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HORMONAL CONTROL OF SEX EXPRESSION
IN BUFFALO GOURD (CUCURBITA POETIDISSIMA HBK.)

by

Joseph Carl Scheerens

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF PLANT SCIENCES
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
WITH A MAJOR IN AGRONOMY AND PLANT GENETICS
In the Graduate College
THE UNIVERSITY OF ARIZONA

1985
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Joseph Carl Scheeren entitled Hormonal Control of Sex Expression in Buffalo Gourd (Cucurbita foetidissima HBK.) and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Signed:

Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation Director

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SIGNED

Joseph C. Schreens
DEDICATION

This manuscript is dedicated to the memory of my mother,

EDITH ADELE MUNSON SCHEERENS,

whose love for plant life and natural ability for its culture was instrumental in the development of my interest in agriculture

and

to my father,

EMIL EDWARD SCHEERENS,

whose natural curiosity and mechanical aptitude continues to stimulate my interest in science.
ACKNOWLEDGMENTS

In the course of graduate study, one usually encounters many ‘rough spots’ which (with luck) are eased by the generous gift of time, effort and/or encouragement from others. I have considered myself lucky indeed to have been associated over the last decade with two excellent departments (the Department of Plant Sciences and the Department of Nutrition and Food Science) and with the many members comprising the Interdisciplinary ‘Buffalo Gourd Research Team’ who have made both work and study a joy.

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In addition, I would like to express special thanks to Dr. James W. Berry, also for many reasons: for encouraging me to continue advanced study, for contributing to my understanding of the 'philosophy' of science, for allowing me to expand my research interests and capabilities as a fledgling food scientist, and for introducing me to the chemistry of everyone's favorite biopolymer (i.e. starch).
I wish to thank the other members of my graduate committee, Drs. John E. Endrizzi, Robert T. Ramage and Charles W. Weber for friendly advice throughout my tenure as a graduate student and for their willingness to help with the 'nuts and bolts' of the degree requirements (i.e. tests, dissertation critique etc.). I would also like to thank Dr. Melvin H. Schonhorst for serving as a last minute substitute during my oral preliminary examination.

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ABSTRACT

Seven field experiments and two in-vitro studies were performed to elucidate hormonal control of staminate flowering in gynoeclous and monoecious buffalo gourd (Cucurbita foetidissima HBK.) sex types. Objectives included development of techniques effecting staminate induction on gynoeclous phenotypes which normally produce abortive stamenless male buds.

Natural and synthetic growth regulants shown to modify sex expression in other cucurbits were surveyed for their masculinizing potential. Several compounds exogenously-applied to apical meristems elicited changes in shoot morphology. However, only aminoethoxyvinylglycine (AVG, an ethylene synthesis inhibitor) effected staminate induction on gynoeclous segregates. Growth rate, patterns of female flowering or ontogeny of stamenless buds differentiated prior to treatment were not influenced by AVG.

AVG was applied at various dosages (0-500 ppm) and produced male buds on all replicates treated at levels of 125 ppm or higher. The mean number of staminate buds induced varied linearly with dosage and averaged from 0-7.5 male flowers/shoot. A control model for staminate induction mediated by endogenous ethylene was advanced and potential benefits of this phenomenon to breeding efforts and/or to hybrid seed production were discussed.

Ethephon (an ethylene releasing compound) was applied at various dosages to monoecious plants in anticipation of simulating the
gynoecious phenotype. Although morphological changes were evident (i.e. reduction in shoot growth rate and floral initiation, increase in floral bud abortion and tissue senescence), ethephon failed to reduce staminate flowering or increase differentiation of antherless buds as expected. Dosage levels employed and/or confounding environmental factors may have contributed to the lack of staminate inhibition.

During in-vitro studies, indirect evidence for ethylene-mediated control of male flowering was obtained by staminate proliferation in buds of gynoecious explants treated with silver nitrate (an inhibitor of ethylene action) and by formation of stamenless buds on monoeclous explants treated with ethephon. However, low levels of floral induction under culture conditions employed rendered these results inconclusive.

An incidental study of segregation ratios among AVG-facilitated self- and cross-pollination progeny upheld the supposition for monogenic inheritance of gynoecy in buffalo gourd.
CHAPTER 1
INTRODUCTION AND RESEARCH PURPOSE

Buffalo Gourd

Biological aspects of buffalo gourd, *Cucurbita foetidissima* HBK., have been presented in publications by several authors (Bailey, 1943; Bemis et al., 1975; Bemis et al., 1978; Bemis, Berry and Weber, 1979). The information has been recently reviewed in detail by Hogan and Bemis (1983) and Gathman and Bemis (1985).

This species, a perennial xerophytic member of the squash genus, is a ruderal colonizer native to the plains and higher deserts (above 1200 m) in northern Mexico and western USA. Feral plants exist in colonies which produce a dense vine cover. Total vine length of individuals can exceed 2000 m (Dittmer and Talley, 1964). At each node, vines contain initials for development of flowers, leaves, tendrils, shoots and adventitious roots.

Flowers (staminate or pistillate) are borne singly. Staminate flowers contain three to five coalesced stamens. Pollen is transferred to stigmatic surfaces mainly by solitary bees (Hurd and Lindsley, 1964). Pistillate flowers are epigynous with subtending ovaries commonly divided into three or four carpels. Flowers reach anthesis near dawn while reduced stigmatic receptivity and pollen viability occur after mid-morning. Once pollinated, gourds (pepos) mature seed within 32 to 38 days (Ba Amer and Bemis, 1968; Costa and Bemis, 1972).
Individual plants can produce substantial fruit yields. These fruit possess a hardened exocarp, fibrous placental tissue and typically mature 200 to 300 seeds which are rich in protein and vegetable oil.

Buffalo gourd is a sexually dimorphic species exhibiting both monoecious (bearing male and female flowers separately) and gynoecious (developing female flowers only) phenotypes. Gynoecy is thought to be the result of a single dominant gene (M) (Dossey, Bemis and Scheerens, 1981). Sexually dimorphic populations are presumed to be composed of two genotypes with respect to sex expression: Mm (gynoecious) and mm (monoecious). Therefore, progeny of gynoecious seed parents segregate in a 1:1 ratio of sex types. There is also evidence to suggest that gynoecious plants produce more fruit than do their monoecious counterparts (Curtis and Rebelz, 1974; Scheerens et al., unpublished data). This effect, however, may not be universal over all background genotypes or in all environments (Wilkins, 1980). Sex expression in buffalo gourd and other cucurbita Is discussed in detail in Chapters 3 through 6.

Although seed production may be adequate for maintenance of the species, feral colony proliferation is predominantly accomplished asexually through the development of adventitious roots. Roots of buffalo gourd are essentially storage organs for water and starch which provide raw materials for vine regrowth after periods of overwintering or drought (Dittmer and Roser, 1963; Dittmer and Talley, 1964). Roots which typically weigh 3 to 4 kg in their second season of growth are protected from desiccation by an impermeable suberized periderm.
Buffalo Gourd Domestication

Although native North Americans utilized the feral plant in various ways (Gathman and Bemis, 1985), steps toward the domestication of this species first occurred by scientific investigation after World War II. Curtis (1946) first suggested the use of buffalo gourd as an oilseed crop. In the decade following this report, several researchers investigated quality parameters of the seed oil (Ault, Swain and Curtis, 1947; Bolley, McCormack and Curtis, 1950; Shahani et al., 1951) but breeding and agronomic development of the species remained virtually unexplored until 1968.

An intensive selection and hybridization program was established in Lebanon in 1968 under the direction of Curtis (Curtis and Rebelz, 1974; Bemis et al., 1975). Emphasis of this program centered on improvement of seed yield per plant and on selection of seedstocks exhibiting high levels of protein and oil. Genetic investigations included the discovery and study of sexual dimorphism within the species.

Since 1974, an interdisciplinary program at the University of Arizona has intensified efforts to domesticate buffalo gourd. Agronomic development of this crop has progressed through a logical sequence of germplasm collection and screening, genetic and pathological studies, selection and hybridization followed by studies of cultural (cropping) techniques and their effect on yield parameters. Concurrent with field research, extensive investigations depicting the physical and chemical nature of buffalo gourd raw products, their potential uses in food and feed, nutritional and toxicological implications of their consumption by man and domestic animals, and their potential as industrial products or
bloenergy sources were conducted by University of Arizona personnel and other research groups (Gathman and Bemis, 1985).

During the course of research, emphasis has shifted from development of buffalo gourd as an oilseed crop to that of its development as a starch-producing crop. Justifications of this change include the following: 1) Inadequate or unpredictable seed yields from currently available genetic stocks (Gathman and Bemis, 1985); 2) the immediate availability of germplasm that when grown at high plant densities (500,000 plants/ha) produces commercial yields of starch (Nelson et al., 1983); and 3) the unique physical and chemical properties of the starch (Dreher et al., 1983; Scheerens et al., 1983). Commercialization of buffalo gourd as a starch crop has been attempted through joint efforts of the University of Arizona and various corporations.

**Buffalo Gourd Improvement Through Plant Breeding**

As buffalo gourd emerges as a commercial crop, a major concern of both researchers and corporate associates will be the evolution of high yielding, improved and perhaps legally protected germplasm. Breeding of high seed producing lines will most likely command as much attention as development of genetic stocks for high starch yields. Establishment of high density buffalo gourd plantings for starch production may require as much as 40 kg of seed/ha (Nelson et al., 1983). High seed yielding lines used to produce planter's seed stocks might help to limit costs of planting starch production acreages. Also, the isolation and development of consistently high seed yielding genotypes might enhance the commercial feasibility of buffalo gourd as an oilseed
crop. Finally, sufficient variation exists within the available germplasm for rapid advancement in seed production rates using a variety of breeding strategies (Scheerens et al., 1978; Scheerens et al., unpublished data; Gethman and Bemis, 1985). Programs for yield improvement through breeding will probably be augmented by selection for culturally desirable traits and disease resistance.

**Sexual Dimorphism and Plant Improvement**

Biological and genetic aspects of buffalo gourd which may enhance or impede breeding progress are listed in Table 1. Sexual dimorphism is considered to provide both advantages and disadvantages to breeders attempting varietal and F1 hybrid development. The existence of gynoecious phenotypes predisposes the potential for F1 hybrid production with minimal hand labor. However, with respect to breeding logistics, constraints imposed by sexual dimorphism in buffalo gourd are analogous to those effected by other systems of genic male sterility in constant backgrounds of sterile cytoplasm. Barriers to self and cross-pollination among gynoecious genotypes may retard progress under improvement schemes which require inbreeding or mating among all selected phenotypes.

As buffalo gourd breeding programs expand in scope, a technique for self- and cross-pollination of gynoecious plants would greatly simplify the development of gynoecious Inbreds or the use of these plants in common breeding schemes. Breeding flexibility obtained by the use of said technique would save time, money, land and human resources.
Table 1: Biological and Genetic Aspects of Buffalo Gourd Advantageous or Disadvantageous to the Process of Plant Breeding

<table>
<thead>
<tr>
<th>Biological/Genetic Aspects</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td><strong>Advantageous Aspects</strong></td>
<td></td>
</tr>
<tr>
<td>Ample genetic variation for important traits</td>
<td>Genetic variation exists within available germplasm for seed yield per plant, root yield, important quality parameters and agronomic characteristics.</td>
</tr>
<tr>
<td>Large unisexual flowers</td>
<td>Large unisexual flowers simplify mechanics of controlled crossing. Male flowers can be detached and transported from plant to plant and from field to field.</td>
</tr>
<tr>
<td>Long flowering period</td>
<td>Flowers are produced over a relatively long time span allowing for multiple self- or cross-pollinations to be made on individual seed parents.</td>
</tr>
<tr>
<td>Perennial growth habit</td>
<td>Perennial regrowth maintains heterozygous (unique) genotypes.</td>
</tr>
<tr>
<td>Sexual dimorphism</td>
<td>Gynoeclous and monoecious genotypes within breeding populations allow the development of hybrids without hand labor.</td>
</tr>
<tr>
<td>Asexual reproduction</td>
<td>Relative ease of asexual propagation enhances flexibility in maintaining or increasing desired heterozygous (unique) germplasm.</td>
</tr>
</tbody>
</table>
Table 1: (Continued)

<table>
<thead>
<tr>
<th>Biological/Genetic Aspects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disadvantageous Aspects</strong></td>
<td></td>
</tr>
<tr>
<td>Large plant size and space requirement</td>
<td>Plant size and indeterminate growth habit limit the number of genotypes which can be incorporated in a breeding program.</td>
</tr>
<tr>
<td>Inbreeding depression</td>
<td>Unlike other cucurbits, buffalo gourd appears to suffer some degree of inbreeding depression. Further study is necessary before the effects of this phenomenon upon breeding programs can be accurately assessed.</td>
</tr>
<tr>
<td>Perennial growth habit</td>
<td>Perennial growth habit delays the expression of seed yield potential. Plants that yield little or no seed in their first year of growth often produce adequate or superior yields in subsequent seasons.</td>
</tr>
<tr>
<td>Sexual dimorphism</td>
<td>The inability to self- and or cross-pollinate gynoecious phenotypes may hamper progress under certain breeding regimes.</td>
</tr>
</tbody>
</table>
In addition, development of this technique would allow the isolation of homozygous gynoecious (MM) seed stocks. Such germplasm would greatly benefit future buffalo gourd seedsmen in several ways. The development of true breeding gynoecious lines would eliminate the necessity of hand roguing monoecious segregates from seed parent populations used for the production of hybrids. Reduction in labor would enhance possibilities for affordable F₁ or population hybrid planter's seed. Also, all hybrid progeny of crosses between gynoecious and monoecious homozygotes would exhibit gynoecious phenotypes. Hybrids could be used to create new seed parent lines for production of three-way crosses. It could also be blended with seed of an appropriate monoecious pollinator (if needed) and used as planter's seed. For commercial acreages producing buffalo gourd as an oilseed, the hybrid:pollinator ratio might be adjusted to optimize the high seed yielding effects of the gynoecious phenotype.

**Research Purpose**

Research objectives have been twofold and interrelated: 1) to evaluate the effects of exogenously applied growth regulants upon sex expression in buffalo gourd; and 2) to elucidate, in part, the nature of hormonal control over sex expression in buffalo gourd. The primary goal of this work was the development of methodology required for staminate flower production on gynoecious plants. This applied study was complemented with basic research efforts to further describe floral biology of buffalo gourd sex types.
CHAPTER 2
GENERAL LITERATURE REVIEW

Salisbury and Ross (1978) described plant development as consisting of two processes: growth and differentiation. Experimental evidence for hormonal influence on most (if not all) developmental functions in plants is substantial. A thorough review of all hormonal effects on growth or differentiation is therefore beyond the scope of this manuscript. Rather, the material presented in this chapter has focused upon general properties of each hormone or hormone group, proposed mechanisms for hormonal regulation of plant development through gene action, and more specifically, upon the role of plant hormones in floral evocation and development. This general survey of publications has been included to augment a more specific review of literature pertaining to sex expression in the Cucurbitaceae and its agronomic implications as presented in Chapter 3 and to provide needed background for discussion of research results.

