 by 13 days ($p < 0.001$). Late sowing affected the vegetative phase (from VE to R2) more strongly than the reproductive phase (from R2 to R5). The cultivar affected the duration of each phase: Satonohohoemi had the longest total (VE to R7), vegetative (VE to R2), and reproductive (R2 to R7) stages when averaged across years and sowing dates. The effect of late sowing on these durations differed substantially among the cultivars, as indicated by significant interactions between sowing date and cultivar ($p < 0.01$). In both years, the decrease in the duration of total growth (VE to R7) was greatest in Enrei (16 days in 2015, 15 days in 2016; Table S3). Previous studies found that a reduction in yield with late sowing is due to a shortened duration from sowing to flowering and to maturity [6,25], which is consistent with our results.

Table 3. Days from emergence (VE) to full flowering (R2), from R2 to the beginning of seed filling (R5), from R5 to the beginning of maturity (R7), and from VE to R7 for three soybean cultivars (Ryuhou, Enrei, Satonohohoemi) with normal and late sowing dates in 2015 and 2016 in the Tohoku region of northern Japan.

		Days from VE to R2	**Days from R2 to R5**	**Days from R5 to R7**	**Days from VE to R7**
Year (Y)	2015	46	20	46	112
	2016	48	19	50	116
Sowing date (S)	Normal	53	20	48	121
	Late	41	19	48	108
Cultivar (Cv)	Ryuhou	45c	18b	45b	107c
	Enrei	47b	20a	45b	113b
	Satonohohoemi	49a	20a	54a	123a
ANOVA	Y	**	**	***	***
	S	***	*	ns	***
	Cv	***	***	***	***
	Y × S	*	ns	ns	**
	Y × Cv	ns	**	ns	**
	S × Cv	**	**	***	***
	Y × S × Cv	**	ns	***	**

Values of a parameter followed by the same letter did not differ significantly between cultivars (ANOVA followed by Fisher's LSD test, $p < 0.05$). ANOVA results: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant.

3.4. Canopy Cover, Dry Matter Production, Radiation Interception, and Use Efficiency

Indices of soybean growth, such as canopy cover [27], crop growth rate (CGR), RIE, and RUE [20], are correlated with seed yield. The changes in percentage canopy cover are shown in Figure S1. The cover was significantly lower in late sowing plots than in normal sowing plots for most of the growth period (DOY 200 to 240) in all cultivars in both years.

ΔAGB, CumIR, and RUE during the reproductive stages (from R2 to R5 and from R5 to R5 + 20 days) are summarized in Table 4. One of the important findings of this study was the reductions of ΔAGB and CumIR from R2 to R5 due to late sowing. When averaged across years, late sowing significantly decreased ΔAGB from R2 to R5 by 30% and CumIR from R2 to R5 by 31% ($p < 0.001$), and for both parameters, the interaction between sowing date and cultivar in the period was significant ($p < 0.05$). The decreases in ΔAGB and CumIR were larger for Satonohohoemi and Enrei than for Ryuhou (Table S4). By contrast, from R5 to R5 + 20 days, the effects of year, sowing date, and cultivar on ΔAGB were not significant, nor were the interactions among them. However, the effects of year, sowing date, and cultivar on CumIR were significant, as were all interactions among them ($p < 0.01$). As shown in Figure S2, the solar radiation after R5 differed between the two years. Therefore, these effects and interactions were due primarily to yearly changes in solar radiation during this period rather than to changes in canopy cover (which is related to RIE), because canopy cover reached approximately 100% at R5 (Figure S1), and there was no difference among all combinations. The effects of year, sowing date, and cultivar on RUE were not significant, nor were the interactions among them from R2 to R5 and from R5 to R5 + 20 days, except for a significant interaction between year and sowing date from R2 to R5 ($p < 0.05$). Our results indicate that the decrease in ΔAGB from R2 to R5 with late sowing was due to the decrease in CumIR rather than that in RUE. The decrease in CumIR that resulted from late sowing in this study was due to both slowed canopy development and a shortened period from R2 to R5.

Table 4. Increase in aboveground biomass (ΔAGB, g m^{-2}), cumulative intercepted irradiation (CumIR, MJ m^{-2}), and radiation use efficiency for dry matter production (RUE, g MJ^{-1}) during the reproductive stages (from R2 to R5 and from R5 to 20 days after R5) of three soybean cultivars (Ryuhou, Enrei, Satonohohoemi) with normal and late sowing dates in 2015 and 2016 in the Tohoku region of northern Japan.

