The Physiological Basis for Cytokinin Induced Increases in Pod Set in IX93-100 Soybeans

Dale R. Carlson*, Daniel J. Dyer, C. Daniel Cotterman, and Richard C. Durley
Monsanto Agricultural Company, St. Louis, Missouri 63198

ABSTRACT

Previous investigations have shown the feasibility of increasing pod number on legumes by the application of 6-benzylaminopurine (BA) directly to the raceme. These investigations were designed to determine what reproductive parameter was affected by cytokinin application, and if these applications were overcoming a deficiency in root-produced cytokinins during late flowering. Five individual main stem racemes on greenhouse grown soybeans (Glycine max L. Merr.) were treated with 2 millimolar BA. A single application of BA when pods appeared at 25 to 50% of the proximal floral positions resulted in a 58% increase in pod set due primarily to a 33% reduction in floral abscission. Applications of BA at later intervals also resulted in significant reductions in total abscission. When three applications of BA were imposed on the upper five nodes of field grown soybeans, total pod number and seed weight were significantly increased in this section of the canopy by 27 and 18%, respectively. Throughout the flowering period, root pressure exudate was sampled for the subsequent separation and quantification of zeatin, dihydrozeatin, zeatin riboside, dihydrozeatin riboside, and isopentenyladenine. Total cytokinin flux peaked from 0 to 9 days after flowering began, and then dropped to one-half of this level by 15 days postanthesis. The probability that a flower would initiate a pod was directly related to the concentration of total cytokinins present in the exudate when the flower opened.

Yield in soybean is determined by the number of seeds per unit area, and the average weight per seed. The number of seeds per unit area is determined by the number of flowers which initiate pods that attain maturity. Soybeans produce an abundance of flowers, but shed a rather large proportion of them before the seeds begin to fill. Estimates vary, but investigators have shown that the abscission of flowers and small pods ranges from 32 to 82% of the total flowers produced (29, 30). Possible factors which contribute to the abscission of flowers and small pods include: (a) vascular constrictions, (b) nutrient deficiencies, mineral or carbohydrate, and (c) abscission inducing factors (i.e., hormones). Application of exogenous BA* to the floral raceme has resulted in increased mature pod number in mung bean (8) and soybean (7, 24). These observations, as well as the circumstantial evidence present in the literature suggest that endogenous cytokinins play a major role in the regulation of pod set in legumes.

Cytokinins and cytokinin-like activity have been reported to increase or attain maximal levels in the root pressure exudate of several species after floral induction (4, 9, 10, 16). Heindl et al. (16) suggested that the flux of ZR and DHZR was involved in the control of reproductive development, since this flux was not directly correlated with specific nodule activity or leaf senescence.

Supportive evidence for this hypothesis is found in reports of increases in the export of cytokinins which follow dis budding or fruit removal treatments (4) (JC Cotterman, personal communication). The second line of evidence is found in studies with grapes (25) and sweet cherries (27), where higher cytokinin-like activity in xylem sap has been correlated with increased yield.

Increases in the concentration of cytokinins or cytokinin-like activity have been shown to occur coincident with early ovule development in lupin (11), wheat (18), barley (22), Phaseolus (23), and soybean (6) (MB Hein, personal communication). An example of the cytokinin dependence of the fertilized ovule during early embryogenesis is the interspecific hybrid cross, P. lunatus × P. vulgaris. This cross results in embryos which cease to divide at the four-celled stage, but can be induced to the preheart stage if the female parent is supplied with cytokinin via hydroponic culture (20). The poor development of P. vulgaris × P. acutifolius hybrid embryos has been correlated with reduced levels of cytokinin-like compounds in the endosperm as compared with selfed embryos of both species (23).

Crosby et al. (7) reported increases in pod set due to exogenous BA in two soybean cultivars. However, increases in pod number were greater for the cultivar Shore than they were for Essex. The authors suggested that the difference in response was related to the lower endogenous level of cytokinin-like activity in Shore ovules as compared to Essex at the stage [R3](14) when BA was applied (6).