Properties of Plant Hormones

Moore (1979) has described plant hormones as organic substances which: are active in very minute concentrations (usually less than $10^{-6}$ M); are often produced in one organ and translocated to sites of activity in other organs; and are regulators of specific biochemical, physiological and/or morphological events which either induce or inhibit
growth or differentiation. Common hormone classifications have included auxins, ethylene, gibberellins, cytokinins and abscisic acid.

Structure, Biosynthesis and Metabolism

**Auxins.** Evidence for the presence of a photosensitive growth promoting stimulus in coleoptiles of canary grass (*Phalaris canariensis*) was first reported by Charles Darwin in 1880 (Wareing and Phillips, 1978; Moore, 1979; Bearder, 1980). Darwin was able to demonstrate curvature of growing coleoptiles in the direction of a unilateral light and to identify the coleoptile apex as the source of the stimulus controlling this phenomenon. In 1926, F. W. Went collected a chemical substance from oat (*Avena sativa*) coleoptiles responsible for their curvature in the presence of unilateral light and devised a bioassay procedure (the avena coleoptile curvature test) which is still in use. Chemical substances which were active in the avena curvature test (termed auxins) were isolated from malted barley (*Hordeum vulare*), human urine and fungal sources by Kogl and other research groups in the 1930s. In 1934, Kogl and coworkers identified the primary auxin as indole-3-acetic acid (IAA). Since then, researchers have demonstrated the presence of IAA in most actively growing plant tissues (Bearder, 1980).

Most authors consider IAA to be the most significant if not the only naturally occurring auxin. However, evidence has been recently accumulated to suggest the endogenous occurrence and auxin-like activity of chlorindeoles (Sembdner et al., 1980) and non-indole auxins such as p-hydroxyphenylacetic acid (Kefell and Kutacek, 1977; Bearder, 1980). The term "bound auxins" has frequently been applied to ester or amide
Linked conjugates of IAA including IAA thioglucosides, such as glucobrassin, glycosyl esters such as IAA-myo-inositol and IAA peptides like IAA-aspartate and IAA-glutamate. IAA conjugates have been considered to be storage forms of IAA (Moore, 1979) and their role in regulation of endogenous IAA levels has been discussed by Muir (1972), and by Bandurski (1979).

Isolation of IAA by Kogl precipitated suggestions that tryptophan was its precursor. Many studies have since substantiated the role of tryptophan in auxin biosynthesis and this common amino acid is now considered to be the primary auxin intermediate (Schneider and Wightman, 1974; Wareing and Phillips, 1978; Moore, 1979; Sembdner et al., 1980; Bandurski, 1982). Biosynthesis of tryptophan from shikimic and anthranilic acids was reviewed by Kefell (1978). Pathway specificity for D-tryptophan was puzzling before the discovery of tryptophan racemase in cell cultures of tobacco (Moore, 1979). Since then, this enzyme which converts the commonly occurring L-enantiomer to the D-form has been viewed as a possible regulator of auxin biosynthesis.

Schneider, Gibson and Wightman (1972) reviewed five pathways for auxin biosynthesis from tryptophan proposed for various species. Two of these pathways are considered to be predominant in higher plants (Figure 1). In pathway #1 elucidated by Wightman and Cohen (1968), tryptophan is first converted to indole-3-pyruvic acid through the action of tryptophan transaminase (a multispecific aminotransferase). Indole-3-pyruvic acid is then decarboxylated to form indole-3-acetaldehyde which in turn is oxidized to produce IAA by the action of NAD-dependent indoleacetaldehyde dehydrogenase. An alternate reversible reaction
Figure 1: Biosynthesis and Metabolism of Indole-3-acetic Acid

A: Tryptophan
B: Indole-3-pyruvic acid
C: Indole-3-acetaldehyde
D: Indole-3-acetic acid (IAA)
E: Indole-3-ethanol
F: Tryptamine
G: Indolenine hydroperoxide
H: Indolenine epoxide
I: 3-Methyleneoxindole
J: 3-Methylindole
K: Indole-3-carboxylic acid
L: Zeanic acid

1Adapted from Sembdner et al. (1980)
Figure 1: Biosynthesis and Metabolism of Indole-3-acetic Acid
reduces indole-3-acetaldehyde to indole-3-ethanol with the employment of an alcohol dehydrogenase. Vickery, Sherwin and Purves (1972) isolated this enzyme from cucumber (Cucumis sativus) seedlings and demonstrated its partial inhibition by IAA. These authors suggested indole-3-ethanol to be a storage form of IAA and proposed a model for the regulation of endogenous level of IAA through feedback inhibition.

Evidence for the operation of pathway #2 in tissues of tomato (Lycopersicon esculentum) and barley was offered by Schneider, Gibson and Wightman (1972). In this scheme, tryptophan was first decarboxylated to form tryptamine through the action of tryptophan decarboxylase. IAA oxidase and other enzymes were thought to deaminate and oxidize tryptamine forming indole-3-acetaldehyde. Although tryptamine was reported to be a natural compound synthesized by many species, its presence was not uncovered universally. It was not, for example, found in species of Cucurbita. However, in barley and tomato both pathways were demonstrated to be operative. The authors suggested further study to reveal predominant pathways used at different stages of growth, in different tissues or under different physiological conditions.

Minor synthetic pathways thought to produce IAA were discussed by Sembdner et al. (1980). Vagrancies in IAA biosynthesis among species were best summarized by Moore (1979).

At the present state of our knowledge, the possibilities must be considered that: (1) there may be, and probably are in particular cases, different pathways of IAA biosynthesis from tryptophan in different species; (2) that more than one pathway may be operative in a single species; and (3) that these pathways may differ in different parts of the same plant or at different stages of growth in the same plant part.
Once produced, IAA can be inactivated by reversible or irreversible formation of IAA conjugates as discussed above, by photooxidation enhanced in the presence of plant pigments and/or redox systems, or by enzymatic oxidation. An in-depth discussion of IAA catabolism has been offered by Sembdner et al. (1980) and Bandurski (1982). These authors reviewed evidence supporting IAA degradation through the formation of IAA free radicals and hydroperoxides by the action of endogenous peroxidases and IAA oxidase. Hydroperoxides were decarboxylated forming epoxides which were subsequently oxidized to form compounds such as 3-methyleneoxindole, 3-methyloxindole and Indole-3-carboxylic acid (Figure 1). Oxidized derivatives of IAA have been isolated from a number of plant sources. Sembdner et al. (1980) also summarized recent evidence for alternate pathways of IAA oxidation without decarboxylation. These pathways terminated with the formation of compounds such as zeanic acid which has been isolated from corn (Zea mays) steep liquor.

Ethylene. Establishment of ethylene as an important endogenous growth regulant has been reviewed by Abels (1973), Moore (1979) and Bearder (1980). Unlike early histories of most phytohormones, effects of ethylene on plants were discovered through 'accidental exogenous application'. In 1864, Girardin first reported the defoliation of trees near a leaking gas main. Scattered reports over the next forty years expounded upon the effects of illumination (lighting) gas on various developing plant organs. Neljubov first determined the active agent in illuminating gas to be ethylene in 1901. He noticed that etiolated pea (Pisum sativum) seedlings grown in laboratory air (containing some illuminating gas) exhibited shortened, swollen and prostrate stems.
Systematic examination of various gas constituents on morphological development of etiolated pea seedlings identified ethylene as the causal agent.

Ethylene was illustrated to be a natural product of plant metabolism by Gane in 1934. He trapped olefinic volatiles from apples (*Malus* spp.) by bromination and observed the presence of ethylene dibromide in the trapped materials. Soon after this discovery, Crocker and coworkers suggested ethylene to be a plant hormone regulating growth and fruit ripening. However, its hormone status remained controversial until the late 1950s when gas chromatographic analysis refined analytical techniques. This refinement allowed accurate identification and quantitation of ethylene from a variety of plant tissues. Since then, ethylene has been well established as an endogenous regulant and many aspects of its metabolism have been explored.

Potential precursors of ethylene which have been initially explored using model systems include the following: linoleic acid, acetate, fumarate, carbohydrates, glycerol, propanal, acrylic acid, β-alanine, β-hydroxypropionic acid, methional, methionine, ethionine and IAA (Yang, 1968; Yang and Baur, 1972; Abels, 1973; Sembdner et al., 1980; Yang et al., 1982). Many of these potential substrates were screened for their ability to produce ethylene in enzyme-free model systems employing oxidants and reductants, peroxidase enzymes or photo-activated electron transfer agents such as flavin mononucleotide (FMN) (Yang, 1968). However, of all possible precursors, methionine has been the only material repeatedly shown to evolve ethylene in-vivo (Yang and Baur, 1972; Lieberman, 1979; Sembdner, 1980).
Subsequent studies with radiolabelled methionine indicated the formation of the following products in vivo from specific carbon atoms of the substrate: CO₂ (C-1), formic acid (C-2), ethylene (C-3 and C-4) and thioester products retained in tissues (C-5 and sulfur) (Abels, 1973). The importance of sulfur retention during ethylene synthesis (as opposed to formation of gaseous sulfur products) for maintenance and reformation of sulfur amino acid pools was discussed by Yang and Baur (1973). Through studies with labelled methionine these authors also determined that all functional groups of the substrate are essential for enzymatic conversion to ethylene. Metabolites of methionine such as methylmethionine, homoserine and α-keto-methylthiolbutyric acid previously considered as potential pathway intermediates were thought to evolve ethylene only after conversion to methionine.

Current evidence suggests the conversion of methionine to ethylene following the pathway outlined by Adams and Yang (1979), Yang (1980), Sembdner et al. (1980) and by Yang et al. (1982) (Figure 2). The discovery of ethylene synthesis inhibition in the presence of 2,4-dinitrophenol (DNP, an inhibitor of oxidative phosphorylation) indicated a pathway requirement for ATP. It was hypothesized and latter proved that ATP was needed to convert methionine to S-adenosylmethionine (SAM). The second step, [cleavage of the methionine moiety between C-4 and sulfur to form methylthioadenosine and an as then unknown ethylene-releasing compound] was catalyzed by a pyridoxal phosphate (vitamin B₆)-dependent mechanism. Labelling studies substantiated the conversion of SAM to methylthioadenosine and its subsequent metabolite methylthiorebose. Further corroboration was demonstrated through the inhibition
Figure 2: Predominant Pathway of Ethylene Biosynthesis

A: Methionine
B: S-Adenosylmethionine
C: Pyridoxal Phosphate (Vitamin B₆, enzyme-bound)
D: Enzyme-bound Schiff base intermediate
E: Enzyme-bound Schiff base intermediate
F: Enzyme-bound Schiff base intermediate
G: Methylthioadenosine
H: Methylthioribose
I: Homoserine
J: 1-Amino-cyclopropane-1-carboxylic acid (ACC)
K: Ethylene

¹Adapted from Sembdner et al. (1980)
Figure 2: Predominant Pathway of Ethylene Biosynthesis
of ethylene synthesis by rhizobitoxine and its ethoxy analog amino-ethoxyvinylglycine (AVG, see Chapter 3). Both compounds were shown previously to be potent inhibitors of pyridoxal phosphate-dependent reactions. Methyllthioribose was demonstrated to transfer its thioester group to homoserine to reform methionine by the use of radiolabelling. The identity of the unknown metabolite formed from cleavage of SAM was first elucidated by Adams and Yang (1979) and recounted soon after by other research groups to be 1-amino-cyclopropane-1-carboxylic acid (ACC). The oxygen-dependent mechanism of ACC decomposition to ethylene, formic acid, NH₃, and CO₂ is still under investigation.

Regulation of this pathway varies from organ to organ and from species to species. Interaction with stress environments often results in increased ethylene synthesis as does the mechanical wounding of tissue (Abeles, 1973). Pathway intermediates as well as other growth regulators either initiate, stimulate or retard ethylene production (Abeles, 1973; Sembdner et al., 1980; Amrhein et al., 1982; Imaseki, Yoshii and Todaka, 1982; Yang et al., 1982). The most studied of these interactions is that of auxins and ethylene (Imaseki, Yoshii and Todaka, 1982). IAA and synthetic auxins stimulate the production of ethylene in most tissues through nuclear induction of RNA synthesis. Increased messenger RNA presumably codes for production of the enzyme complex responsible for conversion of SAM to methyllthioadenosine and ACC. This process is also stimulated by the addition of synthetic cytokinins (see discussion below). "Autocatalysis of ethylene production is a common feature of ripening fruits and other senescing tissues in which a massive increase in ethylene production is triggered by exposure to ethylene above a
threshold level" (Yang et al., 1982). This effect may be based on increased production of ACC synthesizing enzymes or through changes in compartmentalization of its potential substrates (Mayak and Halevy, 1980, see discussion below). Conversely, the proliferation of ACC synthesizing enzymes is halted by ACC accumulation or through the action of abscisic acid (a growth inhibitor) (Imaseki, Yoshii and Todaka, 1982). Enzymes responsible for the conversion of ACC to ethylene are stimulated by added CO₂ (Yang et al., 1982).

Although accumulated evidence for the ethylene synthesis scheme presented above is substantial, it may not represent the only possible pathway for evolution of this hormone. Yoshii, Watanabe and Imaseki (1980) recently offered evidence of additional pathway intermediates of auxin-induced ethylene synthesis in mung bean (Vigna radiata). Also, studies reviewed by Sembdner et al. (1980) have demonstrated evidence for additional or alternate pathways of ethylene synthesis in some species. Baker, Lieberman and Anderson (1976) reported rhizobitoxine insensitive ethylene synthesis in avocado (Persea spp.) which was inhibited by free radical scavengers such as benzoate and propyl gallate. This synthetic mechanism was reminiscent of model systems involving light activated FMN (Yang, 1968) or the mode of ethylene genesis exhibited by some synthetic auxins (Sembdner et al., 1980).

Once produced, little if any ethylene is incorporated into plant tissues. Studies have shown labelled carbons of ethylene to be incorporated into organic acids, benzene, CO₂, and more recently, ethylene oxide and ethylene glycol (Bloomstrom and Beyer, 1980; Sembdner et al., 1980). However, under certain conditions, a substantial proportion of
synthesized ACC was shown to be converted to its N-malonyl conjugate (Amrhein et al., 1982; Yang et al., 1982). Formation of malonyl-ACC was thought to represent a potent ethylene detoxification mechanism in stressed tissues.

**Gibberellins.** This hormone class undoubtedly contains a greater number of compounds, both active and inactive, than any other. All gibberellins (GAs) are tetracyclic diterpenoids composed of an ent-gibberellane structure (Figure 3) which has been oxidized at two or more sites. Most GAs also possess a methylene group at C-16 and may be further unsaturated at the C-1,2 or C-2,3 position of the A ring. Two general groups of GAs exist in nature, those that contain 20 carbon atoms [C(20) GAs] and those that have been transformed to C(19) GAs through the loss of C-20. Aspects of GA metabolism are presented below.