		ΔAGB		**CumIR**		**RUE**	
		(g m^{-2})		**(MJ m^{-2})**		**(g MJ^{-1})**	
		R2–R5	**R5–R5 + 20 d**	**R2–R5**	**R5–R5 + 20 d**	**R2–R5**	**R5–R5 + 20 d**
Year (Y)	2015	217	212	255	203	0.86	1.06
	2016	231	271	256	273	0.89	0.99
Sowing date (S)	Normal	264	247	303	234	0.87	1.08
	Late	184	235	207	242	0.89	0.97
Cultivar (Cv)	Ryuhou	191b	252	235b	247a	0.82	1.03
	Enrei	241a	239	269a	232b	0.91	1.06
	Satonohohoemi	240a	233	261a	233b	0.90	0.99
ANOVA	Y	ns	ns	ns	***	ns	ns
	S	***	ns	***	**	ns	ns
	Cv	**	ns	***	***	ns	ns
	Y × S	ns	ns	**	***	*	ns
	Y × Cv	ns	ns	ns	***	ns	ns
	S × Cv	*	ns	***	***	ns	ns
	Y × S × Cv	ns	ns	ns	***	ns	ns

Values of a parameter followed by the same letter did not differ significantly between cultivars (ANOVA followed by Fisher's LSD test, $p < 0.05$). ANOVA results: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant.

Pearson's correlation coefficients were determined for the relationships between ΔAGB and seed yield and yield components when data were combined across years, sowing dates, and cultivars (Table 5). ΔAGB from R2 to R5 was highly correlated with seed yield (Pearson's $r = 0.639$, $p < 0.001$) and number of nodes ($r = 0.606$, $p < 0.001$) and was correlated with 100-seed weight ($p < 0.05$). However, from R5 to R5 + 20 days, the correlations between ΔAGB and yield components were not significant. Thus, the results support the first hypothesis that the yield reduction with late sowing date is explained by the reduction in CumIR during reproductive stages. Our results reveal that yield reduction with late sowing was due primarily to the reduction in the number of nodes, which in turn resulted from the decrease in the ΔAGB associated with the decrease in CumIR during the early to middle reproductive stage (R2 to R5). Although the appearance of main stem nodes stops soon after R2, branch nodes continue to increase from R1 to R5 in determinate soybean cultivars [28]. Therefore, by stage R5 in determinate soybean cultivars, the total number of nodes is determined. High CumIR, the ΔAGB, and the associated total number of nodes attained by R5 were important factors in preventing yield reduction of soybeans with the late sowing. Although an increase in the sowing rate is widely accepted to prevent yield decline with late sowing [19], the optimal combination of the sowing date with the sowing rate has not been determined. The combined effect of late sowing and dense planting on radiation interception and use in soybean will need to be investigated in future research. Our results are consistent with the well-known premise that the number of pods (seeds) and seed yield depend on assimilate supply during the period before R5 [17,21,29]. However, Shiraiwa et al. [30] found that soybean yield was significantly and positively correlated with CGR (ΔAGB/days) during the 20-day period after R5 in multiple environments (four seasons) in a warm region of Japan (Kyoto and Shiga prefectures), although they did not observe any positive correlations between seed yield and CGR before or after this period that were consistent among the environments. The weather conditions at the experimental sites and the cultivars used are possible explanations for the differences between the two studies. Thus, in future research, more comprehensive investigations of the relationship between yield and dry matter accumulation in many genotypes under different environmental conditions are needed.

Table 5. Pearson's coefficients of correlation of the relationships between the increase in aboveground biomass (ΔAGB, g m^{-2}) and seed yield (g m^{-2}) and yield components (numbers of nodes per plant, pods per node, and seeds per pod, and 100-seed weight, g) during the reproductive stages (from R2 to R5 and from R5 to 20 days after R5) across three soybean cultivars and normal and late sowing dates in 2015 and 2016 (n = 36) in the Tohoku region of northern Japan.

