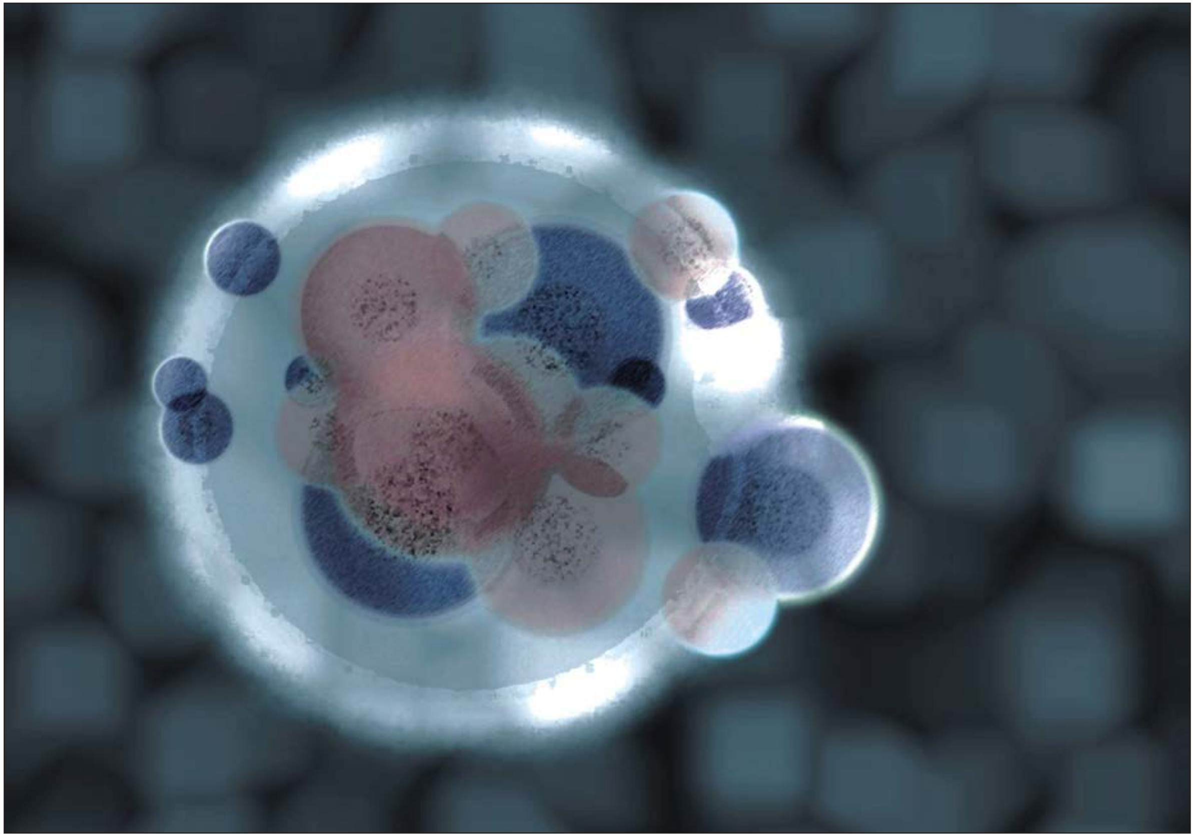

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Nitrogen and Carbohydrate Nutrient Concentrations and Flower Set in Soybean (*Glycine max* (L.) Merr.)

[1]C. Dean Dybing and [2]R. Neil Reese
[1]Department of Plant Science, Faculty of Agriculture, Agricultural Research Service, South Dakota State University, Brookings, SD 57007, USA
[2]Department of Biology and Microbiology, South Dakota State University, Brookings, South Dakota 57007, USA

Abstract: In this study, flowers varying in level of pod set were obtained from the semi-determinate, many-flowered line IX93-100 and analyzed for total soluble sugars, transported N compounds, starch and total N. Correlations between percent pod set and chemical composition varied with method of sampling for some substances. Little evidence of nutrient shortage in aborting flowers was seen. Analysis of raceme tissues separately from flowers revealed little evidence of restricted nutrient removal from raceme into the aborting flower except possibly for the transported N compounds. The best association with abortion appeared to be slow growth of the flower.

Key words: Soybean, flower abortion, flower nutrition, flower abscission, chemical analysis

INTRODUCTION

Events that occur during fruit set in higher plants include pollination, fertilization and development of seeds and fruits. Failure in one or more of these steps leads to reduction in the number of seeds within the fruit or in the proportion of fruits to flowers. Overproduction of reproductive structures and subsequent abortion of the excess number are common in plants and may play a significant role in the reproductive strategy and success of many species (Stephenson, 1981). Mechanisms involved in the regulation of set, abortion and abscission of reproductive structures are not well understood in any species for reasons that include difficulties in identifying and controlling critical events for experimental purposes.