The history of GA research has been reviewed by Moore (1979). The identification of this hormone class began with studies of 'bakanae' (foolish seedling) disease of rice (*Oryza sativa*). This condition, characterized by formation of tall and spindly plants was attributed to fungal infestations of *Gibberella fujikuroi* (*Fusarium moniliforme*) known to be pathogenic in several other crops. During the latter 1930s Japanese workers Yabuta and Sumiki first isolated crude preparations of active materials (termed gibberellins) from fungal cultures. However, these discoveries were not evinced in western societies until the 1950s. Radley (in England) and West and Phinney (in USA) simultaneously demonstrated the endogenous presence of GAs in several species of higher plants. Soon after this disclosure, MacMillan and Suter isolated and identified GA₁ from immature scarlet runner beens (*Phaseolus coccineus*).
Since then, at least 57 endogenous GAs have been isolated and characterized from fungal or higher plant sources (Bearder, 1980).

The biosynthesis and metabolism of GAs have been reviewed by several authors: West and Fall, 1972; Barendse, 1975; MacMillan, 1977; Hedden, 1979; Moore, 1979; Phinney, 1979; Sembdner et al., 1980 and Grabe, 1982. The hormone class was demonstrated to be of terpenoid origin when labelled carbons of acetate and mevalonate fed to cultures of \textit{G. fujikuroi} appeared in various isolated GAs. Biosynthetic steps from acetate to trans-geranylgeranyl pyrophosphate (GGPP) which involve the systematic condensation of 6 carbon isoprenoid units are common to the synthesis of all \textit{di-}, sesqui- and triterpenes (GAs, steroids, carotenoids, etc.) (Figure 3).

The cyclization of GGPP to copalyl pyrophosphate (CPP, dicyclic) and subsequent condensation of CPP to form the tetracyclic compound ent-kaurene were found to be catalyzed by the complex enzyme ent-kaurene synthetase. West and Fall (1972) studied this enzyme system and reported it to possess two active sites, one cyclizing GGPP to CPP (site A) and the other converting CPP to ent-kaurene (site B). The complex was shown to be the site of action of several GA synthesis inhibitors (Corcoran, 1975). Moore and Ecklund (1974) have suggested \textit{in-vivo} regulation of GA synthesis to be a function of ent-kaurene synthetase. Stereospecificity of the (-) enantiomer of kaurene was demonstrated when the addition of (+) kaurene and other isomers to fungal cultures failed to produce labelled GAs.

Grabe (1972) elucidated the oxidative transformation of ent-kaurene to \textit{7\beta}-hydroxy-ent-kaurenolic acid using an ATP-dependent cell-
Figure 3: Pathway of Gibberellin Biosynthesis

A: Mevalonate
B: trans-Geranylgeranyl pyrophosphate
C: Copalyl pyrophosphate
D: ent-Kaurene
E: ent-Kaurenol
F: ent-Kaurenoic acid
G: GA12-aldehyde
H: 7β-Hydroxy-ent-kaurenoic acid
I: GA13
J: GA12

1Adapted from Moore (1979)
Figure 3: Pathway of Gibberellin Biosynthesis
free system isolated from endosperm of *Cucurbita pepo*. The oxidation of ent-kaurene required hydroxylation of the substrate at the C-7 and the C-19 positions by the action of mixed function oxidases. Further oxidation at the C-19 position to form a carboxylic acid was presumably accomplished by NADPH + H\(^+\) dependent dehydrogenases. Oxidative enzymes involved were associated with microsomal membranes whereas enzymes responsible for the synthesis of ent-kaurene were not (Moore and Ecklund, 1974; Sembdner et al., 1980).

Evidence concerning contraction of the B ring at the C-7 position of 7β-hydroxy-ent-kaurenoic acid was summarized by Grabe et al. (1974) and by Sembdner et al. (1980). The Mn\(^{+2}\)-dependent enzyme responsible for ring contraction was found to exhibit low substrate specificity as various hydroxylated kaurenoids and kaurene analogs could also be metabolized by *Gibberella* cultures. Possible products of enzymatic reaction using a cell-free system isolated from *Cucurbita maxima* were shown to be 6β-7β-dihydroxy-ent-kaurenoic acid and GA\(_{12}\)-aldehyde (Grabe et al., 1974). The latter compound was said to be the first in the biosynthetic pathway of GAs to possess the ent-gibberellane structure.

Interconversion of GAs and the effects of structure upon GA activity have been discussed by several authors: Grabe et al., 1974; Grabe, Hedden and MacMillan, 1974; Takahashi, 1974; Durley, Rallton and Pharils, 1974; Rallton, Durley and Pharils, 1974; MacMillan, 1977; Sembdner et al., 1980. In addition to oxidation of the aldehydic group at C-7, common structural modifications of GA\(_{12}\)-aldehyde have been shown to include hydroxylation at the C-2, C-3 and C-13 positions, loss of the C-20 carbon, formation of a C-10,19 lactone and desaturation at either
the C-1,2 or the C-2,3 positions. Difficulties in studying GA interconversion pathways in higher plants have been attributed to the myriad of possible GA forms (over 40 have been isolated from higher plants) and their rapid interconversion from form to form (Sembdner et al., 1980). Also, it has been demonstrated that pathways differ from species to species and from organ to organ within the same species. However, accumulated information from several studies has elucidated some general schemes.

Investigations of C(20) GA metabolism in fungal cultures and higher plants have indicated that conversion of GA\textsubscript{12}-aldehyde to GA\textsubscript{14}-aldehyde by hydroxylation at the C-3 position may precede oxidation of the C-7 aldehydic group in some species. This conversion appeared to occur in pea seedlings as all isolated C(20) GAs were hydroxylated at the C-3 position (Durley, Rallton and Phariss, 1974) (Figure 4). An alternate pathway has been demonstrated to operate in Cucurbita systems as shown by the occurrence of non-hydroxylated C(20) GAs prior to formation of C-3 hydroxylated forms (Grabe, Hedden and MacMillan, 1974; Grabe et al., 1974; Grabe, 1982). Evidence to suggest early hydroxylation at the C-13 position of C(20) GAs in legume systems has been reviewed by Takahashi (1974). Subsequent hydroxylation and lactone formation steps in these systems and/or ultimate defunctionalization at C-20 to form C(19) GAs are illustrated in Figure 4. Elucidation of recent research concerning these pathways has been offered by Grabe (1982).

Conversion of C(20) GAs to C(19) GAs in Gibberella has been confirmed by the feeding of radiolabelled C-7 hydroxy analogs of GA\textsubscript{12} and GA\textsubscript{14}-aldehydes resulting in labelled compounds not possessing C-20
Figure 4: Comparison of C(20) Gibberellin Metabolism In Three Species

\textit{Plum sativum}

A: GA_{12}-aldehyde
B: GA_{14}-aldehyde
C: GA_{14}
D: GA_{18}
E: GA_{28}
F: GA_{23}
G: GA_{1}
H: GA_{6}
Figure 4: Comparison of C(20) Gibberellin Metabolism in Three Species
Figure 4: (Continued)

Cucurbita

I:  \( GA_{12} \)
J:  \( GA_{24} \)
K:  \( GA_{25} \)
L:  \( GA_{36} \)
M:  \( GA_{13} \)
N:  \( GA_{43} \)
O:  \( GA_{15} \)
P:  \( GA_{38} \)

Leguminosae

Q:  \( GA_{12} \)
R:  \( GA_{53} \)
S:  \( GA_{44} \)

Figure 4: (Continued)
(Sembdner et al., 1980). Although extensively studied, the mechanism(s) and direct pathway(s) for the formation of C(19) GAs from their C(20) counterparts have remained elusive. Early hypotheses concerning this subject, derived from structural relationships of C(20) GAs, included the theory of successive oxidation at the C-20 position (CH₃--CHOH--CHO--COOH) followed by decarboxylation and subsequent formation of C(19) GAs. However studies of cell-free systems using radiolabelled substrates failed to indicate the action of this mechanism. Further study of alternative mechanisms have also proved inconclusive, but saturation of the A and B ring has been demonstrated to be a requirement of the process. C(19) GAs initially formed have been proposed to be GA₉, GA₄ and GA₂₀ (Figure 5). Further alterations in these three structures have been shown to be oxidative, the most notable oxidative event being the inactivation of GAs through hydroxylation at C-2 (Reeve and Crozier, 1975; Hedden, 1979).

Although structure-activity relationships have proved to vary among bioassay systems, general aspects of structural modification on relative activity have become apparent: C(19) GAs with γ-lactone structure at C-10,19 are highly active in most assays, with reduced activities realized from C(20) GAs with a C-20 aldehyde or a C-19,20 δ-lactone or lactol; hydroxylation of both C(19) and C(20) GAs at the C-3 and/or C-13 position stimulates biological activity; and hydroxylation in both GA species at the C-2 position inhibits or eliminates growth response in assay plants. C-2 hydroxylation has been hypothesized to be a potent regulatory mechanism of GA activity in-vivo (MacMillan, 1977; Hedden, 1979).
Figure 5: Metabolism of C(19) Gibberellins

A: GA₉
B: GA₄
C: GA₂₀
D: GA₁
E: GA₇
F: GA₃ (proposed pathways)
G: GA₃₄
H: GA₈
I: GA₅₁
J: GA₂₉

Adapted from Sembdner et al. (1980)
Figure 5: Metabolism of C(19) Gibberellins
In addition to inactivation of GAs by C-2 hydroxylation, GA activity in higher plants has been shown to be significantly affected by the formation of GA conjugates. Sembdner et al. (1980) divided known GA conjugates into 3 groups: "(a) hydroxyl-bound GA-O conjugates (glucosyl and acyl derivatives), (b) carboxyl-bound GA-O conjugates (glucosyl and alkyl esters), (c) carboxyl-bound GA-N conjugates (amino acid derivatives)". β-Glucosides were found to be the most prevalent form of naturally occurring GA conjugates. Sembdner et al. (1972) described the rapid metabolism of exogenously applied GA₃/GA₅ to their respective glucosides in several species and confirmed the reversibility of conjugation, reforming active compounds. However, Rappaport et al. (1974) were unable to show the reversibility of GA₅ glucosylation using a dwarf corn assay procedure. Species differences in catalytic activity of conjugates were suggested to be confounding elements in the study of GA function through the use of bioassays. Sembdner et al. (1980) summarized pathways of irreversible GA degradation through the formation of GA-amino acid conjugates and through possible further oxidation of C-2 hydroxyl groups to C-2 carbonyl groups.

Cytokinins. Cytokinins are the only class of plant hormones which contain compounds also synthesized in animal tissues. The metabolism of these compounds is directly linked with that of nucleic acids. All natural plant cytokinins contain an adenine nucleus and all (except one) are alkylated at the N-6 position with an isoprenoid side chain (Bearder, 1980). In plants, these substances can exist as free compounds or as constituents of transfer RNA (t-RNA) species. Cytokinins isolated from animal tissue are found only in association with t-RNA,
whereas various cytokinins isolated from plant cells are only found in the free state. Unbound cytokinin bases can also be conjugated as nucleosides, nucleotides, glycosides or with amino acids (Sembdner et al., 1980).

Cytokinins associated with t-RNA were shown to reside adjacent to the 3' end of the t-RNA anticodon (Moore, 1979; Bearder, 1980). Only certain t-RNA species (among them, those which are coded for transfer of serine, phenylalanine, cysteine, tryptophan, leucine, isoleucine, methionine, threonine and tyrosine) were found to possess a cytokinin constituent. This specificity appeared to be determined by the initial anticodon base (adenine or uracil). Also, cytokinins associated with either adenine or uracil initiated anticodons were specific.

Several studies have ascertained that cytokinins in t-RNAs are functionally significant (Moore, 1979). Fittler and Hall demonstrated that modification of the cytokinin moiety of yeast (Saccharomyces spp.) serine t-RNAs through iodination reduced their ability to bind with ribosomal complexes. These results were substantiated by similar studies of cytokinin-containing and cytokinin-free tyrosine t-RNA species isolated from Escherichia coli by Gefter and Russell, but were not supported by similar investigations of Lactobacillus acidophilus t-RNA activity in cell free systems (Moore, 1979). Cherry and Anderson (1972) speculated that cytokinin moieties in t-RNA species might be recognized by specific ribonuclease. These authors proposed a model of protein synthesis regulation involving the breakdown of cytokinin-containing t-RNAs by specific ribonucleases with the release of free cytokinins. Free cytokinins in cells were thought to compete for active
sites of the nucleases, thus controlling t-RNA destruction through feedback inhibition.

The recent history of cytokinin research was presented by Moore (1979). Discovery of cytokinins as a hormone class was associated with the advent of plant tissue culture techniques. While working with tobacco (*Nicotiana tabacum*) pith cultures, Skoog and others noticed substantial cell enlargement in auxin-treated calli without cytokinesis (cell division). This phenomenon was shown to be rectified by the addition of vascular tissue, and later by the addition of coconut milk, malt extract or yeast extract to the cell cultures. Miller and others isolated the first compound with cytokinin activity (kinetin) from autoclaved herring sperm DNA. This compound was later demonstrated to be formed during autoclaving from DNA decomposition.

Natural plant cytokinins are depicted in Figure 6. The first naturally occurring plant cytokinin (zeatin, $10^6$ Ade) was isolated from immature corn kernels in 1963 by Letham. Soon after the existence of zeatin riboside ($10^6$ Ado) was demonstrated. Since then, these two substances have been shown to be the most common cytokinins of the plant kingdom. Only the cis isomer was found to be associated with t-RNA, whereas free $10^6$ Ade and $10^6$ Ado were found to exist as both cis and trans isomers. Trans isomers proved to be the most physiologically active.

Although $10^6$ Ade/Ado are the most common plant cytokinins, their reduced counterpart $N^6$-(2-isopentenyl) adenine ($1^6$ Ade) and its riboside ($1^6$ Ado) are the most ubiquitous cytokinin compounds found in nature.
Figure 6: Biosynthesis and Metabolism of Plant Cytokins

A: \( R = H \): adenine  
   \( R = \) ribosyl: adenosine  
   \( R = \) ribosyl 5'-phosphate: AMP

B: \( R = H \): N\(^6\)[2-isopentenyl] adenine (1\(^6\) Ade)  
   \( R = \) ribosyl: N\(^6\)[2-isopentenyl] adenosine (1\(^6\) Ado)

C: \( R = H \): zeatin (1\(^6\) Ade)  
   \( R = \) ribosyl: zeatin riboside (1\(^6\) Ado)

D: \( R = H \): 2-methylthio-N\(^6\)[2-isopentenyl] adenine (ms 1\(^6\) Ade)  
   \( R = \) ribosyl: 2-methylthio-N\(^6\)[2-isopentenyl] adenosine (ms 1\(^6\) Ado)

E: \( R = H \): 2-methylthio zeatin (ms 1\(^6\) Ade)  
   \( R = \) ribosyl: 2-methylthio zeatin riboside (ms 1\(^6\) Ado)

F: \( R = H \): dihydrozeatin (H\(_2\) 1\(^6\) Ade)  
   \( R = \) ribosyl: dihydrozeatin riboside (H\(_2\) 1\(^6\) Ado)

G: \( R = H \): 3-hydroxydihydrozeatin

H: \( R = H \): 2,3-dihydroxydihydrozeatin

I: \( R = H \): 6-(3-hydroxy-3-methylbutylamino)-purine

\(^1\)Adapted from Sembdner et al. (1980)
Figure 6: Biosynthesis and Metabolism of Plant Cytokinins
$^{16}\text{Adenine}$ was first extracted from serine t-RNA moieties isolated from yeast by Blemann and coworkers (Moore, 1979).