	Seed Yield	**Number of Nodes**	**Pods Per Node**	**Seeds Per Pod**	**100-Seed Weight**
ΔAGB R2–R5	0.639	0.606	−0.276	0.282	0.415
Significance	***	***	ns	ns	*
ΔAGB R5–R5 + 20 d	0.174	0.239	0.214	−0.035	0.043
Significance	ns	ns	ns	ns	ns

*** $p < 0.001$; * $p < 0.05$; ns, not significant.

3.5. Correlations of Seed Yield, Yield Components, and Growth Parameters with Temperature and Fraction of Available Soil Water

Cool temperatures during the reproductive stage are one of the limiting factors for seed yield in the northern region of Japan [6,31]. Both flooding and drought stress also influence soybean production in Japan [9–11]. As shown in Table 1 and Figure 1 and Figure S2, weather conditions and FASW during the reproductive stages varied across years, sowing dates, and cultivars in this study. Although the dataset was limited from two years at one site, we attempted to elucidate how temperature and FASW influenced seed yields, yield components, and growth parameters in the three cultivars sown on two sowing dates and in two years. The values of Pearson's r were significant and positive for the relationships between mean temperature from R2 to R5 and pods per node ($p < 0.05$) and between mean temperature from R5 to R5 + 20 days and seed yield ($p < 0.05$; Table S5), presumably indicating that cooler temperatures during the reproductive stage due to late sowing (Figure S1) reduced seed yield and yield components. Furthermore, significant and negative correlation coefficients were found for the relationships between mean FASW during 20 days after R5 and both node number and pods per node ($p < 0.05$, Table S5). This result might indicate that excessive soil water reduced yield components. However, significant and negative correlation coefficients were detected for the relationships between mean temperature and mean FASW during the two periods ($p < 0.001$), indicating that excessive soil water because of the high amount of precipitation was associated with cold weather.

The partial correlation coefficients, when mean FASW was held constant, were determined for the relationships between yield components, and growth parameters and mean temperature during the reproductive stage (Table 6). The partial correlation coefficient between mean temperature and seeds per pod with a fixed mean FASW during the 20 days after R5 was 0.864 ($p < 0.001$). Although the number of seeds per pod remains stable under a wide range of temperatures [32], Kurosaki and Yumoto [33] report that low temperature (15.5 °C mean temperature) for four weeks in the flowering season reduced the number of seeds per pod in soybean cultivars in Hokkaido (the northernmost prefecture in Japan). In this study, the mean temperature was 22.6 °C during the period from R2 to R5 and 19.3 °C for the 20 days after R5 in the late-sown soybeans. Those temperatures might not negatively affect the number of seeds per pod. Kurosaki and Yumoto [33] also reported that the response of seeds per pod to low temperature was different among soybean cultivars. Therefore, one plausible explanation for the variation in the number of seeds per pod across years, sowing dates, and cultivars was both temperature variability during the initial seed filling stage and the differential responses of cultivars to low temperatures. Further investigation of the effect of low temperature on yield components in late-sown soybean in different years and sites will be required to optimize the combination between sowing dates and cultivars in cool regions of Japan.

Table 6. Partial correlation coefficients, when mean fraction of available soil water is held constant, for the relationships between mean temperature during the reproductive stage (from R2 to R5 and from R5 to R5 + 20 days) and yield components (numbers of nodes, pods per node, and seeds per pod, and 100-seed weight), change in aboveground biomass (ΔAGB), cumulative intercepted irradiation (CumIR), and radiation use efficiency for dry matter production (RUE) across three soybean cultivars and two sowing dates in 2015 and 2016 ($n = 12$) in the Tohoku region of northern Japan.

	Number of Nodes	Pods Per Node	Seeds Per Pod	100-Seed Weight	ΔAGB	RUE	CumIR
Temperature R2–R5	0.544	0.557	−0.530	0.207	0.108	0.259	−0.094
Significance	ns	ns	ns	ns	ns	ns	ns
Temperature R5–R5 + 20 d	−0.401	−0.329	0.864	−0.290	0.101	−0.133	0.226
Significance	ns	ns	***	ns	ns	ns	ns