Since applications of exogenous cytokinins alleviates some or all of the abscission-inducing factors mentioned previously, we used BA as a tool to ascertain what reproductive parameter was altered by cytokinin treatment. i.e. floral production, pod initiation, or pod abscission. Concurrent with this study, we examined the levels of cytokinins in the root pressure exudate to determine if a relationship existed between the concentration of these root-produced hormones and the probability of reproductive abscission. Further, we evaluated the developmental period during which pod initiation could be altered by exogenous cytokinins, and compared the efficacy of single or multiple applications for increasing pod number and seed weight on plants in a field environment.

MATERIALS AND METHODS

Plant Culture—Greenhouse Studies. IX93-100 soybean (Glycine max L. Merr.) plants were grown in 7.6 L pots in a mixture

Received for publication June 12, 1986 and in revised form January 21, 1987

Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.
of sand:peat:soil (1:1:2,v:v:v). This genotype produces distinct axillary racemes (3–5 cm) on which the flowers open individually and sequentially from the base to the tip at a rate of approximately one flower per day. Seeds of IX93-100 were obtained from C. D. Dybing, USDA-ARS, Brookings, SD, via W. A. Brun, University of Minnesota, St. Paul. IX93-100 was selected from crosses made by D. E. Green, Iowa State University, of A71-5558-1 and L61-344, a semideterminate isolate of 'Harosoy.' The plants were grown in a greenhouse at 25°C/21°C day/night temperatures. A 13.5 h photoperiod was maintained with supplemental metal halide lamps, which supplied a PPF of 300 \( \mu \text{E m}^{-2} \text{s}^{-1} \). Seeds were inoculated with Bradyrhizobium japonicum (Nitragren, Nitragin Co., Milwaukee, WI) at the time of planting. Plants were thinned to one per pot at the unifoliolate leaf stage (V1). All branches below the first main stem raceme (node 9 or 10) were removed to ensure uniform plant material. Plants were fertilized beginning at the V3 stage with 40 ml of a commercial fertilizer containing 16.7 mm NO\(_{3}\), 41.5 mm NH\(_{4}\), 36.8 mm urea, 52.9 mm P, and 76.2 mm K. At 2 week intervals thereafter, each pot was supplied with 150 ml of this nutrient solution. Plants were irrigated with tap water twice daily.

Data and Sample Collection. Flower and pod abscission were monitored on three to five midcanopy main stem racemes. Each individual flower was tagged at anthesis with colored thread. All tagged flowers were then monitored on a daily basis to determine if flower abscission, pod initiation, or pod abscission had taken place. This was done until 6 weeks after cytokinin treatment when pod abscission was complete.

Cytokinin Treatments. Cytokinin and control treatments were formulated according to the method of Crosby et al. (7). The main stem racemes were sprayed with 2 ml of 2 mm BA when pods were present at 25 to 50% of the proximal floral positions (time 0). A pod was defined as any ovary which had elongated sufficiently to extend past the calyx.

Cytokinin Experiments. Three separate experiments were conducted to address the experimental objectives. In the first greenhouse study, flowers were tagged and monitored on five racemes of BA treated and control plants. At five successive intervals before and after treatment, five plants from each treatment group were used for the collection of root pressure exudate. One set of control and treated plants was allowed to develop until 6 weeks after treatment, after all flower and pod abscission was complete. This study was conducted in the months of December through March. In the second greenhouse study, three racemes on three plants were treated with BA at 0, 2, 4, 7, and 11 d after the time previously found to result in significant enhancements in pod number (7). Flowers were also tagged and monitored in this study, and measurements of pod length at each position of the raceme were made 11 d after time 0. This study was conducted in the months of April through July. The third study was conducted in the field, and is described in a separate section.

Collection of RPE. Root pressure exudate (RPE) was collected from the control plants in the manner of Heindl et al. (16). Exudate was collected for 4 h at each sampling time beginning at 1230. Immediately after collection, the exudate from each plant was weighed, frozen, and stored at -20°C. The exudate was lyophilized and stored at -80°C until later analysis for cytokinins.

