SENESCENCE DEFERRAL IN BIG BLUESTEM WITH EXOGENOUS CYTOKININ APPLICATIONS

by

EARL EUGENE TOWNE

B. S., Kansas State University, 1976

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

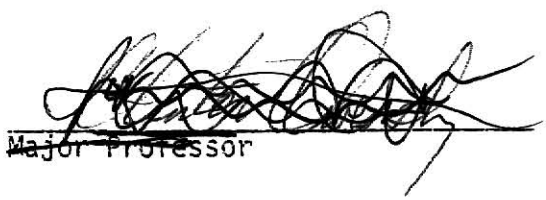
MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1981

Approved by:



Major Professor

TABLE OF CONTENTS

LIST OF FIGURES

SENESCENCE DEFFERAL IN BIG BLUESTEM WITH EXOGENOUS CYTOKININ APPLICATIONS

INTRODUCTION

Maturity has a direct effect on forage quality of range plants. In tallgrass prairie, protein content falls below the subsistence level required for cattle about mid-July (Rao et al. 1973). At that time, organelles are being broken down and exported from senescing leaves to other parts of the plant. Delaying the translocation of metabolites out of leaves could provide higher forage quality to the grazing animal at a time when nutritive values are normally declining.

Richmond and Lang (1957) initially demonstrated that exogenous cytokinins can delay the onset of senescence in detached leaves. That antisenescent effect has been attribituted, in whole or in part, to retarding chlorophyll loss, inhibiting protein degradation, preventing effluent carbohydrate translocation, and mobilizing metabolites. Most studies confirming cytokinin-induced senescence deferral have been with excised leaves in the dark. The physiological response of cytokinins in vivo, however, are often less conspicuous and dramatic than in vitro. For that reason, the antisenescent role of cytokinins have generally been relegated to tissue cultures and postharvest preservation of horticultural products.

Fletcher (1969) demonstrated that cytokinins can retard senescence in intact bean (*Phaseolus vulgaris*) leaves. Since then, other studies have confirmed the ability of cytokinins to delay senescence in vivo, but there is little diversity in plant species examined. We suspected that field applications of exogenous cytokinins could delay the onset of senescence in perennial grass plants.

The senescent leaf is a major source of nitrogen, since its protein is hydrolyzed and redistributed throughout the plant. In annuals, nutrients are translocated acropetally for seed development. Dalling et al. (1976) reported that 80% of the nitrogen in wheat (*Triticum aestivum*) grain originated from senescing leaves. But in perennial grasses, N and other assimilates are mobilized to storage organs for internal cycling (Weinmann 1942). McKendrick et al. (1975) reported that 18% of the total N requirement for growth in big bluestem (*Andropogon gerardi* Vitman) and indiangrass [*Sorghastrum nutans* (L.) Nash] came from an internal nitrogen reserve. Thus, manipulating senescence with exogenous cytokinins could alter internal nutrient cycling, such that it had a deleterious effect on overwintering survival and herbage production the following growing season.

The objectives of this study were to examine the optimum cytokinin concentration and date of application that could delay foliar senescence in big bluestem, and to determine its effects on herbage production the following growing season.

STUDY SITE AND METHODS

The study area was located on Kansas State University pastures in the northern Kansas Flint Hills near Manhattan. Average annual precipitation is 85 cm, 74% of which is received as rain during the May through September growing season. In the 1979 growing season, there were 39 cm of rainfall, 30% below average for the period (Fig. 1). But total moisture for 1979 was only 2 cm below normal. Rainfall in the 1980 growing season was 50% below average, and there were 35 days with temperatures exceeding 38 C.

Soil at the study site was a Benfield-Florence complex in the Udic Argiustolls subgroup of the Mollisols. That loamy upland range site has well-drained, moderately-deep, silty clay loams and cherty silt loams overlying a heavy silty clay loam subsoil.

Botanical census for the study area in 1979, by the modified step-point technique (Owensby 1973), indicated typical vegetation for native tallgrass prairie, with big bluestem and indiangrass the major dominants (Table 1).

The area has been annually burned in late-spring, and in 1978 the study site was fenced to exclude livestock grazing. We partitioned the site into 4.3 m x 6.4 m plots separated by 0.6 m alleys that were intermittently mowed throughout the growing season.

Benzyladenine (BA) was dissolved in a small quantity of 95% ethanol, heated, and mixed with distilled water. That cytokinin stock solution was diluted into 10 1 aliquots of 5, 10, 20, and 40 ppm BA, each containing 10 ml (0.1%) Tween-20 surfactant. All mixtures were formulated one day prior to application.

BA at the four concentrations was applied separately to entire plots via pressurized handsprayer at two-week intervals from mid-June until late-July 1979. Each treatment was replicated three times, and there were six untreated plots in the completely randomized design.

Beginning the first week of August, and every week thereafter until October, big bluestem within 15 cm on either side of a line through the width of every plot was clipped 2 cm above ground level. Each week the line was systematically moved in the plot to prevent sampling any previously-clipped plants.

One-third of the big bluestem harvested from each plot was placed

in a plastic bag, cooled in an ice chest, and stored in a freezer for chlorophyll analysis. The remaining portion of the sample was dried in a forced-air dryer (60 C), ground through a 1 mm mesh screen, and stored in sealed containers for nitrogen analysis.

On 1 December 1979, big bluestem rhizomes were dug in each plot along a previously unclipped transect. After cold water washing, rhizomes were oven-dried for five days at 60 C, ground in a Wiley mill (1 mm mesh screen), and stored in sealed bottles for nitrogen and total nonstructural carbohydrates (TNC) analyses.

The absence of unclipped areas in each plot precluded measuring herbage yields for 1979. But following the 1980 growing season, we determined herbage production from each treatment by clipping all species from three 0.406 m^2 quadrats to ground level in every plot. Herbage was oven-dried and reported as kg dry matter per hectare.

Chlorophyll from the frozen leaf samples was extracted with acetone and measured spectrophotometrically (Arnon 1949). Micro-Kjeldahl nitrogen (N) was determined colorimetrically for all leaf and rhizome samples, and crude protein estimated by N x 6.25. TNC concentration was measured for rhizome samples by enzyme extraction and copperiodometric titration (Smith 1969).

Data were analyzed by analysis of variance, and probability of differences among treatment means reported as $\hat{\alpha}$. Linear contrasts on cytokinin concentration and date of application treatments were also calculated. Cytokinin concentration and date of application interaction means were separated by probability levels without regard to F-ratios from the analysis of variance.

RESULTS AND DISCUSSION

Chlorophyll

As expected, chlorophyll content in untreated big bluestem leaves progressively declined each week (Fig. 2). But there was no difference in chlorophyll levels between BA-treated and untreated leaves ($\hat{\alpha}$=0.16). Although not considered statistically significant, leaves treated with 5 ppm BA had the highest chlorophyll levels of any treatment throughout the sampling period. The lack of significance was likely due to the large variability in chlorophyll content among replications.

Contrary to our findings, other studies on intact plants have shown that cytokinins effectively retard chlorophyll degradation. In bean, BA applied at any developmental stage delayed chlorophyll loss (Fletcher 1969). Naito et al. (1978) observed that BA increased leaf chlorophyll content if applied to intact bean plants at early or middle phenological stages, and delayed chlorophyll breakdown if applied in late stages.

Leaf yellowing is considered an important criterion of senescence, but the inability of BA to significantly retard chlorophyll loss in big bluestem may not be indicative of its antisenescent effectiveness. Spencer and Titus (1973) observed that in excised leaves, chlorophyll loss is the first indication of senescence, but in attached leaves, protein decline precedes chlorophyll loss. Thomas and Stoddart (1975) consider chlorophyll breakdown a secondary effect of protein degradation and of lesser importance in the senescence syndrome.

Crude Protein

Exogenous cytokinin applications generally produced higher crude protein content in big bluestem leaves, but the magnitude depended upon concentration and date of application. Linear contrasts indicated that

leaves treated with any BA concentration except 10 ppm had higher protein levels than untreated plants (Fig. 3). Big bluestem treated with 5 ppm BA had higher crude protein content than plants receiving other cytokinin applications ($\hat{\alpha}<0.01$).

Averaged over all sampling weeks, big bluestem treated with BA any time in July had higher protein levels ($\hat{\alpha}<0.05$) than untreated plants (Fig. 4). The ineffectiveness of mid-June treatments suggests that young non-senescing leaves are not responsive to exogenous cytokinins. That may indicate adequate endogenous cytokinin levels at that time. As the plants mature and approach the onset of senescence, exogenous BA may evoke its antisenescent effect by substituting for deficient internal cytokinins. Richmond et al. (1971) also reported that cytokinins were most effective on bean plants if applied in the summer when endogenous levels were suboptiminum.

There was no difference in crude protein content between BA-treated plants and untreated plants the first week in August. But thereafter, plants treated with 5 ppm BA any time in July had higher protein levels than untreated plants (Fig. 5). The transitory rise in protein content from 10 August to 17 August, corresponded with a week of cool temperatures and abundant rainfall. Mid- and early-July treatments of 5 ppm BA accentuated this rise ($\hat{\alpha}=0.03$ and 0.08 respectively).

Crude protein also increased during the week of 24 August to 30 August, but that was unrelated to favorable climatic conditions. In that week, big bluestem treated with 5 ppm BA any time in July had higher ($\hat{\alpha}<0.10$) crude protein levels than untreated plants. That protein increase coincides with the time when big bluestem in normally translocating metabolites to its rhizomes (McKendrick et al. 1975). Thus, leaves

receiving 5 ppm BA could have been temporarily redirecting nutrients by acting as a stronger sink. Engelbrecht and Mothes (1961) observed that labelled amino acids applied to tobacco (*Nicotiana tobacum*) leaves accumulated in the root tips, but cytokinin added to the leaf prevented this mobilization.

The ability of BA to maintain high protein in big bluestem leaves but unable to retard chlorophyll loss appears contradictory. Morita (1980) observed that 85-95% of the nitrogen released from senescent rice (*Oryza sativa*) leaves originated from chloroplasts. The primary constituent of chloroplast nitrogen is the Calvin cycle enzyme ribulose bisphosphate carboxylase (RuBPCase) (Peterson and Huffaker 1975). Chlorophyll loss from big bluestem leaves suggests that BA was unable to inhibit proteolytic enzymes from hydrolyzing RuBPCase. However, BA evidently was able to defer the nitrogenous degradation products from being translocated out of the leaf. Fletcher et al. (1970) maintain that the ability of cytokinins to prevent effluent translocation of sugars and amino acids out of leaves is responsible for its antisenescent effect.

Rhizome Crude Protein and TNC Content

There was no difference in rhizome crude protein or TNC content of big bluestem receiving any BA applications compared to untreated plants. Since spring growth potential is related to nitrogen and carbohydrate reserves, that indicates the senescence deferring effect of BA did not alter internal nutrient reserve cycles.

Any factor affecting the amount of photosynthetic material is reflected in carbohydrate storage. If chlorophyll degradation had been delayed with BA, we would have expected an increase in rhizome TNC.

Protein degradation and remobilization from the senescent leaf provides an important source of N for internal recycling. Rains et al. (1975) reported that replenishment of nitrogen reserves in big bluestem rhizomes begins in mid-August, coinciding with new root growth. Thus, delaying protein migration out of big bluestem leaves without altering N reserves, indicates that the rhizome could be compensating for this loss with soil nitrogen uptake from new roots.

Alternatively, big bluestem rhizomes could have imported nitrogenous compounds from the leaves at a time past the October sampling date. McKendrick et al. (1975) reported that nitrogen and TNC from big bluestem shoots continued to accumulate in rhizomes after frost, reaching a maximum in December.

Herbage Yield

Applying 5 ppm BA in either mid- or late-July significantly increased ($\hat{\alpha}<0.02$) herbage yields the following year over untreated plots (Fig. 6). Since plants were not segregated after clipping, we do not know if that increased yield response was primarily from big bluestem or from other species. Other cytokinin concentrations and application dates had no effect on herbage production compared to untreated plots.

Biswas and Choudhuri (1977) found that 100 ppm BA applied at any developmental stage in rice increased growth and dry matter accumulation. Evidently, in big bluestem the response to exogenous cytokinin application has a carryover effect into the next year. Seasonal changes in endogenous hormone levels of willow (*Salix babylonica*) indicated that cytokinins were stored and actively translocated to meet physiological requirements of the plant (Van Staden and Brown 1978). That would suggest that synthetic cytokinins can be cycled through the plant storage organs and reactivated with the resurgence of growth.

TABLE 1. Botanical composition (% total basal cover) in 1979 on loamy upland study site in Flint Hills near Manhattan, Kansas.

Species	% Composition
Andropogon gerardi Vitman	26.3
Sorghastrum nutans (L.) Nash	25.0
Andropogon scoparius Michx.	7.9
Sporobolus asper (Michx.) Kunth	5.0
Poa pratensis L.	4.9
Bouteloua curtipendula (Michx.) Torr.	4.8
Other perennial grasses	6.4
Annual grasses	6.6
Carex spp.	8.5
Perennial forbs	3.7
Annual forbs	0.6
Woody species	0.1

Fig. 1. Monthly precipitation totals for Manhattan, Kansas during 1979-1980 period (bars), compared with long-term average for the area (solid line).

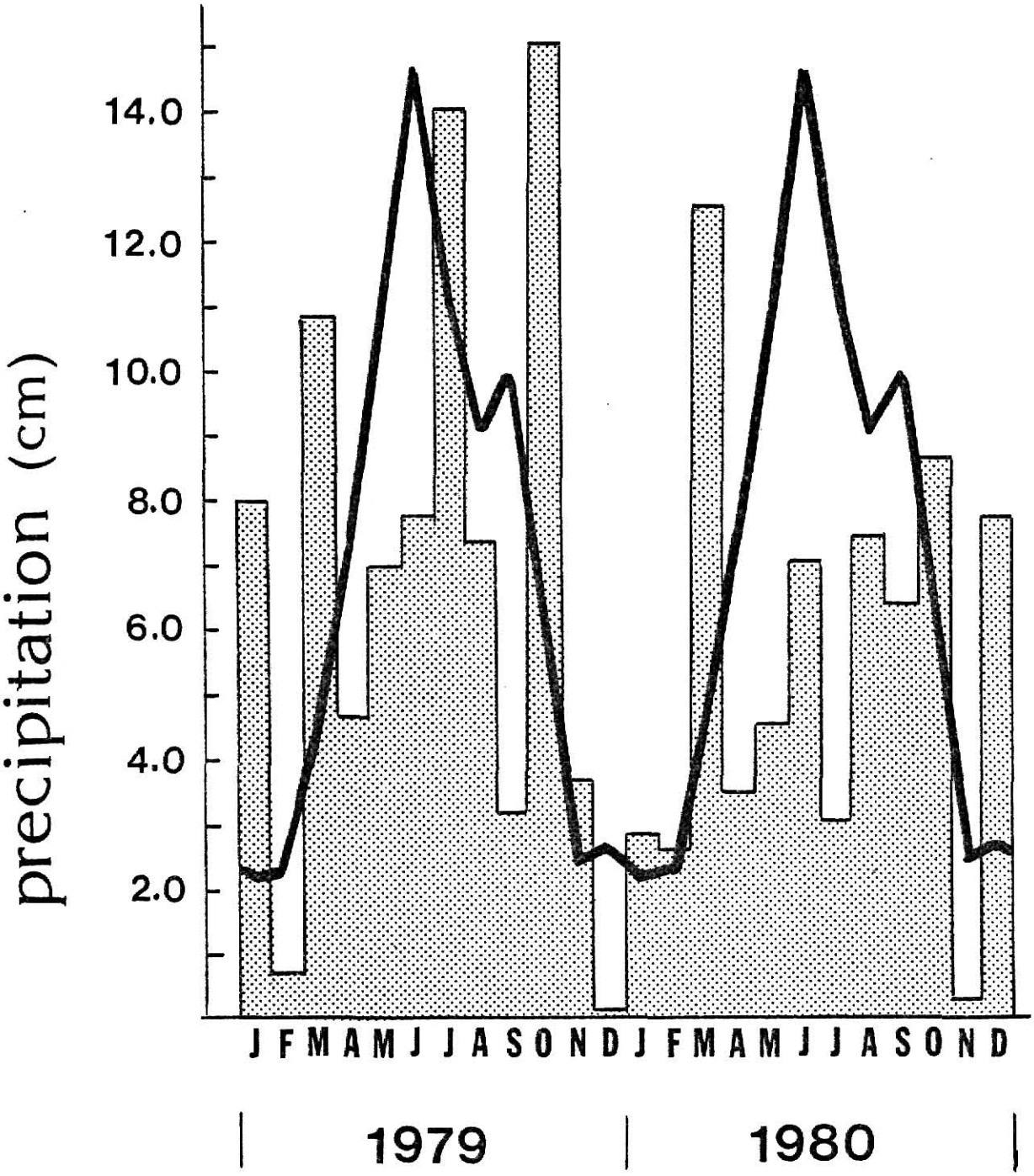

precipitation (cm)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
J F M A M J J A S O N D J F M A M J J A S O N D
1979
1980

Fig. 2. Chlorophyll decline of untreated big bluestem leaves from 1 Aug. through 5 Oct. 1979. Standard deviation for each sampling date bracketed.

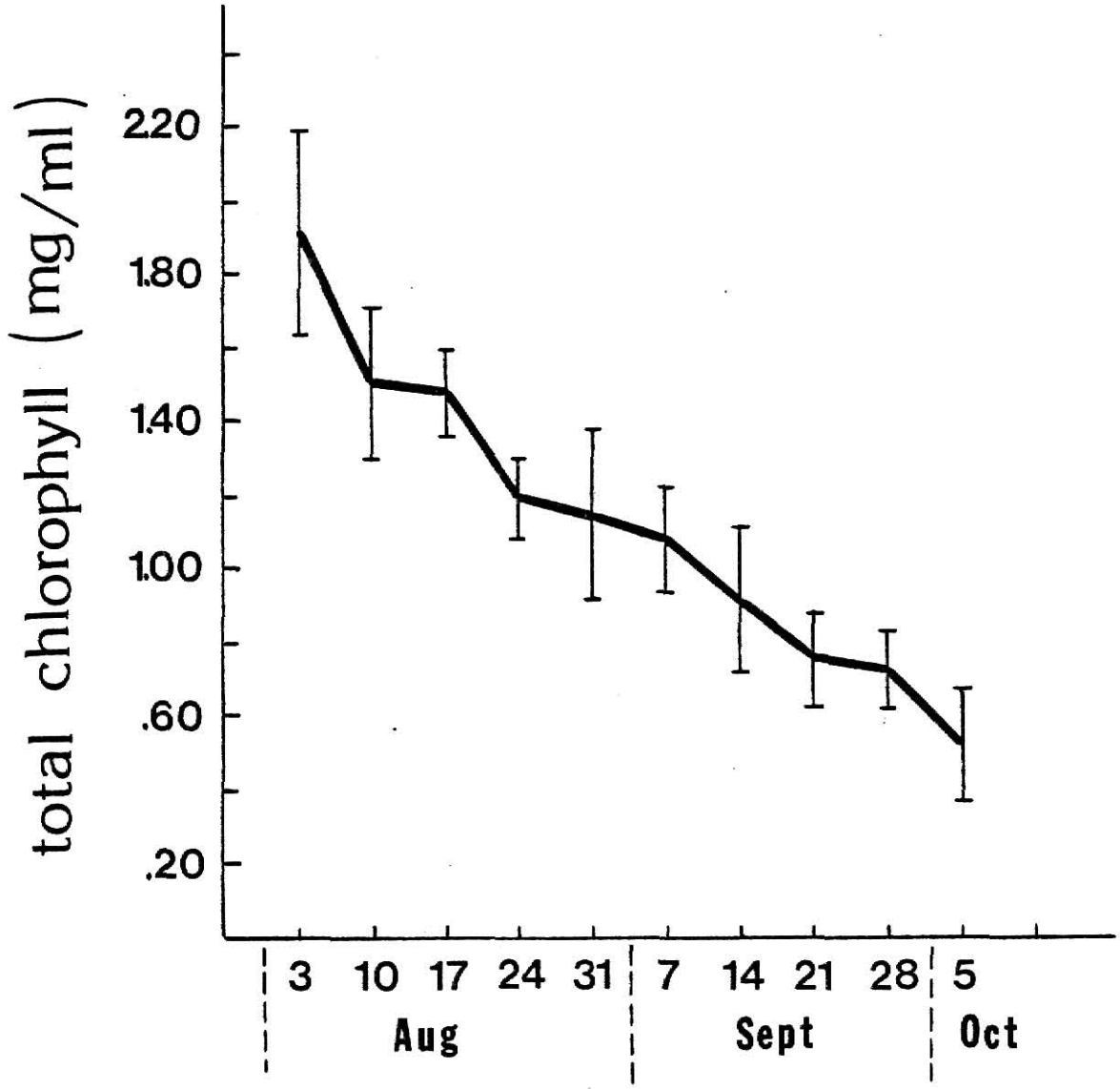

total chlorophyll (mg/ml)
2.20
1.80
1.40
1.00
.60
.20
3
10
17
24
31
7
14
21
28
5
Aug
Sept
Oct

Fig. 3. Effect of BA concentrations on crude protein content of big bluestem leaves. Means with the same letter are not significantly different (P>0.10).

