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Depodding Causes Green-Stem Syndrome in Soybean

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Abstract

Green-stem syndrome in soybean (*Glycine max* L. Merrill) occurs when stems stay green after the pods mature causing problems during harvest. Green-stem syndrome has been linked to disease (primarily viruses), insects [principally stink bugs *Nezara viridula* (L.), *Acrosternum hilare* (Say), and *Euschistus servus* (Say)], and environmental stress, all of which could cause reductions in pod number. We evaluated the relationship between pod removal (25 and 50% of all pods) and the appearance of green-stem symptoms in a two-year field experiment at Lexington, KY (38°N). Depodding treatments were applied at the beginning of growth stage R6 to nine soybean varieties (three each from maturity groups III, IV, and V) grown in single-row plots with two replications. The progression of pod and stem maturation was determined at three-day intervals by visually estimating the proportion of the pods that had reached their mature color and by counting brown stems in a 10-plant sample. Pod removal had almost no effect on pod and seed maturation (delays for depodding were usually < 7 days) or on seed moisture when 95 to 100% of the pods were mature. Stem maturation was always delayed relative to control plants and the delay was larger for the 50% than the 25% treatment. Seventy percent of the variety by year combinations in the 50% treatment were delayed by > 20 days, but for 60% of the variety by year combinations the delay for the 25% treatment was ≤ 10 days. Stem maturation was not complete on some treatments when frost occurred 30 to 50 days after the controls matured. Soluble sugars, starch, and N accumulated in the stems of depodded plants and there was a significant (P = 0.01) linear relationship (r^2 = 0.41) between soluble sugar enhancement (but not starch or N) and the delay in stem maturation. Depodding always created green-stem symptoms in all varieties, but the expression of the symptoms was not consistent across years. These results suggest that disease, insects, and environmental stress may cause green-stem syndrome indirectly by reducing pod load.

Introduction

The green-stem syndrome in soybean occurs when stems fail to mature normally, remaining green when the pods are mature and ready for harvest (12,13). Leaves and petioles may remain on the plant. The presence of green stems may slow harvest and increase seed loss (11) and potentially reduce yield. Green stem is often associated with infection of plants by viruses, most commonly *Bean pod mottle virus* (5,6,12), but *Tobacco ringspot virus* has also been implicated (13). Other possible causes include insect feeding, principally stink bugs (1,2), and environmental stress (13).

The common thread that links these agents is the potential they have to reduce the pod load, altering the source-sink ratio in favor of the source. Reducing pod and seed number and sink size slows mobilization of carbon and N from vegetative tissues to seeds (9,15). The objectives of our work were to (i) determine the relationship between pod load and the development of the green-stem syndrome; (ii) evaluate the progression of stem, pod, and seed maturation on plants exhibiting green-stem symptoms; and finally (iii) assess variety variation in the development of this syndrome.

Reducing the Pod Load

High-yielding soybean varieties (three each from Maturity Group III, IV, and V) were selected using results of the Kentucky Soybean Performance Test. Seed of these varieties were sown in 30-inch rows (approximately 8 seeds per foot of row) in a conventionally-tilled seed bed on June 10, 2002 [Lanton silt loam (fine-silty, mixed, thermic Cumulic Epiaquoll)] and May 24, 2004 [Maury silt loam (fine, mixed, semiactive, mesic Typic Paleudalf)]. We followed University of Kentucky soil fertility recommendations and a combination of herbicides and hand weeding was used for weed control. The plots were irrigated with an overhead sprinkler system as needed to minimize water stress. Each variety was sown in a single 20-foot row with the depodding treatments (0, 25, and 50% pod removal) applied to all plants in randomly-selected 3-foot sections of row. A split-plot arrangement of treatments was used with pod removal treatments randomly assigned to sub-plots and with main plots (varieties) arranged in a randomized block design with two replications. The pod removal treatments were applied once at approximately the beginning of growth stage R6 (4) to minimize additional pod set after the treatments were applied. All pods were removed from alternate nodes on the main stem and branches in the 50% removal treatment and from every fourth node in the 25% treatment. Pods were counted on 4 plants per plot of one variety from each maturity group at maturity to estimate the final reduction in pod load.

