

# Influence of 6-Benzylaminopurine on Fruit-Set and Seed Development in Two Soybean, *Glycine max* (L.) Merr. Genotypes<sup>1</sup>

Received for publication August 8, 1980 and in revised form May 4, 1981

KEVIN E. CROSBY, LOUIS H. AUNG, AND GLENN R. BUSS  
Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

## ABSTRACT

The influence of 6-benzylaminopurine (BA) on the premature abscission of developing soybean, *Glycine max* (L.) Merr. fruits of 2 genotypes was studied. BA was applied during the critical period of fruit-setting. The tested concentration range of BA was from 1 micromolar to 5 millimolar; 2 millimolar was optimal. Spray application of 2 millimolar BA to terminal inflorescences at the R<sub>3</sub> developmental stage of field-grown soybeans significantly increased fruit-set and seed yield of the Shore genotype during three growing seasons. In contrast, the Essex genotype gave significant responses two out of three seasons. The response of Shore was generally more pronounced than that of Essex. The apical fruits on the inflorescences gave the greatest response to BA. Seed weight increase was apparent 3–4 weeks after BA treatment.

Cytokinins are essential for the growth of intact and isolated plant organs and tissues. Their involvement in the processes of cell division, mobilization of inorganic and organic nutrients and senescence are well documented (24). Studies with various species have demonstrated changes in endogenous levels of cytokinins occurring after anthesis (5, 6, 18). The high levels of cytokinins in developing seeds and fruits (6, 16, 25) are indicative of a function of this type of hormone during periods of active cell division and high assimilate demand in the growing embryonic tissues.

Among the legumes, pea, lupin and soybean fruits have been shown to contain cytokinins or cytokinin-like activity (6–9, 17). The endosperm of developing peas or lupins is rich in cytokinin and it has been suggested that this tissue supplies the cytokinins for embryo growth (6, 9). In *Phaseolus coccineus* cytokinins caused significant growth enhancement of young 1- to 2-mm embryos *in vitro* but lesser growth promotion of older 5-mm embryos. It was suggested that cytokinins are required especially for early embryo growth (4). On the other hand, embryos of *Phaseolus vulgaris* and *Phaseolus acutifolius* hybrids fail to develop normal endosperm and have much lower levels of endogenous cytokinin-like substances than selfed *P. vulgaris* embryos. The failure of hybrid embryos has been attributed to an inadequate supply of cytokinins which are regarded as the critical essential factors for successful embryo development (20).

It has long been recognized that soybeans flower abundantly, but for undetermined reasons a high proportion of flowers and fruits abscise prematurely. The premature shedding of reproductive structures diminishes the number of potential "sinks" for storage or accumulation of assimilates and thus may limit final seed yields. Although there is wide variation among soybean

cultivars, the estimated proportion of potential fruits attaining final harvest maturity is about 30% (11, 29). The abscission of flowers has been attributed to a lack of fertilization, but evidence indicates that the significance of this factor to premature flower and fruit abscission may be overrated (1, 14). Other observations indicate that flower and fruit abscission occurs not only between the stages of the 4- to 8-celled proembryo and heart-shaped cotyledon (14), but also occurs during any period of growth until harvest (11).

Soybean seed yield is dependent on fruit and seed numbers (15, 21) which are directly related to successful fruit-set. Therefore, the factors controlling fruit-set in soybean and other crops are of great interest. Work with other legumes (2, 6, 9) suggests that hormones such as cytokinins are important in seed and fruit development and more research efforts need to be directed at soybeans. The terminal inflorescence as a unit was chosen for detailed study which could serve as a model for further analysis of fruit abscission of the whole plant. This paper reports the effects of exogenous cytokinins on fruit-set and seed development of the terminal inflorescence in two field-grown soybean genotypes over three growing seasons.

## MATERIALS AND METHODS

**Plant Growing, Harvesting and Analyses.** Two determinate soybean genotypes (maturity group V), Essex and Shore (26, 27) were grown at the Virginia Polytechnic Institute and State University Horticulture Research Farm at Blacksburg, VA during the growing seasons of 1977, 1978, and 1979. The soil is a Lodi series (typic hapludult) (pH of 6.5–7.0). The soil was fertilized with 90 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 189 kg ha<sup>-1</sup> K<sub>2</sub>O before planting. Seeds were inoculated with a commercial *Rhizobium japonicum* inoculant, and weeds were controlled with 1.8 liter ha<sup>-1</sup> of trifluralin.