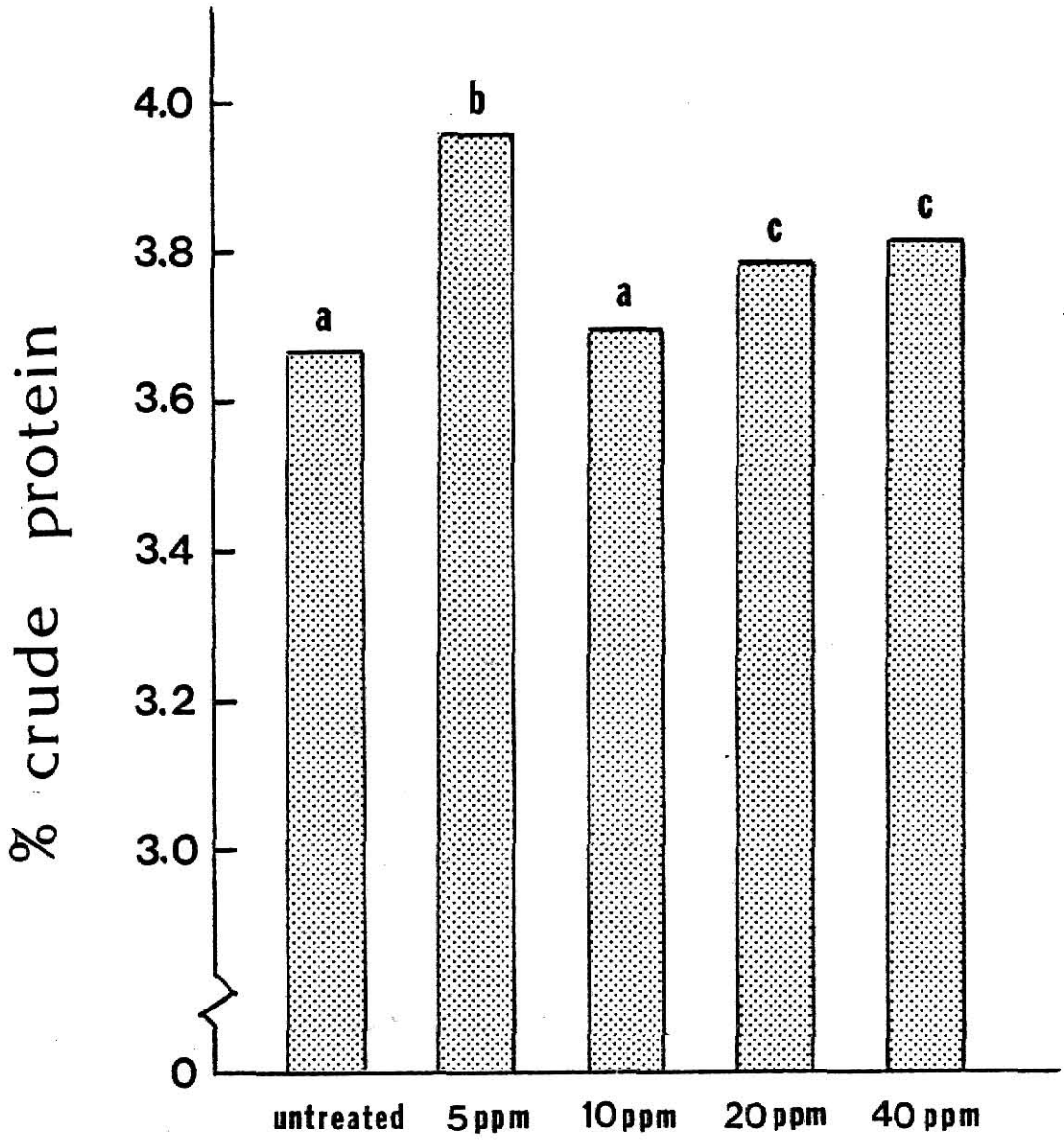

% crude protein
4.0
3.8
3.6
3.4
3.2
3.0
0
a
b
a
c
c
untreated
5 ppm
10 ppm
20 ppm
40 ppm

Fig. 4. Effect of BA applications at different dates on crude protein content of big bluestem leaves. Means with the same letter are not significantly different (P>0.10).

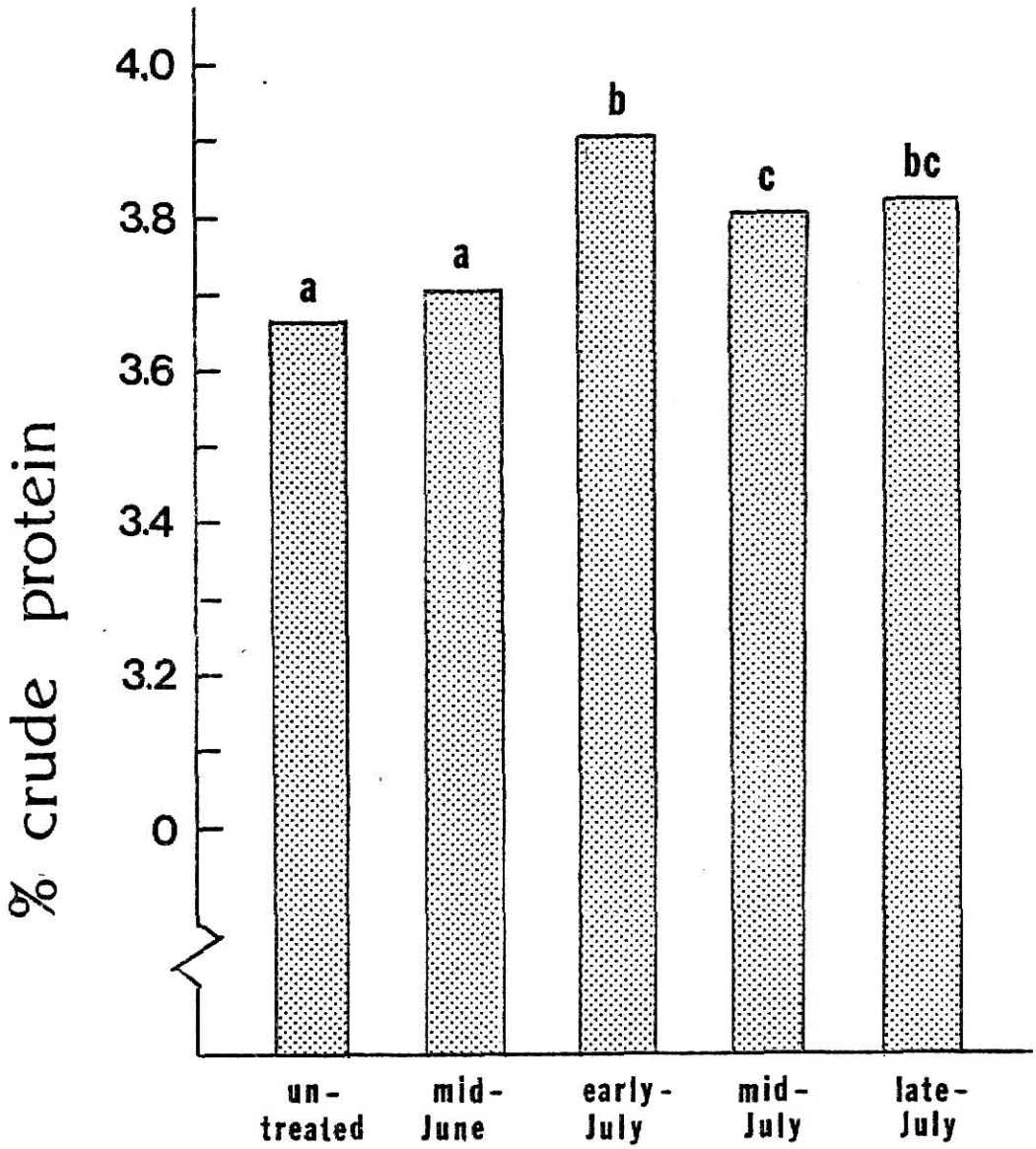

% crude protein
4.0
3.8
3.6
3.4
3.2
0
a
a
b
c
bc
un-treated
mid-June
early-July
mid-July
late-July

Fig. 5. Changes in crude protein content of untreated big bluestem leaves compared to leaves treated with 5 ppm BA applied at different intervals. Asterisk above a given harvest date indicates a significant difference due to BA treatment ($P<0.10$).

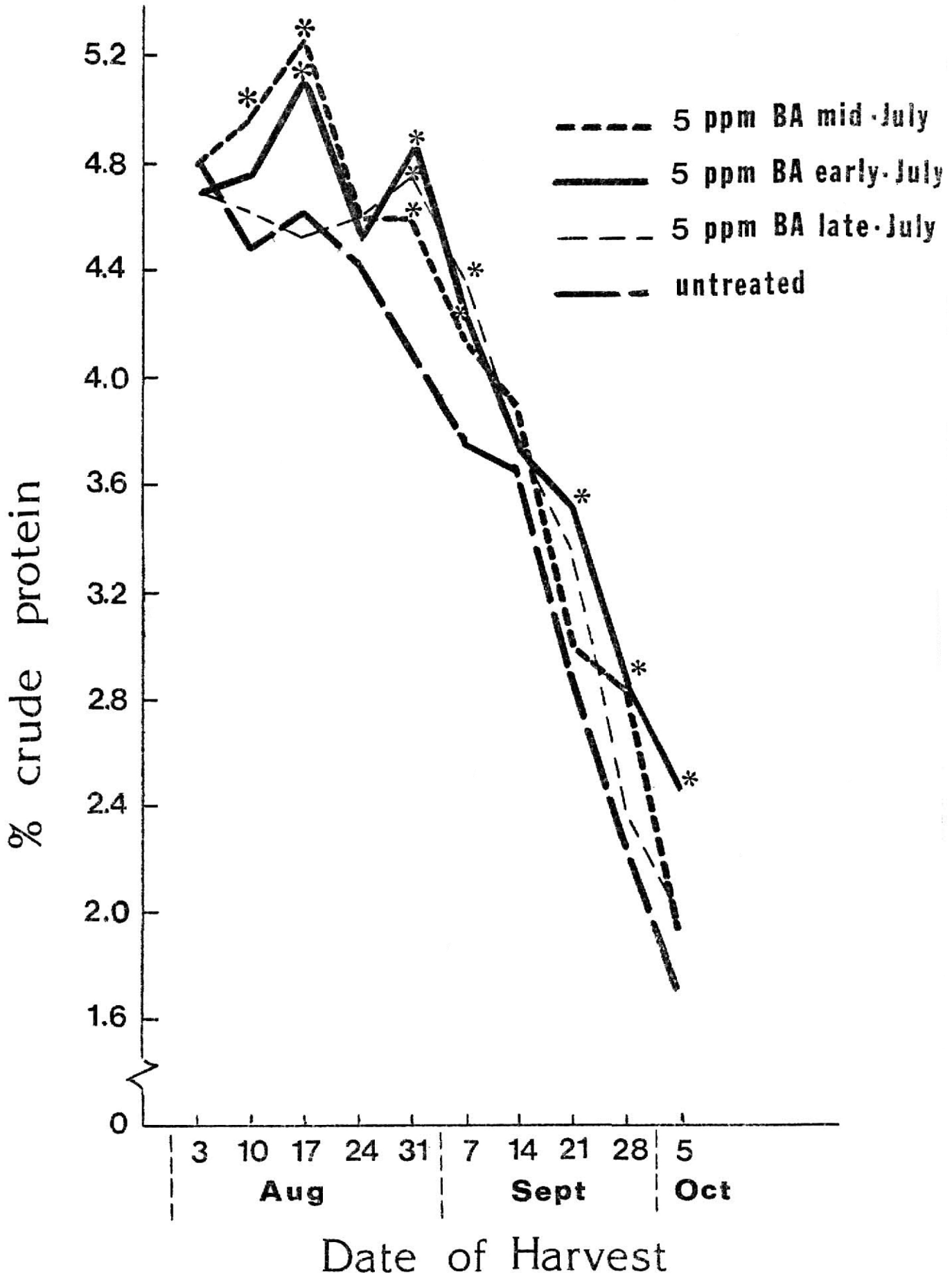
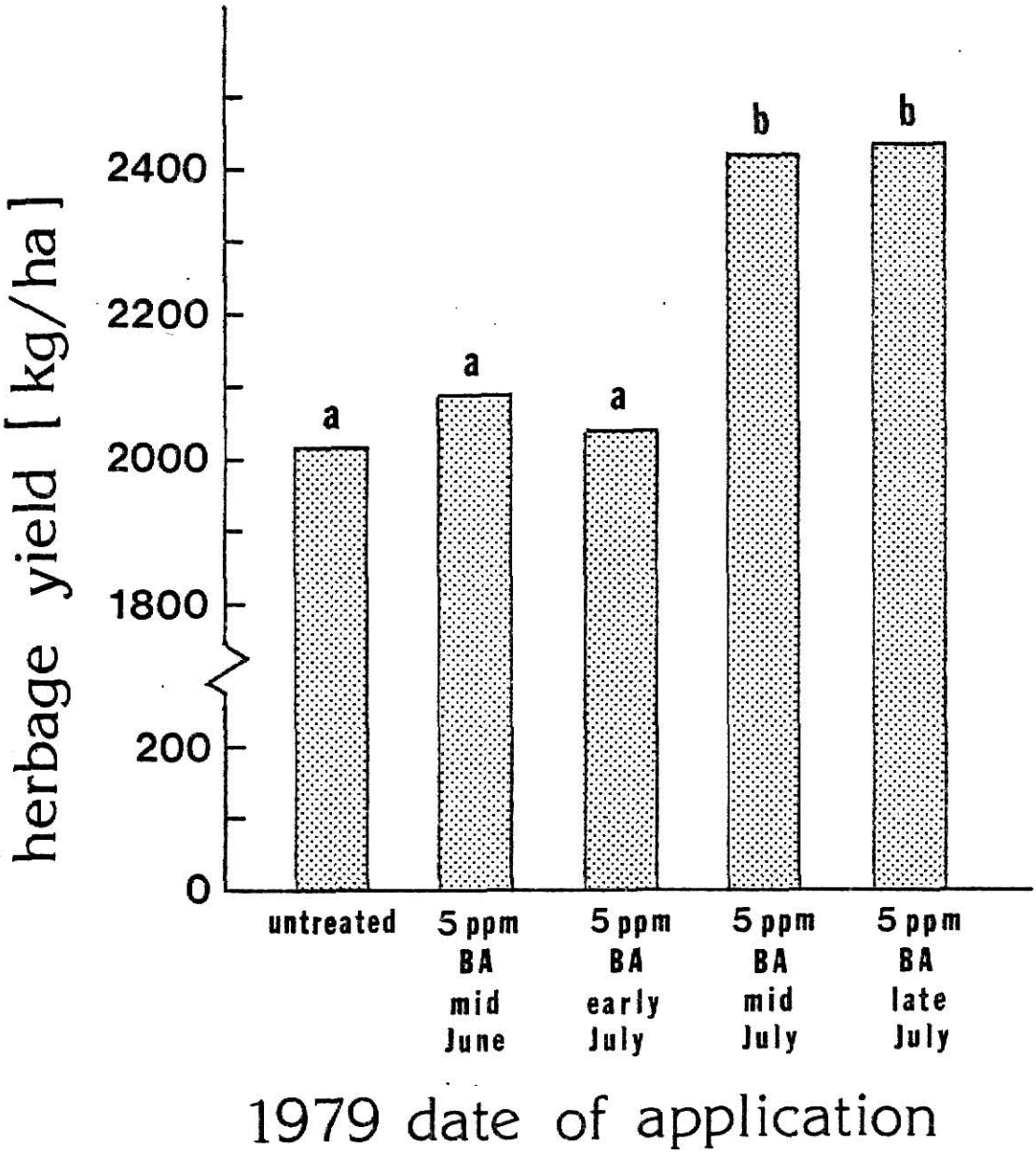

5 ppm BA mid-July
5 ppm BA early-July
5 ppm BA late-July
untreated
% crude protein
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
0
3 10 17 24 31 7 14 21 28 5
Aug
Sept
Oct
Date of Harvest

Fig. 6. Herbage yields (kg D.M./ha) of all species harvested at end of 1980 growing season, one year after BA application. Means with the same letter are not significantly different (P>0.10).


herbage yield [kg/ha]
2400
2200
2000
1800
200
0
a
a
a
b
b
untreated
5 ppm BA mid June
5 ppm BA early July
5 ppm BA mid July
5 ppm BA late July
1979 date of application

LITERATURE CITED

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoleoxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.

Biswas, A.K., and M.A. Choudhuri. 1977. Regulation of leaf senescence in rice by hormones sprayed at different developmental stages and its effect on yield. Indian J. Agric. Sci. 45:38-40.

Dalling, M.J., G. Boland, and J.H. Wilson. 1976. Relation between acid proteinase activity and redistribution of nitrogen during grain development in wheat. Aust. J. Plant Physiol. 3:721-730.

Engelbrecht, L., and K. Mothes. 1961. The effect of kinetin on the development of roots. Plant Cell Physiol. 2:271-276.

Fletcher, R.A. 1969. Retardation of leaf senescence by benzyladenine in intact bean plants. Planta 89:1-8.

Fletcher, R.A., G. Hofstra, and N.O. Adedipe. 1970. Effects of benzyladenine on bean leaf senescence and the translocation of ^{14}C-assimilates. Physiol. Plant. 23:1144-1148.

Hewett, E.W., and P.F. Wareing. 1973. Cytokinins in *Populus* x *robusta*: Qualitative changes during development. Physiol. Plant. 29:386-389.

McKendrick, J.D., C.E. Owensby, and R.M. Hyde. 1975. Big bluestem and indiangrass vegetative reproduction and annual reserve carbohydrate and nitrogen cycles. Agro-Ecosystems 2:75-93.

Morita, K. 1980. Release of nitrogen from chloroplasts during leaf senescence in rice (*Oryza sativa* L.). Ann. Bot. 46:297-302.

Naito, K., H. Tsuji, and I. Hatakeyama. 1978. Effect of benzyladenine on DNA, RNA, protein, and chlorophyll contents in intact bean leaves: Differential responses to benzyladenine according to leaf age. Physiol. Plant. 43:367-371.

Owensby, C.E. 1973. Modified step-point system for botanical composition and basal cover estimates. J. Range Manage. 26:302-303.

Peterson, L.W., and R.C. Huffaker. 1975. Loss of ribulose 1, 5-diphosphate carboxylase and increase in proteolytic activity during senescence of detached primary barley leaves. Plant Physiol. 55:1009-1015.