 Biosynthesis of cytokinins was reviewed by Sembdner et al. (1980) and Chen (1981, 1982). Cytokinin containing t-RNAs were shown to arise by alkylation of adenine previously incorporated in the RNA molecule through studies with radiolabelled mevalonate and adenine precursors. Radiolabelled $^{16}\text{Adenine}$ and $^{10}\text{Adenine}$ isolated from t-RNAs were shown to possess radioactivity only in carbons of the Isoprenoid side chain.

Models of cytokinin activity and its relation to t-RNA turnover such as that presented by Cherry and Anderson (1972), stimulated the hypothesis of free cytokinin genesis resulting from catabolism of t-RNA. Indirect evidence for operation of this biosynthetic mechanism in corn was offered by Klement and Klambt (1974). Indirect evidence for de-novo synthesis of free cytokinins was submitted by Burrows (1978). Analysis of poplar (Populus $\times$ robusta) leaves and lupin (Lupinus luteus) seeds revealed differences in bound and free cytokinins. The t-RNA associated cytokinins were similar in both species consisting of $^{16}\text{Adenine}$, $^{10}\text{Adenine}$ and their 2-methylthio derivatives $^{16}\text{Adenine}$ and $^{10}\text{Adenine}$. Free cytokinins, however, differed from bound cytokinins and between species with dihydrozeatin ($H_2^{10}\text{Adenine}$) predominating in lupin and 6-(2-hydroxybenzylamino)-purine predominating in poplar. Further evidence for de novo cytokinin synthesis was suggested by differences in cellular distribution in zeatin isomers (see above). Confirmation of direct synthesis of free cytokinins was obtained by Chen in 1979 through cell-free synthesis of N-6 Isopentenyl adenosine monophosphate ($^{16}\text{AMP}$) from AMP and Isopentenyl pyrophosphate (Chen, 1981, 1982). From studies with
tobacco calli, Burrows and Fuell (1981) uncovered evidence for the operation of both biosynthetic systems.

Metabolism of cytokinins was discussed by Sembdner et al. (1980), Horgan et al. (1981) and by Laloue and Pethe (1982). From in-vivo experiments using Vinca rosea crown gall tissue (tissue infected with Agrobacterium tumefaciens) Horgan and coworkers reported rapid metabolic turnover of various cytokinins. A metabolic scheme devised from experimental evidence to date was presented by Sembdner et al. (Figure 6). In tobacco and corn, hydroxylation of $1^6$ Ade/Ado to $10^6$ Ade/Ado was demonstrated. Both hydroxylated and non-hydroxylated compounds were found to be modified at the C-2 position to form methylthio derivatives. Saturation of the side chain of $10^6$ Ade/Ado formed $H_2 10^6$ Ade/Ado in bean (Phaseolus vulgaris) axes. Further hydroxylation of the side chain resulted in the di- and trihydroxy analogs of $H_2 10^6$ Ade/Ado. Experimentation with pea and marrow (Cucurbita spp.) suggested the direct transformation of $1^6$ Ade/Ado to 6-(3-hydroxy-3-methylbutylamino) purine. One of the main pathways of cytokinin catabolism was demonstrated to be the breakdown of $1^6$ Ade/Ado and $10^6$ Ade/Ado to adenine and adenine derivatives through the action of cytokinin oxidase.

Sembdner et al. (1980) listed five types of cytokinin conjugates: 1) cytokinin-N-9-ribosides and riboside 5' phosphates; 2) cytokinin-N-(3, 7 or 9)-glucosides; 3) cytokinin-O-4-glucosides; 4) cytokinin-N-9-ribosyl-O-4-glucosides; and 5) cytokinin-N-(3 or 9)-amino acid conjugates. Some cytokinin conjugates displayed physiological activity (ribosides) whereas others were thought to function as storage forms (glucosides). Horgan et al. (1981) described the rapid uptake of $10^6$
Ade into **Vinc**a crown gall cells and its equally rapid conversion to a ribosyl derivative which was later conjugated with a glucose moiety. Conversely, $10^6$ Ado glucoside offered to cells was incorporated at a slower rate than its unconjugated counterpart but was rapidly converted to the active form once inside the tonoplast. Although interconversion of cytokinin glucoside and riboside has been demonstrated, Laloue and Pethe (1982) suggested reversible formation of cytokinin riboside 5' phosphates to most effectively moderate the level of active form within cells.

**Abscisic Acid.** Although most hormone classes have been shown to retard the processes of growth and differentiation when present in certain forms, at specific sites and/or at supraoptimal concentrations, abscisic acid is the only natural hormone generally considered to be an inhibitor. Conversely, among endogenous constituents of plants which have been reported to inhibit developmental processes, abscisic acid is the only compound classified as a hormone (Moore, 1979).

The discovery and characterization of abscisic acid in the 1960s resulted from investigations of three independent research groups (Wareing, 1978; Moore, 1979; Bearder, 1980). Addicott and coworkers extracted compounds from young cotton (*Gossypium spp.*) bolls which stimulated leaf abscission in cotton seedlings. The most active entity, characterized and named abscisin II, was later shown to be identical to a compound determined by Wareing and others to arrest the development of shoot buds in various tree species. Also, Rothwell and Wain demonstrated flower drop in lupine as caused by abscisin II extracted from
the seeds of this species. By popular consent, the name abscisic acid (ABA) was adopted (Addicott et al., 1968).

Like GAs, the biosynthesis of ABA and related compounds is accomplished through the condensation of Isoprenoid units. ABA consists of a six-membered unsaturated ring which exhibits methyl, hydroxyl and carbonyl substitutions, and an unsaturated trans/cis seven-membered side chain which terminates in a carboxylic acid function. Trans/trans isomerization is common in solution, however only the trans/cis configuration has been shown to be physiologically active. The structure of ABA and that of related compounds has been depicted in Figure 7.

The biosynthesis of ABA was thought to result from direct formation of Isoprenoid units, by cleavage of C(40) carotenoids such as violaxanthin and by modification of carotenoid intermediates such as α- or β-ionone (Milborrow, 1974; Kefeli, 1978; Sembdner et al., 1980; Neill et al., 1982). Kefel (1978) summarized evidence for operation of the latter pathway. In-vitro synthesis of ABA from β-ionone has been accomplished and both α- and β-ionone have been shown to inhibit the growth of wheat (Triticum spp.) coleoptiles. Formation of abscisic acid from the oxidative cleavage of violaxanthin [a C(40) carotenoid] has been demonstrated in-vitro (Moore, 1979). This cleavage, which has been shown to require high light intensity or systems involving lipoxygenases, formed several products including xanthoxin. Xanthoxin has been isolated from a number of plant sources, has been shown to be a potent growth inhibitor, and when exogenously supplied, has been converted to ABA in-vivo. Evidence is now sufficient to
Figure 7: Biosynthesis and Metabolism of Abscisic Acid

A: Mevalonate
B: Farnesyl pyrophosphate
C: trans-cis-Abscisic acid (ABA)
D: trans-trans-Abscisic acid
E: Violaxanthin
F: Xanthoxin
G: 6'-Hydroxymethylabscisic acid
H: Phaseic acid
I: Dihydrophaseic acid

Adapted from Moore (1979)
Figure 7: Biosynthesis and Metabolism of Abscisic Acid
suggest operation of this pathway under certain conditions (Sembdner et al., 1980).

Experimental efforts have concentrated upon elucidation of direct ABA biosynthesis from mevalonate (Milborrow, 1974; Sembdner et al., 1980). Application of radiolabelled mevalonic acid to tomato and avocado fruits as well as in other plant tissues resulted in the recovery of labelled ABA. This result suggested hormone synthesis through the well established pathway forming farnesyl pyrophosphate. Subsequent studies with isotopes have identified the fate of mevalonate carbons as they are incorporated in ABA, but experimentation with potential direct precursors of ABA which possess similar carbon skeletons have proved inconclusive. Recent information and hypotheses concerning ABA precursors have been summarized by Neill et al. (1982).

Metabolism of ABA has been studied extensively by Milborrow and coworkers and by other groups (Sembdner et al., 1980; Hartung, Gimmler and Hellmann, 1982). Milborrow reported the catabolic formation of three ABA metabolites (metabolites A, B and C). Metabolite A and B have since been identified as methyl abscisate and glucosyl abscisate respectively, the latter playing an important role in the regulation of ABA concentration within cells. Metabolite C, isolated in crystalline form from tomato shoots, has been characterized as 6'-hydroxymethyl ABA. In beans, this compound cyclized rapidly to produce phaseic acid by formation of a furan ring joining the 6' hydroxyl group and the C-2 position. Phaseic acid has been demonstrated to undergo further oxidation at the C-4 position forming dihydrophaseic acid. Both phaseic and dihydrophaseic acids have been isolated from plant tissue. Aside from experi-
mental Indications that phaseic acid is involved in the regulation of photosynthesis, these compounds have been shown to be deactivated with respect to ABA-like function.

Others. Processes of growth and differentiation have been shown to be affected by other hormone-like compounds [e.g. triacontanol, (Reis and Houtz, 1983)] and by secondary plant products which may or may not be classified as hormones. Secondary plant products demonstrated to influence plant processes have included the following: tannins (Corcoran, 1975); phenolic substances (Kefell and Kutacek, 1977) steroids (Heftman, 1977; Geuns, 1978); pigments, most notably phytochrome (Moore, 1979; Bernier, Kinet and Sachs, 1981a,b); aliphatic di- and polyamines (Bagni and Fracassini, 1974) and other compound classes. General reviews of natural products and their role in plant growth regulation have been published by Mandava (1979) and by Bearder, 1980). The regulatory effects of these compounds have often been found to be mediated through interactions with the major plant hormones discussed above.

In addition to the endogenous levels of plant metabolites, the environment has on many occasions been demonstrated to exert effects upon the growth and differentiation of plants. Environmental influences may be directed through their effect on specific growth substances or on general plant homeostasis.

Sites of Synthesis and Transport

Studies of hormone biosynthetic sites can be divided in two groups, those identifying localized tissues or organs where synthesis occurs, and those examining enzyme systems isolated from subcellular
components. Indirect evidence of hormone biosynthesis is offered by studies of endogenous hormone levels, patterns of distribution in exudates and diffusates, occurrence of hormone biosynthetic intermediates, or by studies of developmental abnormalities following organ excision. More direct proof of synthesis by specific tissues requires demonstration of hormone production from radiolabelled precursors using isolated cell-free enzyme systems (Sembdner et al., 1980).

Effects of apical excision on the growth of oat coleoptiles and those of other grains led early investigators Darwin, Boysen-Jensen and Paal to consider coleoptile apices as sites of hormone synthesis. Subsequent studies of IAA metabolism over several decades have provided convincing evidence for synthesis of this compound in most rapidly growing or meristematic tissues (apical meristems, buds, developing seeds, etc.) In addition, Wightman (1973) offered data indicating substantial IAA synthesis from radiolabelled TRP in the rapidly expanding leaves in proximity with vegetative apices of tomato. Leaves of this species at all developmental stages were shown to possess IAA synthesizing capabilities.

Although IAA biosynthetic enzymes have been isolated from many plant tissues, studies attempting subcellular localization of auxin biosynthesis have been rare (Sembdner et al., 1980). Bower, Brown and Purves (1976) uncovered different forms of indole-3-ethanol oxidases and indoleacetaldehyde reductases (dehydrogenases) isolated from cucumber seedlings indicating IAA metabolic activity at multiple cellular sites. Both NADH and NADPH-specific reductases were isolated, the latter being associated with an unspecified membrane component of the microsomal
fraction. These authors suggested the dual enzyme system affects regulation of auxin biosynthesis. Wightman and Fregaux (1982) offered evidence for IAA synthesis in isolated sunflower (*Helianthus annuus*) chloroplasts and mitochondria. Soluble protein fractions from these organelles were also capable of IAA synthesis. Synthetic capability of chloroplasts and chloroplastic protein fractions were three times higher than that associated with mitochondria.

Ethylene is produced in most plant tissues, although "rates of ethylene production during the development of higher plants vary from organ to organ and time of development" (Abeles, 1973). Evidence was discussed suggesting high levels of ethylene production in meristematic and nodal tissue of pea seedlings. Studies of ethylene production during apple flower and leaf development reveal high levels of synthesis in flower and leaf buds (Blanpled, 1972). Production levels drop as organs develop and increase again as organs abscise. Highest levels of ethylene are in pre-climacteric (ripening) fruit (Abeles, 1973).

Ethylene synthesis was first thought to be associated with mitochondria and chloroplast membranes (Sembdner et al., 1980). However, studies failed to demonstrate ethylene evolution from cell-free preparations of these organelles. Mattoo and Lieberman (1977) investigated ethylene production in cultures of intact apple fruit cells and cell protoplasts. Protoplasts obtained through enzymatic digestion of cell walls were unable to produce ethylene whereas upon cell wall regeneration ethylene synthesis capability was restored. These results led Mattoo and Lieberman (1977) to suggest a cell wall-cell membrane-bound enzyme system as the site of synthesis of this hormone.
Bardense (1975) reviewed data indicating the major sites of GA synthesis: apical shoot buds and subtending expanding leaves, root tips, immature fruit and developing or germinating seeds. Presence of high GA levels in exudates and diffusates was used as criterion suggesting synthetic events in most studies. However, cell-free systems producing GAs in-vitro were found in certain tissues (Grabe, 1972).

Considerable evidence has been published substantiating GA biosynthesis associated with chloroplast or protoplast membranes (Rappaport and Adams, 1978). Cell-free systems isolated from plastids have demonstrated the synthesis of ent-kaurene from GGPP in *Mariah* spp., and pea, of ent-kaurenolic acid from ent-kaurenol in barley and of GAs from ent-kaurenolic acid in *Brassica* spp. GA interconversion has also been reported in isolated, intact pea chloroplasts (Rallitton and Reid, 1974).

Rappaport and Adams (1978) have summarized data indicating phytochrome mediation of GA release from the chloroplast. Although substantial evidence exists for GA formation and metabolism in chloroplasts, these authors have cautioned against acceptance of this organelle as the sole site of synthesis.

Crozier and Reid (1972) suggested the possibility of tissue specific synthesis or interconversion of GAs. By excision of scarlet runner bean root tips, these authors demonstrated a loss of GA and a concomitant increase in levels of GA in leaves, apical buds and sub-apical root remnants. They hypothesized that leaves and apical buds were incapable of transforming the (C-20) GA to the (C-19) GA and that the conversion likely occurred following transport to the root.
apex. The presence of both GA species in cotyledons of treated plants indicated the synthetic autonomy of this tissue.