Cytokinin Analysis. Samples of root pressure exudate were purified by ion exchange and immunoaffinity chromatography. The samples were passed through a DE-52 (anion exchange) column (7 ml), and then directly into two immunoaffinity columns (2 ml each) containing anti-ZR and anti-iPA antibodies. The cytokinin antibodies were polyclonal, raised in rabbits from ZR-, DHZR-, and iPA-BSA conjugates (ribosomal linkage)(12). The IgG fractions of the rabbit antisera were isolated by passing sera over protein A columns according to Davis et al. (12). The IgG fraction was immobilized on a matrix of Affi-gel 10 (Bio-Rad Co., Richmond, CA) via a succinamide ester linkage by mixing for 1 h in coupling buffer (0.1 M NaHCO\(_3\), pH 8.0), centrifuging and blocking with 1.0 M ethanolamine (pH 8.0). Thirty-five to 40 mg of IgG per ml of gel were optimal for maximum cytokinin retention (unpublished data). Root pressure exudate samples were dissolved in 0.5 ml of 40 mm ammonium acetate buffer (pH 7.4). The sample was loaded on the DE-52 column, and the series of three columns were rinsed with 15 ml of the ammonium acetate buffer. After removal of the DE-52 column, the immunoaffinity columns were rinsed with 10 ml of a phosphate buffer-DMSO (0.5 mM NaCl, 75 mM KPO\(_4\) at pH 6.8, with 2% [v/v] DMSO). Both columns were then rinsed with 3 ml of deionized H\(_2\)O at pH 7.0. The bound cytokinins were eluted from both columns with 5 ml of 100% (v/v) methanol. Samples were dried in a Savant rotovaporator (Savant Instruments, Hicksville, NY). HPLC was used to separate and quantify the five cytokinins Z, DHZ, ZR, DHZR, and iPA. Separation was completed on a Beckman Ultrasphere-ODS (Beckman Instruments, Inc.) reverse phase column (25 cm x 4.6 mm i.d.) with a gradient as described in Table I. Samples were dissolved in 500 \( \mu \text{l} \) of 38 mm acetic acid, adjusted to pH 3.30 with distilled triethylamine, and 240 \( \mu \text{l} \) was injected onto the column using a WISP model 710B automatic injector (Waters Associates, Milford, MA). Identification of the individual cytokinins was achieved by retention time, and quantification was based on absorption at 280 nm relative to authentic standards of the five cytokinins. Internal standards were not used, and percent recovery of the hormones was not calculated. All hormone quantities were expressed as pmol plant\(^{-1}\) h\(^{-1}\) to allow comparison of the results without regard to the different molecular weights of the five different cytokinins.

GC-MS. Positive identification of the five cytokinins was made by GC-MS-selected ion monitoring. Samples of cytokinins from each fraction were permethylated by the method of Stafford and Corse (26) prior to injection on the GC-mass spectrometer. Mass spectra were obtained using a Hewlett-Packard GC connected to the 5970 Mass Selective Detector (MSD) with data processing by a Hewlett-Packard 59970B workststution. The samples were chromatographed on a 30 m x 0.25 mm i.d. fused silica capillary containing DB-5 film thickness 0.25 \( \mu \text{m} \) (J&W Scientific) and were eluted with helium. The temperature program was 140°C for 6 min, then ramped at 10°C min to 220°C, and then ramped at 4°C min\(^{-1}\) to 280°C. Injector and transfer lines were at 250 and 280°C, respectively. Ions monitored by the MSD were highly abundant and/or characteristic ions of the individual permethylated cytokinin derivatives. These were as follows: Z: 176(13%), 188(24), 230(100), and 261(8). DHZ: 176(100%), 190(27), 232(19), 248(16), and 263(11). ZR: 174(38%), 202(17), 216(100), 246(16), 348(20), and 390(68). DHZR: 162(100%), 176(58), 234(46), 250(52), 278(26), 336(17), and 392(18). iPA:

| Table I. Gradient Program Used for the Separation and Quantification of Z, DHZ, ZR, DHZR, and iPA |
|---|---|---|
| Time | A* | B* |
| min | | % |
| 0.0 | 90 | 10 |
| 18.0 | 90 | 10 |
| 18.5 | 76 | 24 |
| 35.5 | 76 | 24 |
| 37.5 | 100 | 0 |
| 47.5 | 100 | 0 |
| 49.5 | 90 | 10 |