Rains, J.R., C.E. Owensby, and K.E. Kemp. 1975. Effects of nitrogen fertilization, burning, and grazing on reserve constituents of big bluestem. J. Range Manage. 28:358-362.

Rao, M.R., L.H. Harbers, and E.F. Smith. 1973. Seasonal change in nutritive value of bluestem pastures. J. Range Manage. 26:419-422.

Richmond, A.E., and A. Lang. 1957. Effect of kinetin of protein content and survival of detached *Xanthium* leaves. Science 125:650-651.

Richmond, A.E., B. Sachs, and D.J. Osborne. 1971. Chloroplasts, kinetin and protein systhesis. Physiol. Plant. 24:176-180.

Smith, D. 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Univ. of Wisconsin Res. Rep. No. 41. 11 p.

Spencer, P.W., and J.S. Titus. 1973. Apple leaf senescence: Leaf disc compared to attached leaf. Plant Physiol. 51:89-92.

Thomas, H., and J.L. Stoddart. 1975. Separation of chlorophyll degradation from other senescence processes in leaves of a mutant genotype of meadow fescue (*Festuca pratensis* L.). Plant Physiol. 54:438-441.

Van Staden, J., and N.A.C. Brown. 1978. Changes in the endogenous cytokinins of bark and buds of *Salix babylonica* as a result of stem girdling. Physiol. Plant. 43:148-153.

Weinmann, H. 1942. The autumnal remigration of nitrogen and phosphorus in *Trachypogon plusosus*. J. South African Bot. 8:179-196.

APPENDIX

REVIEW OF CYTOKININ LITERATURE

HISTORICAL

The existence of a specific cell division factor that could be translocated throughout a plant was postulated by Wiesner in 1892 (Steward and Krikorian 1971). Evidence for such a factor was obtained in 1913, when the German botanist Haberlandt discovered that an endogenous compound in phloem tissue, leptohormone, stimulated cork cambium formation in cut potato (*Solanum tuberosum*) (Weaver 1972). Wehnelt later showed that various plant extracts added to bean (*Phaseolus vulgaris*) parenchyma produced wart-like growths in which cell division occurred (Strong 1958). Bonner and English (1938) found that a fatty acid extracted from crushed beans cells caused localized growth on bean pod parenchyma. However, the primary response of this substance, traumatin, was in promoting cell enlargement and not cell division.

In seeking a nutritive supplement for agar medium, Van Overbeek et al. (1941; 1942) discovered that the fluid endosperm of coconut (*Cocos nucifera*) stimulated cell division of embryos grown in vitro. Subsequent work by other investigators indicated that coconut milk, in combination with indoleacetic acid, supplied some undetermined growth substance required for cell division in tissue cultures (Caplin and Steward 1948; Nickell 1950). Steward et al. (1952) developed quantitative assays of cell proliferation using excised carrot (*Daucus carota*) tissue to isolate the growth factor in coconut milk. After fractioning 2,650 L of coconut milk, Shantz and Steward (1952; 1955) isolated 1,3-diphenylurea as the active ingredient. This compound, however, was not universally recognized as the growth-promoting factor

in coconut milk. Although diphenylurea stimulated cell division in tobacco (*Nicotiana tobacum*) callus, the effect was often delayed, sporadic, and responsive only at high concentrations (Strong 1958). Many researchers also felt that diphenylurea was either an artifact or a contaminant from synthesized urea herbicides (Jacobs 1979).

While seeking factors that interact with the growth inhibiting effects of auxin, Skoog and Tsui (1948) observed that both adenine and its nucleoside stimulated cell division in tobacco callus tissue. The discovery that yeast DNA induced cell proliferation in excised pith tissue (Jablonski and Skoog 1954) led to further investigations of other nucleic acids. The active fraction was finally isolated from old herring sperm DNA and named kinetin because of its ability to bring about cytokinesis in cells (Miller et al. 1955b). Kinetin was found to be a degradation product of deoxyribonucleic acid not present in fresh preparations. Apparently, during the transformation, the pentose derivative in deoxyadenosine migrates from the 9-position to the 6-position of the adenine ring (Moore 1976). Hall and deRopp (1955) demonstrated that kinetin could be produced by autoclaving adenine and furfuryl alcohol together. Kinetin's chemical structure was identified as 6-furfuryaminopurine, and subsequently synthesized in crystalline form (Miller et al. 1955a).

Kinetin stimulated cytokinesis in callus tissue with concentrations as low as one part per billion, which was 1000 times more active than any previously know substance. Hence, the name kinin was proposed by Miller et al. (1956) to be the class designation for all substances with similar activity as kinetin. But kinin had been used earlier by animal physiologists to designate a class of polypeptids in venoms and

stings that stimulate smooth muscle movement (Collier 1962). Consequently, other generic terms were proposed to replace kinin, including cytonin, kinetenoid, and phytokinin. To avoid confusion and establish uniformity, Skoog et al. (1965) urged that all previous designations be discontinued and replaced by the term cytokinin. That is now the accepted generic name for all 6-substituted purine derivatives with kinetin-like biological activity.

ANALOGS

Although kinetin was the first isolated cytokinin, it was an artifact formed by the preparation procedure and did not occur naturally. Miller (1961) isolated and partially characterized an active natural cytokinin from corn (*Zea mays*) endosperm. Letham (1963a) identified this substance as 6-(4-hydroxy-3-methyl-*trans*-2-butenylamino) purine, and named it zeatin. This endogenous cytokinin is an adenine derivative with a 5-carbon isoprenoid substitute instead of the furfuryl group on the nitrogen of the 6-amino group (Fig. 1). As a result of this chain substitution, zeatin is 10-100 times more active than kinetin in many bioassays (Letham 1967b). Attachment of a pentose sugar (ribofuranose) to the purine ring at position 9 forms zeatin riboside, which is less active than zeatin (Letham 1972), but is the major translocational form (Gordon et al. 1974). Zeatin and its riboside have been purified from a wide variety of sources and account for most of the cytokinin activity in coconut milk (Letham 1968).

The most widespread natural cytokinin in plants differs only slightly from the chemical structure of zeatin. Isopentenyladenine (iP), and its ribonucleoside (2iP), provide the constituent base for almost all naturally occurring cytokinins (Letham 1978). Whenever

required, enzymatic reactions convert isopentenyl adenosine into zeatin (Miura and Miller 1969). Isopentenyladenine had been synthesized before it had been identified as a natural occurring compound (Leonard and Fujii 1964). It was first isolated as a free base from *Corynebacterium fascians* cultures (Klambt et al. 1966), but has since been found in tRNA preparations from both plant and animal sources (Hall et al. 1966).

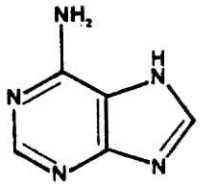
STRUCTURE

Structural requirements for a high level of cytokinin activity generally include an adenine molecule with a N-6 substituent of moderate size (Skoog and Leonard 1968). A variety of ring substitutes in the N-6 position confer cytokinin activity on adenine, but benzyl is the most effective, followed by furfuryl, phenyl, and thenyl rings (Skoog et al. 1967). In all monosubstituted adenines that possess cytokinin activity, the substituent is at the N-6 position, however, the activity of these compounds varies considerably with the length, degree of unsaturation, and substitutions on the side chain (Skoog and Schmitz 1972).

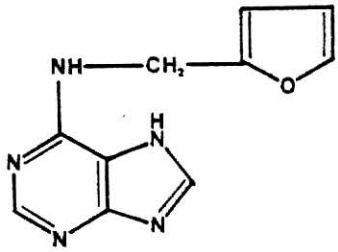
Modification of the adenine moiety by replacing the exocyclic nitrogen at the N-6 position with other atoms markedly reduces activity (Strong 1958). But in bioassays based on senescence or other properties not involving growth, the requirement for an adenine ring appears to be less stringent and substitutions of other ring systems may suffice (Skoog and Armstrong 1970).

There are some nonpurine compounds that have cytokinin activity, the most potent being phenylurea derivatives. Minimum structural requirements for activity are a -NH-CO-NH- grouping and a phenyl ring, but activity is increased by substitution with electronegative groups (Bruce et al. 1965). Although Schantz and Steward (1955) isolated

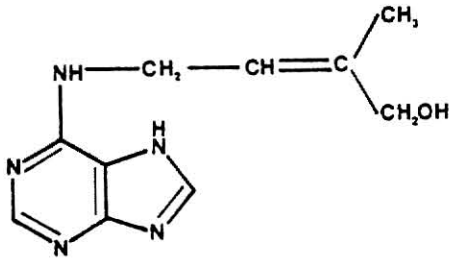
Fig. 1. Structural formulas of some synthetic and naturally occurring cytokinins.



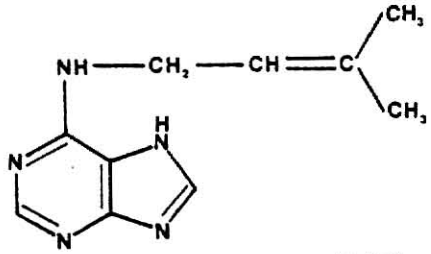
Adenine



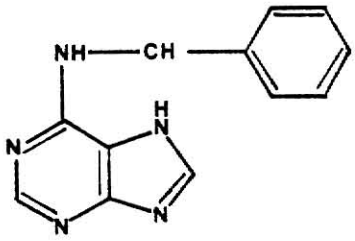
Kinetin



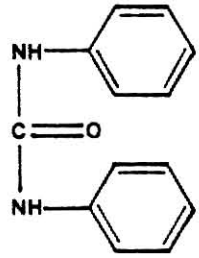
Zeatin



Isopentenyladenine (2iP)



Benzyladenine
(6-Benzylaminopurine)



Diphenylurea

diphenylurea as the fraction in coconut milk responsible for cell division, its activities are weak compared to adenine derivatives. However, urea-type cytokinins and adenine-type cytokinins both show a similar spectrum of biological activities, suggesting that both classes act through similar mechanisms (Kende 1971).

Synthetic cytokinins are usually quantitatively more active than naturally-occurring compounds. Kinetin and benzyladenine (6-benzylaminopurine) (BA) are 100 times more effective than zeatin or 2iP in retarding leaf senescence in oat (*Avena sativa*) (Varga and Bruinsma 1973). Biddington and Thomas (1978) reported that BA was more active and more stable than zeatin in leaf tissue. Synthetic 2iP is ten times more active than kinetin in tobacco bioassays (Rogozinska et al. 1964), but it is less effective for senescence deferral in chlorophyll retention tests (Hamzi and Skoog 1964). BA is also more potent than kinetin (McDonald et al. 1971) and is generally the most commonly used synthetic cytokinin.

METABOLISM

The origin and specific biosynthetic pathways of free cytokinins is not yet resolved. Anabolism is generally assumed to occur via substitution of the side chain onto an adenine moiety and not due to degradation of tRNA (Hall 1973). The conversion of mevalonic acid to isopentenyl pyrophosphate is the initial step in side chain formation of 2iP (Peterkofsky 1968). Transferring isopentenyl to adenine would result in the active cytokinin base. However, this process has been varified only in bacteria tRNA.

After observing that adenine did not overcome the inhibition of cytokinin synthesis caused by hadacidin, Kung-Woo Lee et al. (1974)

concluded that the basic precursor of biosynthesis involved inosine monophosphate rather than adenine. Additionally, Beutelmann (1973) found that only a very low percentage of labelled adenine can be converted to free cytokinin in plant tissue.

Catabolism of active cytokinins can occur by removing the side chain from the adenine moiety to form inactive metabolites. There are at least two enzyme activities in iP that catalyze cleavage of the isopentenyl side chain and the N-glycosylic bond (Paces et al. 1971). Preferential enzymatic degradation of natural cytokinins by cytokinin oxidase is responsible for their reduced activity in plants compared to synthetic cytokinins (Whitty and Hall 1974). But, Henderson et al. (1962) discovered that the enzyme xanthine oxidase can readily degrade synthetic cytokinins to 2,8-dihydroxy derivatives. Fox (1966) found more than 95% of benzyladenine taken up in tissue cultures was degraded by cleavage of the benzyl side chain. In intact bean leaves, BA is metabolized into BA ribotide and riboside, but only the nucleoside is translocated (Ramina 1979). In senescing leaves, labelled BA was readily metabolized into a number of low molecular weight compounds including adenine, guanine, and urea, but the primary metabolite was benzyladenosine (McCalla et al. 1962). The adenine moiety of metabolized BA is scavenged by the tissue, but the remaining metabolites accumulate inside tissues in the form of a detoxification compound (Fox and Chen 1967).

Side chain cleavage is not the only mechanism that inactivates cytokinins. Zeatin is extensively metabolized to form zeatin riboside and dihydrozeatin derivatives, without removing the N-6 side chain (Sondheimer and Tzou 1971). Glucose can attach to either position 7

or 9 on the purine ring (Letham et al. 1975), or to the hydroxyl group on the isopentenyl side chain of zeatin riboside (Horgan 1975). Exogenous zeatin applied to radish (*Raphanus*) roots rapidly metabolized to 7-glucosylzeatin (raphanatin) (Parker et al. 1972). Glycosylation of both natural and synthetic cytokinins at the 7- and 9-position occurs in plants, but 7-glucosides are more metabolically stable (Parker et al. 1973). In most bioassays, glucosides exhibit very low cytokinin activity (Schmitz et al. 1972).

The actual function of cytokinin glucosides is speculative. Their accumulation in senescing leaves indicates that they do not retard senescence, mobilize nutrients, or stimulate cell division. Cytokinin glucosides may be either bound and inactive forms of the active derivative (Gazit and Blumenfeld 1970), or storage compounds which accumulate when active cytokinins are present in amounts above that required for growth (Parker and Letham 1973). Henson and Wareing (1977b) found glucoside levels declined when *Xanthium* buds started developing, suggesting that cytokinin glucosides are hydrolyzed in the meristematic tissues of the plant when needed. Wareing et al. (1977) identified cytokinin glucosides in senescing leaves but did not find any in roots or root exudate. Hewett and Wareing (1973b) theorize that once the fruit tissue ceases to be a sink, cytokinins in the xylem sap are diverted to the leaves and converted to cytokinin glucosides. Any cytokinins remaining in senescent leaves are lost to the plant upon leaf abscision.

Active cytokinins may be converted to inactive glucosides so they can be transported through the phloem without any deleterious effect on plant growth (Van Staden 1976). Transport of active cytokinins through

non-living xylem would not require inactivation.

MODE OF ACTION

Mechanisms of cytokinin action are conjectural. After finding cytokinin bases in tRNA, Skoog et al. (1966) suggested that cytokinins exert their biological effects at the translation level. Fox and Chen (1967) reported incorporation of BA into tRNA where it appeared to occur as a nucleotide. Other investigators, however, failed to observe incorporation of labelled cytokinins into tRNA (Kende and Tavares 1968). Walker et al. (1974) concluded that only one molecule of intact BA per 10,000 tRNA molecules is incorporated. The inability of cytokinin-dependent tissue to grow on cytokinin-free media indicates physiologically insignificant amounts of free cytokinins are produced by tRNA degradation (Burrows et al. 1971). But in vitro and in vivo are not always analagous. Burrows (1976) suggests that the cytokinin-dependent in vitro tissue could have a defective biosynthetic pathway necessary for the alkylation of free adenine, or that the gene for this enzymatic pathway could not be derepressed.

Cytokinins may control at the translation level by binding to ribosomal proteins. Brandes and Kende (1968) initially suggested that cytokinins interact with specific receptor molecules by loose noncovalent bonds. Berridge et al. (1970) described reversible nonenzymatic binding of cytokinins in *Brassica* leaf ribosomes, and found that active cytokinins have a higher affinity for ribosomes than inactive cytokinin analogs. Fox and Erion (1975) identified both high-and-low-affinity cytokinin binding sites on wheat (*Triticum aestivum*) germ ribosomes, and concluded that the high affinity receptor site is a protein. The significance of cytokinin-binding proteins is unknown, but may reflect

the binding to a ribosome-associated protein kinase (Ralph et al. 1972).

OCCURRENCE AND SITES OF SYNTHESIS IN PLANTS

Transfer RNA

In general, cytokinins are widespread in plants, not only as free hormones, but as components of tRNA. Most investigations of cytokinin biogensis have been concerned with the mechanism by which cytokinins originate in tRNA fractions. Cytokinins are unique among phytohormones in that they are structural components of tRNA and have been isolated in bacteria, yeast, corn, mice, humans, and an ubiqutous spectrum of other organisms (Skoog and Armstrong 1970). But only tRNA species corresponding to codons beginning with an uracil base contain cytokinins (Armstrong et al. 1970). In *Escherichia coli*, cytokinin-containing tRNA species correspond to all six amino acids (phenylalanine, leucine, serine, tyrosine, cysteine, and tryptophan) that have codons starting with uridine (Skoog et al. 1966). Only one cytokinin base is present in each tRNA molecule and it's always attached adjacent to the 3' end of the anticodon (Letham 1978).

Although bound cytokinin moieties in tRNA do not function as free cytokinins in the regulation of physiological processes (Kende and Tavares 1968), they are significant. Modifying the cytokinin nucleotides in tRNA impairs ribsomal binding of amino acids (Fittler and Hall 1966). Gefter and Russel (1969) reported that cytokinins in tRNA serve an important regulatory role in the translation step of gene-controlled protein synthesis.

Roots

Meristematic regions of roots are principal sites of cytokinin biosynthesis (Goldacre 1959). Short and Torrey (1972) found over 40

times more free cytokinin in the terminal 0-1 mm segment of pea (*Pisum*) roots than in the next 1-5 mm segment. Older, proximal root regions contain very low cytokinin levels compared to root tips (Weiss and Vaadia 1965). Engelbrecht (1972) detected cytokinins in detached bean leaves only after the petiole developed roots. Cytokinin content in root exudate of sunflower (*Helianthus*) did not decrease for four days after decapitation (Kende and Sitton 1967). The appearance of cytokinins in xylem sap identical to cytokinins in root exucate further indicates that the root is the primary source of cytokinin biosynthesis (Skene 1970).

Buds

Cytokinin activity in apple (*Malus*) xylem sap first appears at bud burst and is maximum at blossom time (Luckwill and Whyte 1968). Alvium et al. (1976) found highest cytokinin levels in willow (*Salix*) at flower bud swelling and again at leaf bud burst. Phillips and Cleland (1972) extracted more cytokinins from phloem sap in flowering plants than in vegetative plants. The concentration of cytokinins in xylem sap of flowering *Perilla* plants was five times that of vegetative plants (Beever and Woolhouse 1973).

Cytokinin activity in roots and stems of sugar maple (*Acer saccharum*) rises in early spring following a resurgence of root growth (Dumbroff and Brown 1976). Henson and Wareing (1977b) demonstrated that detached *Xanthium* buds were unable to maintain the cytokinin levels that occur in intact plants. Van Staden and Brown (1978) regard buds as a site of conversion rather than one of synthesis. When required, cytokinin glucosides are transported up to the lateral buds for hydrolysis by meristematic tissue. These observations suggest that

cytokinins are synthesized in roots and then become available to support bud growth.

There is evidence, however, that root-produced cytokinins are not involved directly with the induction of bud burst, and that buds are sites of cytokinin biosynthesis (Kannangara and Booth 1974). In perennial grasses, root growth does not occur until after initial leaf production commences (McKendrick et al. 1975). Cytokinin levels in buds of excised twigs that lack roots increased at the time of natural bud burst (Hewett and Wareing 1973a). Kung-Woo Lee et al. (1974) determined that the cytokinin required for lateral pea bud development is synthesized in the bud itself. By grafting different apple varieties, Luckwill and Whyte (1968) discovered that the character of the scion, not the stock, determined time of bud burst, indicating that bud burst was not a root controlled phenonmenon. Thus, the physiological significance of increased cytokinins in xylem sap at bud burst may be to either induce the rapid growth that follows or to overcome the inhibitor effects of abscisic acid (Alvim et al. 1976).