In soybean (*Glycine max* (L.) Merr.), a large proportion of fertilized flowers fail to develop fruits with or without environmental stress (Abernethy *et al*., 1977; Dybing *et al*., 1986; Nagel *et al*., 2001; Liu *et al*., 2004a, b, c; Yashima *et al*., 2005). The process is called flower abortion if the ovary of the shed structure does not extend beyond the calyx (Dybing *et al*., 1986; Peterson *et al*., 1992). Inheritance appears to be polygenic with dominance effects for high abortion (Sharma *et al*., 1990) and appears to be associated with the indeterminate wild type ancestral *Glycine soya* (Saitoh *et al*., 2004). Abscission processes during flower abortion may begin very soon after anthesis (Huff and Dybing, 1980; Brun and Betts, 1984; Dybing *et al*., 1986). Compared to setting flowers, sink intensity is lower in aborting flowers as early as 3 days after anthesis (Brun and Betts, 1984; Heitholt *et al*., 1986a). Most proximal flowers set, most distal flowers abort and removal of the proximal flowers can induce set of the distal flowers (Huff and Dybing, 1980; Dybing *et al*., 1986; Wiebold, 1990). Such observations lead to the idea that young, distal soybean flowers cannot compete successfully with the older proximal flowers for limited supplies of either nutrients or hormones. However, no model has been widely accepted as stating the correct mechanism.

Several studies have presented often conflicting data suggesting that nitrogen and carbohydrate availability (photosynthesis) may play a role(s) in the number of flowers formed and the total number of mature soybean fruits and seeds that develop (Sinclair and de Witt, 1976; Streeter and Jeffers, 1979; Antos and Wiebold, 1984; Dybing *et al*., 1986; Stockman and Shibles, 1986; Dybing, 1994; Jiang and Egli, 1993; Hayati *et al*., 1995; Liu *et al*., 2004c; Egli and Bruening, 2005). However, correlations to parameters other than total dry weight are rare and inconsistent. Cytokinins have also been shown to regulate flower number and abortion/abscission rates (Huff and Dybing, 1980; Ghiasi *et al*., 1987; Peterson *et al*., 1990; Mosjidis *et al*., 1993; Wiebold, 1990; Reese *et al*., 1995; Nagel *et al*., 2001; Cho *et al*., 2002; Yashima *et al*., 2005). The objective of the present study

Corresponding Author: R. Neil Reese, Department of Biology and Microbiology, South Dakota State University, Brookings, South Dakota 57007, USA

was to relate pod set to nutrient levels in reproductive tissues and evaluate nutrient competition as a possible cause of soybean flower abortion, in light of the response of these plants to cytokinin treatment (Reese *et al*., 1995). Specifically, the experiments were designed to test the hypothesis that nutrient availability is not the primary factor limiting flower abortion in soybeans.

Glycine max line IX93-100 was used for this research because the long racemes provide a convenient system for examining the flowers at various stages of development in a single raceme at a single point in time. As the flowers develop, the proximal flowers mature first. All of the flowers are pollinated and have the potential to set fruit. More distal flowers tend to abort, but will develop normally if proximal flowers are removed and/or the racemes are treated with cytokinins.

Substances measured were those that might be considered transported nutrients, namely soluble sugars, amides and ureides, as well as stored nutrients present as starch and total nitrogen. In this general plan, the study is similar to earlier studied by Heitholt *et al.* (1986b), where chemical concentration measurements were made for flowers at anthesis and pods at 1 cm of length. It differs from the older work in that flowers were sampled at one location in the canopy rather than at different places and two sampling procedures were compared. Flower and raceme tissues were analyzed separately to detect any gradients that would indicate difficulties of transport into the flower and to examine the relationship between raceme chemical concentrations and formation of the abscission zone (Oberholster *et al*., 1991).