Reproductive growth stages of control plants (10 consecutive plants in the row) were determined at weekly intervals following the system of Fehr and Caviness (4). The appearance of mature pods (visual estimate of the percent of the pods that had completely reached their mature pod color) and brown stems (the number of completely brown stems in 10 consecutive plants in the row) was determined at three-day intervals until all pods and stems were mature or brown or until the first frost. Stem tissue (two consecutive nodes and the subtending internode tissue from the middle of the main stem from the control and 50% depodding treatment and a four node section on the 25% depodding treatment from 2 plants in each plot) and seed samples (10 pods per plot) were taken when the plants had approximately 95 to 100% mature pods. Seeds were removed from the pods and seed moisture levels were determined after drying at 60°C. The stem samples were frozen, freeze dried, ground, and soluble sugars and starch levels were determined as described by Heberer et al. (7). Total N was determined by a Kjeldahl procedure (7,10).

The time to 90% mature pods and 65% brown stems was determined for each individual plot from the time course of mature pod and brown stem appearance. Linear interpolation was used to estimate the date when it occurred between measurements. Brown stem development on the depodded treatments was often not complete when the first frost occurred; consequently 65%, instead of 90%, brown stem development was used as the end point to increase the number of treatment combinations that reached the end point before frost. The time to 90% mature pod data was subjected to a combined analysis of variance across years. The brown stem data from each year were analyzed separately. Only those treatments reaching 65% brown stems before frost were included in the analysis of variance, so the analysis included only 4 varieties in the 25% treatment in 2002 and 5 varieties in the 25 and 50% treatments in 2004.

Pod and Stem Maturation on Depodded Plants

Applying the pod removal treatments at the beginning of growth stage R6 effectively prevented additional pod set after the treatments were applied. Pod loads at maturity were near target levels, averaging 27% below the control in the 25% treatment and 48% for the 50% treatment (*data not shown*).

The development of mature pods usually occurred rapidly in all treatments with only minor delays (typically < 7 days with the largest 18 days) caused by depodding (Fig. 1, Table 1). There were significant ($P = 0.05$) variety, treatment, and year effects on the development of mature pods (Table 1), and all interactions except the variety × treatment interaction were significant. Most variety and treatment effects, however, were too small to have much practical significance and some were not consistent across years.