* A, 38 mm acetic acid adjusted to pH 3.30 with triethylamine. * B, Acetonitrile.
174(100%), 202(82), 216(70), 217(82), 246(9), 348(21), and 391(85). A typical scan for permethylated DHZR is shown in Figure 1. We observed that the DE-52 immunoaffinity purification method resulted in concise, well separated cytokinin peaks observed on HPLC (unpublished data). The estimated quantity of cytokinins observed on HPLC was compared to the quantity observed by subsequent permethylation and examination by GC-MS-selected ion monitoring. For all five cytokinins, both HPLC and GCMS-SIM quantitation compared well. Thus, cytokinin peaks observed by HPLC did not contain extraneous, co-eluting peaks of other material.

Field Studies. In 1985, IX93-100 plants were grown in three 4-row plots spaced 38 cm apart with 76 cm spacings between each plot. Each plot was 24 m in length. Final plant population at harvest averaged 362,775 plants ha⁻¹. IX93-100 seeds were planted on May 22, but were subsequently replanted on June 20 due to defoliation by rabbits. A preemergence application of 5.0 kg of naptalam (sodium N-1-naphthylphthalamate) plus dinoseb butyl-4,6-dinitrophenol) and 2.2 kg of alachlor (2-chloro-2'-6'-diethyl-N-[methoxymethyl]-acetanilide) ha⁻¹ was applied to the Ray silt loam. Treatments consisted of single and triple applications of BA to the upper canopy (five nodes) racemes, with a control corresponding to both. The upper five nodes corresponded to one-quarter to one-third of the mainstem racemes. Treatments were applied to random plants within the two inner rows. The initial application was applied on August 16 when the apex was at 50% bloom. Racemes subject to multiple applications were subsequently treated on August 21 and 27. An evaluation of pod number was made on September 5. Plants were harvested on October 3 for the measurement of pod number, total seed weight, and weight per seed on both the treated and untreated racemes. Treatments were applied in a completely randomized design with 40 replicates of each treatment.

RESULTS

Effects of BA on Floral Parameters. The data in Table II were from the set of plants left to grow for the purpose of observation. The table gives the mean number of reproductive structures produced over the 8 week observation period, and the number which remained 6 weeks posttreatment. Application of BA significantly increased pod initiation by 58%. Pod abscission increased due to treatment, but did not offset the 91% decrease in flower abscission. Six weeks after treatment, the end result was a 101% increase in final pod number at the five nodes treated with BA. These data are presented graphically in Figure 2, a to d. These figures are frequency plots which represent the fate of the reproductive units at various positions within the raceme (position 1 is the basal flower, position 20 is the most distal). The plots were generated by dividing the total number of flowers subject to a particular event (i.e. pod initiation, flower, or pod abscission) by the total number of flowers produced at each raceme position, and for most positions represent the summary of 25 positions. In positions where 100% flower abscission occurred, pod abscission was not calculated. Figure 2a shows how application of BA affected pod initiation 2 weeks after treatment when flowering was complete. The highest probability of pod set on the control racemes was from positions 1 through 8, with a precipitous drop occurring thereafter. Application of BA doubled the number of positions which had a 100% probability of setting pods. Figure 2b shows the cumulative flower abscission which occurred by 6 weeks after treatment. Since the majority of the flower abscission occurred within 2 weeks posttreatment, this graph is almost an inverse image of that in 2a. When BA was applied in this study, the number of flowers which had attained anthesis ranged from 14 on the lower part of the raceme, to 9 on the upper part of the raceme under observation. From the data in Figures 2a and b, it appears that those flowers (or young pods) which were affected by BA application were at the stage from 7 d preanthesis to 5 d postanthesis. From the period 2 to 6 weeks posttreatment, pod abscission was also altered (Fig. 2c). The same pattern of pod abscission which was present on the control plants was pushed to the distal part of the raceme on the treated plants due to an increased frequency of pod initiation in this area. At 6 weeks after treatment, the ultimate result was an increased frequency of pod initiation at the middle portion of the raceme on the treated plants (Fig. 2d). The increased pod initiation from floral positions 7 through 14 resulted in the 100% increase in final pod number shown in Table II.

