Research article

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Cluster and Principle Component Analysis of Soybean Grown at Various Row Spacings, Planting Dates and Plant Populations

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Abstract: Increased light interception (*LI*), along with concomitant increases in crop growth rate (*CGR*), is the main factor explaining how cultural factors such as row spacing, plant population, and planting date affect soybean yield. Leaf area index (*LAI*), *LI*, and *CGR* are interrelated in a “virtuous spiral” where increased *LAI* leads to greater *LI* resulting in a higher *CGR* and more total dry matter per area (*TDM*). This increases *LAI*, thus accelerating the entire physiological process to a higher level. A greater understanding of this complex growth dynamic process could be achieved through use of cluster analysis and principle components analysis (*PCA*). Cluster analysis involves grouping of similar objects in such way that objects in same cluster are similar to each other and dissimilar to objects in other cluster. *PCA* is a technique used to reduce a large set of variables to a few meaningful ones. Seasonal relative leaf area index (*RLAI*), relative light interception (*RLI*), and relative total dry matter (*RTDM*) response curves were determined from the data by a stepwise regression analysis in which these parameters were regressed against relative days after emergence (*RDAE*). Greatest levels of *RLAI*, *RLI* and *RTDM* were observed in soybean planted early on narrow row spacings

and recorded greater plant population. In contrast, lower levels of these parameters occurred on plants with wide row spacings at late planting dates. For farmers, these results are useful in terms of adopting certain cultural practices which can help in the management of stress in soybean.

Keywords: Cluster analysis, principle components, leaf area index, light interception, total dry matter, planting dates and row spacing

Abbreviations

CGR =Crop growth rate (g of dry matter accumulated by the crop per day per square meter)

LAI = Leaf area index (ratio of leaf area to subtending ground area)

LI =Light interception (% of available sunlight intercepted by the crop)

TDM =Total dry matter per area (g of above-ground crop dry matter per square meter) *RLAI* =Relative leaf area index (*LAI* at any time during the growing season relative to the maximal seasonal *LAI*)

RLI =Relative light interception (*LI* at any time during the growing season relative to the maximal level)

RTDM =Relative total dry matter per area (*TDM* at any point during the growing season relative to the maximal *TDM*)

RDAE =Relative days after emergence (days to any time during the growing season as a percentage of the total growing season)

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1 Introduction

Optimal row spacing, planting date, and plant population are three of the most important cultural decisions a

farmer makes (Ball et al. 2000; Board and Kahlon 2011; Costa et al. 1980; Board and Kahlon 2013). Analyses of yield components and crop growth dynamics, combined with prevailing environmental data, have provided a comprehensive picture of how row spacing, planting date, and plant population affect soybean yield in the southeastern USA (Board and Kahlon, 2011). Growth dynamic parameters are rates and levels of TDM, LAI, and LI (Loomis and Connor 1992). Other growth dynamic parameters pertinent to yield formation in soybean and other crops are *CGR* and dry matter partitioning parameters such as harvest index [(yield dry matter per area/total dry matter per area) x 100]. However, the focus of this study is on TDM, LAI, and LI. Yield components are morphological characteristics whose formation is critical to yield. For soybean, yield components which have potential to influence yield are seed number per area, seed size (grams per seed), seed per pod (no.), pod number per area, pod per reproductive node (no.), and reproductive node number per area (a reproductive node is a node that contains at least one viable pod having at least one seed) (Ball et al. 2001; Board and Modali 2005; Lehman and Lambert 1960; Kahlon et al. 2011).

Similar to many other environmental stresses in soybean culture (Egli 1998; Jiang and Egli 1995), non-optimal planting date, sub-optimal plant population, and row spacings, reduce yield through reductions in seed number, number per area (Boerma and Ashley 1982; Board and Modali 2005; Board et al. 2010; Board and Kahlon 2011; Kahlon and Board 2012). Seed number per area is largely determined between emergence and shortly after R5 (stages according to Fehr and Caviness 1977; Pigeaire et al. 1986). The R5 stage is the initiation of seed filling and is indicated by at least one pod among the top four main stem nodes that has one seed at least 0.3 cm long. Because seed number per area, pod number per area, and reproductive node number per area are all strongly linked to TDM by R5 (Board and Modali 2005), *CGR* during the emergence stage to R5 period is very important for final yield determination. Crop growth rate during this period has long been recognized as regulated by *LI* (Shibles and Weber 1966; Board et al. 1990a; Sinclair and Horie 1989). Light interception is largely controlled by LAI and can also be influenced by light interception efficiency (light interception/leaf area index), especially during the early vegetative period (Board 2000). Both LAI and light interception efficiency are influenced by row spacing, plant population, maturity group, and planting date. Greater LI in narrow vs. wide row spacing starts occurring as early as three weeks after emergence and continues throughout this stage up to R5 period (Board