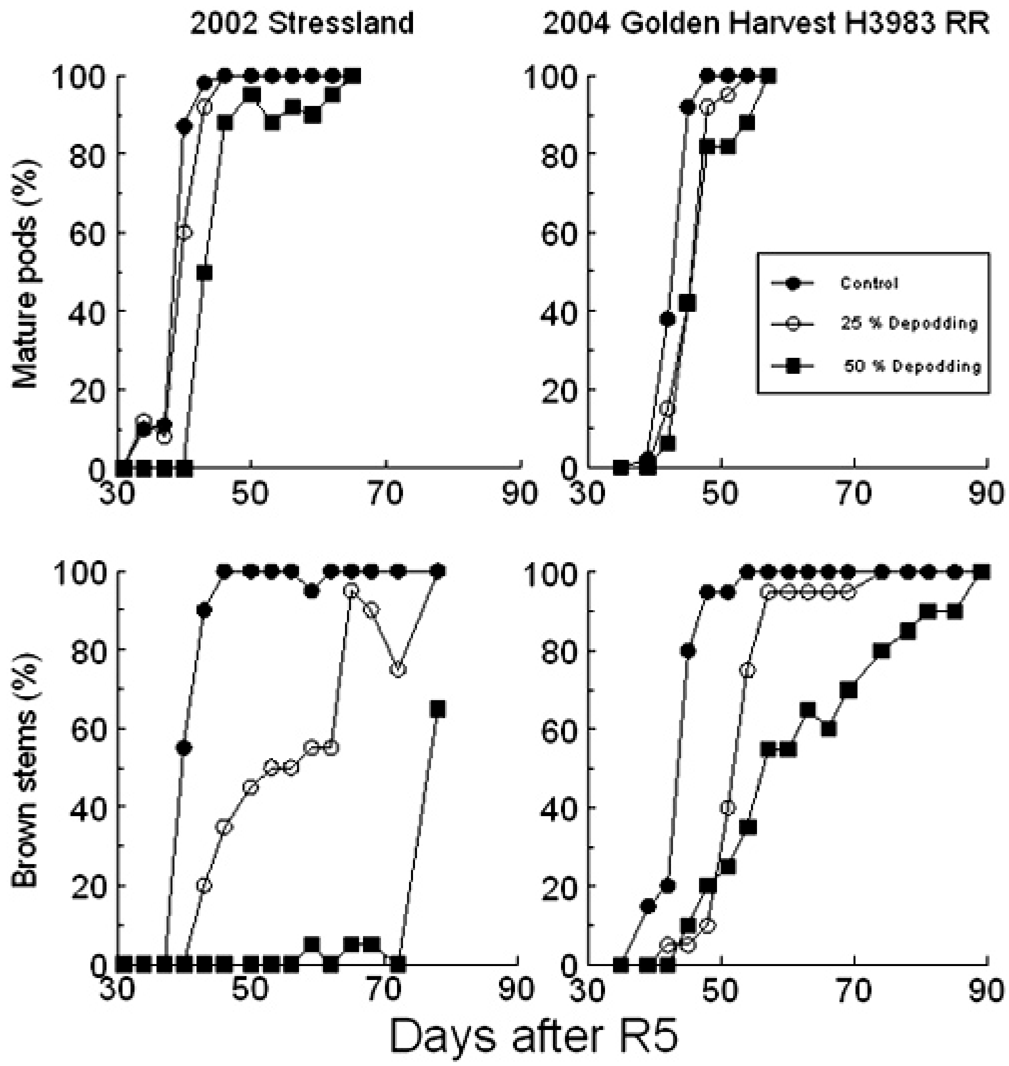

Fig. 1. Typical progression of pod and stem maturation on soybean plants with 0, 25, and 50% of the pods removed early in seed filling (growth stage R6).

Table 1. The effect of depodding on the development of mature pods on nine soybean varieties, 2002 and 2004.

<table>
<tr><th colspan="2" rowspan="3"></th><th colspan="6">Days (after control) to 90% mature pods</th></tr>
<tr><th colspan="3">25% depodding</th><th colspan="3">50% depodding</th></tr>
<tr><th>2002</th><th>2004</th><th>Mean</th><th>2002</th><th>2004</th><th>Mean</th></tr>
<tr><td rowspan="3">MG III</td><td>Stine 3870-0</td><td>4</td><td>1</td><td>2.5</td><td>7</td><td>4</td><td>5.5</td></tr>
<tr><td>Pioneer variety 93B87</td><td>2</td><td>2</td><td>2.0</td><td>3</td><td>7</td><td>5.0</td></tr>
<tr><td>Golden Harvest H-3983RR</td><td>4</td><td>3</td><td>3.5</td><td>18</td><td>7</td><td>12.5</td></tr>
<tr><td rowspan="3">MG IV</td><td>Southern States FFR-439</td><td>4</td><td>4</td><td>4.0</td><td>2</td><td>5</td><td>3.5</td></tr>
<tr><td>LG Seeds C9474</td><td>3</td><td>3</td><td>3.0</td><td>10</td><td>6</td><td>8.0</td></tr>
<tr><td>Stressland</td><td>2</td><td>1</td><td>1.5</td><td>6</td><td>3</td><td>4.5</td></tr>
<tr><td rowspan="3">MG V</td><td>Asgrow AG 5001</td><td>1</td><td>0</td><td>0.5</td><td>6</td><td>0</td><td>3.0</td></tr>
<tr><td>Delta King 5465RR[y]</td><td>--[x]</td><td>1</td><td>--</td><td>--</td><td>3</td><td>--</td></tr>
<tr><td>Hutcheson[y]</td><td>--[x]</td><td>3</td><td>--</td><td>--</td><td>2</td><td>--</td></tr>
<tr><td colspan="2">LSD (0.10)</td><td colspan="3">4</td><td colspan="3">4</td></tr>
</table>

[x] Control plants had not reached 90% mature pods when the first frost occurred.