MATERIALS AND METHODS

Plant culture: *Glycine max* genotype IX93-100 was used because it produces a large number of flowers and percent pod set is readily and predictably manipulated (Dybing *et al*., 1986; Sharma *et al*., 1990). Field plantings were made on Vienna loam soil at Brookings, South Dakota. Rows were 5.2 m long and had 0.76 m spacing. Cultural methods for field plots were typical of commercial production. Additional plantings were made in the greenhouse and growth chamber in nutrient solution with N supplied as nitrate (Dybing *et al*., 1986; Dybing and Yarrow, 1984). Greenhouse air temperature was 25±5°C and photoperiod was maintained at 16 h by supplementation of daylight with lighting from high pressure sodium lamps. The growth chamber environment had 30/21±2°C day/night temperature, 16 h photoperiod, 450±50 μmol m^{-2} sec^{-1} photosynthetic photon flux density and uncontrolled humidity level. Where exogenous cytokinin application was made, the entire

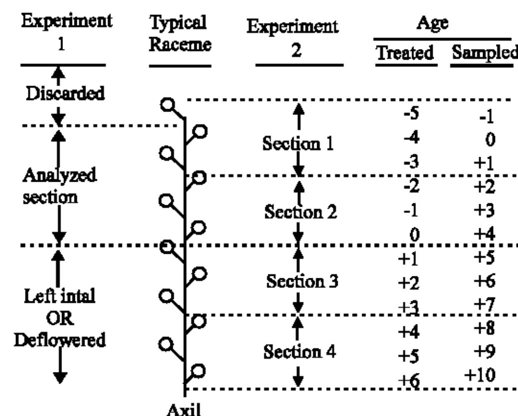

Fig. 1: Experimental treatments and sampling procedures. Diagram shows a typical inflorescence axis with pedicels and flowers from leaf axil to raceme apex. Horizontal lines indicate segmentation of inflorescences by experiment. Sections analyzed for experiment 1 were 5-flower sections starting with the middle flower. Segments analyzed for experiment 2 were obtained by dividing the inflorescence into four sections. Flower ages when BA-treated and sampled are number of days before (-) or after (+) anthesis, assuming that one flower reaches anthesis each day. Each replication consisted of at least 24 inflorescences. Final samples were obtained by dividing each section into flowers and raceme plus pedicels

inflorescence inflorescence was sprayed to runoff using 1 mM N-6 benzyladenine (BA) in 0.075% Tween 80 (Peterson *et al*., 1990). Inflorescences chosen for use always had at least 10 flowers and were chosen at random at early bloom in the upper half of the canopy.

Experiment protocols: Two different procedures were used to obtain samples having high level of pod set for comparison with other samples having lower levels of pod set (Fig. 1). The first employed a single section of the inflorescence and regulated the level of pod set in this section by partial deflowering or by application of cytokinin to the entire inflorescence. The second involved positional sampling with or without cytokinin treatment and relied on the natural decrease in level of pod set that occurs from proximal to distal ends of the inflorescence. These two protocols were considered to be Experiments 1 and 2, respectively.

Plants providing inflorescences for Experiment 1 were sown in the field in 2 replications of 50 rows each and in the greenhouse in 3 replications of 40 plants each. Level of pod set in the sampled section (Fig. 1) was varied

by applying the following treatments: Inflorescence left intact (treatment I); intact but treated with cytokinin (treatment IC); deflowered below the middle flower (treatment D); and deflowered below the middle flower and then treated with cytokinin (treatment DC). These treatments were made at full anthesis of the flower at the middle of each inflorescence (Peterson *et al*., 1992). Four days after treatment the inflorescences were harvested and sectioned. Analysis was conducted to allow calculation of chemical concentrations for the total flower unit (TFU; basis = a single flower plus its pedicel and associated raceme axis section), as well as for two subdivisions identified as raceme (flower pedicel plus the attached inflorescence axis) and flower (calyx, corolla androecium and ovary). This type of tissue division provided greater sample dry weight for the flower sample than if the ovary had been analyzed alone as we have done elsewhere (Dybing *et al*., 1986). It was based on a preliminary experiment showing that concentrations of the substances measured here remained constant in the ovary relative to concentrations in the other flower tissues. The samples were frozen in liquid nitrogen, lyophilized, ground, weighed and stored in a desiccator until extracted. Individual samples for experiment 1 were pooled from 24 to 38 inflorescence sections per replication and they averaged 105 to 212 mg dry tissue. Age of the flowers in the samples was not an experimental variable even though each analyzed section contained five flowers ranging approximately from anthesis to 4 days past anthesis.

Experiment 2 utilized inflorescences having no cytokinin treatment that were obtained from plants grown in the growth chamber (4 replications), as well as inflorescences with and without 1 mM BA treatment from plants sown in the field in 4 replications of 25 rows each. Timing of the experiment again commenced at anthesis of the middle flower, with cytokinin treatment (field only) made then and positional sampling of the inflorescences occurring four days later. These were divided into four sections (Fig. 1) having 3 to 5 flowers each. Structures included in these sections were: Buds, anthesis flowers and immediately post-anthesis flowers (section 1); post-anthesis flowers (section 2); very young pods (section 3); and older pods (section 4). All four sections were separated into flower and raceme tissues as described above. Experiment 2 samples had 43 to 149 inflorescences and averaged 132 to 1028 mg of tissue per replication.