Torrey (1976) reviewed evidence for biosynthesis of cytokinins in mitotically active root tips, considered to be the main source of these hormones in their free state. Other tissues capable of high levels of synthesis included seeds and fruits. Chen and Pitchow (1978) demonstrated the synthesis of $10^6$ Ade/Ado from adenine and other purines in cultures of rootless tobacco plants. Cytokinin-synthesizing systems were hypothesized to be active in the meristematic regions of the tobacco shoot. Sembdner et al. (1980) commented on the dearth of information about intracellular sites of free cytokinin synthesis.

ABA biosynthesis appears to occur in many metabolically active plant tissues as its formation from labelled mevalonate has been reported in leaves, stems, fruits, and various seed parts (Wareing, 1978). Studies of ABA metabolites (phaseelic and dihydrophaseelic acids) in castor bean (Ricinus communis) have intimated mature leaves to be the major site of ABA biosynthesis. Shoot apices quickly metabolized the compound to its inactive forms (Zeevaart, 1977). In addition, these studies have also revealed ABA synthetic capability in young leaves in response to water stress. ABA synthesis in water-stressed leaves has recently been shown to limit ethylene accumulation through inhibition of ACC synthesizing enzymes (Yang et al., 1982).

Sembdner et al. (1980) and Hartung, Gimmler and Hellmann (1982) summarized reports of ABA synthesis in isolates of intact and lysed chloroplasts. A high proportion of ABA present in extracts of non-stressed spinach (Spinacea oleracea) leaves was found in association
with chloroplasts, while extracts of mildly stressed leaves exhibited an eleven-fold increase in cytoplasmic ABA concentration (Loveys, 1977). These results were thought to indicate an increase in chloroplastic ABA synthesis followed by transport to the cell sap through a 'readily permeable chloroplast envelop'. Hartung, Gimmler and Hellmann (1982) advanced evidence for increased extraplastidic ABA prior to de-novo synthesis of the hormone in stressed leaves. The stress-induced leaching of ABA from chloroplasts was thought to result from changes in a cellular pH gradient.

Mechanisms of hormonal transport have been examined at two levels, long range transport of hormones from organ to organ or short range transport from cell to cell within tissues. From studies of xylem and phloem exudates in many species, long range transport has been shown to involve the movement of hormone conjugates (primarily glucosides) which may undergo conversion to active forms upon arrival at the target tissue. Short range transport of IAA and GAs through tissues has also been examined in detail whereas less has been reported about intercellular movement of the other hormone classes.

The study of intercellular movement of IAA through tissues began with Darwin's coleoptile apex excision experiments and was further advanced by other early studies of this type (Thimann, 1977). Using sub-apical coleoptile segments, Went first demonstrated the polar nature of auxin transport. IAA donor and receiver agar blocks were placed on the cut surfaces. When the donor block was situated at the acropetal surface, IAA was shown to be transported to the receiver block; when the segment was inverted (donor block placed at the basipetal surface),
transport of the hormone was halted. A series of trials by van der Welj determined the velocity of polar auxin transport to be faster than could be accounted for by diffusion. They also revealed transport to function against a concentration gradient and transport velocity to be independent of segment length.

Work of van der Welj suggested active transport of auxins. General features of active transport systems and their applicability as elements in a model of auxin movement were discussed by Leopold and de la Fuente (1968). In addition to velocity and movement against a concentration gradient, the auxin transport mechanism was shown to be chemically specific, to possess binding (pumping) sites at the plasma membrane, and to be subject to substrate saturation. Use of radiolabeled IAA in later experiments elucidated the pulsating nature of IAA transport, a phenomenon substantiated by measurement of an oscillating electric field generated around the coleoptile shaft (Thimann, 1977).

The action of the IAA transport mechanism has been shown to be dependent upon tissue age, enhanced by the presence of IAA, inhibited by high concentrations of ethylene, and mediated through the action of phytochrome (Thimann, 1977). The presence of GAs also promotes auxin movement, perhaps by stimulating the production of or maintaining elements of the auxin transport mechanism (Jacobs and Case, 1965). The exact nature of this mechanism is still under investigation.

Bardense (1975) summarized evidence for both polar and nonpolar transport of GAs. Early experimentation with corn coleoptiles and potato (Solanum tuberosum) tubers indicated intercellular transport to be nonpolar. These experiments, however, were criticized for their use
of GA concentrations far in excess of physiological levels. Rudich, Sell and Baker (1976) described nonpolar transport of \(^{3}\text{H}\)GA\(_1\) in hypocotyl segments of two cucumber sex types. These authors reported enhanced GA transport with the addition of IAA or synthetic auxins. Although gynoeclous seedlings transported a greater volume of GAs than did their androeclous counterparts, sex expression was thought to be mainly influenced by GA synthesis rates.

Basipetal polar movement of GAs was elucidated in petioles of \textit{Coleus blumei} and root segments of corn by Jacobs and Kaldewey (1970) and Jacobs and Pruett (1973) respectively. Hartung and Phillips (1974) and Phillips and Hartung (1974) studied apparent polar transport of GAs in root segments and stem internodes of scarlet runner beans. Results indicated polarity to be due to movement of GAs within the vascular tissue or intercellular movement in the direction of hormonal sinks.

Movement of ethylene through tissues, whether living or necrotic, occurs by diffusion (Abeles, 1973). Concentration of this hormone within individual cells of a given organ is dependent in part upon the synthetic rate and upon the concentration of diffusing gas surrounding the cell. The rate of diffusion from the tissues to the atmosphere is dependent upon organ morphology. Thus, ethylene is more readily lost from leaves than less porous fruit tissues.

Wareing (1978) summarized evidence for nonpolar intercellular transport of ABA at low volumes and rates. A study of intercellular cytokinin movement heretofore has not been initiated.
Function of Plant Hormones

Although many aspects about hormonal regulation of plant growth and differentiation have been uncovered, complete elucidation of even a single process at the cellular level has yet to be brought forth (Zeroni and Hall, 1980). In contrast to animal systems, study of plant hormonal mechanisms has been impeded by: the existence of relatively few active compounds, each involved in many regulatory events; the delocalized synthesis of these compounds which complicates studies of exogenous hormonal application; and the synergism and/or antagonism of hormones, rendering their effects upon cellular (and ultimately organismic) processes a function of their relative concentrations within the cell (Biswas and Roy, 1978).

Clearly, hormonal regulation of plant development is mediated through the control of enzyme synthesis and/or function at the cellular level. Classic examples of such regulation include the auxin-stimulated increase of enzymes responsible for cell wall extension (Zeroni and Hall, 1980) and the GA-induced synthesis and secretion of $\alpha$-amylase and other enzymes by the aleurone layer of germinating barley embryos (Mann, 1975; Varner and Ho, 1977). There is also evidence for direct hormonal effects on membrane structure and activity as is suggested by the proposed auxin activation of plasma membrane-bound ATPase (active in cell wall extension) (Venls, 1977; Biswas and Roy, 1978) and by the alteration of membrane permeability initiated through GA action (Stoddart and Venls, 1980).

These regulatory processes and others involve the complexation of hormones with receptor molecules (proteins) followed by the initia-
tion of hormone-specific responses. Stimulation or repression of membrane-bound enzyme systems is thought to occur rapidly after formation of hormone-receptor complexes whereas changes in endogenous enzyme levels are delayed, suggesting a dependence on transcriptional (and perhaps translational) events (Venls, 1977). Published reports concerning in-vitro and in-vivo hormonal regulation of RNA synthesis [heterogeneous nuclear RNA (hn-RNA), messenger RNA (m-RNA) and ribosomal RNA (r-RNA)] as well as regulation of RNA polymerases are plentiful (Jacobsen, 1977).

Hormone-Receptor Protein Complexes

Characteristics of plant hormone interactions with receptor proteins were reviewed by several authors (Jacobsen, 1977; Venls, 1977; Bıswas and Roy, 1978; Ram Chandra, Muthukrishnan and Maxwell, 1979; Stoddart and Venls, 1980; Flrn and Kerns, 1982; Fox and Gregerson, 1982; Hall et al., 1982; Kende et al., 1982; Stoddart, 1982). Although much of the published information concerning these interactions is fragmentary (especially evidence describing ABA-receptor or ethylene-receptor complexes), general characteristics of these associations could be summarized by these authors. A model of hormone mediated responses was described by Kende et al. (1982) as follows: first, active hormones (derived from precursors or conjugated forms bind with receptors; binding of hormone and receptor then elicits a "primary response"; physiological responses arise from the primary response only after a complex series of events. For all hormone classes, the nature of the "primary response" remains the most elusive and simultaneously the most
sought after research prize. However, recently acquired information links the formation of IAA, GA and ethylene hormone-receptor complexes, their "primary response" and hormone metabolism (oxidation, etc.) suggesting the simultaneous action and inactivation of the hormone molecule (Bandurski, 1982; Hall et al., 1982; Stoddart, 1982).

Precise structural and functional characteristics of hormones necessary for biological activity suggest a 'lock and key' concept of hormone receptor complexes (Stoddart and Venls, 1980). At physiological pH, IAA, highly active synthetic auxins and auxin inhibitors all possess an ionized carboxylic acid moiety separated by 55 nm from partial positive charges within the molecule. Comparison of various GA and GA analog structures in relation to their relative biological activity indicates the necessity for an ionized carboxyl function at C-7 and the advantage of both C-10,19 lactone formation and hydroxylation at C-3 and C-13. Three dimensional structural analysis of the most biologically active GAs shows "a two-fold axis of symmetry involving the D-ring and the lactone group projected on opposite sides of the molecule and the C-3 and C-13 substituents diametrically opposed" (Stoddart and Venls, 1980). Potent activity of natural plant cytokinins requires an intact purine ring with an N6 unsaturated side chain of 5 carbon atoms. Ethylene receptors are only responsive to molecules possessing unsaturation adjacent to the terminal carbon and are stimulated in inverse proportion to the molecular size of the stimulator. Loss of activity through cis-trans isomerization such as noted for trans-ABA, through optical isomerization as displayed by non-functional auxin analogs, or through modification of functional groups as experienced with GA and
cytokinin analogs are additional examples of molecular precision required for hormone action. Indeed, the 'precision fit' of hormones and their receptors is of paramount importance if these interacting molecules are to regulate discrete and specific processes of growth and differentiation (Biswas and Roy, 1978; Venis, 1978; Ram Chandra, Muthukrishnan and Maxwell, 1979; Stoddart and Venis, 1980).

A thorough review of hormone receptor proteins and their binding characteristics was accomplished by Venis (1977) and by Stoddart and Venis (1980). An ideal receptor protein was described as: hormone specific; exhibiting a high affinity for its specific hormone counterpart; displaying binding kinetics associated with substrate-enzyme complexes (i.e. exhibiting finite and reversible binding capacity at physiological concentrations of the substrate (hormone); initiating a specific biological response following formation of the hormone-receptor complex; and possessing the ability to bind with hormones, hormone analogs, synthetic growth regulants and inhibitors in a competitive manner in proportion to the biologic response elicited by each. No receptor system has yet been described which adequately meets all criteria for an ideal receptor protein (Biswas and Roy, 1978; Stoddart and Venis, 1980).

Over the last 10 years, a multitude of studies have used a variety of techniques to demonstrate hormone binding at several discrete cellular sites and to isolate and characterize the protein factors of these binding complexes (Stoddart and Venis, 1980; Firn and Kerns, 1982; Fox and Gregerson, 1982; Hall et al., 1982; Kende et al., 1982; Stoddart, 1982). Investigations have centered on proteins of auxin-
receptor complexes which mediate auxin-induced cell elongation. However, information about other hormone-receptor proteins which regulate additional plant functions has also been reported. The majority of studies have been concerned only in the demonstration and characterization of binding and not with the identification of cellular processes affected by the formation of complexes.

Receptor proteins have been classified as belonging to one of two groups according to their cellular location and proposed mode of action (Venis, 1977). Soluble receptors have been isolated from protein fractions of both cytoplasmic and nuclear sources. Hormone-soluble receptor complexes formed from these proteins have been shown to affect the cellular complement of enzymes mainly through regulation of transcription (RNA synthesis) (Jacobsen, 1977). Interaction of activated plant hormone-receptor complexes with DNA or chromatin (DNA and associated proteins) have been suggested to alter template availability generally or at specific chromatin sites in a manner similar to inducible or repressible systems in prokaryotes or to mechanisms of transcription control by animal steroid hormones. Indirect evidence for the operation of this control mode in plant cells has included changes in transcription rate and qualitative differences in RNA fractions following hormone treatment, and the required presence of non-histone proteins for hormone-induced modifications of transcription in-vitro (Biswas and Roy, 1978). Hormone-soluble receptor complexes have also been demonstrated to alter the activity (synthetic rate) of RNA polymerases (Jacobsen, 1977; Ram Chandra, Muthukrishnan and Maxwell, 1979) with changes in production of RNA from single-copy DNA template (RNA
polymerase II) followed by stimulation or depression of r-RNA production by RNA polymerase I.

The second group of receptor proteins, those which are membrane-bound, have been characterized (Jacobsen, 1977; Venis, 1977; Stoddart and Venis, 1980). As with their soluble counterparts, the binding characteristics of these receptors have been studied. However, the regulatory functions of hormone-(membrane-bound protein) receptor complexes have remained a matter of conjecture. Hormone-(membrane-bound protein) receptor complexes have been thought to alter membrane permeability or to activate or repress membrane-bound enzyme systems.

Available information concerning hormonal control of cellular processes which regulate plant development has been summarized by Zeroni and Hall (1980). Plant processes in which hormones act upon cells with a predetermined pattern of development (such as growth responses in hypocotyl tissue) have been contrasted with those in which hormones and their relative concentrations determine the pattern of development (such as in the transition from a vegetative to a floral meristem). In the first case, it has not been difficult to envision control schemes in accordance with current knowledge of hormone-receptor interactions. In the second case, regulatory mechanisms have been hypothesized to be more complex as they must provide for manipulation of a large portion of the genome.

The question which needs to be resolved is whether the nature of the primary action of the hormone is different in kind when it is acting as a determining factor than when it is initiating a predetermined response. At this stage in the state of the field we can only speculate, since it is possible to construct consistent hypotheses to fit either case (Zeroni and Hall, 1980).
Hormonal Regulation of Growth and Differentiation

To reiterate, all facets of plant development are thought to be influenced by endogenous hormone levels. As a myriad of studies both theoretical and applied have demonstrated the effects of endogenous hormone concentrations or exogenous hormone treatments upon plant developmental processes, a thorough review of this subject is not practical in this format. However, hormonal regulation of growth and differentiation is summarized in Table 2. This compendium is not intended to be exhaustive and should be considered to hold information which is valid in general. Studies of specific germplasm or environmental conditions may contradict data presented. Citations include studies of physiological effects of endogenous hormone levels, of plant response to growth regulator application and of cell behavior in tissue culture.