et al. 1996). Greater LAI in late versus early maturity groups is usually achieved because of longer periods to R5 [i.e. longer periods for vegetative growth and leaf expansion (Board et al. 1996; Board and Kahlon 2012a)]. Increased plant population creates greater LI compared with low plant population occurring during the early stages of the growing season. However, if environmental conditions are optimal, low plant populations can achieve yields equivalent to optimal plant populations (Board 2000). Late planting with its restricted emergence to R5 period tend to have lower leaf area index and LI compared to normal planting dates simply because of less time available for leaf area development (Board and Settimi 1986). Late planting in the southeastern USA reduces crop yield by restricting the emergence to R5 period because of the shortening daylengths commencing in late June/early July (Board and Settimi 1986). Essentially this is due to limited time available to the crop to optimize TDM by R5 and consequently produces a suboptimal number of pods and seed per area, resulting in lower yields (Board et al. 1990b; Kahlon and Board 2011; Board and Kahlon 2012b; Saeki 1960; Rahman and Islam 2006).

In this study, seasonal growth dynamic parameters (RTDM, RLAI, and RLI) were studied for soybean using cluster analysis and PCA across a range of Maturity Groups, environments and cultural practices (planting dates, plant populations, and row spacings). Our objectives were to:

1. Organize treatment combinations across the data sets used into cluster groups and determine the seasonal patterns of RTDM, RLAI, and RLI within the clusters.
2. Demonstrate how the data analysis can be used to help farmers cope with crop stress problems that reduce yield.

2 Materials and Methods

2.1 Cultural Conditions

The current study uses data sets obtained from three soybean field experiments conducted across a seven-year period near Baton Rouge, Louisiana (USA) (30°N Lat). The first study was conducted for three years (1986-1988) and involved: two cultivars (maturity group V and VI), two planting dates (optimal and late), two plant populations (normal and high), and two row spacings (50 and 100 cm) (Board et al. 1990a; Board et al. 1990b). The second study was conducted for two years (1989 and 1990) and involved: one cultivar (maturity group VI), and four row spacings (25 cm, 50 cm, 75 cm, 100 cm) planted at a late date (Board et al. 1992). The third field experiment was done for two

years (1994 and 1995), planted at an optimal planting date and involved one cultivar (maturity group V), and four plant populations (70,000, 164,000, 189,000, and 234,000 plants per hectare (Carpenter and Board 1997). Specific details for these field studies and treatments are shown in Table 1 and Table 2. In this study, LI, TDM, and LAI were sampled for all studies throughout the growing season. Phenological data for the major reproductive stages were also taken according to the methods described in Fehr and Caviness (1977). These included first flowering (R1 stage), pod initiation (R3 stage), seed initiation (R5 stage), and the end of seed filling (also known as physiological maturity) (R7 stage). After the R7 stage, all experimental units in all studies were sampled for various yield components described in the introduction. Plot yield at harvest maturity was determined by combine harvest of interior rows (6.5 square meters) of each plot that had been end-trimmed to 4.3 meters and corrected to 130 g per kg moisture content.

2.2 Statistical Methodology

Experimental design in the first study (Board et al. 1990a) was a randomized complete block in a split-split plot arrangement with four replications and three years as blocking factors. The main plots were characterized by the two planting dates; normal (May) and late (July). Split plots were characterized by soybean cultivars Forrest (maturity group V) and Centennial (maturity group VI). Split-Split plots were characterized three row widths at 100 cm (wide) row width at 200,000 plants ha⁻¹, 50 cm (narrow) row width at 220,000 plants per ha⁻¹, and 50

cm row width at 325,000 plants ha⁻¹ (narrow row/ high density). For this study, response curves for RLI, RTDM, and RLAI were determined by a stepwise regression analysis. Experimental design in the second study (Board et al. 1992) was a randomized complete design in a split-plot arrangement with two years and four replications as blocking factors. Main plots were characterized by the four plant populations and split plots indicated the nine sampling dates. Data were subjected to the same regression analysis as for study one.

The third study (Carpenter and Board 1997) was also a randomized complete block in split-plot arrangement. The study was conducted across two years (1994 and 1995) and had four replications. Main plots were characterized by four plant population and split plots were characterized by sampling dates. Data analyses were similar as in studies *one and two*.