Level of pod set for the analyzed samples could not be known in either experiment because destructive sampling occurred near anthesis of the middle flower. Therefore,

$$\text{Percent pod set} = \frac{\text{No. of pods}}{\text{No. of flowers}} \times 100$$

for each sample was estimated by identifying and making similar manipulations on additional inflorescences and analyzing them about two weeks after final blossoming when flowers had been replaced either by pods or by abscission scars (Sharma *et al*., 1990). Number of inflorescences used to determine percent pod set varied from 6 to 18 per replication in experiment 1 and 5 to 10 per replication in experiment 2.

Tissue analysis: Two to four 25 mg subsamples of dry tissue per replication were extracted with 80% ethanol for determination of soluble sugars, starch, ureides and the amides asparagine and glutamine in each experiment. Total N was determined on separate subsamples. Procedures used for sugars, starch and total N have been described previously (Dybing, 1994). Ureides and the amides were determined by procedures of Vogels and van der Drift (1970) and Fiedler and Plaga (1987), respectively; their sum is herein called transported N. All determinations were expressed on either concentration (mg g^{-1}) or content (mg per tissue unit) basis. Statistical analysis was by general linear models procedures (SAS, 1982) with separation of means by t-test following a significant F-test. Experimental design was considered to be a randomized complete block with nesting of subsamples within replications. Relationships between percent pod set and chemical composition were tested by correlation using replication means. Treatments in experiment 1 and section positions in experiment 2 entered only as levels of pod set in these analyses.

RESULTS

Analysis based on the total flower unit: Levels of pod set varied from 1 to 80% in experiment 1 and 1 to 84% in experiment 2 (Table 1). Greenhouse plants in Experiment 1 were higher in percent pod set in all treatments than field plants. The cytokinin and deflowering treatments of experiment 1 significantly increased pod set in both environments. In experiment 2, pod set increased basipetally and cytokinin treatment significantly increased pod set in the upper middle section of the inflorescence.

Correlations of percent pod set with characteristics measured for the TFU tissues were consistent in sign for some characteristics and inconsistent for others (Table 2). Noteworthy for showing consistent relationship to pod set in both experiments were TFU dry weight and concentration of soluble sugars. The relationship to pod set was positive for dry weight, but for sugar concentration it was negative. In contrast, sign of r were varied by experiment and within experiments for starch,

Table 1: Percent pod set in two experiments with varied environments

Experiment 1			Experiment 2			
Treatment[a]	Greenhouse[b]	Field[b]	Raceme Section[a]	Growth chamber[b]	Field[b]	Field + BA[b]
I	37.3[c]	1.1[c]	1	9.4[d]	1.2[d]	5.3[d]
IC	56.3[c]	36.4[c]	2	26.1[c]	24.5[c]	37.5[c]*
D	74.4[a]	43.8[c]	3	53.1[c]	59.3[c]	62.0[c]
DC	80.0[a]	63.3[a]	4	66.4[a]	80.7[a]	83.7[a]

[a] Experiment 1: I = intact inflorescence treated with 1 mM BA; D = proximal half of inflorescence deflowered; DC = half-deflowered inflorescence treated with BA. Experiment 2: 1 = 4 = distal, upper middle, lower middle and proximal inflorescence sections, respectively. [b]Means for a characteristic within a column having the same letter do not differ significantly at p = 0.05; mean for BA-treated section marked *: differs significantly at p = 0.05 from mean for corresponding untreated section in the field in experiment 2

Table 2: Correlation of percent pod set with tissue weight and chemical composition in soybean

	Experiment 1[b]		Experiment 2[b]		
Associated characteristics	Greenhouse	Field	Growth chamber	Field	Field + BA
Tissue dry weight (mg TFU^{-1})	0.50	0.71*	0.34	0.93**	0.94**
Soluble sugars (mg g^{-1})	-0.29	-0.76*	-0.29	-0.72**	-0.75**
Transported N compounds (mg g^{-1})	-0.76**	-0.37	0.37	0.77**	0.80**
Starch (mg g^{-1})	0.03	0.72*	-0.38	0.52**	0.82**
Total N (mg g^{-1})	-0.64*	-0.89**	0.18	0.35	-0.18

[a]TFU = Total Flower Unit consisting of flower, pedicel and associated raceme axis. [b]Correlation is significant at p = 0.05 and p = 0.01 (* and **, respectively); n = 12 for Greenhouse in experiment I, 8 for Field in experiment 1 and 16 for all units of experiment 2