Several comprehensive aspects of hormonal regulation of growth and differentiation can be perceived from the information compiled in Table 2. Most important perhaps is that most plant processes are sensitive to regulation by two or more hormones and often both promoting and inhibiting substances are present in affected tissues at the same time. Growth regulators act in concert and interact with environmental stimuli resulting in a physiological response. Obviously, more is known about the effects of auxin on plant development than about other hormone groups. This hormone class almost universally promotes activities responsible for growth and differentiation and inhibits processes of senescence. A notable exception to the trend in auxin action is the suppression of axillary bud development in favor of apical dominance. GAs and cytokinins are also, in general, promoters of plant development. ABA,
Table 2: Functions of Endogenous Plant Hormones\(^1,2\)

<table>
<thead>
<tr>
<th>Function</th>
<th>Auxins</th>
<th>Ethylene</th>
<th>GAs</th>
<th>Cytokinin</th>
<th>ABA</th>
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<tr>
<td><strong>Subcellular Aspects</strong></td>
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<td>DNA synthesis</td>
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<td>RNA synthesis</td>
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<tr>
<td>Polysome formation</td>
<td>⚫</td>
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<tr>
<td>Synthesis of specific enzymes</td>
<td>⚫</td>
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<td>Plastid development</td>
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<td>Cytoplasmic streaming</td>
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<td><strong>Cellular Aspects</strong></td>
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<td>Cell cycle interval</td>
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<td>S phase</td>
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<tr>
<td>Mitosis</td>
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<tr>
<td>Cell division</td>
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<td>Membrane permeability</td>
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<tr>
<td>Ion influx</td>
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<tr>
<td>Water uptake and turgor maintenance</td>
<td>⚫</td>
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<tr>
<td>Cell elongation</td>
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<tr>
<td><strong>Organic Aspects</strong></td>
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<td>Seed germination</td>
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<tr>
<td>Hypocotyl or epicotyl growth</td>
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<tr>
<td>Vascular system development</td>
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Table 2: (Continued)

<table>
<thead>
<tr>
<th>Function</th>
<th>Aux-Ins</th>
<th>Ethylene</th>
<th>GAs</th>
<th>Cyto-kinins</th>
<th>ABA</th>
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<tr>
<td>Root Development</td>
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<tr>
<td>Root growth</td>
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<td>Axillary root initiation</td>
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<tr>
<td>Adventitious root initiation</td>
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<td>Mycorrhiza formation</td>
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<tr>
<td>Geotropism</td>
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<tr>
<td>Shoot development</td>
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<td>Dormancy</td>
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<td>Apical growth</td>
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<td>Stem growth</td>
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<td>Axillary bud growth</td>
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<tr>
<td>Phototropism</td>
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<tr>
<td>Plagiotropism</td>
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<tr>
<td>Leaf development and metabolism</td>
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<tr>
<td>Leaf expansion</td>
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<td>Stomatal opening</td>
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<td>Leaf senescence</td>
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<tr>
<td>Leaf abscission</td>
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</table>
Table 2: (Continued)

<table>
<thead>
<tr>
<th>Function</th>
<th>Aux-Ins</th>
<th>Ethylene</th>
<th>GAs</th>
<th>Cytokinins</th>
<th>ABA</th>
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<tbody>
<tr>
<td>Reproductive development³</td>
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<tr>
<td>Fruit set</td>
<td>⭐️</td>
<td>⭐️</td>
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<td>⭐️</td>
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<tr>
<td>Seed development</td>
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<tr>
<td>Fruit development</td>
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<tr>
<td>Fruit maturation</td>
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<td>⭐️</td>
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<tr>
<td>Fruit abscission</td>
<td>⭐️</td>
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</tbody>
</table>

1Hormonal promotion or stimulation ⭐️ Hormonal inhibition ⚫

2References:
Abeles, 1973
Apelbaum and Burg, 1972
Bearder, 1980
Biswa and Roy, 1978
Burg, Apelbaum and Kang, 1972
Burstrom and Svensson, 1972
Davies, 1976
Elliot, 1977
Esashi and Leopold, 1968
Goldthwaite, 1972
Gorrier, 1972
Hasmann and Onder, 1972
Jacobson, 1977
Kaska, 1972
Kende, 1983
Kende and Hanson, 1977
Laetsch and Boasson, 1972

Laloue, 1978
Lieberman, 1979
Lieberman and Kunishi, 1972
Marre, 1977
Martin and Northcote, 1983
Moore and Ecklund, 1975
Morgan, Durham and Lipe, 1974
Osborne, 1977
Pead-Lenoel, 1977
Sembdner et al., 1980
Stoddart, 1972
Stoddart and Venis, 1980
Tang et al., 1974
Thilmann, 1977
Wareing and Phillips, 1978
Weaver and Sachs, 1968
Yamada, Yasuda and Yajima, 1974
Zeroni and Hall, 1980

³Hormonal influence upon floral initiation, development and senescence discussed in detail (Chapters 2 and 3)
In most instances, antagonizes the effects of growth promoting hormones and serves to moderate their action. Ethylene is variable in its influence on developmental processes. IAA and ethylene often act in conjunction, due in part to auxin stimulation of ethylene biosynthesis (see discussion above). Ethylene is perhaps most noted for its role in maturation, senescence and abscission.

**Role of Plant Hormones in Floral Biology**

Due to its tremendous agricultural and economic importance, reproduction has perhaps been one of the most studied processes in plant development. Biological investigation has indicated the reproductive process to be influenced by plant hormones (Table 2) as well as by other cellular components and by environmental stimuli. Agricultural experimentation undertaken to enhance crop production through chemical manipulation of flowering, fruiting and seed development has added significantly to the understanding of reproductive regulation through hormone action. Although a wealth of documentation has accrued, a complete schematic of hormonal influences throughout the reproductive cycle has remained elusive for even a single species indicating the complex nature of the developmental process. Study has been centered on discrete aspects of the process such as floral evocation (initiation), floral development, anthesis, pollination/fertilization and floral senescence.

Most germane to the present study is the subject of floral development, and in particular, development of sex organs (Chapter 3). However, reproductive development from floral initiation to fruit maturation should be viewed as an integrated sequential process.
Floral Evocation

... the overall flowering process involves many steps, usually starting with perception of an environmental factor and terminating with the differentiation of three-dimensional structures, the flower primordia (Zeevaart, 1976).

Flowering is the end result of physiological processes, biochemical sequences, and gene action, with the whole system responding to the influence of environmental stimuli and the passage of time (Murfet, 1977).

The process of evocation starts with an initial event altering a formerly vegetative meristem and terminates when anatomical and physiological changes have irreversibly committed the meristem to flower (Bernier, Kinet and Sachs, 1981b).

More scientific curiosity has been generated and more treatises have been prepared concerning the biological aspects of floral evocation than about any other phase of floral development. The selective advantage of floral evocation expediently timed with and stimulated by environmental factors for survival of feral species has been discussed by Murfet (1977). One of the most studied of environmental factors stimulating evocation has been that of daylength. Species have been described as long day plants (LDP) requiring long days for floral initiation, short day plants (SDP) reacting favorably to short days, combinations of the above (LSDP, SLDP) which require a sequential change in daylength and day neutral plants (DNP) which do not respond to changes in daylength.

Early experimentation with photoinductive phenomenon revealed leaves to be the primary "target organ" which when stimulated by an inductive day-night cycle released a chemical inducer translocated to meristematic regions. The leaf component receiving the photoperiodic message was identified in 1959 as phytochrome, a membrane-bound water
soluble chromoprotein possessing a tetrapyrrole chromophore (Moore, 1979). This pigment was found to exist in two distinct and reversible conformations, an active species ($P_{fr}$) formed by exposure to red light (660 nm) and an inactive counterpart ($P_r$) formed by the action of far red light (730 nm) or reversion during periods of darkness. Response to the level, duration and sequence of $P_{fr}$ activity dependent upon dark reversion and other destructive mechanisms was presumed to differ in photoperiodic species (Zeevaart, 1976; Moore, 1979). How phytochrome mediates the production and transport of a chemical signal to potential floral meristems has yet to be elucidated.

Although daylength (nightlength) has received the bulk of attention, other environmental factors such as temperature (chilling or heat requirements), water balance and mineral nutrition have also been found to control the onset of flowering (Zeevaart, 1976; Murfet, 1977). In addition, Murfet (1977) has reviewed evidence for environmental control over other phases of the flowering cycle.

The identity of a floral stimulus (florigen) presumably produced in the leaves and translocated to the apex has been sought after ever since Challakhyan proposed its existence in 1936 (Challakhyan, 1977; Cleland 1978, 1982; Challakhyan, 1982; Cleland and Ben-Tal, 1983). Attempts to isolate florigen or other compounds specific to the flowering process have failed for reasons discussed by Cleland (1978) and Bernier, Kinet and Sachs (1981b). Alternate theories have proposed the triggering mechanism to involve changes in concentration of common plant hormones present in preinduced meristems (Miginiac, 1978; Bernier, Kinet and Sachs, 1981b; Challakhyan, 1982, Cleland and Ben-Tal, 1983).
However, the lack of suitable microanalytical techniques have prevented a detailed survey of changes in endogenous homonal levels in inducible meristems (Bernier, Kinet and Sachs, 1981b).

Bernier, Kinet and Sachs (1981b) state "... despite the enormous diversity in environmental parameters affecting flower initiation and kinds of reproductive structures to be constructed, the [anatomical] features of floral transition seem fairly universal". These authors discussed the cellular and subcellular as well as the histological and macromorphological events occurring in meristems undergoing floral transition (Table 3). These events could be related generally to an increase meristem metabolic activity (increased respiratory function, increased transcriptional and translational events, increased rate and regulation of cell division) which in turn influenced morphological changes in meristem arrangement and that of the subtending tissue (telescopic condensation of appendage formation and changes in their phyllotaxis, precocious initiation of axillary meristems, vacuolation of pith-rib meristem). These authors viewed this array of changes not as serial events but rather as a number of parallel and interacting sequences each possibly affected in a discrete manner by internal and environmental stimuli. The specificity of floral evocation was thought to "reside primarily in the way the many changes involved are timed and integrated" (Bernier, Kinet and Sachs, 1981b).

Most of what is known about hormonal influence over evocational events has been provided by monitoring effects of exogenously-applied growth regulants even though the natural course of Induction may not be directly correlated to these effects (Bernier, Kinet and Sachs, 1981b).
Table 3: Influence of Hormones and Metabolites on Evocational Events\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Evocational Events</th>
<th>Auxins</th>
<th>GAs</th>
<th>Cytokinins</th>
<th>Carbohydrates</th>
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<tbody>
<tr>
<td><strong>Subcellular</strong></td>
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<tr>
<td>Increased respiratory substrates</td>
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<tr>
<td>Increased respiration rate</td>
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<tr>
<td>Increased RNA and protein synth.</td>
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<tr>
<td>Increased enzymatic activity</td>
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<tr>
<td>Changes in protein complement</td>
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<tr>
<td><strong>Cellular</strong></td>
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<tr>
<td>Cell synchronization</td>
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<tr>
<td>Increased cell division rate</td>
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<tr>
<td><strong>Histological</strong></td>
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<tr>
<td>Meristem reorganization</td>
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<tr>
<td>Vacuolation of pith-rib meristem</td>
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<tr>
<td><strong>Macromorphological</strong></td>
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<tr>
<td>Precocious initiation of axillary meristems</td>
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<tr>
<td>Increased rate of appendage formation</td>
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<tr>
<td>Phyllotactic changes</td>
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</table>

\textsuperscript{1}Modified from Bernier, Kinet and Sachs, 1981b

\textsuperscript{2}Function influenced by hormone(s) or carbohydrate \*
Ability of regulants to promote as well as inhibit flowering in different species and/or under varying experimental conditions suggested the specificity of hormonal balance for optimum induction. Auxins, GAs and cytokinins appeared to assist in control of a number of inductive sequences (Table 3). High carbohydrate levels also proved to enhance inductive events dependent upon growth whereas substances such as ethylene, ABA, phenolics, steroids, α-tocopherol (Vitamin E), amino acids and others have been found to affect the process in a limited number of species or in minor ways.

Genetic effects and environmental interactions influencing floral evocation were discussed by Murfet (1977). Substantial genetic diversity in evocation potential among wild populations was said to be masked by stable gene combinations and environmental effects. Phenotypic expression of this diversity could be achieved by growth under continuous non-inductive conditions. The existence of an oligopolygenic system of control (control by both major genes and polygenic effects) was noted. Epistatic factors were thought to be extremely important in a control system of this type as the ultimate response depends upon regulation of many endogenous substances.

Floral Bud Development, Flowering and Senescence

The post-inductive meristem continues to be metabolically active as primordia for floral organs (bracts, sepals, petals, stamens and pistils) begin to differentiate. Decades ago, Wardlaw (1957) published hypotheses concerning the course of organogenesis in the induced meristem which with minor changes due to increased knowledge of genetic
regulation are still thought to be valid (Frankel and Galun, 1977). Wardlaw commented that the sub-distal region of terminal vegetative meristems contain "growth centers", or areas of specialized metabolism which in response to physiological conditions, gave rise to leaf primordia in an organized repeating pattern. Changes in growth center physiology upon evocation (including alteration of endogenous hormone levels) were thought to alter the pattern and spatial arrangement of primordia as well as induce specific genetic action. Additional changes in meristem physiology through gene expression were presumed to moderate sequential development of floral organs from primordia (calyx—corolla—androecium—gynoeclum) with the differentiation of each successive organ being affected by the physiological activity of those previously formed.

Floral organogenesis controlled by interaction among endogenous hormones and environmental stimuli can be inferred. However, information concerning hormone influence on differentiating floral tissue is scant. Most of what is known can be derived from investigations of floral bud development in floricultural crops with additional information available from applied studies of developmental control in tree fruits and vegetables.

Wertzlov, Plotnikova and Alexandrova (1978) found IAA levels of apple buds to be reduced by 50% during primordia differentiation and to continue declining during bud development. In contrast, Jeffcoat and Cockshull (1972) uncovered peak levels of IAA and GAs early in morphogenesis of chrysanthemum (Chrysanthemum morifolium) floral buds. Subsequent changes in growth promoter levels during the developmental
process coincided with fluctuations in bud growth rates. These authors identified GA₃, GA₄, GA₅ and GA₈ as floral components of this species. Menhenett (1978) maintained that GAs increased assimilates available to the developing chrysanthemum floral bud. This contention was substantiated by the earlier work of Harris, Jeffcoat and Garrod (1969) with carnation (Dianthus caryophyllus) floral buds. When plants were exposed to ^{14}CO₂, treatment of buds with GA₃ resulted in increased percentage of radiolabel associated with floral tissue (36%) over that found in control flower buds (26%). GA treatment also promoted the growth of sepals and petals and hastened anthesis. High endogenous levels of GAs were suggested to cause bud abortion in tomato inflorescences grown under suboptimal environmental conditions (Abdul, Canham and Harris, 1975). This effect could be alleviated by treatment with (2-chloroethyl)trimethylammonium chloride (CCC), a GA synthesis inhibitor.

Dathe and Sembdner (1980) analyzed broad bean (Vicia faba) floral buds for endogenous growth substances. Free GAs (predominantly GA₂₀) and GA glycosides were detected during all phases of development and in all floral organs studied. These compounds were present at higher concentrations in the developing androecium and perianth, but just prior to anthesis, levels were highest in the gynoecium. High concentrations of ABA and unknown inhibitors were also uncovered in developing floral parts with maximum levels detected in the gynoecium during the latter stages of development.