Cluster analysis and PCA were used to rationalize this large data set (48 treatments) into similar cluster groups. The purpose of cluster analysis is to organize the data into groups based on measured characteristics that have the same or similar value. In this study we used the complete linkage method of cluster analysis. This method defines the distance between two clusters by the farthest-neighbor distance, i.e., the distance between the two farthest inter-cluster observations or units. This method tends to produce clusters that are compact, and relatively similar observations can remain in separate clusters until the analysis is complete. The data were analyzed with cluster analysis and then studied to potentially pool into 2 or 3 groups. The data were also analyzed with PCA. This method analyzes the data set to identify the most important factors influencing the total variance. In our study we included

Table 1: A listing of journal articles from which data were obtained for the current study with various cultural, environmental, and genotype data for individual studies conducted for soybean grown near Baton Rouge, LA, 1986 through 1996

Reference	Years	Planting dates	Cultivars (Maturity Group))	Row spacing (cm)	Plant Population (plants ha ⁻¹)
Boards et al. 1990	1986	15 May 1986	Centennial (VI)	50	220 000
		2 July 1986	Forest (V)	100	325 000
	1987	22 May 1987			
	1988	12 July 1987			
		24 May 1988 6 July 1988			
Board et al. 1992	1989	26 July 1989	Centennial (VI)	100	
	1990	10 July 1990		75	
				50	
				25	
Carpenter and Board 1997	1994	25 May 1994	DP415 (V)	75	234 000
	1995	16 May 1995			189 000
					164 000
					70 000

figures of variance shown by the first two principle components. The analysis was done in R software and the data were mainly grouped into two categories for RLAI, *RLI*, and RTDM regressed on RDAE. The RLAI, RLI and RTDM are the values of LAI, LI and TDM and are on 0 to 100 scale. All LI, LAI and TDM values in the data set were converted to a 0 to 100 scale, with the lowest value considered to be 0 and the maximum value to be 100 and all other values given a relative value between the minimum and maximum value. This was also done for DAE. Data were pooled from all the treatments in two or three cluster groups. The pooled data were analyzed with regression analysis by regressing RLAI, RLI, and RTDM on RDAE.

3 Results and Discussion

3.1 Leaf Area Index

The various row spacing, plant population, maturity groups and planting dates affected the LAI through LI and TDM accumulation and the, leaf area ratio (LAR, LAI/TDM). The cluster analysis resulted in identification of three cluster groups and two main clusters were selected based on PCA (Figure 1). Cluster group 1 consisted of 26

similar treatments, while cluster group 2 contained 20 treatments. Cluster group 3 contained only 2 treatments. Treatments consolidated into cluster group 1 represented a wide diversity of genetic, cultural, and environmental factors. All 3 data sets analyzed in the study were included in this cluster. This represents a data consolidation of 7 years of field studies. Both Maturity Group V and VI cultivars are represented in the cluster. Most surprising of all, the cluster contained treatments equally representing the poorest-yielding (late plantings at wide row spacings) and highest-yielding (optimal planting date at narrow rows) environments. Thus, cluster 1 truly provided a broad range of treatment factors for developing seasonal growth dynamic patterns. In contrast to cluster group 1, cluster group 2 predominately represented treatments from late planting dates (66% of treatments were late planted). Consequently, only about 1/3 of treatments came from a high-yielding environment (optimal planting date having normal plant population and optimal row spacing). Both Maturity Groups V and VI were equally represented in Cluster Group 2. Regression of RLAI on RDAE within both cluster groups displayed similar patterns of seasonal RLAI (Figure 2). Both cluster groups showed a quadratic relationship, where LAI gradually increased after emergence in a sloping fashion, reaching the maximal

Table 2: A listing of treatment details used for the current study with various cultural, environmental, and genotype data for individual studies conducted for soybean grown near Baton Rouge, LA, 1986 through 1996