[y] Delta King 5465 RR and Hutcheson were not included in the ANOVA. Variety, treatment and year effects and all of the interactions (variety × treatment, treatment × year, and variety × treatment × year) except variety × year were significant at $P = 0.05$. The LSD is for comparison of any two variety × treatment × year means.

The development of brown stems usually occurred rapidly on the control plants (Fig. 1), but depodding caused delays that were usually larger on the 50% than on the 25% depodding treatment (Table 2). None of the varieties reached 65% brown stems before the first frost (November 2) in the 50% depodding treatment in 2002, probably as a result of the late planting date (June 10). However, five varieties reached 65% brown stems in the 50% treatment in 2004. It was difficult to thoroughly evaluate variety effects on stem maturation given that none of the varieties reached 65% brown stems in both depodding treatments and both years, precluding a combined analysis of variance across years. There were significant varietal differences in 2004 in the subset of varieties included in the statistical analysis (Table 2). Combining the results of the statistical analysis with an evaluation of the trends in all of the data suggests that the variation among years may be as large as the variation among varieties.

Table 2. The effect of depodding on the development of brown stems on nine soybean varieties, 2002 and 2004.

		Days (after control) to 65% brown stems				
		25% depodding			50% depodding	
		2002	2004	Mean	2002	2004
MG III	Stine 3870-0	4	4	4.0	(37)[w]	24
	Pioneer variety 93B87	(37)	15	--	(37)	(46)
	Golden Harvest H-3983RR	6	8	7.0	(41)	16
MG IV	Southern States FFR-439	23	8	15.5	(25)	(37)
	LG Seeds C9474	(27)	29	--	(27)	22
	Stressland	15	5	10.5	(37)	30
MG V	Asgrow AG 5001	-[x]	14	--	--	(20)
	Delta King 5465RR	--	5	--	--	(14)
	Hutcheson	--	6	--	--	7
LSD (0.10)		ns[y]	17[z]	--	--	--

[w] Depodded plants had not reached 65% brown stems when the last sample was taken shortly after the first frost. The number in parentheses represents the days after the control reached 65% brown stems when frost occurred and data collection stopped.

[x] Control plants had not reached 65% brown stems when the first frost occurred.

[y] ANOVA for 2002 included only the 25% treatment and the four varieties that reached 65% brown stems at the last sample.

[z] The ANOVA for 2004 included only the five varieties that reached 65% brown stems before the last sample in both treatments. Variety (P = 0.10) and treatment (P = 0.05) effects were significant, but the variety × treatment interaction was not significant (P = 0.05). LSD is for comparison of any two means in 2004.

Depodding consistently increased soluble sugar, starch, and N levels in the stems when most of the pods were brown (Table 3). The magnitude of the increase varied among varieties (*data not shown*), but the treatment effects were consistent so only the average across varieties within a maturity group is shown in Table 3. The delays in stem maturation increased linearly (r^2 = 0.41, n = 19, P = 0.01) as the levels of soluble sugars (expressed relative to control levels) increased (Fig. 2), but there was no significant relationship with starch (r = 0.39, ns) or N (r = 0.44, ns). Seeds were relatively dry (< 25%) when most pods were brown and moisture levels were generally higher in 2002 than in 2004. Depodding had no significant (P = 0.05) effect on seed moisture levels in 2004, but there was a reduction in MG III in 2002 (Table 3).

Table 3. The effect of cultivar and depodding on stem composition and seed moisture levels. Each plot was sampled when 95 to 100% of the pods were mature.