Growth promoting substances have been shown to influence fully developed floral buds (as in studies of Dathe and Sembdner discussed above), flowering, pollen growth, post-fertilization ovary development
and subsequent fruit set whereas (presumed auxin-stimulated) ethylene production has been indicated in processes of floral senescence and abscission. GA₃ has been isolated and quantified in the petals and stamens of *Cassia fistula* (Sircar et al., 1970). IAA levels have been reported to be highest in mature buds and young flowers of daffodil (*Narcissus pseudonarcissus*) (Edelbluth and Kaldewey, 1976). In contrast, Kalhara and Takimoto (1983) have reported exogenous application of ABA to hasten anthesis of *Pharbitis nil* floral buds under adverse conditions whereas IAA surpressed the process. These authors suggested ABA to be a controlling factor of ion influx of petal mid ribs. Increased turgor pressure in mid ribs was said to result in anthesis.

Lund (1956) has reported pollen growth to stimulate enzymatic activity responsible for IAA synthesis both in pollen tubes and styles of tobacco. He has suggested that elevated IAA levels generated during the process of fertilization promoted ovarian development and enhanced subsequent fruit set. Hall and Forsyth (1967) have shown both pollination and auxin application to simulate ethylene production in tobacco floral tissue. A cyclic diurnal pattern of ethylene evolution from mature cotton buds which preceded pollination and peaked at anthesis has been characterized by Morgan, Durham and Lipe (1974). Ethylene levels in post-anthesis floral tissue remained high in plants exhibiting poor fruit set and declined in phenotypes displaying adequate or good fruit set. Exogenous treatment of the latter plants with ethylene caused flower bud and young fruit abscission.

Floral (petal) senescence has been studied in detail, perhaps due to its relevance to floral industries. A thorough review of the subject
has recently been proffered by Mayak and Halevy (1980) whereas the publication of Pratt and Goeschl (1969) has reviewed early research outlining the role of ethylene in regulation of this process. Intracellular and metabolic changes in senescent petal tissue have been reported to include the following: a disappearance of ribosomes and polysomes, transient elevation in respiration rate, changes in carbohydrate and protein metabolism, increased synthesis of hydrolyzing enzymes and destruction of cellular components, increased peroxidase activity stimulating the production of ethylene, changes in transport of nutrients from petals to ovary (ethylene mediated), loss of turgor in petal tissue, changes in pH and alterations in the form and concentration of corolla pigments.

Petal senescence is currently viewed as a programmed sequential process mediated by endogenous hormone levels (especially ethylene) and available carbohydrates (Mayak and Halevy, 1980). Reports of applied studies to delay the onset or retard the progress of floral senescence in economically important floral crops abound in literature. Successful techniques center on methodology to retard the effects of ethylene through the action of ethylene synthesis inhibitors, antioxidants, or storage conditions. Reduced ethylene levels are also experienced by the addition of sucrose and/or cytokinins.

The biosynthesis of ethylene and changes in membrane permeability in senescing floral tissue was studied in a series of experiments performed by Hanson, Kende and coworkers (Hanson and Kende, 1975; Hanson and Kende, 1976; Kende and Hanson, 1976; Kende and Hanson, 1977). These researchers chose a tissue system consisting of rib sections from mor-
ning glory (*Ipomea tricolor*) petals excised at or before anthesis and cultured in a liquid media. Senescence could be visually monitored in this system as during the aging process, these sections curled or rolled and darkened in color, reminiscent of their behavior in intact corollas. In addition, this system facilitated efforts to study the influence metabolite levels and changes in membrane permeability upon the process.

Initially, the effects of exogenous ethylene on membrane permeability and curling were examined in pre- and post-anthesis segments (Hanson and Kende, 1975). In bud rib sections, ethylene treatment increased the efflux of radiolabelled ions from cell vacuoles over the level effluxed from controls and in the same time frame, caused the curling effect. Both effects were noted in treated and untreated flower rib sections although ethylene treatment hastened both processes. These results indicated changes in membrane permeability and turgor pressure associated with the curling process perhaps mediated by ethylene evolution. A later study (Kende and Hanson, 1976) confirmed that curling coincided with increased ethylene levels within cells. However, treatment of tissue with rhizobitoxine or AVG (ethylene synthesis inhibitors) only delayed the curling process indicating that changes in membrane permeability precede the evolution of ethylene in petal tissue aging. The role of increased ethylene synthesis was thought to be an integral part of the aging process but not its triggering mechanism.

Subsequent experimentation by Kende and Hanson using radiolabelled methionine supplied to tissues indicated its rapid transformation into S-methyl methionine in both pre- and post-anthesis rib segments.
S-Methylmethionine interacted with methyl acceptors in pre-senescent tissue generating various methylated products and reforming methionine. However, in floral rib segments undergoing senescence, S-methylmethionine reacted mainly with homocysteine to form two molecules of methionine, thus raising the endogenous level of the products and 'fueling' the synthesis of ethylene. Ethylene synthesis was presumably prevented in pre-senecent tissue by compartmentalization of homocysteine. Following membrane changes during the early stages of senescence, the ethylene synthesizing system was thought to contact stored homocysteine initiating the production of ethylene. In their model, the ethylene produced increased membrane permeability and thus its synthesis became autocatalytic.

Although ethylene was not indicated as the triggering mechanism at the cellular level, Kende and Hanson (1977) suggested its synthesis in a few prematurely aged cells could result in the induction of senescence at the organ level. Diffusion of this hormone from tissues of high concentration to those of low concentrations has been well-documented (Abeles, 1973).
Sexual reproduction is the prevalent mode of propagation in higher plants. The biological significance of sexual reproduction in diploid and polyploid plants lies in pooling of genetic information carried by the individuals of an interbreeding population. Phenotypic uniformity must not be sacrificed by storage of a large amount of genetic variability in such a population (Frankel and Galun, 1977).

Notwithstanding various deviations, it is evident enough that certain basic elements of the sexual reproductive systems and its adjuncts—...—are virtually universal among the flowering plants. Overlying these strata of broadly uniform structure and behavior there are countless variations in the devices by which the consummation of the sexual union—initiated by the actual physical contact of the male and female gametophytes—is eventually attained (Heslop-Harrison, 1983).

Sex expression in flowering plants was reviewed by Heslop-Harrison (1957, 1963, 1972, 1983) and Frankel and Galun (1977). Types of sex expression common in flowers, plants and plant populations are presented in Table 4.

On several occasions over a century ago, Darwin discussed the importance of cross-pollination to the maintenance of vigor in natural plant populations (Frankel and Galun, 1977). Floral structure of a given species was thought to be evolutionarily significant and to reflect functional considerations necessary for promotion of adequate outcrossing levels. However, the obvious diversity among species for required cross-pollination has led to several theories concerning the nature of hybrid vigor. Current ideas have included those of Mather as
Table 4: Common Types of Sex Expression in Flowering Plants

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Flowers</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Monoclinous</strong></td>
<td></td>
</tr>
<tr>
<td>Hermaphroditic-</td>
<td>containing both stamens (male organs) and carpel(s) (female organs)</td>
</tr>
<tr>
<td><strong>Diclinous</strong></td>
<td></td>
</tr>
<tr>
<td>Stamine-</td>
<td>possessing only stamens</td>
</tr>
<tr>
<td>Pistillate-</td>
<td>possessing only carpels</td>
</tr>
<tr>
<td><strong>Individual Plants</strong></td>
<td></td>
</tr>
<tr>
<td>Hermaphroditic-</td>
<td>only hermaphroditic flowers present</td>
</tr>
<tr>
<td>Monoeclous-</td>
<td>both staminate and pistillate flowers present</td>
</tr>
<tr>
<td>Androeclous-</td>
<td>only staminate flowers present</td>
</tr>
<tr>
<td>Gynoeclous-</td>
<td>only pistillate flowers present</td>
</tr>
<tr>
<td>Andromoneoecious-</td>
<td>having both staminate and hermaphroditic flowers</td>
</tr>
<tr>
<td>Gynomoneoecious-</td>
<td>having both pistillate and hermaphroditic flowers</td>
</tr>
<tr>
<td>Trimoneoecious-</td>
<td>having staminate, pistillate and hermaphroditic flowers</td>
</tr>
<tr>
<td><strong>Plant Populations</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Monomorphic</strong></td>
<td></td>
</tr>
<tr>
<td>Hermaphroditic-</td>
<td>consisting of only hermaphroditic plants</td>
</tr>
<tr>
<td>Monoeclous-</td>
<td>consisting of only monoeclous plants</td>
</tr>
<tr>
<td><strong>Dimorphic</strong></td>
<td></td>
</tr>
<tr>
<td>Dioecious-</td>
<td>consisting of androecious and gynoeclous plants</td>
</tr>
<tr>
<td>Androdioecious-</td>
<td>containing hermaphroditic and androecious plants</td>
</tr>
<tr>
<td>Gynodioecious-</td>
<td>containing hermaphroditic and gynoeclous plants</td>
</tr>
</tbody>
</table>

1Adapted from Frankel and Galun (1977)
summarized by Heslop-Harrison (1983). Predominantly inbreeding species were considered to possess 'functional genic balance' within the genome whereas their predominantly outbreeding counterparts were thought to exhibit this balance within and between genomes. Conservation of breeding mechanisms was said to be necessary for continued maintenance of genetic balance and subsequently, fitness.

Floral morphology and pollination systems which promote outcrossing were outlined by Heslop-Harrison (1983), who classified the controlling features as: dependent on interaction of pollen and pollen tube (self-incompatibility systems); dependent on developmental timing (protandry and/or protogyny); or dependent on structural adaptations (such as, characteristics affecting behavior of pollen vectors, features influencing the distribution of pollen and the development of dicious [unisexual] flowers). Of these controlling features, the development of dicious flower structures appeared to be the most evolutionarily radical mechanism for promotion of cross-pollination (Heslop-Harrison, 1983). Polymorphic species (about half of which are dicious) were found to comprise only 6 to 7% of all higher plants whereas monoeious species composed less than 20% of the total. In contrast, hermaphroditic sex expression was found to be most prevalent in angiosperms.

Although evolutionary solutions to specific pollination needs have resulted in great diversity of floral structures, these structures were thought to share a common ancestry and their development was said to be controlled by highly conserved genetic mechanisms (Heslop-Harrison, 1957, 1963). These theories corresponded well with the Wardlaw model of floral development (see Chapter 2) in which organo-
genesis was said to proceed in a sequential and predictable pattern. Diversity was presumed to arise from a superimposed complex of secondary genetic and environmental controlling elements which modify growth rates of specific floral organ primordia during 'plastic phases' of development. Diclinous flowers, representing highly altered floral forms, were thought to result from complete (or nearly complete) suppression of stamen or pistil primordia development through the action of one or more secondary controlling elements (Frankel and Galun, 1977).

Heslop-Harrison (1972) described two variations in diclinous flower development: those which progress through a visible bisexual stage prior to suppression of either antheridium or pistillodium and those in which a bisexual stage is never visible. Precisely timed manipulation of the ontogenetic process through the use of growth regulators or modification of environmental conditions was found to modify diclinous floral development in some species. Modifications commonly resulted in the formation of hermaphroditic flowers or unisexual flowers of the alternate sex types, perhaps reflecting the totipotent nature of plant cells. Both the failure to modify the developmental progression through exogenous manipulation experienced with some species and variations in response to outside stimuli witnessed in others exemplified the complexity of genetic control over floral development (Heslop-Harrison, 1957, 1972; Frankel and Galun, 1977).
Flowering Habits

Variation in Floral Development

Development of hermaphrodite flowers in some cucurbits has been reported; however, dicialinous flowers predominate in most Cucurbitaceae species. The ontogeny of staminate and pistillate cucumber flowers was outlined in detail by Helmlich (1927) and Judson (1929) respectively. Later, dicialinous floral development of both sex types was compared to that of hermaphroditic cucumber buds by Atsmon and Galun (1960). Floral buds were found to differentiate in axils of immature leaves. The succession of primordia development differed between sex types in dicialinous buds with differentiation of calyx and corolla from the perianth tube occurring later in the staminate buds. However, all forms exhibited morphological bisexuality during early stages of enlargement at which differentiating stamindium and pistillodium appeared equal in development. Inhibition of appropriate organ development in dicialinous buds following this initial period was said to result in staminate and pistillate flowers containing at most vestiges of the reproductive organs of the opposite sex type.

In contrast to the pattern found in cucumber, Pereira (1968) failed to uncover a morphological bisexual stage in developing staminate buds of Acorn squash (Cucurbita pepo). Pistillate buds developed stamen initials but cell division in this tissue was shown to be restricted in very early stages of bud growth.

Normal staminate flower development in leaf axils of buffalo gourd was demonstrated by Yousef (1976) to resemble that reported for buds of Acorn squash. Rapid growth of stamen initials was evident when
buds reached 1 mm in size and staminate tissue filled the urn-shaped receptacle after an additional 2 mm of growth. Pistillate flower development in buffalo gourd has yet to be studied in detail.

Hermaphroditic flowers have not been previously reported in this species. However, functional flowers of this type were found borne on a single segregate developed in a breeding program to improve root shape (Nelson, unpublished data). Also, recent observations on sex expression within various Mexican accessions uncovered staminate flowers containing developed stigmatic and stylar tissue. This pistillate tissue was encased within the coalesced staminate structure (Gathman, Scheerens and Ralowicz, unpublished data). Histological examination of these 'pseudo-hermaphroditic' staminate buds in various stages of maturation failed to reveal the presence of ovarian tissue.

Qualitative Sex Expression

Sex expression in the cucurbits historically has been viewed as two separate but related plant characteristics: qualitative sex expression (i.e. differences in presence or distribution of hermaphroditic, pistillate and staminate flowers among and within individuals and populations); and quantitative sex expression (i.e. differences in the ratio of flower types and developmental pattern among individuals displaying the same qualitative sex expression).

Qualitative variation in sex expression among individuals and among breeding populations abounds within the Cucurbitaceae; perhaps more combinations are found in this family than in any other. Whitaker, (1931) citing Yampolski, indicated monoecy to be the most common expres-
among plants and populations within the family, with solely hermaphroditic species occurring infrequently. However, he also cited Correns who suggested hermaphroditism to be the original plant form from which others evolved. Androecious and gynoecious plants were thought to arise through andromonoecious and gynomonoecious intermediate forms whereas monoecious individuals were presumed to be formed from trimonoecious intermediates (refer to Table 4). Phylogenetic emergence of sexual dimorphism or polymorphism occurs predominantly although not exclusively within formerly hermaphroditic or monoecious populations (Charlesworth and Charlesworth, 1978a,b; Ross, 1982). The resulting breeding populations (termed subdioecious) contain both unisexual (gynoecious or androecious) and bisexual (hermaphroditic, andromonoecious, gynomonoecious, trimonoecious and/or monoecious) individuals (Ross, 1982). Dioecious populations are presumed to result from at least two independent mutations affecting sexual development and are said to be the most evolutionarily advanced (Charlesworth and Charlesworth, 1978a).