Leaves

Cytokinin activity in plant leaves suggests that they too may be a site of biosyntheses. Detached sunflower leaves placed in a complete mineral nutrient solution can produce active cytokinins (Wareing et al. 1977). Alvim et al. (1976) found an increasing cytokinin content in willow leaves was not due to xylem transportation. Translocation of cytokinins to the leaves via phloem, however, could account for that increase. Girdling of willow stems caused a fall in the leaf cytokinin content and an accumulation of cytokinin activity below the ring, indicating that leaf cytokinins are not synthesized de novo but

originate in the root (Van Staden and Brown 1977). The rapid decline of cytokinin activity in detached leaves further indicates that intact leaves are dependent on imported cytokinins (Henson and Wareing 1977a).

Leaf senescence is likely brought about by a decrease in the level of endogenous cytokinins. Highest levels and greatest cytokinin diversity occur in young, expanding leaves, and as leaves mature and senesce, the number and level of cytokinins decrease (Hewett and Wareing 1973b). But in *Ginkgo* (Van Staden 1976) and willow (Van Staden 1977) the lowest cytokinin level was in expanding leaves and the concentration increased over the growing season. Ilan and Goren (1979) also detected more cytokinin activity in mature and senescing lemon (*Citrus limon*) leaves than in young leaves. Cytokinin utilization in cell enlargement could be responsible for the low cytokinin levels in developing leaves at a time when the highest levels are being transported.

Stems

If all adventitious roots are removed, the shoot apex of asparagus (*Asparagus officinalis*) can synthesize a small amount of cytokinins, but the yield is less than one-sixth of that produced by the root tips (Kodo and Okazawa 1980). The authors suggest that the cytokinin producing capacity, which is inherent in all plant embryonic cells, is gradually monopolized by the root tip during normal plant development, and removing the roots restores cytokinin synthesis to the shoot apex.

Seeds and Fruits

Peterson and Fletcher (1973) found fruit and seed development in many plants that had all roots removed. Pea seeds excised after anthesis and grown in vitro, independently produced cytokinins and

developed to maturity (Hahn et al. 1974). Parthenocarpic tomato (*Lycopersicum esculentum*) fruits have retarded development and very low cytokinin levels compared with seeded fruits (Varga and Bruinsma 1974). Letham and Williams (1969) concluded that the dividing tissue of the seed is the principal source of cytokinins in apple fruit.

Developing fruits are rich in cytokinins. Maximum cytokinin activity in xylem sap occurs at the time of rapid fruit growth (Beever and Woolhouse 1973). In apple and plum (*Prunus*) extracts, Letham (1963b) found cytokinin activity peaked during the period of rapid cell division following fertilization, and then declined before the cessation of division. But cytokinins detected in fruit are not necessarily synthesized there. Fruit development creates a new center of intense meristematic activity that acts as a strong sink for metabolites. Davey and Van Staden (1978) found cytokinins present in fruits that originated from other regions of the plant. The source of cytokinins in developing fruits of many plants appears to be the root system (Beever and Woolhouse 1973).

OCCURRENCES IN OTHER ORGANISMS

Free cytokinins are found in organisms other than higher plants. The pathogen, *Corynebacterium fascians*, can synthesize the cytokinin 2iP that releases lateral buds from apical dominance, producing a witches broom in infected trees (Thimann and Sachs 1966). Crown gall formation is caused by the release of 2iP from the causal organism, *Agrobacterium tumefaciens* (Upper et al. 1970). Phillips and Torrey (1970) isolated a cytokinin from *Rhizobium japonicum* that induces nodule formation in pea roots. Tien et al. (1979) found that *Azospirillum brasilense* (formerly *Spirillum lipoferum*), a nitrogen-fixing bacterium

in the rhizosphere of various grass species, produced cytokinin, gibberellin, and indole acetic acid. Some species in the plant genus *Ardisia* have lost the ability to produce cytokinins, but have bacterial symbionts which live intercellularly and provide the host plant with needed cytokinins (Pereira et al. 1972).

Mycorrhizal fungi produce cytokinins that cause enlargement of root cortical cells (Miller 1967). Rust fungi produce cytokinins that not only retard leaf senescence around the infection site with formation of "green islands", but also mobilizes nutrients into the area of infection (Pozsar and Kiraly 1966). Powdery mildew (*Erysiphe graminis*) produces cytokinins that form "green islands" and inhibit root development in infected barley (*Hordeum vulgare*) plants (Vizarova 1974).

Cytokinins have been detected in many species of marine algae. Pedersen (1973) found that sea water surrounding brown algae (*Fucus*) contained varying levels of cytokinins. Part of the highly stimulating effect of sea water on algae growth could thus depend on its content of cytokinins. Low concentrations of commercial seaweed extract has promoted greater growth than kinetin in tissue cultures (Brain et al. 1973).

Some insects also have the ability to synthesize cytokinins. Mining caterpillars (*Stigmella*) produce cytokinins that are responsible for "green islands" in senescing tree leaves (Engelbrecht et al. 1969). Cynipid wasp larvae have vestigial mouthparts but secrete cytokinins that stimulate surrounding leaf cells into forming highly specialized galls (Van Staden 1975).

FACTORS AFFECTING ENDOGENOUS CYTOKININ LEVELS

Nutrition

The nutritional status of plants has a profound effect on levels of endogenous phytohormones. Cytokinins are nitrogen-containing compounds whose production is inevitably linked with nitrogen metabolism of the plant. Endogenous cytokinin levels in birch (*Betula pendula*) leaves significantly dropped when seedlings were transferred to nitrogen deficient conditions (Horgan and Wareing 1980). Wagner and Michael (1971) found a sharp decrease in cytokinin activity in xylem sap of sunflower plants deprived of nitrogen, and renewed cytokinin activity when nitrogen was restored. Nitrate fertilizer causes a substantial increase of zeatin in rice (*Oryza sativa*) leaves at a time when cytokinin levels are normally decreasing (Yoshida and Oritani 1974). Although nitrate increases cytokinin activity in sunflower, ammonium or ammonium nitrate does not markedly affect the cytokinin level (Salama and Wareing 1979).

Because of its role in nucleic acid structure, phosphorus deficiencies also reduce cytokinin activity. Menary and Van Staden (1976) showed that phosphorus starvation reduced cytokinin activity in tomato plants. Increasing phosphorus levels, up to a point, stimulates cytokinin activity in sycamore (*Platanus*) (Dhillon 1978). A deficiency in other essential nutrients may also reduce cytokinin levels. Their effect may be direct by affecting other phytohormones that interact with cytokinins, or it may be indirect by inhibiting root growth or impairing photosynthate transport.

Moisture

Leaf and root cytokinin activity is rapidly reduced in plants

suffering from water stress (Itai and Vaadia 1971). When water stress is relieved, the cytokinin level in xylem sap increases (Browning 1973). Increasing levels of abscisic acid (ABA) have been detected in leaves (Wright and Hiron 1969) and in phloem sap (Hoad 1973) after drought, indicating that ABA may be the hormonal signal responsible for supressing cytokinin production and transport. Waterlogging induces similar physiological changes in a plant as drought stress. Flooding reduces cytokinin and gibberellin levels in xylem sap, and increases auxin, ethylene, and ABA levels (Goodwin et al. 1978).

Temperature

Changes in root temperature affect cytokinin levels in xylem sap. In maize roots, export of cytokinins and gibberellins were greatest, and levels of inhibitors lowest, at the optimum temperature for shoot growth (Atkin et al. 1973). Decreasing root temperatures increased the export of inhibitors and lowered the cytokinin content. Menhenett and Wareing (1975) also concluded that low root temperatures affect the supply of hormones in tomato sap.

High temperatures also decrease endogenous hormone levels. Cytokinin activity in leaves is markedly reduced when subjected to heat stress (Ben-Zioni and Itai 1975). Itai et al. (1973) found that heat treatments at 46 C for two minutes on bean and tobacco roots decreased cytokinin and increased ABA levels. In ungrafted apple rootstocks, high root temperatures suppressed growth and reduced cytokinin content in both roots and leaves (Gur et al. 1972).

Light

Light quality and photoperiod markedly alter cytokinin activity in plant tissues. Cytokinin levels in attached *Populus* leaves has a

diurnal cycle, with levels declining through the night and rising to peak at dawn, followed by a further decline and slow rise during the course of the day (Hewett and Wareing 1973c). Treatment with red light causes a pronounced increase in the level of cytokinin riboside (Thompson et al. 1975). Red light also causes rapid increases in endogenous cytokinin levels in light-requiring *Rumex* seeds (Van Staden and Wareing 1972). The action of phytochrome requires a supply of cytokinin, and both are interdependent even though they interact through different modes (Feierabend 1969a).

TRANSPORT THROUGHOUT THE PLANT

Changes in levels of cytokinins in plant parts indicate active translocation. But exogenous cytokinins on the surface of leaves do not move appreciably from the site of application (Skoog et al. 1965). Sachs and Thimann (1964) noted that kinetin stimulated growth only when applied directly to lateral buds and not to neighboring tissues.

Although not emphasized by them, Richmond and Lang (1957) first demonstrated that cytokinins could be translocated through cut petioles. Radioactive BA injected into severed petioles moved down the stem but not up it (Chvojka et al. 1961). This basipetal polarity was enhanced by auxin, which also exhibits basipetal movement (Black and Osborn 1965). In decapitated bean plants, exogenous cytokinins move down the stump only in the presence of IAA (Seth et al. 1966). Lagerstedt and Langston (1966) confirmed the polar movement of kinetin, but found that uptake and direction of transport depended upon plant species, age of the tissue, and concentration of auxin.

Basipetal movement of cytokinins, however, may not be a general phenomenon. Most studies showing basipetal transport were performed

with excised segments. Letham (1978) theorized that reported basipetal transport in cultures may be due to cytokinin concentrations greater than physiologically optimum. Fox and Weis (1965) were unable to detect any polar transport with or without auxin. Pilet (1968) demonstrated that while no polarity of cytokinin transport occurred in vitro, a highly acropetal polarized transport was evident in vivo. In intact bean plants, no basipetal translocation of BA occurred, but BA was easily absorbed by the roots and translocated acropetally (Ramina et al. 1979).

Lagerstedt and Langston (1967a) found that kinetin moved distal in the vascular system if applied directly over a main vein. Entry of cytokinins into the transpiration stream results in acropetal movement. The passive transportation of cytokinins from root tips to other parts of the plant is well documented (Kende 1964; Mozes and Altman 1977). Thus, changes in the levels of cytokinins in xylem sap and factors that affect the ascent of sap, would have a profound effect on shoot development and physiology.

Cytokinins also have been discovered in phloem (Kursanov 1963). Aphids feeding on phloem sap of *Xanthium* plants excrete honeydew containing cytokinins (Phillips and Cleland 1972). Accumulation of cytokinins in the bark below a girdle in willow stems reflected phloem transport from the roots (Van Staden and Brown 1978). Cytokinin content of grape (*Vitis*) vines decreased after girdling (Skene 1972), further indicating that xylem was not the primary transporter of cytokinins.

But preferential movement of BA may be in response to sink activity regardless of the direction. Gersani et al. (1980) noted that BA moves and is unloaded into the appropriate sink, and not in any given direction. They concluded that sinks which develop in response to

cytokinins may have a requirement for the hormone.

Zeatin riboside is the major form of cytokinin translocated in xylem sap of intact radish seedlings (Gorden et al. 1974). Davey and Van Staden (1976) observed fluctuations in concentrations of zeatin and zeatin riboside translocated from tomato roots. They suggested that individual cytokinins may undergo phasic changes in order to initiate a particular developmental response in the shoot.

PHYSIOLOGICAL ROLES

Cell Division and Growth

The ability to promote cell division in tissue cultures led to the discovery of cytokinins as a distinct class of phytohormones. Bioassays measuring cell proliferation became the standard technique to estimate cytokinin concentrations (Miller et al. 1956). In the presence of exogenous cytokinins, callus tissue develops by division of polyploid parenchyma cells (Torrey 1961). Although cytokinins, by definition, promote cell division in tissue cultures, they may not fulfill the same function in vivo. There is a correlation between cytokinin content and the rate of cell division in meristematic tissue (Goldacre 1959), but high cytokinin levels may be due to de novo synthesis rather than stimulating cytokinesis.

Cytokinins also promote cell enlargement (Miller 1956). Growth from kinetin in leaf discs is due to cell expansion, not cell enlongation, and this response is enhanced with IAA (Kuraishi and Okhumura 1956). Kinetin suppresses auxin-stimulated cell enlongation in pea stems, but increases segment thickness due to cell expansion (Katsumi 1962). Exogenous cytokinin applied to intact bean plants increases leaf area by extending the period of leaf expansion (Jacoby and

Dagan 1970). This stimulatory action on leaf expansion is the basis for various bioassays to quantitatively measure cytokinin concentration (Kuraishi 1959).

A ratio-specific interaction of cytokinins and auxins control morphogenesis in cultured tobacco callus tissue; high cytokinin to auxin ratios induce shoot formation, and low cytokinin to auxin ratios produce roots (Skoog and Miller 1957). No cell division occurs with cytokinins in the absence of auxin. Increasing the auxin level increases the level of cytokinin required to achieve a particular response (Skoog et al. 1967). Thus, the physiological basis for the control of meristematic differentiation is the balance between cytokinin and auxin levels, as well as other hormones, metabolites, and nutrients that affect their activity.

Root growth is ordinarily inhibited by exogenous cytokinins (Kaminek 1967). Johnston and Jeffcoat (1977) found that root applications of 1, 5, and 25 ppm cytokinin on cereal seedlings arrested root growth and caused necrotic lesions on the leaves. Wittwer and Dedolph (1963) reported that any concentration of cytokinins applied to leaves of intact plants inhibited root growth. Low concentrations of cytokinins, however, stimulate root initiation (Torrey 1962). Meredith et al. (1970) doubled the rooting of terminal *Feijoa* cuttings with 0.1 ppm exogenous cytokinin. Auxin and cytokinin concentration gradients along the longitudinal axis of roots are responsible for regulating the initiation of lateral roots (Bonnett and Torrey 1965).

Cytokinins normally inhibit elongation of isolated stem segments (Fox 1964). Applied to etiolated peas, kinetin temporarily enhances ethylene production, and inhibits shoot growth (Fuchs and Lieberman 1968).

In intact green plants, exogenous cytokinin inhibits stem growth (Wittwer and Dedolph 1963). But low concentrations of cytokinins promote stem growth in stressed plants (Railton and Reid 1973).

Stem swelling is often stimulated with cytokinins. In nutsedge (*Cyperus*), cytokinins promote tuberization in darkness by substituting for light (Chetram and Bendixen 1974). Kinetin-treated potato stolons induce immediate starch activation preceding tuber formation (Smith and Palmer 1970). In most plants, tuber formation is associated with high cytokinin and low gibberellin levels (Booth and Lovell 1972).

Lateral Bud Development

Cytokinins release lateral buds from apical dominance. Axillary buds are regulated by a correlative interaction between auxins produced in the apex and cytokinins synthesized in roots. Exogenous kinetin overcomes the IAA inhibition and promotes lateral bud development (Wickson and Thimann 1958). Even in highly dominant plants, cytokinins applied to lateral buds antagonize apical dominance (Sachs and Thimann 1964). Woolley and Wareing (1972) suggest that gibberellin is also involved by antagonizing the action of cytokinin in the initial release of axillary buds, but then promoting lateral shoot growth once cytokinin-induced release is achieved.

Cytokinins supplied to either the whole plant or to individual buds, can stimulate growth of repressed tillers in various grass species (Jewiss 1972). At any developmental stage in wheat, 6 ppm kinetin promoted tiller bud elongation (Langer et al. 1973). Johnston and Jeffcoat (1977) concluded that in Gramineae, cytokinins stimulate growth of axillary tillers by diverting assimilates to the bud. However, the response is small if other metabolites are limiting (Sharif

and Dale 1980).

Bud dormancy can sometimes be terminated with exogenous cytokinins. BA applied to dormant *Opuntia* lateral buds activates the meristem and causes it to develop leaves instead of spines (Mauseth 1976). Exogenous cytokinin also releases grape buds (Weaver 1963), and potato tuber buds (Hemberg 1970) from dormancy. Weinberger (1969) reported that 200 ppm cytokinin terminates bud rest in peach (*Prunus*), but only after the chilling requirement is partially satisfied.

Seed Production

Cytokinins are involved in the ripening process of grain since they can influence filling period length and dry matter accumulation (Seth and Wareing 1967). Applying kinetin during the seed filling period increased barley grain yield 10% (Barnsley 1964). Higher individual kernel weight and greater production from smaller tillers, increased barley yields 57% after pre-heading applications of BA (Williams and Cartwright 1980). Ruckenbauer and Kirby (1973) sprayed 20 ppm kinetin on barley and observed a larger main shoot with longer ears bearing more grains. In rice, 100 ppm kinetin applied at any growth stage substantially increased grain yield over untreated plants (Biswas and Choudhuri 1977).

In wheat, a decrease in ribulose bisphosphate carboxylase (RuBPCase) during flag leaf senescence corresponds with grain filling (Hall et al. 1978). That may be a significant factor in limiting crop yield. However, cytokinins applied to wheat ears before anthesis did not affect grain dry weight or number of grains per ear, indicating that endogenous cytokinins in the ear are adequate for normal growth (Wheeler 1976).

Transpiration

Cytokinins stimulate stomatal opening and hence increase the transpiration rate of leaves (Livne and Vaadia 1965). That effect was first noticed when Kemp et al. (1957) observed kinetin-treated plants wilted more than untreated plants on hot days. Tal et al. (1970) concluded that excess cytokinin activity in a tomato mutant was responsible for its resistance to stomata closure and the resultant proneness to wilt. In the grass species *Anthephora pubescens*, Incoll and Whitelam (1977) separated the epidermis from the mesophyll and noticed a 70% increase in transpiration when kinetin was added. A wide range of synthetic and natural-occurring cytokinins also promote in vivo stomatal opening in the same species (Jewer and Incoll 1980).

Low concentrations of BA increased transpiration 112% over nontreated tomato plants, but high concentrations of BA decreased transpiration because of higher root resistance to water uptake (Tal and Imber 1971). In leaves with few stomata, cytokinins have a more profound effect on total transpiration than on species with a large number of stomata (Kuraishi 1976). The increased transpiration from cytokinins is the basis for a rapid bioassay that is 10 times more sensitive than chlorophyll retention tests (Luke and Freeman 1967).

There are diverse hypotheses for the mode of action of cytokinins in promoting stomatal opening. Pallas and Box (1970) suggest that cytokinins affect stomatal aperture by decreasing turgor of epidermal and mesophyll cells causing the guard cells to have an osmotic advantage and swell open. Since cytokinins stimulate absorption of potassium ions (Ilan 1971), the guard cells could selectively receive an influx of K^+ that would maintain their turgor. Meidner (1967)

concluded that cytokinin effect on stomatal opening was a consequence of decreased carbon dioxide concentration in the vicinity of the aperture.

Respiration

The climacteric rise in respiration associated with leaf senescence is inhibited by cytokinins. Reduced respiration of cytokinin-treated *Brassica* leaves is correlated with decreased hexokinase and pyruvic kinase activity (Tuli et al. 1964). Inhibiting pyruvic kinase would repress entry of pyruvate into the Krebs cycle and thus reduce respiration (Moore and Miller 1972). Although both phosphoglucoisomerase and glucose-6-phosphate dehydrogenase decrease in the presence of kinetin, Simpkins and Street (1970) found that kinetin increased the content of glucose and reduced the content of galactose and xylose in extracellular hemicellulose, indicating that the inhibition of respiration by kinetin was due to supression of glucose conversion to other sugars. Shaw et al. (1965) concluded that kinetin inhibits oxygen uptake by preserving the integrity of the mitochrondria, and thus preventing oxidative phosphorylation uncoupling.