*** $p < 0.001$; ns, not significant.

The effect of water stress on soybean yield depends not only on the duration and intensity of the stress but also on the timing [34]. Drought stress during the reproductive stage increases the rate of soybean flower and pod abortion [35] and reduces seed size [36]. Frederick et al. [37] report that drought stress during the period from R1 to R7 decreases seed number and yield on branches, because most branch growth occurs between R1 and R5 in determinate soybean cultivars. Brevedan and Egli [38] found a lower photosynthetic rate in water-stressed soybean, which contributed to earlier maturity. However, according to Wani et al. [39], soybean yields are not substantially affected until root zone soil has been depleted below 0.6 FASW and leaf area development has not been hampered by prolonged mild water stress during the vegetative phase. Soltani and Sinclair [14] show that the threshold FASW for leaf area development is 0.31 and that for growth is 0.25. Although FASW occasionally fell below 0.5, at which transpiration in soybean is inhibited [24], around R2 (Figure 1), no positive linear relationship was detected between mean FASW during reproductive stages and yield, yield components, or growth parameters (Table S5), indicating that lower FASW in the plow layer at least did not affect soybean growth and yield. We did not detect the threshold FASW for inhibiting growth and yield in this study. These results did not support our second hypothesis that the yield variations among the combinations of years, sowing dates, and cultivars are explained by variations in SMC. However, it is possible that using the dataset from two years and two sowing dates prevented the present study from detecting the effects of drought stress. Moreover, soil physic characteristic was site-specific within the Tohoku region [40]. Therefore, further investigation under different years (weather) and sites (soil) will be needed to test this hypothesis in the future. Another possible explanation for the absence of a drought stress effect in this study could be that water flowed from the subsoil below the plow layer. Generally, the taproot of soybean cannot penetrate a hardpan (a compacted layer) immediately below the plow layer, and therefore the effective root zone is limited in converted paddy fields in Japan [40]. We found that the taproot length was approximately 14 cm in the two years (data not shown), which was equivalent to the plow depth. Therefore, uptake of water from the subsoil below the hardpan by taproots seems unlikely. However, capillary rise (upward water flow from subsoil) is a feature of Andosols [41], and thus taproots near the hardpan might avoid drought stress. To better understand water flow in soil and the effects on soybean growth, further investigations are also necessary.

4. Conclusions

The results from two years of field experiments in northern Japan show that sowing approximately three weeks late decreased soybean yields. The yield reduction was partially caused by reductions in the number of nodes, ΔAGB, and CumIR due to slower canopy development during vegetative and early reproductive stages. The number of seeds per pod was one of the major determinants of the variation in yield. Low temperatures caused by the delay in sowing date during the 20 days after R5 likely explained the reduction in the number of seeds per pod. Our study also showed that a lower FASW during reproductive stages did not affect seed yield, yield components, or growth parameters.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/1/66/s1: Table S1: Soil characteristics of the experimental soybean fields at TARC, NARO, Morioka, Iwate Prefecture, Japan, in 2015 and 2016; Table S2: Aboveground biomass (g m^{-2}), seed yield (g m^{-2}), harvest index (HI), and yield components (numbers of pods per plant, seeds per pod, nodes per plant, and pods per node, and 100-seed weight, g) of three soybean cultivars (Ryuhou, Enrei, Satonohohoemi) with normal and late sowing dates in 2015 and 2016 in the Tohoku region of northern Japan; Table S3: Days from emergence (VE) to full flowering (R2), from R2 to the beginning of seed filling (R5), from R5 to the beginning of maturity (R7), and from VE to R7 for three soybean cultivars (Ryuhou, Enrei, Satonohohoemi) with normal and late sowing dates in 2015 and 2016 in the Tohoku region of northern Japan; Table S4: Increase in aboveground biomass (ΔAGB, g m^{-2}), cumulative intercepted irradiation (CumIR, MJ m^{-2}), and radiation use efficiency for dry matter production (RUE, g MJ^{-1}) during reproductive stages (from R2 to R5 and from R5 to 20 days after R5) of three soybean cultivars (Ryuhou, Enrei, Satonohohoemi) with normal and late sowing dates in 2015 and 2016 in the Tohoku region of northern Japan; Table S5: Pearson's coefficients of correlation of the relationships between mean temperature and the FASW (fraction of available soil water) during reproductive stages (from R2 to R5 and from R5 to R5 + 20 days) and seed yield (g m^{-2}), yield components (numbers of pods per plant, seeds per pod, nodes per plant, pods per node, and 100-seed weight, g), increase in aboveground biomass (ΔAGB, g m^{-2}), cumulative intercepted irradiation (CumIR, MJ m^{-2}), and radiation use efficiency for dry matter production (RUE, g MJ^{-1}) across three soybean cultivars and two sowing dates in 2015 and 2016 (n = 12) in the Tohoku region of northern Japan; Figure S1: Changes in the canopy cover (%) of three soybean cultivars (Ryuhou, Enrei, Satonohohoemi) growing after normal and late sowing during the (A) 2015 and (B) 2016 seasons in the Tohoku region of northern Japan. DOY, day of the year. Values represent the mean (n = 3); Figure S2. Changes in daily mean temperature and solar radiation from June to October during the soybean growing season in (A and C) 2015 and (B and D) 2016 in the Tohoku region of northern Japan. DOY, day of the year. R2, beginning of flowering; R5, beginning of seed filling stage; | normal sowing date; ⁝ late sowing date; arrows indicate means of the three cultivars.

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