Seeds were planted in the first week of June. Rows were 5 m long and spaced 0.9 m apart with 4 cm between plants in the row. A randomized complete block design of 5–7 replicates was used during the 3 years.

Fruits on the terminal inflorescence were harvested when fully mature (10). Fruits were sorted into one-, two- and three-seeded classes, dried at 80 C for 72 h, and weighed. In 1978 and 1979, the fruits were further sorted by position on the inflorescence. Fruits in the axil of the inflorescence were termed accessory, fruits at the first four nodes of the inflorescence were termed basal, and all other remaining fruits were termed apical.

Plot means were used for analysis of variance and treatment means were compared by Tukey's test (28).

**Cytokinins Preparation and Application.** All cytokinins were purchased from Sigma Chemical Co. In experiments done in 1977, BA,<sup>2</sup> 6-( $\gamma,\gamma$ -dimethylallylamino)purine, 6-( $\gamma,\gamma$ -dimethylallylamino)-9- $\beta$ -D-ribofuranosylpurine, 6-furfurylamino purine, 6-(4-hy-

<sup>1</sup> Work was supported in part by United States Department of Agriculture, Science and Education Administration Grant 701-15-27.

<sup>2</sup> Abbreviations: BA, 6-benzylaminopurine or bzl<sup>6</sup>Ade.

Table I. *The Effects of Five Cytokinins on Fruit-Set and Seed Yield of Essex and Shore Soybeans*  
Data of 1977 growing season; values are means of five replicates (five plants replicate<sup>-1</sup>).

Genotype and Observations <sup>a</sup>	Cytokinin <sup>b</sup>					
	Control	bzl <sup>6</sup> Ade <sup>c</sup>	c,t-io <sup>6</sup> Ade	i <sup>6</sup> Ade	i <sup>6</sup> Ado	fr <sup>6</sup> Ade
Shore						
Seed dry wt (g)	0.4	1.1 <sup>d</sup>	0.7	0.7	0.4	0.7
Fruit number	1.7	3.9 <sup>d</sup>	2.9	3.0	1.7	2.8
Seed number	3.0	7.6 <sup>d</sup>	5.0	5.3	3.5	5.6
Essex						
Seed dry wt (g)	1.5	1.3	1.6	1.4	1.3	1.6
Fruit number	5.2	5.2	5.7	5.2	5.2	5.5
Seed number	10.8	9.7	11.5	10.2	10.2	11.0

<sup>a</sup> Observations are expressed on a per inflorescence basis.

<sup>b</sup> Five-tenths milliliter of 0.1 mM cytokinin applied inflorescence<sup>-1</sup>.

<sup>c</sup> bzl<sup>6</sup>Ade=BA, 6-benzylaminopurine; i<sup>6</sup>Ade, 6-( $\gamma,\gamma$ -dimethylallylamino)purine; i<sup>6</sup>Ado, 6-( $\gamma,\gamma$ -dimethylallylamino)-9- $\beta$ -D-ribofuranosylpurine; fr<sup>6</sup>Ade, 6-furfurylaminopurine; t-io<sup>6</sup>Ade, 6-(4-hydroxy-3-methyl-*trans*-2-butenyl)aminopurine; c-io<sup>6</sup>Ade, 6-(4-hydroxy-3-methyl-*cis*-2-butenyl)aminopurine.

<sup>d</sup> Denotes significant difference from control at the 5% level of probability.

Table II. *The Influence of 6-Benzylaminopurine on Fruiting and Seed Weight of Field-grown Essex and Shore Soybeans*

Values are means of six replicates (five plants replicate<sup>-1</sup>).