MG	Depodding (%)	Soluble sugars		starch		N		seed moisture	
		2002	2004	2002	2004	2002	2004	2002	2004
		(%)							
III	Control[w]	11.4	9.9	2.6	2.1	3.1	0.5	22.5	9.9
	25	17.4	14.1	5.5	3.0	4.1	1.0	21.5	13.2
	50	33.1	27.8	14.4	18.1	8.0	4.9	13.3	10.3
IV	Control	9.6	10.4	1.6	1.6	3.0	1.5	21.8	8.4
	25	15.6	14.2	3.5	3.4	5.0	1.8	19.1	8.0
	50	30.4	22.4	31.5	8.2	11.7	7.0	19.2	9.8
V	Control	10.3[x]	13.6	4.1[x]	2.4	3.4[x]	0.7	22.6[x]	19.3
	25	43.1	18.1	14.9	4.8	8.0	3.2	23.0	22.5
	50	53.2	34.3	23.0	14.9	9.5	1.7	23.6	24.2
LDS (0.05)[y]		10.0	8.4	9.1	6.5	2.4	3.0	5.4	-
Cultivar		**[z]	**	ns	**	ns	*	**	**
Treatment		***	***	***	***	***	**	*	ns
Cult. × Treat.		ns	ns	**	**	ns	ns	ns	ns

[w] Average of three cultivars per maturity group.

[x] Only Asgrow AG 5001. The other cultivars did not reach 95 to 100% mature pods before frost.

[y] LSD is for the comparison of any two means.

[z] *,**,*** significant at *P* = 0.05. 0.01, and 0.001, respectively.