Relationship of Pod Initiation with Endogenous Cytokinin Flux. Figure 3 compares the root-produced cytokinin flux with the probability that a flower opening on the days indicated would initiate a pod. Total cytokinin flux consisted of 85 to 90% ZR and DHZR; the free bases made up a lesser percentage, and iP was insignificant and highly variable amounts. The quantities of all the cytokinins isolated from the root-pressure exudate were summed for further interpretation. This was possible, because the ribosides represented the majority of the cytokinins observed, and because there was no consistent differences in the flux of either ZR or DHZR. The ribosides are thought to be the major transport form of cytokinins because of their presence in root-pressure exudate. Despite the fact that Z has been shown to be more effective in at least one bioassay, Z and DHZ were equally active in promoting increases in pod set when applied directly to the raceme (13). Total cytokinin flux was at its maximum from 0 to 9 after flowering began (Fig. 3). Cytokinin concentration of

### Table II. Cumulative Effects of BA Application on Five Main Stem IX93-100 Racemes

<table>
<thead>
<tr>
<th>Cultivar (Treatment)</th>
<th>Total Production</th>
<th>Total Abscession</th>
<th>Final Pod Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flowers</td>
<td>Pods</td>
<td>Flowers</td>
</tr>
<tr>
<td>IX93-100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>76.0 ns</td>
<td>48.4 ***</td>
<td>27.6 **</td>
</tr>
<tr>
<td>+ BA</td>
<td>80.0</td>
<td>76.6</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Not significantly different. **Significantly different at the P = 0.05, and 0.001 levels of probability, respectively.
the exudate decreased to one-half of this maximum from d 15 to 23. The probability that a flower would initiate a pod was directly related to the concentration of cytokinin ribosides in the exudate when each flower opened. An estimate of the total cumulative cytokinin flux for the subsequent 14 d period was calculated for each day after flowering from the data in Figure 3 (Fig. 4). From these data it is apparent that a very strong relationship also exists between the amount of root-produced cytokinins a particular flower was exposed to throughout reproductive development and the probability that the flower would produce a mature pod.

**Effect of time of BA Application.** Crosby *et al.* (7) reported significant increases in pod number of the apical racemes when treatment was applied at late-flowering (R3). The results of our experiment, in which BA was applied at several times after this time, are given in Figure 5 and Table III. One point should be made before the results of this experiment are described. Because of the time of the year that these plants were grown in the greenhouse, very little flower abscission occurred on this set of plants. Water and nutrient conditions were the same as in the first experiment, but PAR and temperature were more favorable than in the first quarter of the year. Only 10 positions per raceme were initiated on this set of plants, and pod initiation ranged

---

**Fig. 3.** Relationship between the probability of pod initiation and the cytokinin flux within the root pressure exudate of IX93-100 soybean on the day of anthesis of each raceme position.

**Fig. 4.** Relationship between the probability of a flower initiating a pod which attains maturity and the cumulative cytokinin flux from the roots during reproductive development.

---

**Fig. 2.** Effect of BA on the flower and podding activity of each floral position of IX93-100 soybean: a, frequency of pod initiation 14 d after BA treatment; b, flower abscission 6 weeks after BA treatment; c, pod abscission 6 weeks after BA treatment; d, final pod set 6 weeks after BA treatment.
BA had 22.0 and 24.5 pods in the upper five nodes for the single and triple applications, respectively. This was a significant increase of 22 and 38% over their respective controls, which both had 18.5 pods in the same section of the canopy (data not shown). The effect of exogenous BA application on the yield components of field-grown 1X93-100 soybeans is given in Table IV. Since some pod abscission had occurred within the interval between the last evaluation and maturity, a single application of BA did not result in an increase in pod number in the upper canopy section at that time. However, there was a significant decrease in both pod number and seed weight in the untreated lower section of the canopy. The cause of this decrease is not clear, but it does not appear to be compensatory. Three applications of BA significantly increased pod number and total seed weight in the upper canopy section by 27 and 18%, respectively. In the lower section of the canopy, no significant decreases in either of these components was observed. The BAP-induced increase in the upper section of the canopy did not result in significant alterations in pod number or total seed weight when compared to the control on a whole plant basis. None of the treatments had a significant effect on 100 seed weight. There was also no significant difference between any of the parameters for the controls of the single and triple applications.