Treatment abbreviations	Treatment details
86MV20/40, 86MV40, 86MVI20, 86MVI40, 86JV20, 86JV40, 86JVI20, 86JVI40	Treatment from 1986 (86); May Planted (M) and July Planted (J); Maturity Group V (V) and Maturity Group VI (VI); Row Spacing 20" (50 cm) and 40" (100 cm).
87MV20, 87MV20H, 87MV40, 87MVI20, 87MVI20H, 87MVI40 87JV20, 87JV20H, 87JV40, 87JVI20, 87JVI20H, 87JVI40	Treatment from 1987 (87); May planted (M) and July planted (J); Maturity Group V (V) and Maturity Group VI (VI); Row spacing 20" (50 cm) and 40" (100 cm) with 220 000 plants/ha; Row spacing 20H" (50 cm) with 325 000 plants/ha;
88MV20, 88MV20H, 88MV40, 88MVI20, 88MVI20H, 88MVI40 88JV20, 88JV20H, 88JV40, 88JVI20, 88JVI20H, 88JVI40	Treatment from 1988 (88); May planted (M) and July planted (J); Maturity Group V (V) and Maturity Group VI (VI); Row spacing 20" (50 cm) and 40" (100 cm) inches (cm) with 220 000 plants/ha; Row spacing 20"H (50 cm) with 325 000 plants/ha;
89VRSVI10, 89VRSVI20, 89VRSVI30, 89VRSVI40	Treatment from 1989 (89); Variable Row Spacing(VRS) 10" (25 cm), 20" (50 cm), 30" (75 cm) and 40" (100 cm)
90VRSVI10, 90VRSVI20, 90VRSVI30, 90VRSVI40	Treatment from 1990 (90); Variable Row Spacing(VRS) 10" (25 cm), 20" (50 cm), 30" (75 cm) and 40" (100 cm)
94POP25, 94POP64, 94POP75, 94POP113	Treatment from 1994 (94); Population(POP) 25,000 plants/acre (25), 64,000 plants/acre (64), 75000 plants/acre (75) and 133,000 plants/acre (133);
95POP25, 95POP64, 95POP75, 95POP113	Treatment from 1995 (95); Population(POP) 25,000 plants/acre (25), 64,000 plants/acre (64), 75000 plants/acre (75) and 133,000 plants/acre (133);

Figure 1: The cluster analysis (Panel A) and principle components (Panel B) of relative leaf area index of 7 years of study with various row spacing, plant populations and time of planting of Soybean at Ben Hur, near Baton Rouge

Figure 2: Relationship between relative leaf area index and relative days after emergence of 7 years of study with various row spacing, plant populations and time of planting of Soybean at Ben Hur, near Baton Rouge

level shortly before the midpoint of the growing season. Relative LAI then stayed at a plateau level for about 30 days, and then began to decline as senescence set in and maturity approached. Similar results have also been reported in other studies. (Worku and Astatke 2011; Cox and Cherney 2011; Alblett et al. 1991; Holshouser and Whittaker 2002; Blumenthal et al. 1988)

3.2 Light Interception

The cluster analysis resulted in identification of four cluster groups (Figure 3) and two main clusters were selected based on PCA (Figure 3). Cluster group 1 consisted of 28 similar treatments, while cluster group 2 combined 16 treatments.

Cluster group 3 contained 3 treatments and cluster group 4 contained only 1 treatment. Data for RLI separated into two main cluster groups, mainly distinguished by yield potential. Cluster group 1 was comprised of narrow-row treatments planted at optimal and late planting dates, and having normal plant populations, whereas cluster group 2 contained mainly late-planted treatments with wide row spacings and low populations (Figure 3). Narrow-row spacing in cluster group 1 gave these treatments greater early-season RLI compared to cluster group 2, thus resulting in canopy closure (95% RLI) at about 35% RDAE for the first cluster. In contrast, treatments in cluster group 2 did not reach canopy closure but attained 90% RLI at 60% RDAE (Figure 4). Such early-season RLI advantages demonstrated in cluster group 1 have been shown to be

Figure 3: The cluster analysis (Panel A) and principle components (Panel B) of relative light interception of 7 years of study with various row spacing, plant populations and time of planting of Soybean at Ben Hur, near Baton Rouge

Figure 4: Relationship between relative light interception and relative days after emergence of 7 years of study with various row spacing, plant population and time of planting of Soybean at Ben Hur, near Baton Rouge

major yield-enhancing factors in soybean production (Gasper and Conley 2014; Board et al. 1992). Both cluster groups showed seasonal quadratic responses as maturity progressed (Figure 4). Relative light interception increased between emergence and 50% RDAE; remained relatively constant until about 80% RDAE and then fell as the crop neared maturity. Essentially, the seasonal RLI pattern was similar for RLAI (Figure 2). One difference between the two clusters was that early-season light interception was greater for cluster group 1 than for cluster group 2, possibly due to the greater concentration of narrow-row treatments in that cluster. Narrow-row soybean culture has demonstrated accelerated early-season light interception relative to wide-row culture, which in some cases results in superior yield (Board et al. 1992). For both clusters, 95% RLI was reached at about the seasonal midpoint. Similar

results have also been reported in other studies conducted previously (Shibles and Weber 1965, 1966; Taylor et al 1982; De Bruin and Pedersen 2008; Lee et al. 2008).