The net effect of reduced respiration from exogenous cytokinins is senescence deferral. Applying 1 ppm BA on broccoli (*Brassica*) after harvest reduced respiration and retarded leaf senescence (MacLean et al. 1963). Suppressing respiration with BA preserves freshness and prolongs shelf life in celery (*Apium*) stalks (Wittwer et al. 1962) and strawberry (*Fragaria*) fruits (Dayawon and Shutak 1967). However, after observing that BA retarded the respiration but not the senescence of daffodil (*Narcissus*) flowers, Ballantyne (1966) concluded that the

cytokinin-induced decrease in respiration is not important in delaying tissue senescence.

Membrane Permeability

Cytokinins have the ability to influence membrane permeability by selectively affecting ion uptake. Cells actively absorb potassium and rubidium in the presence of kinetin (Ilan et al. 1971), but sodium uptake is inhibited (Jacoby and Dagan 1970). Ilan (1971) suggests that cytokinins activate a cation pump that carries potassium and sodium in opposite directions. Cytokinins also stimulate an influx of calcium ions that delay cell senescence by maintaining membrane integrity (Lau and Yang 1975). Exogenous calcium inhibits membrane protein phosphorylation, suggesting that cytokinins might act, at least in part, by increasing the availability of calcium ions (Ralph et al. 1976). After finding no change in the lipid bilayer, Feng (1973) suggested that kinetin affects the permeability of substances passing through an "aqueous" channel of proteins in the membrane.

In senescing leaf discs, 10 ppm kinetin delayed the onset of permeability changes, but auxin was more effective in maintaining membrane integrity (Sacher 1967). Richmond et al. (1971) felt that the major effect of kinetin in senescence was delaying permeability changes in chloroplast membranes.

Cytokinins also stimulate lipid synthesis. Releasing *Cicer* axillary buds from apical dominance with cytokinins caused a rapid increase in total lipid levels (Usciati et al. 1974). Cytokinins enhanced methylation of neutral and polar lipids, especially phosphatidylcholine, in dormant tobacco buds (Schaeffer and Sharp 1971). Additionally, cytokinins caused a significant reduction of

lipoxygenase activity in intact pea plants (Grossman and Leshem 1978). That enzyme catalyzes oxidation of unsaturated fatty acids, which are major constituents in the phospholipid component of the plant membrane (Simon 1974). Cytokinins mimic the endogenous antioxidant, α-tocopherol, in the depression of lipoxygenase activity (Leshem et al. 1979). Maintaining membrane integrity by depressing lipoxygenase levels with cytokinins would thus contribute to retarding senescence.

Mobilization

Mothes and Engelbrecht (1961) first observed that localized kinetin treatments on detached tobacco leaves mobilized metabolites from surrounding, untreated tissue. In addition to amino acids, the cytokinin-treated locus creates a sink in excised leaves that attracts C^{14}-labelled photosynthate (Gunning and Barkley 1963), phosphorus (Muller and Leopold 1966a), and auxin (Langerstedt and Langston 1967b). But labbelled Na, Rb, I, and Cl are not drawn toward kinetin-treated areas (Muller and Leopold 1966b). Mobilization requires a cytokinin locus to serve as a sink. If all cells receive cytokinin treatment, then they all will have improved ability to attract metabolites from a source and mobilization will stop (Muller and Brautigam 1973).

Draining nutrients from other parts of the leaf retards senescence in the cytokinin-rich locus, but accelerates senescence in the untreated parts (Leopold and Kawase 1964). However, McHale and Dove (1968) maintain that cytokinins operate directly on treated areas, and do not depend upon accumulation of metabolites from untreated tissue to exert their effect. Von Abrams and Pratt (1967) found that increased mobilization and accumulation of materials in cytokinin-treated areas did not delay senescence in detached leaves. They suggest that

senescence is an autonomous event, and that increased metabolite retention is a secondary response to cytokinins.

In decapitated pea stems, kinetin does not induce transport of labelled phosphorus, but exogenous auxin does (Davies and Wareing 1965). Seth and Wareing (1967) showed that either kinetin or auxin alone does not enhance isotope movement, but cytokinins mixed with IAA produced a synergistic mobilization. Thus, detached leaves evidently contain sufficient amounts of endogenous auxin for exogenous cytokinins to produce the mobilizing effect.

Mobilization of nutrients in intact plants is a more intricate phenomenon than in detached leaves. Kulaeva (1962) observed no mobilization when one-half of an attached leaf was treated with kinetin, and thus concluded that cytokinins have no effect on intact plants. Applying BA to primary leaves of intact bean plants did not attract labelled phosphorus and sucrose from other leaves (Adedipe and Fletcher 1970). In attached corn leaves, kinetin did not cause mobilization or accumulation of phosphorus isotope (Muller and Leopold 1966a). The lack of directed transport in the intact plant may be due to competing parts. Engelbrecht and Mothes (1961) noted that radioactive α-amino-isobutyric acid applied to a tobacco leaf accumulated in the root tips, but kinetin added to the leaf prevented this translocation.

Quinlan and Weaver (1969) reported that BA on intact grape leaves attracted labelled carbon from lower leaves, but mobilization was increased if the competition from other sinks was diminished by darkening the leaves. If cytokinins are applied to the roots of 10-day old oat seedlings, a strong sink is created and a "reverse mothes effect" occurs in which senescence of the leaves is promoted (Thimann

et al. 1974). In the intact plant, roots and buds are strong mobilizing sinks that accumulate metabolites (Adedipe and Fletcher 1971). The absence of potent sinks in detached leaves would thus explain mobilization to cytokinin-treated areas.

Metabolites are mobilized short distances to the cytokinin locus by cell-to-cell movement from surrounding areas against a concentration gradient (Skoog and Schmitz 1972). But exogenous cytokinin can also direct movement through the vascular tissue (Gunning and Barkley 1963). In detached corn leaves, kinetin-induced transport occurs in the axial direction through the pholem (Muller and Leopold 1966b). Other non-metabolites would be swept along to the locus by mass flow.

The antisenescent effect of cytokinins in intact plants are probably independent of mobilization of metabolites from untreated areas. Cytokinins delay senescence by preventing the effluent translocation of sugars and amino acids rather than mobilizing their immigration (Fletcher et al. 1970). Adedipe and Fletcher (1971) concluded that retarding leaf senesecence by BA is not dependent on mobilization, but is accomplished by metabolic self-sustenance associated with high retention of photosynthate.

Photosynthate

Cytokinins increase the net photosynthetic activity in leaves. Adedipe et al. (1971) found that BA increased photosynthate in intact leaves, which was responsible for retarding leaf senescence. Kinetin significantly increased photosynthetic rate in maize seedlings by stimulating the regulatory enzymes of photosynthetic carbon reduction (Wareing et al. 1968). Activity of the Calvin cycle enzymes, ribulose bisphosphate carboxylase (RuBPCase) and glyceraldehyde-3-phosphate

dehydrogenase, are both increased by cytokinins (Feierabend 1969b). Harvey et al. (1974) concluded that BA increases RuBPCase activity by stimulating its synthesis, but high glyceraldehyde-3-phosphate dehydrogenase activity is due to either enzyme activation or stabilization. Since RuBPCase is synthesized by the ribosomes of developing plastids (Ireland and Bradbeer 1971), it is directly interrelated with chloroplast synthesis.

Cytokinins also increase starch assimilation independent of its effect on carbohydrate metabolism. Mittelheuser and Van Steveninck (1972) found a massive increase in both size and number of starch grains after kinetin treatment to detached wheat leaves. In intact bean leaves treated with BA, starch assimilation was 100% higher after six weeks than in untreated leaves (Fletcher and Adedipe 1972). Tasseron-De Jong and Veldstra (1971) concluded that BA has a favorable effect on starch accumulation not connected with its effect on growth rate.

Chlorophyll

Cytokinins affect chlorophyll production in a variety of ways. They induce production of aminolevulinic acid dehydratase, the rate limiting enzyme in chlorophyll biosynthesis (Fletcher et al. 1973). Kinetin treatment lowers the level of chlorophyllase, the enzyme responsible for the degradation of chlorophyll, in detached barley leaves, and prevents its rise in detached oat leaves (Sabater and Rodriquez 1978). Cytokinins also are necessary for the organization of stroma lamellae into functional grana (Stetler and Laetsch 1965). Kinetin induced an increase in the amount of endoplasmic reticulum and ribosomes, and delayed the degeneration of cellular fine structure (Shaw and Manocha 1965). In senescent leaves, kinetin stimulated the

formation of grana and delayed the loss of chloroplast ribosomes (Mlodzianowski and Kwintkiewicz 1973). Mittelheuser and Van Steveninck (1972) reported kinetin maintained chloroplast ribosomes when other organelles were showing signs of advanced senescence.

The effect of cytokinins on chloroplast development often depends upon the physiological state of the tissue. In young leaves, exogenous cytokinins were not effective in promoting chloroplast formation (Alberte and Naylor 1975). Dennis et al. (1967) found that BA accelerates the senescence process if applied to young expanding leaves. They concluded that adding cytokinins at a time when endogenous levels are high apparently over-stimulates the chloroplast, causing enlargement and excessive membrane synthesis, resulting in breakdown. Jacoby and Dagan (1970) found that BA on young intact bean leaves initially lowered chlorophyll content, but net chlorophyll synthesis continued when mature untreated leaves were senescing. Fletcher (1969) reported that a single application of BA was effective in preventing chlorophyll loss in intact bean leaves irrespective of leaf age, and that repeated applications increased chlorophyll content progressively. Naito et al. (1978) also concluded that continuous BA applications beginning at the early stage keeps bean leaves young and able to respond to the hormone even at later stages.

In mature leaves, cytokinins can either promote chloroplast development or delay its degradation. Sugiura (1963) found that cytokinins promoted chlorophyll synthesis in detached bean leaves. In excised *Brassica* leaves, kinetin increased chlorophyll content (Mlodzianowski and Kwintkiewicz 1973). Kursanov et al. (1964) observed chlorophyll restoration in detached yellow leaves after treatment with

exogenous cytokinins. Applying BA to intact bean leaves enhanced or maintained the chlorophyll-synthesizing capacity (Adedipe et al. 1971).

In contrast, most reports indicate that cytokinins only delay chlorophyll degradation. Richmond and Lang (1957) initially observed that kinetin delayed chlorophyll loss in detached leaves. Since that time numerous studies have documented the ability of cytokinins to prevent chlorosis in leaves. Postharvest applications of BA has maintained high chlorophyll content in lettuce, asparagus, cabbage (*Brassica oleracea capitata*), and a wide variety of other vegetables (Weaver 1972).

The ability to retard chlorophyll loss is the basis for bioassays measuring cytokinin activity (Letham 1967a). Although these tests are rapid, they are ineffective in measuring naturally occurring cytokinins in plant extracts (Varga and Bruinsma 1973). Additionally, cytokinin analogs offer varying degrees of effectiveness in chlorophyll retention on different tissues (Kuhnle et al. 1977). In detached cereal leaves, kinetin is more active than BA in retarding chlorophyll loss, but in legume leaves, the order of activity is reversed (Mishra and Misra 1973).

Leaf-yellowing is considered the universal symptom of senescence, and the antisenescent effect of cytokinins may be dependent upon its ability to prevent chlorophyll loss. Srivastava (1963) demonstrated that cytokinin treatments have no effect in retarding senescence in albino barley leaves. The retention of chloroplast genomes in yellow leaves is the major factor in determining their ability to re-green following cytokinin treatment (Dyer and Osborne 1971). In oat leaves, once chlorophyll loss begins, there is no restitution by kinetin, and the normal sequential events of senescence proceed (Tetley and Themann 1974).

Hall et al. (1978) reported that chlorophyll content in the intact flag leaf of wheat plants decreased by more than 50% before any ribulose bisphosphate carboxylase (RuBPCase) protein loss occurred. But in senescing barley leaves, chlorophyll breakdown does not begin until after RuBPCase loss and the concomitant decline in photosynthesis (Friedrich and Huffaker 1980). In rice, the majority of leaf nitrogen is concentrated in chloroplasts, and accounts for most of the protein translocated during senescence (Morita 1980). Spencer and Titus (1973) observed that in excised leaves, chlorophyll loss is the first indication of senescence proceeded by protein loss, but in attached leaves protein decline precedes chlorophyll loss. Thomas and Stoddart (1975) concluded that chlorophyll loss is not an obligatory part of the senescence process.

Protein

Richmond and Lang (1957) originally demonstrated that the protein level in kinetin-treated detached leaves declined more slowly than in untreated leaves. Guttman (1957) showed that kinetin caused a rapid increase in the amount of RNA in excised tissues. The enhancement of protein content in *Rhoeo* leaf sections was dependent on kinetin-induced synthesis in all fractions of RNA (Sacher 1968). Sugiura et al. (1962) found that kinetin increased incorporation of adenine into ribosomal RNA, and caused a net increase in RNA and protein synthesis. After finding that kinetin promoted the incorporation of labelled amino acids into nucleic acids, Osborne (1962) concluded that the antisenescent effect of kinetin was due to stimulating RNA and protein synthesis.

But, incorporating amino acids into proteins or measuring total amount of nucleic acid and proteins after cytokinin treatments may not

be reliable measures of protein synthesis (Shibaoka and Thimann 1970). Chibnall and Wiltshire (1954) observed that detached leaves do not lose the ability to synthesize proteins even in the absence of a hormonal stimulus. Hardwick and Woolhouse (1968) showed conclusively that the cut edge of a leaf disc is the main site for uptake of labelled substances, thus negating any firm conclusions concerning rates of protein syntheses. Tavares and Kende (1970) maintain that only if protein breakdown is negligible and the size of the protein precursor pool does not change, can amino acid incorporation estimate protein synthesis.

In detached *Tropoeolum* leaves, kinetin decreases protein degradation rather than increase protein systhesis (Mizrahi et al. 1970). The action of cytokinins in *Brassica* leaf discs (Kuraishi 1968), duckweed (*Lemna*) (Trewavas 1972), corn tissue (Tavares and Kende 1970), and wheat leaves (Tung and Brady 1972), all indicate that senescence is retarded by inhibiting protein breakdown and not by stimulating protein synthesis.

Cytokinins delay protein breakdown by depressing the level of free amino acids and hydrolytic enzymes. The content of soluble α-amino nitrogen, indicative of free amino acids, increases in detached leaves, but is inhibited by kinetin treatment (Anderson and Rowan 1966). Cytokinins arrested the general decline in total and soluble protein in detached leaves by inhibiting protease activity (Kar and Mishra 1977). Atkin and Srivastava (1969) found that kinetin prevents an increase in the activity of protease and RNase. In both light and dark, kinetin depressed RNase in wheat leaves but did not change RNase activity in barley (Sodek and Wright 1969). Catalase, which is positively correlated with the decline of chlorophyll during senescence, decreases with kinetin treatment (Kar and Mishra 1976). The net effect of

depressing these oxidative enzymes with cytokinins is senescence deferral.

Shibaoka and Thimann (1970) believed that the primary effect of kinetin on preventing senescence in detached leaves was to inhibit proteolysis. The dual action of kinetin in preventing proteolysis and preserving chlorophyll is antagonized by L-serine, which serves as the active center of many proteinlytic enzymes (Martin and Thimann 1972). Wittenbach (1978) observed that the major proteinases have cysteine at the active site and exhibit a high affinity for ribulose bisphosphate carboxylase (RuBPCase) in comparison with other soluble proteins.

Although protease is an integral part of senescence, its activity is not related to the onset or rate of senescence (Beevers 1968). Wittenbach (1978) reported a decline in soluble protein, but no increase in proteolylic activity during the first stage of wheat senescence. Frith et al. (1975) reported that the high specific activity of acid proteinase in senescing tissue was predominantly due to a lower protein content rather than increased proteolytic activity. Kar and Mishra (1977) found protease activity increased very slowly in detached leaves, with or without kinetin.

Most studies of cytokinin effect on hydrolytic enzymes have used excised leaves or leaf discs. But the senescent process of leaves removed from plants may not be the same as in attached leaves. Spencer and Titus (1973) reported that leaf discs accumulate amino acids during senescence, while attached leaves lose amino acids. Unlike the case for excised leaves, cytokinin raised the activities of RNase, DNase, and protease in intact bean leaves (Naito et al. 1979). Fletcher (1969) also showed that RNase activity was higher in intact leaves treated

with BA than in untreated leaves. This increase in hydrolase activities would reduce amino acid levels, and so cytokinins may simultaneously stimulate synthesis in order to maintain protein content.

Regulating RuBPCase plays a key role in controlling plant senescence. During the reversible stage of wheat senescence, RuBPCase accounted for 80% of the total loss of soluble protein (Wittenbach 1978). Peterson and Huffaker (1975) reported that kinetin delays senescence in detached barley leaves by inhibiting RuBPCase breakdown. Wittenbach (1977) observed that kinetin reduces RuBPCase loss in intact wheat seedlings and maintains the tissues capacity for protein recovery.

Nitrogen Metabolism

In addition to its role in nucleic acids and proteins, cytokinins have a direct effect in other aspects of nitrogen metabolism. Spraying BA on seedlings subject to nitrogen deficiency resulted in a rapid leaf regreening (Horgan and Wareing 1980). Exogenous cytokinins, however, do not act as a non-specific nitrogen source to the plant (Fletcher 1969).

Dilworth and Kende (1974) found that cytokinins enhance nitrate reductase activity in *Agrostemma* embryos. Cytokinins have also increased nitrate reductase activity in wheat, barley, and corn (Knypl 1979). The mechanism involved in increasing nitrate reductase is unrelated to substrate induction, since cytokinins do not affect nitrate levels or nitrite reductase (Kende et al. 1974). Rijven and Parkash (1971) suggest that kinetin operates on the transcriptional level of control in stimulating nitrate reductase. But Kende et al. (1971) found that exogenous BA induces nitrate reductase directly through mobilization of nitrate from a metabolically inactive pool.

In legumes, cytokinins play a role in nitrogen fixation by affecting the nodule development. Exogenous cytokinins released by *R. japonicum* and *R. leguminosarum* are sufficient to initiate cortical cell divisions for forming a root nodule (Phillips and Torrey 1972). In clover (*Trifolium*), low concentrations (10^{-9} M) of kinetin had no effect in nodule development, but high concentrations (10^{-8} M) inhibited nodule formation (Kefford et al. 1960). Huang (1977) found that 20 and 40 ppm kinetin inhibited nodule initiation in soybean (*Glycine max*) seedlings and decreased nitrogen fixation over 40%. He concluded that kinetin alters the concentration or function of leghaemoglobin, which alters the anaerobic condition in the nodule and thus suppresses nitrogenase activity.

Adding kinetin to the growth medium was responsible for forming nodules on excised tobacco roots (Arora et al. 1959). Cytokinins have also induced intact tomato and cucumber (*Cucumis*) roots to form pseudonodules (Wittwer and Dedolph 1963). Highest levels of cytokinin activity in alder (*Alnus*) is from zeatin glucosides prior to the recommencement of nitrogen fixation, indicating that nodule cells normally store rather than export excess cytokinins (Wheeler et al. 1979). Low levels of cytokinins stimulate pseudonodule formation in alder roots, but no nodules are formed in cytokinin-free cultures (Rodriguez-Barrueco and De Castro 1973).

SENESCENCE DEFERRAL IN THE WHOLE PLANT

Leaf senescence is a programmed process. A large part of the biochemical apparatus for senescence pre-exists in the leaf in a latent form that is inevitable activated by mRNA transcripts (Thomas and Stoddart 1977). Dyer and Osborne (1971) indicated that the pattern

of senescence prior to the first stages of leaf autolysis and dehydration is species-specific. Consequently, there is no one reliable indicator of the senescence syndrome, but some of the biochemical changes normally taking place in senescent *Festuca* leaves are: loss of total chlorophyll, total protein, Fraction 1 protein (RuBPCase) and total RNA (Thomas and Stoddart 1975). The role of growth regulators in retarding senescence may be in sustaining the metabolic state of the leaf so that some critical metabolite is prevented from inducing the latent senescence process (Thomas and Stoddart 1980).