Growing Season	Treatment	Essex			Shore		
		Fruit	Seed	Seed wt	Fruit	Seed	Seed wt.
				mg			mg
1978	Control	7.4	17.2	1950	7.6	16.3	2130
	BA	10.1	22.4	2540	10.7 <sup>b</sup>	21.6 <sup>b</sup>	2770 <sup>b</sup>
1979	Control	6.7	14.0	1320	6.0	10.0	1080
	BA	7.1	14.7	1570	8.0 <sup>b</sup>	13.3 <sup>b</sup>	1530 <sup>b</sup>

<sup>a</sup> Five-tenths milliliter of 2 mM BA applied inflorescence<sup>-1</sup>.

<sup>b</sup> Denotes significant difference from control at 5% level of probability; fruit and seed number and weight are expressed on a per inflorescence basis.

droxy-3-methyl-*trans*-2-butenyl)aminopurine, and 6-(4-hydroxy-3-methyl-*cis*-2-butenyl)aminopurine were dissolved in 2% ethanol with a small amount of 1 N KOH and diluted to 0.1 mM with 16 mM K-phosphate buffer (pH 6.4) containing 0.05% (v/v) Tween-80. BA solutions ranging from 1  $\mu$ M to 5 mM were also prepared. In 1978 and 1979 BA was dissolved in a minimal amount of 1 N KOH and diluted to 2 mM with the same buffer. Control plants received the carrier solution without cytokinins.

Chemicals were applied as an aerosol spray to the terminal inflorescence (0.5 ml inflorescence<sup>-1</sup>). Treatments were made when the basal fruits on the inflorescence were less than 2 cm long and the apical flowers had faded, corresponding to developmental stage R<sub>3</sub> of Fehr and Caviness (10).

## RESULTS

Of the five cytokinins tested at 0.1 mM concentration in 1977, BA was found to be most effective in promoting fruit-set (Table I). Shore was more responsive to BA than Essex. Essex and Shore both had significant increases in seed weight on the terminal inflorescence in response to BA (8). Based upon these and other initial observations (8), subsequent experiments were performed with BA at 2 mM concentration.

Spray applications of 2 mM BA to the terminal inflorescence of field-grown plants significantly increased fruit-set and seed yield of Shore in 1978 and 1979. In contrast, Essex showed only a significant increase in fruit number in 1978 (Table II). The responses of Shore to BA were generally more pronounced than

Table III. *The Influence of 6-Benzylaminopurine on Fruit and Seed Development of Essex and Shore Soybeans*

Values are means of 6 replicates (5 plants replicate<sup>-1</sup>).

Genotype and Observation	1978		1979	
	Control	BA	Control	BA
Essex				
Seed weight (mg)	1950	2540	1320	1570
Pericarp weight (mg)	940	1360 <sup>a</sup>	570	670
Seed number inflorescence <sup>-1</sup>	17.2	22.4	14.0	14.7
Fruit number inflorescence <sup>-1</sup>	7.4	10.1 <sup>a</sup>	6.7	7.1
Number seeded fruits				
1-seeded	0.7	1.0	0.4	0.4
2-seeded	4.4	6.3	3.3	3.7
3-seeded	2.3	2.8	3.0	3.0
Shore				
Seed weight (mg)	2130	2770 <sup>a</sup>	1080	1530 <sup>a</sup>
Pericarp weight (mg)	1000	1510 <sup>a</sup>	610	910 <sup>a</sup>
Seed number inflorescence <sup>-1</sup>	16.3	21.6 <sup>a</sup>	10.0	13.3 <sup>a</sup>
Fruit number inflorescence <sup>-1</sup>	7.6	10.7	6.0	8.0 <sup>a</sup>
Number seeded fruits				
1-seeded	0.5	1.1	0.5	0.6
2-seeded	5.0	7.3 <sup>a</sup>	4.5	6.0 <sup>a</sup>
3-seeded	2.1	2.3	1.0	1.4

<sup>a</sup> Denotes significant difference from control at the 5% level of probability; 0.5 ml of 2 mM BA applied inflorescence<sup>-1</sup>; data are expressed on a per inflorescence basis.

Essex (Table III).

Further examination of the BA influence on fruit-set showed that the apically located fruits on the inflorescence were affected (Table IV). Fruit-set at the basal and accessory positions of Essex and Shore was unaffected by BA.

BA significantly increased seed yield of Shore in 1978 and 1979, but not of Essex. Most of the increase came from the extra apical fruits, with only small increases in the basal and accessory fruits. The effect of BA on seed yield was evident 3–4 weeks after treatment (Figs. 1, 2).