3.3 Total Dry Matter

The cluster analysis divided all treatments into two cluster groups largely based on row spacing and both clusters were selected based on PCA (Figure 5). Cluster Group 1 contained 19 treatments, 18 of which were grown on narrow-row spacings of 75 cm or less. In contrast, about 70% of the 26 treatments in cluster group 2 were wide-row spacings of 75-cm or more. Thus, the treatments in cluster 2 were more prone to inadequate light interception relative to cluster group 1. Both cluster groups demonstrated

Figure 6: Relationship between total dry matter and days after emergence of 7 years of study with various row spacing, plant population and time of plant of Soybean at Ben Hur, near Baton Rouge

quadratic seasonal patterns of TDM accumulation (Figure 6). Shortly after emergence, the rate of TDM accumulation (i.e. CGR) reached a linear constant rate until about the midpoint of the growing season. After this point, CGR then slowed and TDM reached a plateau shortly before the end of the growing season. This decrease in CGR after 60% RDAE is due to leaf abscission starting after the midpoint of the seed filling period and also is related to the fact that much of TDM accumulation after 60 RDAE is into seed weight which has a much higher energy cost for its manufacture compared with other plant parts; thus, resulting in less TDM accumulated per use of plant photosynthate. This explains why the TDM accumulation curve reaches a plateau value at about 80% RDAE.

Data for cluster group 1 displayed a more rapid CGR between emergence and 60% RDAE than for cluster group 2, as evidenced by a greater linear regression coefficient (1.98 vs. 1.4, respectively, Figure 6). This is explained by the greater number of narrow-row treatments consolidated into cluster group 1 compared with cluster group 2. Thus treatments would have had greater light interception during the emergence to 60% RDAE period in cluster group 1 and CGR would consequently have been higher. Such relationships have frequently been demonstrated in row spacing and plant population studies (Shaw and Weber 1966; Willcott et al. 1984; Board et al. 1992; Board 2000; Asanome and Ikeda 2008; Beurelein 1988).

3.4 Practical Implications for Farmers and Researchers

Seasonal RLI patterns presented in Figure 4 provide farmers and researchers with a diagnostic tool for assessing yield-limiting factors. Failure to achieve 95% RLI within about 1 month after emergence is an indication that genotypic and/or cultural manipulations need to be employed to improve yield. Although once a laborious process, RLI can now accurately, easily, and rapidly be determined using digital photographic methods (Purcell 2000). These methods can be applied in commercial soybean fields with relatively few recordings. Research demonstrates that in a 57-acre field, only three randomly placed light recordings were required for reliable RLI determinations (Singer et al. 2011). Failure to achieve 95% RLI within 30-35 RDAE indicates that farmers can improve yield by narrowing row spacing, increasing plant population, alleviating any stress factor that would limit early-season growth (e.g. low soil pH, waterlogging, drought, insect attacks), and/or growing cultivars with a rapid early-season CGR.

Seasonal patterns of RTDM accumulation for cluster group 1 (Figure 6) have potential use to researchers and farmers for diagnosing stress problems. Previous studies have demonstrated that TDM levels for soybean at first flowering (R1) and the start of seed filling (R5) can be used as benchmarks to indicate potential for optimizing yield (Board and Modali 2005). Data in the current study can be applied to this same process. The RTDM seasonal pattern shown in Figure 6, cluster group 1, represents optimal growing conditions for a given environment. Such a pattern could be useful for scenarios as described below:

Assume that researchers at a local research station are working with a new soybean cultivar that has high yield potential for the area, 60 bu/a (4000 kg/ha), associated with a maximal TDM of 800 g per square meter. Using the quadratic regression model for cluster group 1 in Figure 6, anyone could calculate the RTDM the farmer/researcher should achieve by any given date (early, middle and/or late season) to have the potential to achieve optimal yield. This percentage can then be multiplied by the maximal TDM (800 g square meter), to calculate what TDM should be expected by that date to be on course for optimizing yield. Occurrences of normal vs. sub-normal TDM at given times during the growing season helps identify potential stress problems that could be restricting final yield. For example, if the farmer had optimal TDM at 30 RDAE, but did not achieve optimal TDM by 60 RDAE, it would indicate stress occurred during the period between 30-60 RDAE. The resulting final yield loss would then be identified as

some stress that occurred during this period. Although determination of TDM from large commercial soybean fields is a laborious and time-consuming task, research has shown that TDM levels can be accurately predicted by canopy LI levels, which is a much easier parameter to measure than TDM itself.

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