**DISCUSSION**

The results of our experiments are consistent with previous reports of increases in pod number and total seed weight induced by the application of BA directly to the raceme (7, 24). Applications of cytokinins to the foliage for the purpose of preventing abscission have been ineffective (3, 19). Metabolism studies have shown that the majority of foliarly applied BA or zeatin is rapidly metabolized into side chain glucosylated conjugates (17, 28) which do not appear to be exported to developing flowers or fruits (17). Work in our laboratory indicates that foliarly applied cytokinin analogs may have the potential to increase pod number at the nodes subtending treated leaves (13).

The results of the field study confirm previous observations in mung beans (8) and determinate soybeans (24) that multiple applications of BA may be required to attain increased pod number and seed weight. Kinetic et al. (19) reiterated this conclusion and suggested that a continuous supply of this growth regulator is an important aspect of its action based on the evidence from many different species. For soybeans, the physiological basis for this supposition appears to lie in the ontogenetic variation in the amount of cytokinins exported from the shoot.

### Table IV. Effect of BA on the Yield Components of Field-Grown 1X93-100 Soybeans

<table>
<thead>
<tr>
<th>Location</th>
<th>Type of Application</th>
<th>Pod number</th>
<th>Total seed wt.</th>
<th>Wt/100 seed</th>
<th>Pod number</th>
<th>Total seed wt.</th>
<th>Wt/100 seed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper</strong></td>
<td>Control</td>
<td>18.1 **</td>
<td>5.64</td>
<td>14.3</td>
<td>17.1 **</td>
<td>5.38 **</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>18.9</td>
<td>6.35</td>
<td>14.9</td>
<td>21.7</td>
<td>6.36</td>
<td>14.4</td>
</tr>
<tr>
<td><strong>Lower</strong></td>
<td>Control</td>
<td>35.4 **</td>
<td>11.93 **</td>
<td>15.6</td>
<td>33.3</td>
<td>11.51</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>29.4</td>
<td>9.99</td>
<td>15.7</td>
<td>32.1</td>
<td>10.32</td>
<td>15.2</td>
</tr>
</tbody>
</table>

* Upper section was treated with BA, lower was not. ** Indicates significance at the P = 0.05 level of probability. Differences between treatment means within a plant location were detected using the LSD.
roots during reproductive development (16). Our results show that the probability that a flower would initiate a pod was directly related to the concentration of total cytokinins present in the xylem when that flower opened. The decrease in the total cytokinin flux within the xylem xylem exudate during late flowering is indicative of why later and/or multiple applications of BA were more effective in increasing pod retention.

The fact that multiple applications of BA on field-grown plants did not result in the same effect that resulted from single applications of BA on greenhouse-grown plants may be due to the favorable field environment. From June through September 1985, temperatures averaged 0.6°C less than normal, and 15.6 cm more precipitation was received than on the average. As a result, yields in this county increased from an average of 3.4 Mg ha\(^{-1}\) to 4.0 Mg ha\(^{-1}\) on these types of soil, a 18% increase. The estimated control yield in our test plot was 5.1 Mg ha\(^{-1}\)—this difference due to the use of narrow rows. Although data were not collected for percent flower and pod abscission, the prevailing evidence indicated that the controls retained a higher number of pods than would occur in an average year. Since application of BA at this stage of growth resulted in decreased floral abscission in our greenhouse study, the decreased efficacy of this treatment in the field may be explained by conditions which resulted in increased pod set in all plants, and subsequently, decreased abscision.