The seed size of apical fruits was significantly less than basal and accessory fruits (Fig. 3). Genotype, growing season, and BA were also shown to influence seed size.

## DISCUSSION

The responses of Essex and Shore soybeans to exogenous BA strongly indicate the importance of cytokinins for soybean fruit-

Table IV. The Influence of 6-Benzylaminopurine on the Pattern of Fruit-set of Essex and Shore Soybean Inflorescence

Values are means of six replicates (five plants replicate<sup>-1</sup>).

Genotype and Inflorescence Position	Fruit No Inflorescence <sup>-1</sup>			
	1978		1979	
	Control	BA	Control	BA
<b>Essex</b>				
Apical	1.3	3.8*	1.0	1.7
Basal	3.6	3.6	3.7	3.6
Accessory	2.5	2.7	2.0	1.8
Total	7.4	10.1*	6.7	7.1
<b>Shore</b>				
Apical	0.9	3.7*	0.4	2.0*
Basal	3.8	3.9	2.7	3.0
Accessory	2.9	3.1	2.9	3.0
Total	7.6	10.7*	6.0	8.0*

\* Denotes significant difference from control at the 5% level of probability; 0.5 ml of 2 mM BA applied inflorescence<sup>-1</sup>.

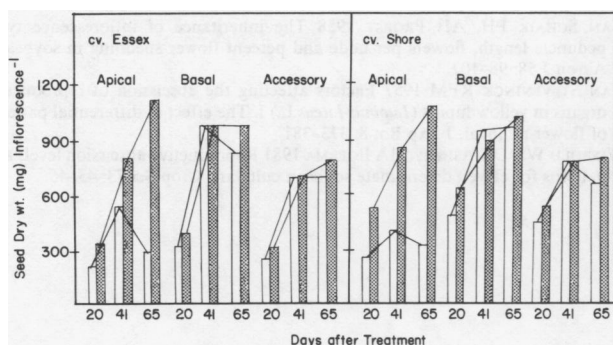


FIG. 1. The influence of 2 mM BA on changes in Essex and Shore seed dry weights of fruits at 3 positions on the terminal inflorescence 20, 41 and 65 days after treatment; ■, BA; □, control (1978 data).

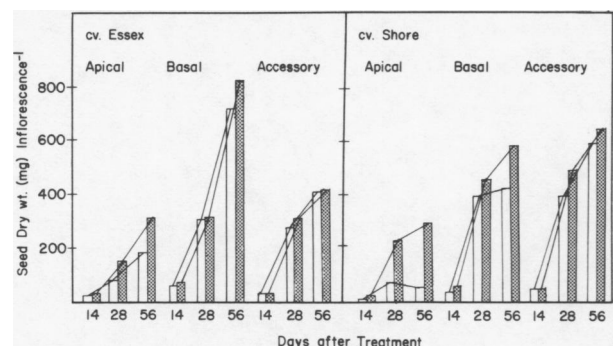


FIG. 2. The influence of 2 mM BA on changes in Essex and Shore seed dry weights of fruits at 3 positions on the terminal inflorescence 14, 28 and 56 days after treatment; ■, BA; □, control (1979 data).

set and seed development. Both genotypes responded to exogenous BA, suggesting that fruit abscission may be caused by a lack of endogenous hormone. However, Shore responded more to BA than Essex. This may be related to the lower levels of endogenous cytokinin-like activity in Shore ovules compared to Essex at the stage ( $R_3$ ) when BA was applied (7). Genotypic differences in endogenous cytokinins or response to exogenous BA are not known to be related to differences in fruit-set. Furthermore, whole plant responses to BA may be different from those of the terminal inflorescence, and this needs further study.