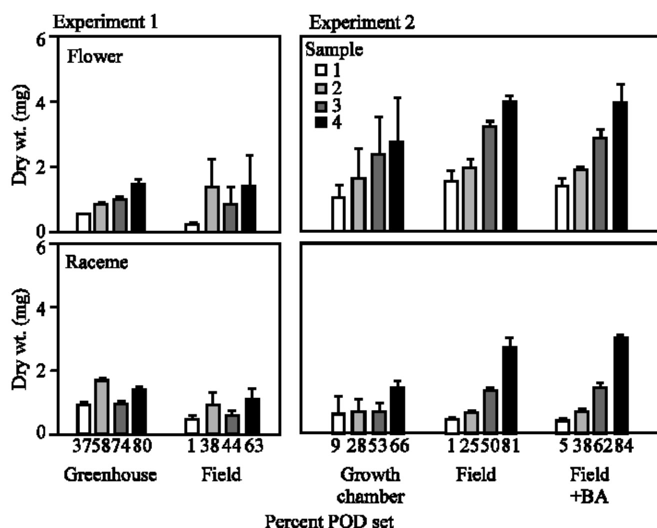

Fig. 2: Mean dry weight of flower (calyx, corolla androecium and ovary) and raceme (flower pedicel plus associated inflorescence axis segment) as related to percent pod set. Samples 1-4 in experiment 1 are upper middle section from intact, intact + cytokinin, deflowered and deflowered + cytokinin treated inflorescenses, respectively. Samples 1-4 in experiment 2 are distal, upper middle, lower middle and proximal inflorescence sections, respectively. Error bars are standard deviations

transported N compounds and total N. Values of r were generally higher for field than for greenhouse or growth chamber plants.

Separate analysis of flowers and racemes: Dry weights of flowers generally increased as percent pod set increased in both experiments (Fig. 2). For raceme tissues, dry weight was closely associated with pod set only in Experiment 2. This was because the deflowering treatment of Experiment 1 increased pod set and flower weight without affecting raceme weight. Cytokinin treatment increased dry weights of both flowers and racemes in Experiment 1 (IC compared with I) but not in Experiment 2 (field + BA compared to field).

Flower sugar concentrations ranged from 46 to 95 mg g^{-1}, whereas raceme levels varied from 22 to 81 mg g^{-1} (Fig. 3). Flower sugar concentrations in samples having lowest pod set were equal to or greater than concentrations in samples having highest pod set in both experiments and all environments within experiments.

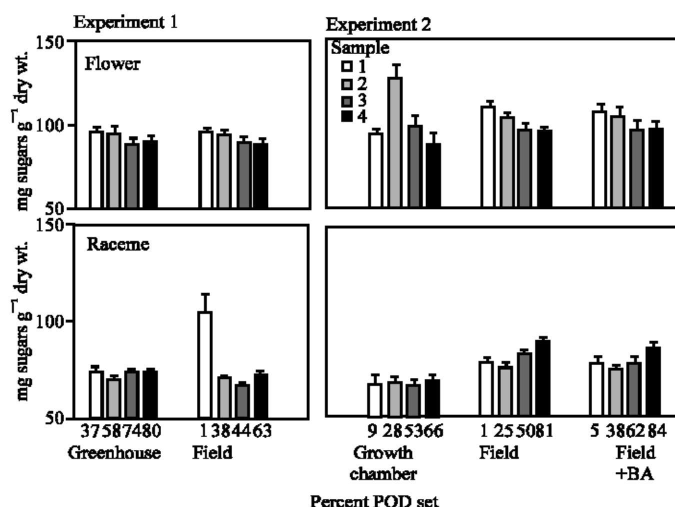

Fig. 3: Concentrations of soluble sugars in flower and raceme as related to percent pod set. Samples 1-4 are intact, intact + cytokinin, deflowered and deflowered + cytokinin treatments in experiment 1 and distal, upper middle, lower middle and proximal raceme sections in experiment 2. Error bars are standard deviations