In a comparison of flowers with known probabilities of abscission, Brun and Betts (5) reported that normally setting flowers gained sink strength shortly after anthesis, while normally abscising flowers were weak sinks. The increase in sink strength of normally setting over abscising flowers was due to a greater sink intensity of the normally setting flowers, and not due to greater sink size. They concluded that the determination of floral abscission occurs at or very near the day of anthesis. In soybean, the prevailing evidence indicates that the majority of abscission occurs after fertilization, and before the pods are 2 cm in length (1, 30). Abernathy et al. (1) reported that ovules of abscising flowers ceased development at the four to eight cell stage, and suggested that this may be due to a “reduced level of cell-division mediating factors.” The results of our studies, and the evidence cited in this paper, indicate that soybean abscission is due in large part to a deficiency of root-produced cytokinins during late-flowering and early pod development (R3–R4).

Limitations in current or stored assimilate have been implicated as a major factor regulating the magnitude of reproductive sink yield in mung bean (8) and soybean (2). In tomato, continuous application of BA plus GA to the inflorescence resulted in an increase in the accumulation of \(^{14}\)C-assimilate at the expense of the apical shoot (21). The increased assimilate accumulation occurred 1 d after treatment, and preceded any morphological change in the reproductive structure. Treatment of the inflorescence did not affect \(^{14}\)CO\(_2\) fixation of the source leaf, or the proportion of \(^{14}\)C-assimilate exported from that leaf. The application of BA plus GA to tomato appeared to reverse the decline in RSA which was associated with reproductive abscission in the study by Brun and Betts (5). The duration of the BA induced alteration of partitioning in tomato continued throughout the imposition of treatment. It is probable that a similar alteration of assimilate partitioning occurred when soybean flowers and pods were treated with BA. This would further explain why continuous or multiple applications of exogenous cytokinins result in larger increases in pod number than do single applications.

Our evidence showed that root-produced cytokinins were involved in pod and seed growth through at least 23 d postanthesis. In this study, we did not measure cytokinin flux from the root system past 23 d postanthesis. However, Heindl et al. (16) reported that once the concentration of cytokinins in soybean root pressure exudate declines, it remains at a low level during late reproductive growth. Similar patterns for root-exported cytokinins have been reported in five different soybean cultivars (16) (JC Cotterman, personal communication; DR Carlson, unpublished data). All profiles indicate that peak concentrations of cytokinins occur sometime during the period that encompasses full bloom (R2) to early pod development (R4). This peak is followed by a decline throughout seed development. In indeterminate soybeans, this decline corresponds to the time that late flowers are opening on all nodes, and pod set is occurring on the upper nodes. Previously recorded patterns of flowering and reproductive abscission have indicated that these structures have a high probability of abscission (15). Flowers and pods in the lower part of the canopy also have a high probability of abscission (15, 30). It is conceivable that two or more mechanisms may be responsible for mediating flower set and subsequent reproductive development. Increased abscission in the lower canopy could be mediated by decreased irradiance and a deficit in assimilate availability (2). Increased abscission of late flowers and pods in the mid to upper part of the canopy (15) may then be explained by a decrease in the concentration of cytokinins in the xylem sap. Our data indicate that the probability that a flower will form a mature pod is dependent upon the total amount of cytokinins available to that flower throughout reproductive development. In the context of this discussion, the cytokinins may only be able to regulate reproductive development in the presence of adequate nutrition (i.e. carbohydrates and/or reduced N) such as that which would exist in the mid to upper canopy. Observations with determinate soybeans add another degree of complication. Weibold et al. (30) have reported that 53% of the pods present at harvest were produced in the upper part of the canopy. Although these genotypes have been shown to be responsive to the application of BA, the relationship between root-exported cytokinins and reproductive development deserves an examination in soybeans with this type of growth habit. Evidence to date would indicate that our conclusions may only be applicable for soybeans with an indeterminate growth habit.

Acknowledgments—The authors would like to thank Mary Vosevich and Margaret Skouby for their excellent technical assistance throughout these experiments.

LITERATURE CITED


13. DYER DJ, DR CARLSON, CD COTTERMAN, JA SKORSKI, SL DITSON 1987
Basis for BA induced increases in soybean pod set

27. Stevens GA Jr, MN Westwood 1984 Fruit set and cytokinin-like activity in the xylem sap of sweet cherry (Prunus avium) as affected by rootstock. Physiol Plant 61: 464-468