Fruits at various stages of development on an inflorescence

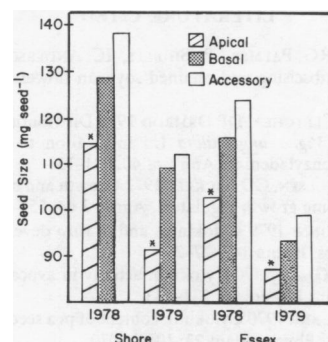


FIG. 3. Positional and seasonal influences on seed size of Essex and Shore soybeans; \* denotes significant difference at 5% level of probability.

require adequate hormones and nutrients for proper development to maturity. If either factor is inadequate, the young developing fruits fail to develop or abscise and consequently fruit-set is reduced. The timing of BA application is, therefore, critical. Application of BA during flowering has been ineffective in increasing seed yield of field-grown soybeans (3). If application is late, the fruits already would have abscised. Soybean fruits up to 2 cm long are very prone to abscise. Wiebold *et al.* (31) found 87% of total abscission occurred before the fruits reached 2 cm in length in 11 soybean cultivars. Also, the apically located fruits on an inflorescence usually abscise resulting in little or no fruit-set at the distal portion of the inflorescence. In lupin, fruits were produced in the basal portion of the inflorescence and poorly in the apical region (30), while basal flower removal promoted apical fruit-set. Similarly, Huff and Dybing (13) found that basal flowers of soybean inflorescences caused the abscission of apical ones, and they implied that the induction of fruit abscission has a hormonal basis. However, the nature of any abscission promoter(s) in lupin and soybean is unknown. In lupin, ABA is present at high levels in flowers during flower abscission, but exogenous ABA failed to stimulate abscission (22). Also, fruits of *Phaseolus* (12) and soybean (23) contain high levels of ABA during early stages of development, but no causal relationship has been established between ABA and fruit abscission in these species.

Some studies (1, 29) have indicated that poor fruit-set of soybeans results from shortages of hormones. Our results indicate that cytokinins may be one class of chemicals limiting soybean fruit-set. The enhanced BA-induced fruit-set of apical fruits of soybean inflorescences lends credence to this view. BA may promote fruit-set of soybeans by: (a) counteracting abscission promoters, (b) augmenting endogenous cytokinin levels, or (c) both. Essex and Shore both exhibit relatively low levels of endogenous cytokinin-like activity during the early stages of fruit growth (fruits 1–2 cm long) when BA application is effective (7). Thus, BA may supplement the endogenous cytokinin supply and promote fruit-set.

BA may act by increasing the ability of the treated fruits to competitively mobilize nutrients. Shortages of assimilates, particularly during the period of fruit-set, may intensify nutrient competition between developing fruits and vegetative organs. This might cause abscission of young fruits deficient in substrate or hormones. Cytokinins are known to attract nutrients to sites of application (19). Adedipe *et al.* (2) showed that BA application increased the amount of [<sup>14</sup>C]sucrose imported into fruits of a high-abscising cultivar of cowpea but had no effect on sucrose transport into fruits of a low-abscising cultivar. The results suggested that cytokinin effects on fruit abscission in cowpea may be related to the ability of the fruits to attract nutrients. This mechanism may also operate in soybeans.

**Acknowledgments**—The authors thank Mr. Allan Shaver for technical assistance and Mrs. Joyce Shelton for typing the manuscript.