Senescence in the whole plant is associated with endogenous phytohormone levels as affected by time, environment, and plant species. In sunflower, cytokinin content in xylem sap increases during rapid growth, but then decreases when root growth stops, suggesting that reduced cytokinin transport from roots to leaves is a significant factor contributing to shoot senescence (Sitton et al. 1967). Young, expanding leaves often do not respond to exogenous cytokinin, suggesting that non-senescing tissues have an adequate endogenous cytokinin content (Muller and Leopold 1966a). Fletcher (1969) reported that the most efficient time to apply BA for senescence deferral in intact bean plants, was after the onset of flowering when endogenous levels were declining. Richmond et al. (1971) also concluded that kinetin was most effective on bean plants if applied in the summer when endogenous cytokinin concentrations are suboptiminum.

But the mechanism for senescence deferral may not be as simplistic as substituting cytokinins when endogenous levels are low. Naito et al. (1978) surmised that the concentration of endogenous cytokinins is far below saturation level even in young, growing leaves. Lindoo and Nooden

(1978) concluded that since cytokinins delay, but do not prevent monocarpic senescence in intact soybeans, an endogenous cytokinin deficiency is probably a secondary cause of foliar senescence. Undoubtedly, cytokinins are intricate participants of a hormonal complex which regulates senescence.

Indeed, cytokinins are not universal senescence inhibitors, nor are they the only growth regulators that delay senescence. Osborne (1965) reported that auxin (2,4-D) will retard senescence in cherry (*Prunus*) leaves, but kinetin is ineffective. Exogenous gibberellins retard senescence in intact dandelion (*Taraxacum officinale*) particularly if applied when endogenous levels are low (Fletcher et al. 1969). A combination of BA and auxin (α-naphthaleneacetic acid) synergistically reacted in intact soybeans to halt leaf export of nitrogen, starch, and chlorophyll two months beyond normal senescence (Nooden et al. 1979).

Additionally, endogenous phytohormones such as abscisic acid and ethylene counteract the effects of cytokinin and promote senescence (Nooden and Leopold 1978). Thus, the physiological state of a leaf is determined by a balance among phytohormones, and the triggering of senescence is brought about by a change in the level of one of these (Ilan and Goren 1979). But, Thomas and Stoddart (1980) claim that neither a decline in endogenous levels of senescence-retarding hormones, nor increases in senescence promoters, are the primary inducers of senescence, although they may be part of a more complex induction system.

The effectiveness of exogenous cytokinin in retarding senescence in the whole plant varies according to physiological stage of development, plant species, environmental conditions, and the level of other

endogenous hormones. These factors may alter sensitivity of the plant tissue and evoke varying responses. But whether the effect is primary or secondary, exogenous cytokinins delay senescence by one or more of the following processes: inhibiting protein (primarily RuBPCase) breakdown, retarding chlorophyll loss, retaining photosynthate, preventing metabolite export, or maintaining membrane integrity.

LITERATURE CITED

Adedipe, N.O., and R.A. Fletcher. 1970. Benzyladenine-directed transport of carbon-14 and phosphorus-32 in senescing bean plants. J. Exp. Bot. 21:968-974.

Adedipe, N.O., and R.A. Fletcher. 1971. Retardation of leaf senescence by benzyladenine in bean plants is not dependent on mobilization. Can. J. Bot. 49:59-61.

Adedipe, N.O., L.A. Hunt, and R.A. Fletcher. 1971. Effects of benzyladenine on photosynthesis, growth and senescence of the bean plant. Physiol. Plant. 25:151-153.

Alberte, R.S., and A.W. Naylor. 1975. The role of cytokinins in chloroplast lamellar development. Plant Physiol. 55:1079-1081.

Alvim, R., E.W. Hewett, and P.F. Saunders. 1976. Seasonal variation in the hormone content of willow. I. Charges in abscisic acid content and cytokinin activity in the xylem sap. Plant Physiol. 57: 474-476.

Anderson, J.W., and K.S. Rowan. 1966. The effect of 6-furfurylamino-purine on senescence in tobacco-leaf tissue after harvest. Biochem. J. 98:401-404.

Armstrong, D.J., P.K. Evans, W.J. Burrows, F. Skoog, J.F. Petit, T. Stewart, J. Strominger, N.J. Leonard, S.M. Hecht, and J. Occolowitz. 1970. Cytokinins: Activity and identification in *Staphyloccus epidermidis* transfer RNA. J. Biol. Chem. 245:2922-2926.

Arora, N., F. Skoog, and O.N. Allen. 1959. Kinetin-induced pseudo-nodules on tobacco roots. Am. J. Bot. 46:610-613.

Atkin, R.K., G.E. Barton, and D.K. Robinson. 1973. Effect of root-growing temperature on growth substances in xylem exudate of *Zea mays*. J. Exp. Bot. 24:475-487.

Atkin, R.K., and B.I.S. Srivastava. 1969. The changes in soluble protein of excised barley leaves during senescence and kinetin treatment. Physiol. Plant. 22:742-750.

Ballantyne, D.J. 1966. Respiration of floral tissue of the daffodil (*Narcissus pseudonarcissus* Linn.) treated with benzyladenine and auxin. Can. J. Bot. 44:117-119.

Barnsley, G.E. 1964. N^6-benzyladenine as a senescence inhibitor. Chem. Abstr. 61:13,816.

Beever, J.E., and H.W. Woolhouse. 1973. Increased cytokinin from root system of *Perilla frutescens* and flower and fruit development. Nature New Biology 246:31-32.

Beevers, L. 1968. Growth regulator control of senescence in leaf discs of nasturtium (*Tropaeolum majus*), p. 1417-1435. *In*: F. Wightman and G. Setterfield (eds.), Biochemistry and Physiology of Plant Growth Substances. Range Press, Ottawa.

Ben-Zioni, A., and C. Itai. 1975. Preconditioning of tobacco and bean leaves to heat shock by high temperature or NaCl. Physiol. Plant. 35:80-84.

Berridge, M.V., R.K. Ralph, and D.S. Letham. 1970. The binding of kinetin to plant ribosomes. Biochem. J. 119:75-84.

Beutelmann, P. 1973. Studies on the biosynthesis of a cytokinin in callus cells of moss sporophytes. Planta 112:181-190.

Biddington, N.L., and T.H. Thomas. 1978. Influence of different cytokinins on the transpiration and senescence of excised oat leaves. Physiol. Plant. 42:369-374.

Biswas, A.K., and M.A. Choudhuri. 1977. Regulation of leaf senescence in rice by hormones sprayed at different developmental stages and its effect on yield. Indian J. Agric. Sci. 47:38-40.

Black, M.K., and D.J. Osborne. 1965. Polar transport of benzyladenine, adenine, and IAA in petiole segments of *Phaseolus vulgaris*. Plant Physiol. 40:676-680.

Bonner, J., and J. English. 1938. A chemical and physiological study of traumatin, a plant wound hormone. Plant Physiol. 13:331-348.

Bonnett, H.T., and J.G. Torrey. 1965. Chemical control of organ formation in root segments of *Convolvulus* cultured *in vitro*. Plant Physiol. 40:1228-1236.

Booth, A., and P.H. Lovell. 1972. The effect of pre-treatment with gibberellic acid on the distribution of photosynthate in intact and disbudded plants of *Solanum tuberosum* L. New Phytol. 71:795-804.

Brain, K.R., M.C. Chalopin, T.D. Turner, G. Blunden, and P.B. Wildgoose. 1973. Cytokinin activity of commercial aqueous seaweed extract. Plant Sci. Lett. 1:241-245.

Brandes, H., and H. Kende. 1968. Studies on cytokinin-controlled bud formation in moss protonemata. Plant Physiol. 43:827-837.

Browning, G. 1973. Flower bud dormancy in *Coffea arabica* L. II. Relation of cytokinins in xylem sap and flower buds to dormancy release. J. Hort. Sci. 48:297-310.

Bruce, M.I., J.A. Zwar, and N.P. Kefford. 1965. Chemical structure and plant kinin activity--the activity of urea and thiourea derivatives. Life Sci. 4:461-466.

Burrows, W.J. 1976. Mode of Action of N,N′-diphenylurea: The isolation and identification of the cytokinins in the transfer RNA from tobacco callus grown in the presence of N,N′-diphenylurea. Planta 130:313-316.

Burrows, W.J., F. Skoog, and N.J. Leonard. 1971. Isolation and identification of cytokinins located in the transfer ribonucleic acid of tobacco callus grown in the presence of 6-benzylamino purine. Biochem. 10:2189-2194.

Caplin, S.M., and F.C. Steward. 1948. Effect of coconut milk on the growth of explants from carrot root. Science 108:655-657.

Chetram, R.S., and L.E. Bendixen. 1974. Phytochrome controlled basal bulb formation in purple nutsedge. Weed Sci. 22:269-272.

Chibnall, A.C., and G.H. Wiltshire. 1954. A study with isotopic nitrogen of protein metabolism in detached runner-bean leaves. New Phyto. 53:38-43.

Chvojka, L., L. Veres, and J. Kozel. 1961. The effect of kinins on the growth of apple tree buds and on incorporation of radioactive phosphate. Biol. Plant. 3:140-147.

Collier, H.O.J. 1962. Kinins. Sci. Amer. 207:111-118.

Davey, J.E., and J. Van Staden. 1976. Cytokinin translocation: changes in zeatin and zeatin riboside levels in root exudate of tomato plants during their development. Planta 130:69-72.

Davey, J.E., and J. Van Staden. 1978. Cytokinin activity in *Lupinus albus*. III. Distribution in fruits. Physiol. Plant. 43:87-93.

Davies, C.R., and P.F. Wareing. 1965. Auxin-directed transport of radio-phosphorus in stems. Planta 65:139-156.

Dayawon, M.M., and V.G. Shutak. 1967. Influence of N^6-benzyladenine on the postharvest rate of respiration of strawberries. Hort. Science 2:12.

Dennis, D.T., M. Stubbs, and T.P. Coultate. 1967. The inhibition of brussels sprout leaf senescence by kinins. Can. J. Bot. 45:1019-1024.

Dhillon, S.S. 1978. Influence of varied phosphorus supply on growth and xylem sap cytokinin level of sycamore (*Platanus occidentalis* L.) seedlings. Plant Physiol. 61:521-524.

Dilworth, F.M., and H. Kende. 1974. Control of nitrite reductase activity in excised embryos of *Agrostemma githago*. Plant Physiol. 54:826-828.

Dumbroff, E.B., and D.C.W. Brown. 1976. Cytokinin and inhibitor activity in roots and stems of sugar maple seedlings through the dormant season. Can. J. Bot. 54:191-197.

Dyer, T.A., and D.J. Osborne. 1971. Leaf nucleic acids. II. Metabolism during senescence and the effect of kinetin. J. Exp. Bot. 22:552-560.

Engelbrecht, L. 1972. Cytokinins in leaf cuttings of *Phaseolus vulgaris* L. during their development. Biochem. Physiol. Pflanzen. 163:335-343.

Engelbrecht, L., and K. Mothes. 1961. The effect of kinetin on the development of roots. Plant Cell Physiol. 2:271-276.

Engelbrecht, L., U. Orban, and W. Heese. 1969. Leaf-miner caterpillars and cytokinins in the "green islands" of autumn leaves. Nature 223:319-321.

Feierabend, J. 1969a. Formation of the photosynthetic apparatus during germination and its control. Progr. Photosyn. Res. 1:280-283.

Feierabend, J. 1969b. Influence of cytokinins on the formation of photosynthetic enzymes in rye seedlings. Planta 84:11-29.

Feng, K.A. 1973. Effects of kinetin on the permeability of *Allium cepa* cells. Plant Physiol. 51:868-870.

Fittler, F., and R.H. Hall. 1966. Selective modification of yeast seryl-t-RNA and its effect on acceptance and binding functions. Biochem. Biophys. Res. Commun. 25:441-446.

Fletcher, R.A. 1969. Retardation of leaf senescence by benzyladenine in intact bean plants. Planta 89:1-8.

Fletcher, R.A., and N.O. Adedipe. 1972. Hormonal regulation of leaf senescence in intact plants, p. 571-580. *In*: D.J. Carr (ed.), Plant Growth Substances, 1970. Springer-Verlag, New York.

Fletcher, R.A., G. Hofstra, and N.O. Adedipe. 1970. Effects of benzyladenine on bean leaf senescence and the translocation of ^{14}C-assimilates. Physiol. Plant. 23:1144-1148.

Fletcher, R.A., T. Oegema, and R.F. Horton. 1969. Endogenous gibberellin levels and sensecence in *Taraxacum officinale*. Planta 86:98-102.

Fletcher, R.A., C. Teo, and A. Ali. 1973. Stimulation of chlorophyll synthesis in cucumber cotyledons by benzyladenine. Can. J. Bot. 51:937-939.

Fox, J.E. 1964. Indoleacetic acid-kinetin antagonism in certain tissue culture systems. Plant Cell Physiol. 5:251-254.

Fox, J.E. 1966. Incorporation of a kinin, N,6-benzyladenine into soluble RNA. Plant Physiol. 41:75-82.

Fox, J.E., and C. Chen. 1967. Characterization of labeled ribonucleic acid from tissue grown on ^{14}C-containing cytokinins. J. Biol. Chem. 242:4490-4494.

Fox, J.E., and J.L. Erion. 1975. A cytokinin binding protein from higher plant ribosomes. Biochem. Biophys. Res. Commun. 64:694-700.

Fox, J.E., and J.S. Weis. 1965. Transport of the kinin, N^6-benzyl-adenine: non-polar or polar? Nature 206:678-679.

Friedrich, J.W., and R.C. Huffaker. 1980. Photosynthesis, leaf resistances, and ribulose 1, 5-bisphosphate carboxylase degradation in senescing barley leaves. Plant Physiol. 65:1103-1107.

Frith, G.J.T., D.G. Bruce, and M.J. Dalling. 1975. Distribution of acid proteinase activity in wheat seedlings. Plant Cell Physiol. 16:1085-1091.

Fuchs, Y., and M. Lieberman. 1968. Effects of kinetin, IAA, and gibberellin on ethylene production and their interactions in growth of seedlings. Plant Physiol. 43:2029-2036.

Gazit, S., and A. Blumenfeld. 1970. Cytokinin and inhibitor activities in the avocado fruit mesocarp. Plant Physiol. 46:334-336.

Gefter, M.L., and R.L. Russel. 1969. Role of modifications in tyrosine transfer RNA: A modified base affecting ribosome binding. J. Mol. Biol. 39:145-157.

Gersani, M., S.H. Lips, and T. Sachs. 1980. The influence of shoots, roots, and hormones on the distribution of leucine, phosphate, and benzyladenine. J. Exp. Bot. 31:777-782.

Goldacre, P.L. 1959. Potentiation of lateral root induction by root initials in isolated flax roots. Aust. J. Biol. Sci. 12:388-394.

Goodwin, P.B., B.I. Gollnow, and D.S. Letham. 1978. Phytohormones and growth correlations, P. 215-249. *In*: D.S. Letham, P.B. Goodwin, and T.J.V. Higgins (eds.), Phytohormones and Related Compounds: A Comprehensive Treatise. Vol.II. Phytohormones and the Development of Higher Plants. Elsevier/North-Holland Biomedical Press, Amsterdam.

Gorden, E.M., D.S. Letham, and C.W. Parker. 1974. The metabolism and translocation of zeatin in intact radish seedlings. Ann. Bot. 38:809-825.

Grossman, S., and Y.Y. Leshem. 1978. Lowering of endogenous lipoxygenase activity in *Pisum sativum* foliage by cytokinin as related to senescence. Physiol. Plant. 43:359-362.

Gunning, B.E.S., and W.K. Barkley. 1963. Kinin-induced directed transport and senescence in detached oat leaves. Nature 199:262-265.

Gur, A., B. Bravdo, and Y. Mizrahi. 1972. Physiological responses of apple trees to supraoptimal root temperature. Physiol. Plant. 27:130-138.

Guttman, R. 1957. Alternations in nuclear ribonucleic acid metabolism induced by kinetin. J. Biophys. Biochem. Cytol. 3:129-132.

Hahn, H., R. deZacks, and H. Kende. 1974. Cytokinin formation in pea seeds. Naturwiss. 61:170.

Hall, N.P., A.J. Keys, and M.J. Merrett. 1978. Ribulose 1, 5-diphosphate carboxylase protein during flag leaf senescence. J. Exp. Bot. 29:31-37.

Hall, R.H. 1973. Cytokinins as a probe of developmental processes. Ann. Rev. Plant Physiol. 24:415-444.

Hall, R.H., and R.S. deRopp. 1955. Formation of 6-furfurylaminopurine from DNA breakdown products. J. Amer. Chem. Soc. 77:6400.

Hall, R.H., M.J. Robins, L. Stasink, and R. Thedford. 1966. Isolation of N^6-(γ, γ-dimethylally) adenosine from soluble ribonucleic acid. J. Amer. Chem. Soc. 88:2614-2615.

Hamzi, Q.H., and F. Skoog. 1964. Kinetin-like growth-promoting activity of 1-substituted adenines [1-benzyl-6-aminopurine and 1-(γ, γ-dimethylallyl)-6-aminopurine]. Proc. Nat. Acad. Sci. U.S. 51:76-83.

Hardwick, K., and H.W. Woolhouse. 1968. Foliar senescence in *Perilla frutescens* (L.) Britt. The mechansim of [2-^{14}C] glycine uptake and incorporation into protein by leaf discs. New Phytol. 67:241-246.

Harvey, B.M.R., B.C. Lu, and R.A. Fletcher. 1974. Benzyladenine accelerates chloroplast differentiation and stimulates photosynthetic enzyme activity in cucumber cotyledons. Can. J. Bot. 52:2581-2586.

Hemberg, T. 1970. The action of some cytokinins on the rest period and the content of acid growth-inhibiting substances in potato. Physiol. Plant. 23:850-858.

Henderson, T.R., C.G. Skinner, and R.E. Eakin. 1962. Kinetin and kinetin analogues as substrates and inhibitors of xanthine oxidase. Plant Physiol. 37:552-555.

Henson, I.E., and P.F. Wareing. 1977a. Cytokinins in *Xanthium strumarium* L.: The metabolism of cytokinins in detached leaves and buds in relation to photoperiod. New Phytol. 78:27-33.

Henson, I.E., and P.F. Wareing. 1977b. An effect of defoliation on the cytokinin content of buds of *Xanthium strumarium*. Plant Sci. Lett. 9:27-31.

Hewett, E.W., and P.F. Wareing. 1973a. Cytokinins in *Populus* x *robusta*: Changes during chilling and bud burst. Physiol. Plant. 28:393-399.

Hewett, E.W., and P.F. Wareing. 1973b. Cytokinins in *Populus* x *robusta*: Qualitative changes during development. Physiol. Plant. 29:386-389.

Hewett, E.W., and P.F. Wareing. 1973c. Cytokinins in *Populus* x *robusta* (Schneid): Light effects on endogenous levels. Planta 114:119-129.

Hoad, G.V. 1973. Effect of moisture stress on abscisic acid levels in *Ricinus communis* L. with particular reference to phloem exudate. Planta 113:367-372.

Horgan, J.M., and P.F. Wareing. 1980. Cytokinins and the growth responses of seedlings of *Betula pendula* Roth. and *Acer pseudoplatanus* L. to nitrogen and phosphorus deficiency. J. Exp. Bot. 31:525-532.

Horgan, R. 1975. A new cytokinin metabolite. Biochem. Biophys. Res. Commun. 65:358-363.

Huang, C.Y. 1977. Effect of plant growth regulators on the nitrogen fixing (acetylene reduction) activity of soybean plants. Taiwani 22:80-90.

Ilan, I. 1971. Evidence for hormonal regulation of the selectivity of ion uptake by plant cells. Physiol. Plant. 25:230-233.