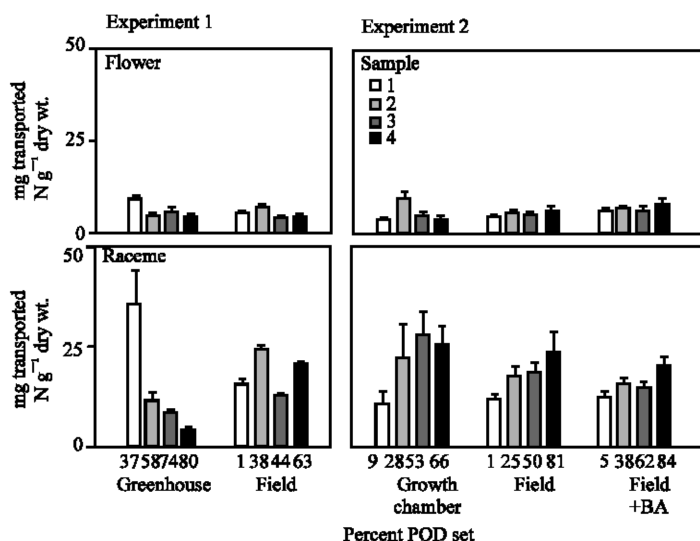

Fig. 4: Concentrations of transported N compounds in flower and raceme as related to percent pod set. Samples 1-4 are intact, intact + cytokinin, deflowered and deflowered + cytokinin treatments in experiment 1 and distal, upper middle, lower middle and proximal raceme sections in experiment 2. Error bars are standard deviations

Raceme sugar concentrations also were relatively high in samples with relatively low pod set in all except the field environment of experiment 2. Therefore, very low sugar concentrations that presumably could be representative of starvation conditions where pod set is low were not observed in either tissue. Raceme sugar levels were reduced as a result of cytokinin treatment, especially in experiment 1, but flower sugar levels were unaffected. The deflowering and deflowering + cytokinin treatments significantly reduced flower sugar concentrations in both environments of experiment 1.

Levels of transported N compounds generally were about three times higher in racemes than in flowers, averaging 16.9 and 5.2 mg g^{-1}, respectively (Fig. 4). No relationship between percent pod set and transported N compounds in flowers was detected in either experiment. In racemes, transported N compounds increased as pod set increased for cytokinin treated inflorescences in the field in experiment 1 and all components of experiment 2. However, the greenhouse component of Experiment 1 gave the opposite result for racemes.

Starch concentrations were mostly higher in experiment 1 than experiment 2, with only minor

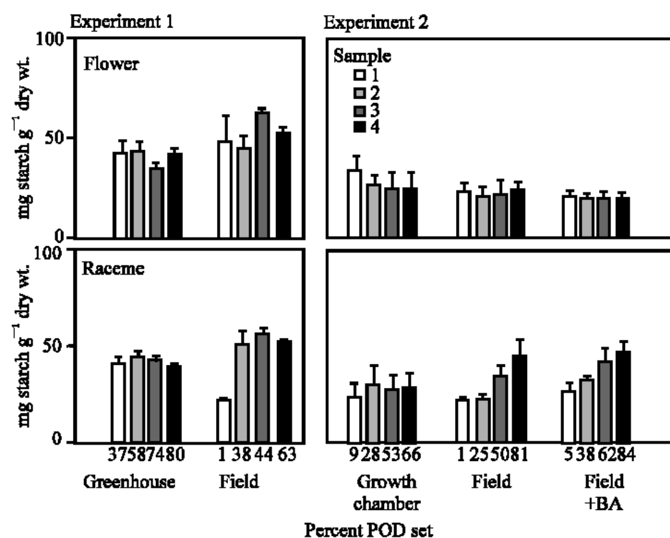

Fig. 5: Concentrations of starch in flower and raceme as related to percent pod set. Samples 1-4 are intact, intact cytokinin, deflowered and deflowered + cytokinin treatments in experiment 1 and distal, upper middle, lower middle and proximal raceme sections in experiment 2. Error bars are standard deviations

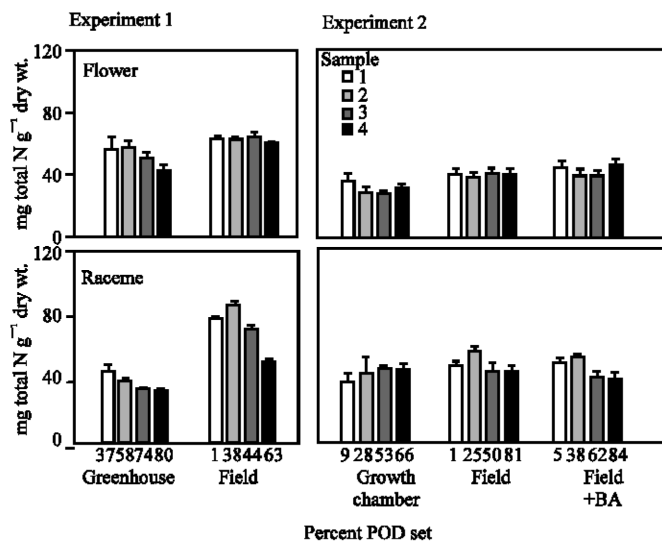

Fig. 6: Concentrations of total N in flower and raceme as related to percent pod set. Samples 1-4 are intact, intact + cytokinin, deflowered and deflowered + cytokinin treatments in experiment 1 and distal, upper middle, lower middle and proximal raceme sections in experiment 2. Error bars are standard deviations

differences in starch concentrations of flowers compared to racemes (Fig. 5). In the field component of each experiment, raceme starch concentrations increased as pod set increased; in the greenhouse and growth chamber components this trend was not pronounced. No significant differences in flower starch concentration were observed in either experiment.