## LITERATURE CITED

1. ABERNETHY RH, RG PALMER, R SHIBLES, IC ANDERSON 1977 Histological observations on abscising and retained soybean flowers. *Can J Plant Sci* 57: 713-716
2. ADEDIPE NO, RA FLETCHER, DP ORMROD 1976 Distribution of [ $^{14}$ C] assimilates in the cowpea (*Vigna unguiculata* L.) in relation to fruit abscission and treatment with benzyladenine. *Ann Bot* 40: 731-737
3. BASNET BS, GM PAULSEN, CD NICKELL 1972 Growth and composition responses of soybeans to some growth regulators. *Agron J* 64: 550-552
4. BENNICI A, PG CIONNI 1979 Cytokinins and *in vitro* development of *Phaseolus coccineus* embryos. *Planta* 147: 27-29
5. BLUMENFELD A, S GAZIT 1970 Cytokinin activity in avocado seeds during fruit development. *Plant Physiol* 46: 331-333
6. BURROWS WJ, DJ CARR 1970 Cytokinin content of pea seeds during their growth and development. *Physiol Plant* 23: 1064-1070
7. CROSBY KE 1979 Cytokinins on soybean *Glycine max* (L.) Merr. fruit and seed growth. MS Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA
8. CROSBY KE, LH AUNG, GR BUSS 1978 Natural and synthetic cytokinins on soybean development and yield. 5th Proceedings Plant Growth Regulator Working Group, Blacksburg, VA
9. DAVEY J, J VAN STADEN 1978 Cytokinin activity in *Lupinus albus* III Distribution in fruits. *Physiol Plant* 43: 87-93
10. FEHR WR, CE CAVINESS 1977 Stages of soybean development. Special Report 80, Cooperative Extension Service, Iowa State University, Ames, IA
11. HANSEN WR, R SHIBLES 1979 Seasonal log of the flowering and podding activity of field-grown soybeans. *Agron J* 70: 47-50
12. HSU FC 1979 Absciscic acid accumulation in developing seeds of *Phaseolus vulgaris* L. *Plant Physiol* 63: 552-556
13. HUFF A, CD DYBING 1980 Bioassays to detect chemical effects on flower abortion in soybean. In: F. T. Corbin, ed World Soybean Research Conference II Abstracts, Raleigh, NC
14. KATO I, S SAKAGUCHI 1954 Studies on the mechanism of occurrence of abortive grains and their prevention in soybeans, *Glycine max*. *Tokai-Ginki Natl Agr Exp Sta Bull* 1:115-132
15. LEHMAN WF, JW LAMBERT 1960 Effects of spacing of soybean plants between and within rows on yield and its components. *Agron J* 52:84-86
16. LETHAM DS 1963 Inhibitors and stimulants of cell division in developing fruits: their properties and activity in relation to the cell division period. *NZ J Bot* 1: 336-350
17. LINDOO SJ, LD NOODÉN 1978 Correlation of cytokinins and abscisic acid with monocarpic senescence in soybeans. *Plant Cell Physiol* 19: 997-1006
18. MICHAEL G, H SEILER-KELBITSCH 1972 Cytokinin content and kernel size of barley grain as affected by environmental and genetic factors. *Crop Sci* 12: 162-165
19. MOTHES K, L ENGLEBRECHT 1961 Kinetin-induced directed transport of substances in excised leaves in the dark. *Phytochemistry* 1: 58-62
20. NESLING FAV, DA MORRIS 1979 Cytokinin levels and embryo abortion in interspecific *Phaseolus* crosses. *Z Pflanzenphysiol* 91: 345-358
21. PANDY JP, JH TORRIE 1973 Path coefficient analysis of seed yield components in soybean (*Glycine max* (L.) Merr.). *Crop Sci* 13: 505-507
22. PORTER NG 1977 The role of abscisic acid in flower abscission of *Lupinus luteus*. *Physiol Plant* 40: 50-54
23. QUEBEDEAUX B, PB SWEETSER, JC ROWELL 1976 Absciscic acid levels in soybean reproductive structures during development. *Plant Physiol* 58: 363-366
24. SKOOG F, DJ ARMSTRONG 1970 Cytokinins. *Annu Rev Plant Physiol* 21: 359-384
25. SMITH AR, J VAN STADEN 1979 Cytokinins in excised embryos and endosperm of *Zea mays* grown under aseptic conditions. *Z Pflanzenphysiol* 93: 95-103
26. SMITH TJ, HM CAMPER 1973 Registration of Essex soybean. *Crop Sci* 13: 495
27. SMITH TJ, HM CAMPER, JA SCHILLINGER 1975 Registration of Shore soybean. *Crop Sci* 15: 100
28. STEEL RGD, JH TORRIE 1960 Principles and Procedures of Statistics. McGraw-Hill Book Co., NY
29. VAN SCHAIK PH, AH PROBST 1958 The inheritance of inflorescence type, peduncle length, flowers per node and percent flower shedding in soybeans. *Agron J* 58: 98-102
30. VAN STEVENINCK RFM 1957 Factors affecting the abscission of reproductive organs in yellow lupins (*Lupinus luteus* L.) I. The effect of differential patterns of flower removal. *J Exp Bot* 8: 373-381
31. WIEBOLD WJ, DA ASHLEY, HA BOERMA 1981 Reproductive abscission levels and patterns for eleven determinate soybean cultivars. *Crop Sci* 73: 43-46