Ilan, I., T. Gilad, and L. Reinhold. 1971. Specific effects of kinetin on the uptake of monovalent cations by sunflower cotyledons. Physiol. Plant. 24:337-341.

Ilan, I., and R. Goren. 1979. Cytokinins and senescence in lemon leaves. Physiol. Plant. 45:93-95.

Incoll, L.D., and G.C. Whitelam. 1977. The effect of kinetin on stomata of the grass *Anthephora pubescens* Ness. Planta 137:243-245.

Ireland, H.M.M., and J.W. Bradbeer. 1971. Plastid development in primary leaves of *Phaseolus vulgaris*. Planta 96:254-261.

Itai, C., A. Ben-Zioni, and L. Ordin. 1973. Correlative changes in endogenous hormone levels and shoot growth induced by short heat treatments to the root. Physiol. Plant. 29:355-360.

Itai, C., and Y. Vaadia. 1971. Cytokinin activity in water stressed plants. Plant Physiol. 47:87-90.

Jablonski, J., and F. Skoog. 1954. Cell enlargement and cell division in excised tobacco pith tissue. Physiol. Plant. 7:16-24.

Jacobs, W.P. 1979. Plant Hormones and Plant Development. Cambridge University Press, New York. 339 p.

Jacoby, B., and J. Dagan. 1970. Effects of ^{6}N-benzyladenine on primary leaves of intact bean plants and on their sodium absorption capacity. Physiol. Plant. 23:397-403.

Jewer, P.C., and L.D. Incoll. 1980. Promotion of stomatal opening in the grass *Anthephora pubescens* Nees by a range of natural and synthetic cytokinins. Planta 150:218-221.

Jewiss, O.R. 1972. Tillering in grasses--its significance and control. J. Br. Grassland Soc. 27:65-82.

Johnston, G.F.S., and B. Jeffcoat. 1977. Effects of some growth regulators on tiller bud elongation in cereals. New Phytol. 79:239-245.

Kaminek, M. 1967. Root formation in pea stem sections and its inhibition by kinetin, ethionine, and chloramphenicol. Biol. Plant. 9:86-91.

Kannangara, T., and A. Booth. 1974. Diffusible cytokinins in shoot apices of *Dahlia variabilis*. J. Exp. Bot. 25:459-467.

Kar, M., and D. Mishra. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. 57:315-319.

Kar, M., and D. Mishra. 1977. Protease activity during rice leaf senescence. Biol. Plant. 19:365-369.

Katsumi, M. 1962. Physiological effects of kinetin. Effect of thickening on etiolated pea stem sections. Physiol. Plant. 15:115-121.

Kefford, N.P., J. Brockwell, and J.A. Zwar. 1960. The symbiotic synthesis of auxin by legumes and nodule bacteria and its role in nodule development. Aust. J. Biol. Sci. 13:456-467.

Kemp, H.T., R.G. Fuller, and R.S. Davidson. 1957. Inhibition of plant growth by root-drench application of kinetin. Science 126:1182.

Kende, H. 1964. Preservation of chlorophyll in leaf sections by substances obtained from root exudate. Science 145:1066-1067.

Kende, H. 1971. The cytokinins. Int. Rev. Cytol. 31:301-338.

Kende, H., M. Fukuyama-Dilworth, and R. deZacks. 1974. On the control of nitrate reductase by nitrate and benzyladenine in *Agrostemma githago* embryos, p. 675-682. *In*: Plant Growth Substances, 1973. Hirokawa Publishing Co., Tokyo.

Kende, H., H. Hahn, and S.E. Kays. 1971. Enhancement of nitrate reductase activity by benzyladenine in *Agrostemma githago*. Plant Physiol. 48:702-706.

Kende, H., and D. Sitton. 1967. The physiological significance of kinetin- and gibberellin-like root hormones. Ann. N.Y. Acad. Sci. 144:235-243.

Kende, H., and J.E. Tavares. 1968. On the significance of cytokinin incorporation into RNA. Plant Physiol. 43:1244-1248.

Klambt, D., G. Thies, and F. Skoog. 1966. Isolation of cytokinins from *Corynebacterium fascians*. Proc. Nat. Acad. Sci. U.S. 56:52-59.

Knypl, J.S. 1979. Hormonal control of nitrate assimilation: Do phytohormones and phytochrome control the activity of nitrate reductase?, p. 541-556. *In*: E.J. Hewitt and C.V. Cutting (eds.), Nitrogen Assimilation of Plants. Academic Press, New York.

Kodo, Y., and Y. Okazawa. 1980. Cytokinin production by asparagus shoot apex cultured *in vitro*. Physiol. Plant. 49:193-197.

Kuhnle, J.A., G. Fuller, J. Corse, and B.E. Mackey. 1977. Antisenescent activity of natural cytokinins. Physiol. Plant. 41:14-21.

Kulaeva, O.N. 1962. The effect of roots on leaf metabolism in relation to the action of kinetin on leaves. Soviet Plant Physiol. 9:182-189.

Kung-Woo Lee, P., B. Kessler, and K.V. Thimann. 1974. The effect of hadacidin on bud development and its implications for apical dominance. Physiol. Plant. 31:11-14.

Kuraishi, S. 1959. Effect of kinetin analogues on leaf growth. Sci. Pap. Coll. Gen. Educ., Univ. Tokyo. 9:67-104.

Kuraishi, S. 1968. The effect of kinetin on protein level of *Brassica* leaf disks. Physiol. Plant. 21:78-83.

Kuraishi, S. 1976. Ineffectiveness of cytokinin-induced chlorophyll retention in hypostomatous leaf discs. Plant Cell Physiol. 17:875-885.

Kuraishi, S., and F.S. Okhumura. 1956. The effect of kinetin on leaf growth. Bot. Mag. (Tokyo). 69:300-306.

Kursanov. A.L. 1963. Metabolism and transport of organic substances in the phloem. Advan. Bot. Res. 1:209-274.

Kursanov. A.L., O.N. Kulayeva, I.N. Steshnikova, E.A. Popova, Y.P. Bolyakina, M.L. Klyachko, and I.P. Vorobyova. 1964. Restoration of cellular structures and metabolism in yellow leaves under the action of 6-benzylaminopurine. Soviet Plant Physiol. 11:710-719.

Lagerstedt, H.B., and R.G. Langston. 1966. Transport of kinetin-8-^{14}C in petioles. Physiol. Plant. 19:734-740.

Lagerstedt, H.B., and R.G. Langston. 1967a. Translocation of radioactive kinetin. Plant Physiol. 42:611-622.

Lagerstedt, H.B., and R.G. Langston. 1967b. The mobilizing force of kinetin. Life Sci. 6:145-149.

Langer, R.H.M., P.C. Prasad, and H.M. Laude. 1973. Effects kinetin on tiller bud elongation in wheat (*Triticum aestivum* L.). Ann. Bot. 37:565-571.

Lau, O.L., and S.F. Yang. 1975. Interaction of kinetin and calcium in relation to their effect on stimulation of ethylene production. Plant Physiol. 55:738-740.

Letham, D.S. 1963a. Zeatin, a factor inducing cell division isolated from *Zea mays*. Life Sci. 2:569-573.

Letham, D.S. 1963b. Regulators of cell division in plant tissues. I. Inhibitors and stimulants of cell division in developing fruits. Their properties and chemical activity in relation to the cell division period. N.Z.J. Bot. 1:336-350.

Letham, D.S. 1967a. Chemistry and physiology of kinetin-like compounds. Ann. Rev. Plant Physiol. 18:349-364.

Letham, D.S. 1967b. Regulators of cell division in plant tissues. V. A comparison of the activities of zeatin and other cytokinins in five bioassays. Planta 74:228-242.

Letham, D.S. 1968. A new cytokinin bioassay and the naturally occurring cytokinin complex, p. 19-31. *In*: F. Wightman and G. Setterfield (eds.), Biochemistry and Physiology of Plant Growth Substances. Runge Press, Ottawa.

Letham, D.S. 1972. Cytokinin activities of compounds related to zeatin. Phytochem. 11:1023-1025.

Letham, D.S. 1978. Cytokinins, p. 205-263. *In*: D.S. Letham, P.B. Goodwin, and T.J.V. Higgins (eds.), Phytohormones and Related Compounds: A Comprehensive Treatise. Vol.I. The Biochemistry of Phytohormones and Related Compounds. Elsevier/North-Holland Biomedical Press, Amsterdam.

Letham, D.S., and M.W. Williams. 1969. Regulators of cell division in plant tissues. VIII. The cytokinins of the apple fruit. Physiol. Plant. 22:925-936.

Letham, D.S., M.M. Wilson, C.W. Parker, I.D. Jenkins, J.K. MacLeod, and R.E. Summons. 1975. Regulators of cell division in plant tissues. XXIII. The identity of an unusual metabolite of 6-benzylaminopurine. Biochim. Biophys. Acta 399:61-70.

Leonard, N.J., and T. Fujii. 1964. The synthesis of compounds possessing kinetin activity. The use of a blocking group at the 9-position of adenine for the biosynthesis of 1-substituted adenines. Proc. Nat. Acad. Sci. U.S. 51:73-75.

Leopold, A.C., and M. Kawase. 1964. Benzyladenine effects on bean leaf growth and senescence. Amer. J. Bot. 51:294-298.

Leshem, Y.Y., S. Grossman, A. Frimer, and J. Ziv. 1979. Endogenous lipoxygenase control and lipid-associated free radical scavenging as modes of cytokinin action in plant senescence retardation, p. 193-198. *In*: L.A. Appelquist and C. Liljenberg (eds.), Advances in the Biochemistry and Physiology of Plant Lipids. Elsevier/North-Holland Biomedical Press, Amsterdam.

Lindoo, S.J., and L.D. Nooden. 1978. Correlation of cytokinins and abscisic acid with monocarpic senescence in soybeans. Plant Cell Physiol. 19:997-1006.

Livne, A., and Y. Vaadia. 1965. Stimulation of transpiration rate in barley leaves by kinetin and gibberellic acid. Physiol. Plant. 18: 658-664.

Luckwill, L.C., and P. Whyte. 1968. Hormones in the xylem sap of apple trees. Soc. Chem. Ind. Monogr. No. 31:87-101.

Luke, H.H., and T.E. Freeman. 1967. Rapid bioassay for phytokinins based on transpiration of excised oat leaves. Nature 215:874-875.

MacLean, D.C., R.R. Dedolph, and S.H. Wittwer. 1963. Respiratory responses of broccoli (*Brassica oleracea* var. Italica) to pre- and post-harvest treatments with N^6-benzyladenine. Proc. Am. Soc. Hort. Sci. 81:484-487.

Martin, C., and K.V. Thimann. 1972. The role of protein synthesis in the senescence of leaves. I. The formation of protease. Plant Physiol. 49:64-71.

Mauseth, J.D. 1976. Cytokinin- and gibberellic acid-induced effects on the structure and metabolism of shoot apical meristems in *Opuntia polyacantha* (Cactaceae). Amer. J. Bot. 63:1295-1301.

McCalla, D.R., D.J. Morre, and D.J. Osborne. 1962. The metabolism of a kinin, benzyladenine. Biochim. Biophys. Acta 55:522-528.

McDonald, J.J. N.J. Leonard, R.Y. Schmitz, and F. Skoog. 1971. Cytokinins: Synthesis and biological activity of ureidopurines. Phytochem. 10:1429-1439.

McHale, J.S. and L.D. Dove. 1968. Mobilization--independent effects of a cytokinin on senescing tomato leaves. Naturwiss. 55:141.

McKendrick, J.D., C.E. Owensby, and R.M. Hyde. 1975. Big bluestem and indiangrass vegetative reproduction and annual reserve carbohydrate and nitrogen cycles. Agro-Ecosystems 2:75-93.

Meidner, H. 1967. The effect of kinetin on stomatal opening and the rate of intake of carbon dioxide in mature primary leaves of barley. J. Exp. Bot. 18:556-561.

Menary, R.C., and J. Van Staden. 1976. Effect of phosphorus nutrition and cytokinins on flowering in the tomato, *Lycopersicon esculentum* Mill. Aust. J. Plant. Physiol. 3:201-205.

Menhenett, R., and P.F. Wareing. 1975. Possible involvement of growth substances in the response of tomato plants (*Lycopersicon esculentum* Mill.) to different soil temperatures. J. Hort. Sci. 50:381-397.

Meredith, W.C., J.N. Joiner, and R.H. Biggs. 1970. Influences of indole-3-acetic acid and kinetin on rooting and indole metabolism of *Feijoa sellowiana*. J. Amer. Soc. Hort. Sci. 95:49-52.

Miller, C.O. 1956. Similarity of some kinetin and red light effects. Plant Physiol. 31:318-319.

Miller, C.O. 1961. A kinetin-like compound in maize. Proc. Nat. Acad. Sci. U.S. 47:170-174.

Miller, C.O. 1967. Zeatin and zeatin-riboside from a mycorrhizal fungus. Science 157:1055-1056.

Miller, C.O., F. Skoog, F.S. Okumura, M.H. von Saltza, and F.M. Strong. 1955a. Structure and synthesis of kinetin. J. Amer. Chem. Soc. 77: 2662-2663.

Miller, C.O., F. Skoog, F.S. Okumura, M.H. von Saltza, and F.M. Strong. 1956. Isolation, structure, and synthesis of kinetin, a substance promoting cell division. J. Amer. Chem. Soc. 78:1375-1380.

Miller, C.O., F. Skoog, M.H. von Saltza, and F.M. Strong. 1955b. Kinetin, a cell division factor from deoxyribonucleic acid. J. Amer. Chem. Soc. 77:1392.

Mishra, D., and B. Misra. 1973. Retardation of induced senescence of leaves from crop plants by benzimidazole and cytokinins. Expt. Geront. 8:235-239.

Mittelheuser, C.J., and R.F.M. van Steveninck. 1972. Effects of ABA and kinetin on ultrastructure of senescing wheat leaves, p. 618-623. *In*: D.J. Carr (ed.), Plant Growth Substances, 1970. Springer-Verlag, Berlin.

Miura, G.A., and C.O. Miller. 1969. 6-(γ, γ-dimethylallylamino) purine as a precursor of zeatin. Plant Physiol. 44:372-376.

Mizrahi, Y., J. Amir, and A.E. Richmond. 1970. The mode of action of kinetin in maintaining the protein content of detached *Tropoeolum majus* leaves. New Phytol. 69:355-361.

Mlodzianowski, F., and M. Kwintkiewicz. 1973. The inhibition of kohlrabi chloroplast degeneration by kinetin. Protoplasma 76:211-226.

Moore, T.C. 1976. Biochemistry and Physiology of Plant Hormones. Springer-Verlag, Berlin. 274 p.

Moore, T.S., and C.O. Miller. 1972. Effects of cytokinins on the respiration of soybean callus tissue. Plant Physiol. 50:594-598.

Morita, K. 1980. Release of nitrogen from chloroplasts during leaf senescence in rice (*Oryza sativa* L.). Ann. Bot. 46:297-302.

Mothes, K., and L. Engelbrecht. 1961. Kinetin-induced directed transport of substances in excised leaves in the dark. Phytochem. 1:58-62.

Mozes, R., and A. Altman. 1977. Characteristics of root-to-shoot transport of cytokinin 6-benzylaminopurine in intact seedlings of *Citrus aurantium*. Physiol. Plant. 39:225-232.

Muller, E., and E. Brautigam. 1973. Symplasmic translocation of α-aminoisobutyric acid in *Vallisneria* leaves and the action of kinetin and colchicine, p. 555-562. *In*: W.P. Anderson (ed.), Ion Transport in Plants. Academic Press, New York.

Muller, K., and A.C. Leopold. 1966a. Correlative ageing and transport of P^{32} in corn leaves under the influence of kinetin. Planta 68: 167-185.

Muller, K., and A.C. Leopold. 1966b. The mechanism of kinetin-induced transport in corn leaves. Planta 68:186-205.

Naito, K., A. Iida, H. Suzuki, and H. Tsuji. 1979. The effect of benzyladenine on changes in nuclease and protease activities in intact bean leaves during ageing. Physiol. Plant. 46:50-53.

Naito, K., H. Tsuji, and I. Hatakeyama. 1978. Effect of benzyladenine on DNA, RNA, protein, and chlorophyll contents in intact bean leaves: Differential responses to benzyladenine according to leaf age. Physiol. Plant. 43:367-371.

Nickell, L.G. 1950. Effect of coconut milk on the growth *in vitro* of plant virus tumor tissue. Bot. Gaz. 112:225-228.

Nooden, L.D., G.M. Kahanak, and Y. Okatan. 1979. Prevention of monocarpic senescence in soybeans with auxin and cytokinin: An antidote for self-destruction. Science 206:841-843.

Nooden, L.D., and A.C. Leopold. 1978. Phytohormones and the endogenous regulation of senescence and abscission, p. 329-369. *In*: D.S. Letham, P.B. Goodwin, and T.J.V. Higgins (eds.), Phytohormones and Related Compounds: A Comprehensive Treatise. Vol.II. Phytohormones and the Development of Higher Plants. Elsevier/North-Holland Biomedical Press, Amsterdam.

Osborne, D.J. 1962. Effect of kinetin on protein and nucleic acid metabolism of *Xanthium* leaves during senescence. Plant Physiol. 37:595-602

Osborne, D.J. 1965. Interaction of hormonal substances in the growth and development of plants. J. Sci. Food Agr. 16:1-13.

Paces, V., E. Werstiuk, and R.H. Hall. 1971. Conversion of N^6-(Δ^2-isopentenyl) adenosine to adenosine by enzyme activity in tobacco tissue. Plant Physiol. 48:775-778.

Pallas, J.E., and J.E. Box. 1970. Explanation for the stomatal response of excised leaves to kinetin. Nature 227:87-88.

Parker, C.W., and D.S. Letham. 1973. Regulators of cell division in plant tissues. XVI. Metabolism of zeatin by radish cotyledons and hypocotyls. Planta 114:119-218.

Parker, C.W., D.S. Letham, D.E. Cowley, and J.K. MacLeod. 1972. Raphanatin, an unusual purine derivative and a metabolite of zeatin. Biochem. Biophys. Res. Commun. 49:460-466.

Parker, C.W., M.M. Witson, D.S. Letham, D.E. Cowley, and J.K. MacLeod. 1973. The glucosylation of cytokinins. Biochem. Biophys. Res. Commun. 55:1370-1376.

Pederson, M. 1973. Identification of a cytokinin, 6-(3 methyl-2-butenylamino) purine, in sea water and the effect of cytokinins on brown algae. Physiol. Plant. 28:101-105.

Pereira, A.S.R., P.J.W. Houwen, H.W.J. Deurenberg-Vos, and E.B.F. Pey. 1972. Cytokinins and the bacterial symbiosis of *Ardisia* species. Z. Pflanzenphysiol. 68:170-177.

Peterkofsky, A. 1968. The incorporation of mevalonic acid into the N^6-(Δ^2-isopentenyl) adenosine of transfer ribonucleic acid in *Lactobacillus acidophilus*. Biochem. 7:472-482.

Peterson, C.A., and R.A. Fletcher. 1973. Formation of fruits on rootless plants. Can. J. Bot. 51:1899-1905.

Peterson, L.W., and R.C. Huffaker. 1975. Loss of ribulose 1, 5-diphosphate carboxylase and increase in proteolytic activity during senescence of detached primary barley leaves. Plant Physiol. 55: 1009-1015.

Phillips, D.A., and C.F. Cleland. 1972. Cytokinin activity from the phloem sap of *Xanthium strumarium*. Planta 102:173-178.

Phillips, D.A., and J.G. Torrey. 1970. Cytokinin production by *Rhizobium japonicum*. Physiol. Plant. 23:1057-1063.

Phillips, D.A., and J.G. Torrey. 1972. Studies on cytokinin production by *Rhizobium*. Plant Physiol. 49:11-15.

Pilet, P.E. 1968. *In vivo* and *in vitro* auxin and cytokinin translocation, p. 993-1004. *In*: F. Wightman and G. Setterfield (eds.), Biochemistry and Physiology of Plant Growth Substances. Runge Press, Ottawa.