Total N concentrations for the two experiments averaged 42.2 mg g^{-1} in flowers and 50.4 mg g^{-1} in racemes, but the difference between tissues was significant at $p = 0.05$ only for the field component of experiment 1 (Fig. 6). Total N declined as pod set increased in both tissues in Experiment 1 but not in experiment 2.

Relationships between percent pod set and chemical composition were also determined for data expressed on content basis (i.e., as mg soluble sugar, transported N, starch, or total N per flower or per raceme). These

calculations showed that chemical content generally increased with increasing percent pod set for all of the substances (data not shown) due to the large changes simultaneously occurring in dry weight of both types of tissue. This result held true for both experiments and all environments within experiments.

DISCUSSION

The two experimental protocols allowed examination of flower and raceme tissues: 1) During the period of time when the processes leading to flower abortion were being initiated (Experiment 1) or; 2) Through a series of developmental stages from the point in time where processes leading to flower abortion would normally just be initiating (sec 1) to the point when the processes causing flower abortion had terminated (sec 4, Experiment 2). In both experiments, levels of abortion were manipulated by removal of proximal flowers and/or cytokinin treatments. This approach allowed comparison of nutritional availability in flower and raceme tissues under conditions leading to both aborting and setting of flowers, at the time at which these events occur (Reese *et al*., 1995). Differences in developmental stages (Experiment II) provided a kinetic study of events, as development of the tissues in the various raceme sections follows the same basic pattern (Abernethy *et al*., 1977; Oberholster *et al*., 1991), while the experimental protocol induced changes in abortion rates (Peterson *et al*., 1990; Wiebold, 1990; Mosjidis *et al*., 1993; Reese *et al*., 1995; Yashima *et al*., 2005). Furthermore, monitoring of flower set and pod development to maturation allowed confirmation of the long term effects of the treatments.

The simplest regulatory model that can be evoked for the shedding of surplus soybean flowers is a death and withering mechanism (Kozlowski, 1972) in which they starve due to inability to compete for a limited supply of nutrient, then they wither and fall without formation of an abscission zone. The actual mechanism must be more complex, however, because an abscission zone has been demonstrated for aborting soybean flowers (Oberholster *et al*., 1991). Regulation of such zones may involve one or more signals that initiate (Osborne, 1989) or inhibit (Addicott, 1982) zone cell differentiation. Competition for limited nutritional (or hormonal) supplies could still be involved if the signals provide information on nutritional (or hormonal) status (Osborne, 1989) relative to the level that is required for set to occur. The research question, therefore, is to directly or indirectly demonstrate such signals and model their actions into a differentiation program for the abscission zone cells.

This study used chemical concentration in the flower itself and in the total flower unit as an index of nutritional status (Cottrell *et al*., 1985). Lines of evidence that might allow one to invoke a nutritional mechanism from chemical concentration data would be a strong, positive correlation between % pod set and one of the nutrient measures or, even better, a zero or very low nutrient concentration in aborting flowers. Neither condition occurred in this study. Correlations between pod set and chemical composition did not suggest a relationship between any component and flower abortion because they varied in magnitude and sign, depending on the method of sampling and environment. Nor were zero or very low nutrient concentrations observed in flowers having high likelihood of aborting. In fact, soluble sugar levels were high, not low, in young aborting flower and raceme tissues, a result that may reflect nectary activity (Erickson, 1975). These data and the results of Egli and Bruening (2002) are consistent with their observations that assimilate availability may affect the growth rate of pods and seeds, but it does not directly control their survival.

Analysis of raceme tissues separately from flower tissues was done on the assumption that increases in raceme starch indicate abscission zone formation is occurring in aborting flowers. These measurements did not reveal the increase in raceme starch level that would be predicted from anatomical studies showing starch grain deposition in developing abscission zones (Oberholster *et al*., 1991). This may mean that the abscission zone is too small a tissue component of the racemes to affect composition measurements for the pedicel/raceme axis segment used here. It may also indicate that the sampling time was too early to detect the stage of abscission zone development having maximum starch deposition.