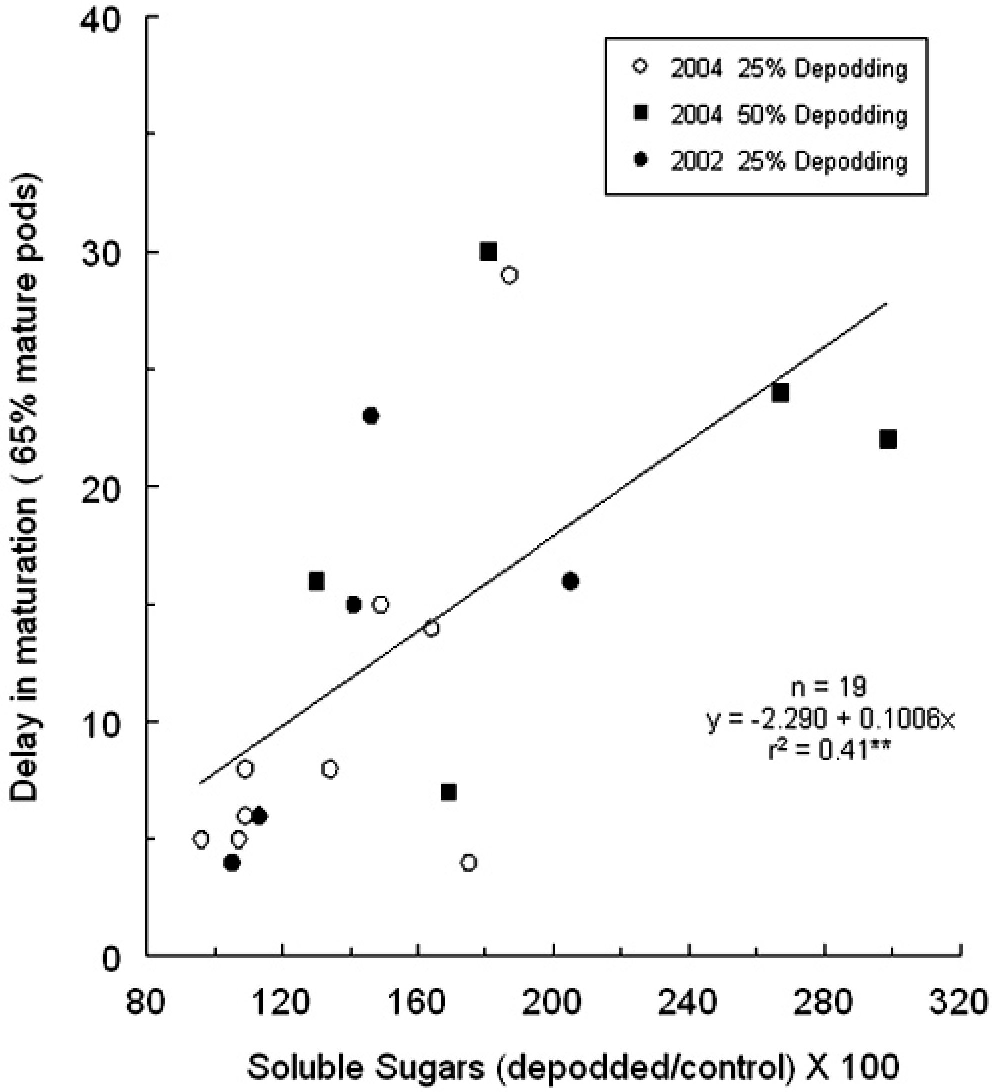

Fig. 2. The association between soluble sugar levels in stems and the delay in stem maturation. The soluble sugar levels represent, for the depodded treatments, the increase over control levels (i.e., depodded/control × 100). Stem maturation is expressed as days later than the control. Only the treatment combinations reaching 65% brown stems before frost are included. The regression analysis was significant at P = 0.01.

Pod Removal and Green-Stem Syndrome

Removing pods early in the seed-filling period created the symptoms normally associated with green-stem syndrome: stems remained green while leaves and petioles abscised, pods matured, and seeds dried to harvestable moisture levels (12). The symptoms developed on all varieties when only 25% of the pods were removed, but they were much more pronounced with 50% removal. The appearance of brown stems on depodded plants was highly variable with the delay, relative to the control, ranging from as few as 4 days to a month or longer.

The green stems were associated with higher stem concentrations of soluble sugars, starch, and N. Starch and N are normally mobilized from stems during seed filling and this movement is known to be limited by reductions in sink size (9). Long delays in stem maturation were associated with the accumulation of high levels of soluble sugar levels in the stem, relative to the control. It is not clear if this relationship represents a direct effect of soluble sugars on stem maturation or if it is only an indirect association with the depodding treatment. There were substantial variety differences in the degree of enhancement of stem soluble sugars within a depodding treatment and there was also some overlap among treatments (Fig. 2). These differences were not related to maturity group and, given the consistent level of depodding, they were probably not related to the magnitude of the sink reduction. It is not clear what caused this variation, but it is difficult to conclude, given the inconsistencies among treatments and years, that varieties differ in their response to pod removal.

Delays in the development of mature pods on depodded plants were much smaller (usually < 7 days) than delays in stem maturation. These results are consistent with previous reports (3) indicating that pod and seed maturation does not require maturation of the vegetative plant. Seed moisture levels are closely associated with the stage of pod and seed development (3), so depodding had minimal affects on the levels in mature pods.

The rapid pod and seed maturation and the delays in stem maturation associated with depodding clearly define the dilemma faced by a producer with green-stem syndrome. Delays of 3 or 4 weeks in stem maturation were relatively common and delaying harvest by this much might result in exceptionally low seed moisture levels, greatly increasing the chances of mechanical damage to the seed (14). Delaying harvest may also reduce seed germination and vigor (14), and increase shattering and harvest losses. Unfortunately, the delay in stem maturation was quite variable (4 to at least 46 days for 65% brown stems) making it difficult to predict when delaying harvest might cause problems. The most realistic solution may be to harvest when pods are mature and simply endure the problems created by green stems.

Our results indicating that green-stem syndrome can be created by reductions in pod number provide an explanation for the many proposed causes of this problem. Any mechanism that reduces the pod load (i.e., disease, insects, environmental stress) could be responsible for green stem; thus the syndrome would only be indirectly related to the many causal agents identified in the literature. There have been suggestions that some varieties may be more prone to green stem than others (8), but we could not, with our limited comparison of varieties, identify consistent variety differences in the response to pod removal. It's possible that variety variation may occur only in response to the cause of the pod reduction (especially disease or insects infestations), not as an effect of pod reduction itself.

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