Pozsar, B.I., and Z. Kiraly. 1966. Phloem-transport in rust infected plants and the cytokinin-directed long-distance movement of nutrients. Phytopathol. Z. 56:297-309.

Quinlan, J.D., and R.J. Weaver. 1969. Influence of benzyladenine, leaf darkening, and ringing on movement of ^{14}C-labeled assimilates into expanded leaves of *Vitis vinifera* L. Plant Physiol. 44:1247-1252.

Railton, I.D., and D.M. Reid. 1973. Effects of benzyladenine on the growth of waterlogged tomato plants. Planta 111:261-266.

Ralph, R.K., S. Bullivant, and S.J. Wojcik. 1976. Effects of kinetin on phosphorylation of leaf membrane proteins. Biochim. Biophy. Acta 421:319-327.

Ralph, R.K., P.J.A. McCombs, G. Tener, and S.J. Wojcik. 1972. Evidence for modification of protein phosphorylation by cytokinins. Biochem. J. 130:901-911.

Ramina, A. 1979. Aspects of [8-^{14}C] benzylaminopurine metabolism in *Phaseolus vulgaris*. Plant Physiol. 63:298-300.

Ramina, A., F. Pimpini, A. Bonioto, and F. Bergamasco. 1979. [8-^{14}C] benzylaminopurine translocation in *Phaseolus vulgaris*. Plant Physiol. 63:294-297.

Richmond, A.E., and A. Lang. 1957. Effect of kinetin on protein content and survival of detached *Xanthium* leaves. Science 125:650-651.

Richmond, A.E., B. Sachs, and D.J. Osborne. 1971. Chloroplasts, kinetin and protein synthesis. Physiol. Plant. 24:176-180.

Rijven, A.H.G.C., and V. Parkash. 1971. Action of kinetin on cotyledons of fenugreek. Plant Physiol. 47:59-64.

Rodriguez-Barrueco, C., and F.B. De Castro. 1973. Cytokinin-induced pseudonodules on *Alnus glulinosa*. Physiol. Plant. 29:277-280.

Rogozinska, J.H., J.P. Helgeson, and F. Skoog. 1964. Tests for kinetin-like growth promoting activities of triacanthine and its isomer, 6-(γ, γ-dimethylallylamino) purine. Physiol. Plant. 17:165-176.

Ruckenbauer, P., and E.J.M. Kirby. 1973. Effects of kinetin on the growth and development of barley and its interaction with root size. J. Agric. Sci. 80:211-217.

Sabater, B., and M.T. Rodriguez. 1978. Control of chlorophyll degradation in detached leaves of barley and oat through effect of kinetin on chlorophyllase levels. Physiol. Plant. 43:274-276.

Sacher, J.A. 1967. Studies of permeability, RNA and protein turnover during ageing of fruit and leaf tissue. Symp. Soc. Exp. Biol. 21: 269-303.

Sacher, J.A. 1968. Senescence: Effects of auxin and kinetin on RNA and protein synthesis in subcellular fractions of fruit and leaf tissue sections, p. 1457-1477. *In*: F. Wightman and G. Setterfield (eds.), Biochemistry and Physiology of Plant Growth Substances. Runge Press, Ottawa.

Sachs, T., and K.V. Thimann. 1964. Release of lateral buds from apical dominance. Nature 201:939-940.

Salama, A.M.S., and P.F. Wareing. 1979. Effects of mineral nutrition on endogenous cytokinins in plants of sunflower (*Helianthus annuus* L.). J. Exp. Bot. 30:971-981.

Schaeffer, G.W., and F.T. Sharpe, Jr. 1971. Cytokinin function: Increase in [methyl-^{14}C] metabolism to phosphatidylcholine and a decrease in oxidation to carbon dioxide-^{14}C. Physiol. Plant. 25:456-460.

Schmitz, R.Y., F. Skoog, S.M. Hecht, R.M. Bock, and N.J. Leonard. 1972. Comparison of cytokinin activities of naturally occurring ribonucleosides and corresponding bases. Phytochem. 11:1603-1610.

Seth, A.K., C.R. Davies, and P.F. Wareing. 1966. Auxin effects on the mobility of kinetin in the plant. Science 151:587-588.

Seth, A.K., and P.F. Wareing. 1967. Hormone-directed transport of metabolites and its possible role in plant senescence. J. Exp. Bot. 18:65-77.

Shantz, E.M., and F.C. Steward. 1952. Coconut milk factor: the growth-promoting substances in coconut milk. J. Amer. Chem. Soc. 74:6133.

Shantz, E.M., and F.C. Steward. 1955. The identification of compound A from coconut milk as 1, 3-diphenylurea. J. Amer. Chem. Soc. 77: 6351-6353.

Sharif, R., and J.E. Dale. 1980. Growth-regulating substances and the growth of tiller buds in barley: Effects of cytokinins. J. Exp. Bot. 31:921-930.

Shaw, M., P.K. Bhattacharya, and W.A. Quick. 1965. Chlorophyll, protein, and nucleic acid levels in detached, senescing wheat leaves. Can. J. Bot. 43:739-746.

Shaw, M., and M.S. Manocha. 1965. Fine structure in detached, senescing wheat leaves. Can. J. Bot. 43:747-755.

Shibaoka, H., and K.V. Thimann. 1970. Antagonisms between kinetin and amino acids. Plant Physiol. 46:212-220.

Short, K.C., and J.G. Torrey. 1972. Cytokinins in seedling roots of pea. Plant Physiol. 49:155-160.

Simon, E.W. 1974. Phospholipids and plant membrane permeability. New Phytol. 73:377-420.

Simpkins, I., and H.E. Street. 1970. Studies on the growth in culture of plant cells. J. Exp. Bot. 66:170-185.

Sitton, D., C. Itai, and H. Kende. 1967. Decreased cytokinin production in the roots as a factor in shoot senescence. Planta 73:296-300.

Skene, K.G.M. 1970. Cytokinins in bleeding sap of the grape vine, p. 476-483. *In*: D.J. Carr (ed.), Plant Growth Substances, 1970. Springer-Verlag, Berlin.

Skene, K.G.M. 1972. The effect of ringing on cytokinin activity in shoots of the grape vine. J. Exp. Bot. 23:768-774.

Skoog, F., and D.J. Armstrong. 1970. Cytokinins. Ann. Rev. Plant Physiol. 21:359-384.

Skoog, F., D.J. Armstrong, J.D. Cherayil, A.E. Hempel, and R.M. Bock. 1966. Cytokinin activity: Localization in transfer RNA preparations. Sci. 154:1354-1355.

Skoog, F., H.Q. Hamzi, A.M. Szweykowska, N.J. Leonard, K.L. Carraway, T. Fujii, J.P. Helgeson, and R.N. Loeppky. 1967. Cytokinins: Structure/activity relationships. Phytochem. 6:1169-1192.

Skoog, F., and N.J. Leonard. 1968. Sources and structure: Activity relationships of cytokinins, p. 1-18. *In*: F. Wightman and G. Setterfield (eds.), Biochemistry and Physiology of Plant Growth Substances. Runge Press, Ottawa.

Skoog. F., and C.O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. Exp. Biol. 11:118-131.

Skoog, F., and R.Y. Schmitz. 1972. Cytokinins, p. 181-213. *In*: F.C. Steward (ed.), Plant Physiology. Vol.VIB. Physiology of Development: The Hormones. Academic Press, New York.

Skoog, F., F.M. Strong, and C.O. Miller. 1965. Cytokinins. Science 148:532-533.

Skoog, F., and C. Tsui. 1948. Chemical control of growth and bud formation in tobacco stem segments and callus cultured *in vitro*. Amer. J. Bot. 35:782-787.

Smith, O.E., and C.E. Palmer. 1970. Cytokinin-induced tuber formation on stolons of *Solanum tuberosum*. Physiol. Plant. 23:599-606.

Sodek, L., and S.T.C. Wright. 1969. The effect of kinetin on ribonuclease, acid phosphatase, lipase and esterase levels in detached wheat leaves. Phytochem. 8:1629-1640.

Sondheimer, E., and D-S. Tzou. 1971. The metabolism of hormones during seed germination and dormancy. Plant Physiol. 47:516-520.

Spencer, P.W., and J.S. Titus. 1973. Apple leaf senescence: Leaf disc compared to attached leaf. Plant Physiol. 51:89-92.

Srivastava, B.I.S. 1963. Investigation of purine-like compounds in immature maize kernels, germinating barley seeds and yeast. Arch. Biochem. Biophys. 103:200-205.

Stettler, D.A., and W.M. Laetsch. 1965. Kinetin-induced chloroplast maturation in cultures of tobacco tissue. Science 149:1387-1388.

Steward, F.C., S.M. Caplin, and F.K. Millar. 1952. Investigations on growth and metabolism of plant cells. I. New techniques for the investigation of metabolism, nutrition and growth in undifferentiated cells. Ann. Bot. 16:57-77.

Steward, F.C., and A.D. Krikorian. 1971. Plants, Chemicals, and Growth. Academic Press, New York. 323 p.

Strong, F.M. 1958. Topics in Microbial Chemistry. Wiley & Sons, Inc., New York. 166 p.

Sugiura, M. 1963. Promotion of chlorophyll synthesis by kinetin. Bot. Mag. (Tokyo) 76:309-310.

Sugiura, M., K. Umemura, and Y. Oota. 1962. The effect of kinetin on protein level of tobacco leaf disks. Physiol. Plant. 15:457-464.

Tal. M., and D. Imber. 1971. Abnormal stomatal behavior and hormonal imbalance in *flacca*, a wilty mutant of tomato. III. Hormonal effects on the water status in the plant. Plant Physiol. 47:849-850.

Tal, M., D. Imber, and C. Itai. 1970. Abnormal stomatal behavior and hormonal imbalance in *flacca*, a wilty mutant of tomato. I. Root effect and kinetin-like activity. Plant Physiol. 46:367-372.

Tasseron-De Jong, J.G., and H. Veldstra. 1971. Investigations on cytokinins. I. Effect of 6-benzylaminopurine on growth and starch content of *Lemna minor*. Physiol. Plant. 24:235-238.

Tavares, J., and H. Kende. 1970. The effect of 6-benzylaminopurine on protein metabolism in senescing corn leaves. Phytochem. 9:1763-1770.

Tetley, R.M., and K.V. Thimann. 1974. The metabolism of oat leaves during senescence. I. Respiration, carbohydrate metabolism, and the action of cytokinins. Plant Physiol. 54:294-303.

Thimann, K.V., and T. Sachs. 1966. The role of cytokinins in the "fasciation" disease caused by *Corynebacterium fascians*. Amer. J. Bot. 53:731-739.

Thimann, K.V., R.R. Tetley, and T.V. Thanh. 1974. The metabolism of oat leaves during senescence. II. Senescence in leaves attached to the plant. Plant Physiol. 54:859-862.

Thomas, H., and J.L. Stoddart. 1975. Separation of chlorophyll degradation from other senescence processes in leaves of a mutant genotype of meadow fescue (*Festuca pratensis* L.). Plant Physiol. 56:438-441.

Thomas, H., and J.L. Stoddart. 1977. Biochemistry of leaf senescence in grasses. Ann. Appl. Biol. 85:461-463.

Thomas, H., and J.L. Stoddart. 1980. Leaf senescence. Ann. Rev. Plant Physiol. 31:83-111.

Thompson, A.G., R. Horgan, and J.K. Heald. 1975. A qualitative analysis of cytokinin using single-ion-current-monitoring. Planta 124:207-210.

Tien, T.M., M.H. Gaskins, and D.H. Hubbell. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl. Envir. Micro. 37:1016-1024.

Torrey, J.G. 1961. Kinetin as a trigger for mitosis in mature endomitotic plant cells. Exp. Cell Res. 23:281-299.

Torrey, J.G. 1962. Auxin and purine interactions in lateral root initiation in isolated pea root segments. Physiol. Plant. 15:177-185.

Trewavas, A. 1972. Control of the protein turnover rates in *Lemna minor*. Plant Physiol. 49:47-51.

Tuli, V., D.R. Dilley, and S.H. Wittwer. 1964. N^6-benzyladenine: Inhibitor of respiratory kinases. Science 146:1477-1479.

Tung, H.F., and C.J. Brady. 1972. Kinetin treatment and protein synthesis in detached wheat leaves, p. 589-597. *In*: D.J. Carr (ed.), Plant Growth Substances, 1970. Springer-Verlag, Berlin.

Upper, C.D., J.P. Helgeson, J.D. Kemp, and C.J. Schmidt. 1970. Gas-liquid chromatographic isolation of cytokinins from natural sources: 6-(3-methyl-2-butenylamino) purine from *Agrobacterium tumefaciens*. Plant Physiol. 45:543-547.

Usciati, M., M. Codaccioni, P. Mazliak, and J. Guern. 1974. Lipogenesis modifications induced by application of 6-benzylaminopurine to inhibited axillary buds of *Cicer arietinum* L. plants. Plant Sci. Letters 2:295-301.

Van Overbeek, J., M.E. Conklin, and A.F. Blakeslee. 1941. Factors in coconut milk essential for growth and development of very young *Datura* embryos. Science 94:350-351.

Van Overbeek, J., M.E. Conklin, and A.F. Blakeslee. 1942. Cultivation *in vitro* of small *Datura* embryos. Amer. J. Bot. 29:472-477.

Van Staden, J. 1975. Cytokinins from larvae in *Erythrina latissima* galls. Plant Sci. letter. 5:227-230.

Van Staden, J. 1976. Season changes in the cytokinin content of *Ginkgo biloba* leaves. Physiol. Plant. 38:1-5.

Van Staden, J. 1977. Seasonal changes in the cytokinin content of the leaves of *Salix babylonica*. Physiol. Plant. 40:296-299.

Van Staden, J., and N.A.C. Brown. 1977. The effect of ringing on cytokinin distribution in *Salix babylonica* L. Physiol. Plant. 39: 266-270.

Van Staden, J., and N.A.C. Brown. 1978. Changes in the endogenous cytokinins of bark and buds of *Salix babylonica* as a result of stem girdling. Physiol. Plant. 43:148-153.

Van Staden, J., and P.F. Wareing. 1972. The effect of light on endogenous cytokinin levels in seeds of *Rumex obtusifolius*. Planta 104:126-133.

Varga, A., and J. Bruinsma. 1973. Effects of different cytokinins on the senescence of detached oat leaves. Planta 111:91-93.

Varga, A., and J. Bruinsma. 1974. The growth and ripening of tomato fruits at different levels of endogenous cytokinins. J. Hort. Sci. 49:135-142.

Vizarova, G. 1974. Level of free cytokinins in susceptible and resistant cultures of barley infected by powdery mildew. Phytopathol. Z. 79: 310-314.

Von Abrams, G.J., and H.K. Pratt. 1967. The effect of kinetin and naphthalenacetic acid upon localized accumulation as related to senescence in detached leaves. Planta 76:306-308.

Wagner, H., and G. Michael. 1971. The influence of varied nitrogen supply on the production of cytokinins in the roots of sunflower plants. Biochem. Physiol. Pflanzen. 162:147-158.

Walker, G.C., N.J. Leonard, D.J. Armstrong, N. Murai, and F. Skoog. 1974. The mode of incorporation of 6-benzylaminopurine into tobacco callus transfer ribonucleic acid. A double labelling determination. Plant Physiol. 54:737-743.

Wareing, P.F., R. Horgan, I.E. Henson, and W. Davis. 1977. Cytokinin relations in the whole plant, p. 147-153. *In*: P.E. Pilet (ed.), Plant Growth Regulation. Springer-Verlag, Berlin.

Wareing, P.F., M.M. Khalifa, and K.J. Treharne. 1968. Rate-limiting processes in photosynthesis at saturating light intensities. Nature 220:453-457.

Weaver, R.J. 1963. Use of kinin in breaking rest of buds of *Vitis vinifera*. Nature 198:207-208.

Weaver, R.J. 1972. Plant Growth Substances in Agriculture. W.H. Freeman and Co., San Francisco. 594 p.

Weinberger, J.H. 1969. The stimulation of dormant peach buds by a cytokinin. Hort. Sci. 4:125-126.

Weiss, C., and Y. Vaadia. 1965. Kinetin-like activity in root apices of sunflower plants. Life Sci. 4:1323-1326.

Wheeler, A.W. 1976. Some treatments affecting growth substances in developing wheat ears. Ann. Appl. Biol. 83:459-462.

Wheeler, C.T., I.E. Henson, and M.E. McLaughlin. 1979. Hormones in plants bearing actinomycete nodules. Bot. Gaz. 140 (Suppl.):S52-S57.

Whitty, C.D., and R.H. Hall. 1974. A cytokinin oxidase in *Zea mays*. Can. J. Biochem. 52:789-799.

Wickson, M., and K.V. Thimann. 1958. The antagonism of auxin and kinetin in apical dominance. Physiol. Plant. 11:62-74.

Williams, R.H., and P.M. Cartwright. 1980. The effect of applications of a synthetic cytokinin on shoot dominance and grain yield in spring barley. Ann. Bot. 46:445-452.

Wittenbach, V.A. 1977. Induced senescence of intact wheat seedlings and its reversibility. Plant Physiol. 59:1039-1042.

Wittenbach, V.A. 1978. Breakdown of ribulose bisphosphate carboxylase and change in proteolytic activity during dark-induced senescence of wheat seedlings. Plant Physiol. 62:604-608.

Wittwer, S.H., and R.R. Dedolph. 1963. Some effects of kinetin on the growth and flowering of intact green plants. Am. J. Bot. 50:330-336.

Wittwer, S.H., and R.R. Dedolph, V. Tuli, and D. Gilbart. 1962. Respiration and storage deterioration in celery (*Apium graveolens* L.) as affected by postharvest treatments with N^6-benzylaminopurine. Proc. Amer. Soc. Hort. Sci. 80:408-416.

Woolley, D.J., and P.F. Wareing. 1972. The interaction between growth promoters in apical dominance. II. Environmental effects of endogenous cytokinin and gibberellin levels in *Solanum andigena*. New Phytol. 71:1015-1025.

Wright, S.T.C., and R.W.P. Hiron. 1969. (+)-ABA. The growth inhibitor induced in detached wheat leaves by a period of wilting. Nature 224: 719-720.

Yoshida, R., and T. Oritani. 1974. Studies on nitrogen metabolism. 13. Effects of nitrogen topdressing on cytokinin content in the root exudate of rice plant. Proc. Crop Sci. Soc. Japan. 43:47-51.

SENESCENCE DEFERRAL IN BIG BLUESTEM WITH EXOGENOUS CYTOKININ APPLICATIONS

by

EARL EUGENE TOWNE

B. S., Kansas State University, 1976

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERISTY
Manhattan, Kansas

1981

ABSTRACT

Different concentrations of the synthetic cytokinin benzyladenine (BA) were applied to ungrazed tallgrass prairie near Manhattan, Kansas in 1979 on four biweekly dates beginning in mid-June. Changes in chlorophyll and crude protein levels of big bluestem (*Andropogon gerardi* Vitman) leaves from the different treatments were monitored weekly from August until early-October. In December 1979, big bluestem rhizomes were dug and analyzed for crude protein and total nonstructural carbohydrates (TNC). Total herbage production of all species was determined after the 1980 growing season.

BA did not significantly delay chlorophyll breakdown in big bluestem, but leaves sprayed with 5 ppm BA averaged higher chlorophyll content than other treatments throughout the sampling period. Big bluestem receiving 5, 20, and 40 ppm BA applied in July had significantly higher crude protein levels than untreated leaves, but 10 ppm BA had no effect on protein content. Applying any BA concentration in mid-June was ineffective in maintaining high crude protein levels.

BA did not alter protein or TNC levels in big bluestem rhizomes, indicating that senescence deferral had no deleterious effect on internal nutrient reserve cycles. Applying 5 ppm BA at either mid- or late-July significantly increased herbage yields the following year over untreated plots.