Comparison of raceme nutrients with levels in flowers also might indicate whether restriction of movement into the flower causes abortion. Assuming this would be indicated by high levels in the raceme tissues and low levels in the flowers, we saw no such evidence for soluble sugars, starch, or total N. However, levels of the transported N compounds were low in the flowers compared to the raceme/pedicel tissues in both experiments. At the same time the aborting flowers were not especially low in transported N compounds compared to setting flowers. The higher levels of ureides and amides in raceme tissues, therefore, may indicate temporary pools resulting from the ability of the system to oversupply N requirements, as observed by Layzell and LaRue (1982), rather than revealing restriction in nutrient movement as a cause of abortion through starvation of the flower.

For chemical concentration, then, a comprehensive picture of nutrients in soybean reproductive tissues has been drawn by this and past studies for the time when abortion is occurring, assuming that abscission begins soon after anthesis (Huff and Dybing, 1980; Brun and Betts, 1984; Dybing *et al*., 1986) and rescue of aborting flowers can occur up to 11 days after anthesis (Wiebold, 1990). The only results in these studies hinting at nutritional limitations as a cause of abortion are low nonstructural carbohydrate levels at lower canopy positions (Antos and Wiebold, 1984) and low (but not zero) starch levels in aborting ovaries at a date near their separation from the raceme (Dybing *et al*., 1986). In contrast, Streeter and Jeffers (1979) measured total nonstructural carbohydrates in leaves, flowers and pods from the end of flowering through seed development and they concluded that soybean reproductive load did not appear limited by carbohydrate levels. Stockman and Shibles (1986) found no apparent nutrient limitations when measuring water soluble carbohydrate in flower and raceme tissues during flowering. Heitholt *et al*. (1986b) found little difference for concentration of carbohydrates and N in early (setting) and late (aborting) flowers at anthesis and pods at 1 cm of length. Finally, the present study provides scant evidence of nutrient limitation for transported and stored carbohydrate and N in aborting reproductive structures about 2 days after anthesis (Experiment I) and from pre-anthesis to early pod ages (Experiment II). We conclude, therefore, that chemical concentration measures support the hypothesis that nutritional limitation is not the factor that regulates the initial sequences leading to flower abortion in soybeans.

Besides studies showing that nutrient concentrations do not clearly indicate nutritional regulation of abortion, measures of sink activity also have given ambiguous results. Sink intensity for ^{14}C has been found to be low in aborting compared to setting flowers as early as 3 day after anthesis (Brun and Betts, 1984; Heitholt *et al*., 1986a) and movement of ^{32}P into ovules has been found to be stimulated by exogenous BA at about the same age (Mosjidis *et al*., 1993). However, these differences may be occurring during the time when rescue from abortion is possible (up to day 11), but not at the time when abscission initiates (about 1 day after anthesis). Moreover, the aborting flower was still seen to be importing C^{14} and P^{32} in these studies, just as it has been shown to be metabolically active nearly up to the date of separation by in vitro measures (Ghiasi *et al*., 1987). If inadequate nutrition causes flower abortion, then these results for nutrient concentration and sink activity may indicate that the mechanism involves some means of determining that level [or flux (Charles-Edwards, 1984) of nutrient exceeds a critical level.

The best association with percent pod set in the present work was for tissue dry weight, since for the TFU, the flower, or the raceme it showed strong positive correlation to pod set throughout our studies. This leads us to a hypothesis based on growth rate similar to one of Mason *et al*. (1988) for a different soybean system: ovary growth rate is the signal for gene activation and enzyme synthesis/activation at a location remote from the ovary, namely the abscission zone (Osborne, 1989). The aborting ovary is growing (Dybing *et al*., 1986; Zheng *et al*., 2003) and metabolizing slowly (Ghiasi *et al*., 1987), has little ovule growth (Abernethy *et al*., 1977; Tischner *et al*., 2003) and can be increased in ovule and pod growth by cytokinin (Mosjidis *et al*., 1993). Slow ovary growth, by this hypothesis, results in signals that either promote or fail to inhibit abscission zone development. Reasons for low growth rate in abscising flowers are unknown. Certainly, the studies using nutrient concentrations or movement summarized above do not provide strong evidence that it is due to inability to compete for nutrients. Mechanisms by which ovary growth rate can be sensed and signaled to regulate the abscission zone are also unknown. Future studies should address the regulation of ovary growth rate (Jahnke *et al*., 1989; Estruch and Beltran, 1991) and the detection of activities in raceme tissues/abscission zones that may be evidence of signal responses (Reese *et al*., 1995; Tucker and Milligan, 1990).

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