Characteristic qualitative sex expression of individuals and populations in some cucurbit species is displayed in Table 5. Entries represent common cultivated cucurbits and also lesser known family members for which data concerning sex expression have been generated.

Buffalo gourd was said to be a subdioecious (mono-gynodioecious) species consisting of gynoecious and monoecious individuals (Curtis and Rebelz, 1974; Bemis et al., 1978). The occurrence of the gynoecious phenotype was found to be widespread in feral populations as nearly half of 47 accessions collected in southwestern USA and northern Mexico.
Table 5: Qualitative Sex Expression In Selected Cucurbit Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Sex Variants</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bryonia alba</td>
<td></td>
<td>monoecious</td>
<td>2</td>
</tr>
<tr>
<td>Bryonia dioica</td>
<td></td>
<td>gynoecious</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>androecious</td>
<td></td>
</tr>
<tr>
<td>Citrullus vulgaris</td>
<td>watermelon</td>
<td>monoecious</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>andromonoecious</td>
<td>20</td>
</tr>
<tr>
<td>Cucumis anguria</td>
<td></td>
<td>monoecious</td>
<td>9, 18</td>
</tr>
<tr>
<td>Cucumis melo</td>
<td>muskmelon, cantaloupe</td>
<td>gynoecious</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(unstable)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>gynoecious (stable)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gynomonoecious</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hermaphroditic</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>monoecious (stable)</td>
<td>19, 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>andromonoecious</td>
<td>19, 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>androecious</td>
<td>5</td>
</tr>
<tr>
<td>Cucumis sativus</td>
<td>cucumber</td>
<td>gynoecious</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(prodrom. pist.)</td>
<td>12, 13, 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gynoecious (multiple pist.)</td>
<td>6, 17, 26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hermaphroditic</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>monoecious (with pist. phase)</td>
<td>12, 13, 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(with mixed phase)</td>
<td>20, 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>andromonoecious</td>
<td>20, 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>androecious</td>
<td>7, 15, 21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trimonoecious</td>
<td>16</td>
</tr>
<tr>
<td>Cucurbita foetidissima</td>
<td>buffalo gourd</td>
<td>gynoecious</td>
<td>3, 4</td>
</tr>
<tr>
<td>Cucurbita maxima</td>
<td>squash (various cult.)</td>
<td>monoecious</td>
<td>3, 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27</td>
</tr>
</tbody>
</table>
Table 5: (Continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Sex Variants</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cucurbita moschata</em></td>
<td>squash (various cult.)</td>
<td>monoecious</td>
<td>27</td>
</tr>
<tr>
<td><em>Cucurbita pepo</em></td>
<td>squash, pumpkin (various cult.)</td>
<td>monoecious</td>
<td>27</td>
</tr>
<tr>
<td><em>Echinocystis ocularis</em></td>
<td>squirting cucumber</td>
<td>monoecious</td>
<td>10</td>
</tr>
<tr>
<td><em>Lagenaria sicariaria</em></td>
<td>bottle gourd</td>
<td>monoecious</td>
<td>22</td>
</tr>
<tr>
<td><em>Lagenaria sicariaria</em></td>
<td>kettle gourd</td>
<td>monoecious</td>
<td></td>
</tr>
<tr>
<td><em>Luffa acutangula</em></td>
<td>loofah</td>
<td>monoecious</td>
<td>1</td>
</tr>
<tr>
<td><em>Luffa cylindrica</em></td>
<td>dishcloth gourd</td>
<td>monoecious</td>
<td>25</td>
</tr>
<tr>
<td><em>Momordica charantia</em></td>
<td>bitter gourd</td>
<td>monoecious</td>
<td>8</td>
</tr>
<tr>
<td><em>Sicyos angulatus</em></td>
<td></td>
<td>monoecious</td>
<td>24</td>
</tr>
</tbody>
</table>

1References:

1. Bose and Nitsch, 1970
2. Challakhyan, 1979
3. Curtis and Rebelz, 1974
4. Dossey, Bemis and and Scheerens, 1978
5. Foster and Bond, 1967
6. Fugleda et al., 1982
7. George, 1970
8. Ghosh and Basu, 1963a
9. Hall, 1949
10. Kopcowicz, 1971
11. Kubicki, 1966
13. Kubicki, 1969a
14. Kubicki, 1969b
15. Kubicki, 1969d
17. Nandgaonkar and Baker, 1981
18. Nitsch et al., 1952
19. Poole and Grimbell, 1959
20. Rosa, 1926
21. Scott and Baker, 1975
22. Sharma, Jyotishl and Agrawal, 1980
23. Shifriss, 1961a
24. Takahashi, Salto and Suge, 1982
25. Takahashi, Suge and Salto, 1980
26. Uzcategui and Baker, 1979
27. Whitaker, 1951
produced progeny which segregated for the gynoeclous trait (Bemis et al., 1978). Curtis and Rebelz (1974) first described abortive staminate buds on gynoeclous plants as antherless and defined three classes of antherless buds dependent on corolla size at the time of abortion. However, field observations during the course of buffalo gourd domestication at this institution did not support the existence of well defined classes (Scheerens et al., unpublished data). Bud abortion was also found to occur at various stages of corolla development (most commonly before buds reached 2 cm in length) and in rare cases, antherless flowers reached anthesis. Also, timing of bud abortion at successive nodes of an individual often varied. Yousef (1976) histologically examined abortive staminate buds and reported normal stamen primordia development in early stages of growth which ceased when buds reached approximately 2 mm in length. Whether bud abortion and cessation of staminate primordia development are controlled by the same factor(s) or constitute a 'cause and effect' relationship has not been investigated.

Curtis and Rebelz (1974) also reported the existence of an additional mutant in which anthers developed but failed to produce pollen. This phenotype was not recovered in populations studied at the University of Arizona.

Quantitative Sex Expression

According to Shiffriss and Galun (1956) the entire monoecious cucumber plant can be considered a compound inflorescence which gradually undergoes a transition from male to female expression. The flowering pattern along a given stem can be divided into three sequential phases:
exclusively staminate flowering—staminate and pistillate flowering—
exclusively pistillate flowering. Acorn squash \textit{(Cucurbita pepo)} dis-
plays additional phases, sequentially producing: underdeveloped male
flowers—normal male flowers—normal male and female flowers—giant
female flowers and inhibited male flowers—parthenocarpic female flowers
(Nitsch et al., 1952). Flowering patterns similar to the examples given
exist in most sex types of these and other cucurbits with male expres-
sion preceding that of female development (Whitaker, 1931). Differen-
tes in the duration or floral productivity of these phases among and
within species constitute quantitative modifications in sex expression.
Variation in expression can be attributed to differences in number of
flowers produced per node; to differences between main and lateral stems
(Currence, 1932; Frankel and Galun, 1977); to the presence of developing
fruit (Tiedjens, 1928; Scott, 1933); and to genetic, environmental,
nutritional and hormonal influences as discussed below.

Several methods of quantitation have been used, but data have
most often been presented in terms of the sex ratio (the number of
staminate flowers/number of pistillate flowers). Whitaker (1931) used
this criteria to exemplify differences in sex expression of 49 varieties
of cultivated cucurbits and reported variation in expression among
genera, among species and among varieties within species, with produc-
tion of staminate flowers greatly outnumbering pistillate flowers in all
cases. Sex ratios ranged from 5.8 to 22.4. Scott (1933) also used
this criterion to characterize three varieties of \textit{Cucurbita pepo} (White
Bush Scallop, Zucchini and Giant Summer Crookneck). Sex ratios were
generally lower than those reported by Whitaker (presumably due to removal of immature fruit) and ranged from 2.0 to 9.9.

The use of node number (number of nodes to first female flower, also termed female tendency) was advocated as a more easily obtainable and a more accurate measure of quantitative sex expression than the determination of sex ratio as it pinpoints the onset of phase change (Shiffriss and Galun, 1956). Determinations of node number identified more significant differences among cucumber varieties than did measures of sex ratio and could be used to indicate relative earliness of cucumber lines. Earlier, Currence (1932) employed this criterion to differentiate among three cucumber varieties.

Nitsch et al. (1952) developed an additional quantitative measure of sex expression which more completely characterized the gradual shift from male to female flowering tendency. They recorded changes in the number of female flowers in sequential short vine segments (10 consecutive nodes per segment) which rose sharply upon termination of the male phase. A plot of the data was obtained with a line of best fit intercepting the abscissa at the onset of female flowering; the slope of the line indicated the strength of female expression with the angle of interception being termed the 'Index of feminization'. Feminization indices from 0 to 80° were determined for Acorn squash populations grown under different environmental conditions.

Quantitative nature of buffalo gourd sex expression was studied by Yousef (1976). The mean node number of 195 two year old gynoe- cious plants was 15.4 +/- 5.3 nodes whereas 105 two year old monoecious
plants generally required greater vine growth for female development (mean node number 23.4 +/- 7.6 nodes).

Populations of first season plants examined by Yousef (1976) contained many individuals (up to 41%) which failed to produce female flowers. This trend has been substantiated by Curtis and Rebelz (1974), Dossey et al. (1981) and by Scheerens et al. (unpublished data).

In the study by Yousef (1974), ten two year old gynoecious plants and ten monoecious plants of the same age were chosen for detailed examination. Flowering pattern of monoecious plants developed as follows: vegetative phase--male phase--mixed (male and female) phase--vegetative phase. The pattern displayed by their gynoecious counterparts developed similarly: vegetative phase--abortive male phase--mixed (female and abortive male) phase--vegetative phase. Some plants began to repeat the flowering cycle. Sex ratios of monoecious plants declined as the season progressed with seasonal values of whole plants, major stems and secondary branches reaching 26.8, 35.0 and 25.5 respectively.

Progressive development of buffalo gourd sex expression was determined for the 20 selected plants using a modified Index of feminization. For both sex types, the number of female flowers versus segment number (10 nodes per segment) defined a curvilinear relationship with female development in gynoecious plants surpassing that of monoecious plants in all segments. Node numbers of female flowers borne on side branches were influenced by nodes of branch initiation. Scatter diagrams revealed a linear relationship between parameters in both sex types.
Environmental Influences upon Quantitative Sex Expression

In general, environmental influences upon sex expression in cucurbits affect only the duration of each flowering phase whereas genetic effects often alter the production of flower types or suppress one or more phases of the flowering pattern. A notable exception occurs in gynoecious cucumber hybrids (heterozygous at loci affecting sex expression, see discussion below) which occasionally produce a few staminate flowers under some environmental conditions. These cultivars by virtue of their genotypes are considered to be predominantly pistillate rather than monoecious. Economic importance of these cultivars has precipitated their inclusion in recent experimentation examining environmental effects on sex expression.

Environmental factors mediate their influence on sex expression by altering relative growth rates. These rates affect the physiology of the meristematic region through modification of hormonal or nutritional balances which in turn, dictate development of floral primordia. Environmental factors of importance include: light intensity, photoperiod, temperature, nutrient availability, plant density and mechanical stress.

A classic treatise concerning environmental effects on cucurbit sex expression was composed by Tiedjens (1928). Shading monoecious cucumber varieties greatly reduced female flower development. Later experiments by Cantilffe (1981) substantiated evidence for reduced pistillate flower formation under low light intensity in a monoecious cucumber cultivar, a gynoecious breeding line and four gynoecious pickling varieties. Cantilffe also reviewed studies of other authors indicating increased pistillate flowering in monoecious cultivars as-
sociated with high light intensity. Staminate flowering in gynoecious (predominantly pistillate) hybrids varied with genotype and was generally greatest at intermediate light intensities (Cantliffe, 1981). Extremes in light intensity reduced staminate production in monoecious cucumber studied by Cantliffe whereas shading was reported by Tiedjens to slightly increase the number of male flowers formed in this sex type.

Since photoperiodism is exhibited by some varieties of cucumber and other cucurbit species, the effects of photoperiod on sex expression have been examined thoroughly. Increased daylength through use of supplemental light increased sex ratio of monoecious cucumber by increasing the total number of staminate flowers (Tiedjens, 1928). Examining several cucumber varieties, Cantliffe (1981) found no differences in staminate or pistillate flower production under several photoperiodic regimes. However this author recapitulated evidence from three independent studies suggesting female flower production to be enhanced under short day conditions. Increased daylength was also found to increase staminate floral development in Acorn squash and gherkin (Cucumis anguria) presumably through reduction of specific endogenous hormones associated with this condition (Nitsch et al., 1952). In contrast, Hail (1949) reported long days to reduce total flowering in gherkins but to have no effect upon their sex ratio. Long days were associated with increased flowering of andromonoecious muskmelon (Cucumis melo) and with a low ratio of staminate to perfect flowers (Brantley and Warren, 1960). Hormonal balance during floral differentiation under long days was said to be optimal for the expression of perfect flowers. Takahashi, Salto and Suge (1983) found endogenous
hormone concentrations in two varieties of cucumber to be similar but their maximum female expression to differ with respect to photoperiod. These results intimated the involvement of additional substances in the regulation of female floral ontogeny.

Nitsch et al. (1952) delineated the effects of temperature on bud development in Acorn squash. High temperatures promoted the differentiation of male buds and prohibited anthesis of all flowers whereas low temperatures increased the index of feminization and enhanced opportunity for production of parthenocarpic fruit. High night temperatures had profound effects upon sex expression, reducing the index of feminization and increasing the node number. Temperature variation had pronounced effects upon sex expression in cucumber (Cantliffe, 1981), with temperature extremes inhibiting both growth and total floral production. Monoeclous and predominantly pistillate varieties exhibited a greater number of male blossoms when grown at 26-30 °C. A review of previously published data by these authors indicated high temperatures to promote male flower production in monoeclous cucumber phenotypes. Pistillate flowering in the monoeclous cultivar was unaffected by temperature variation whereas the gynoeclous breeding line studied exhibited maximum female flower development at temperatures between 22-26 °C.

Low soil fertility was shown to reduce total floral production in cucumber (Tiedjens, 1928), but was revealed to have little effect upon the sex ratio. Low levels of nitrogen also reduced total flowering in gherkin and muskmelon whereas high nitrogen applications increased the production of female flowers and lowered the sex ratio exhibited by these species (Hall, 1949; Brantley and Warren, 1960). Similar results
from studies of Butternut squash (*Cucurbita moschata*) were reported by Hopp (1962). High levels of applied nitrogen lowered the sex ratio due to rapid vine expansion resulting in greater development of the mixed (male and female) phase. Node number was constant over all fertility treatments which substantiated results of Shifriss and Galun (1956).

Plant density had no significant effect on flowering habits of gynoecious inbreds (Lower, Smith and Ghaderi, 1983). However, their predominantly pistillate hybrids exhibited a reduction in female flowers and an increase in male flowers formed at higher plant densities. Predominantly pistillate hybrids grown at equal densities but variable within-row spacings displayed similar changes in floral patterns with increased crowding (Nienhuis, Lower, and Miller, 1984). The extent of variability in male floral production among hybrids could also be attributed (in part) to their respective pollen parent genomes.

Recently, attempts to mimic adverse field conditions causing cotyledonary damage have fostered experiments studying the effects of mechanical stress or removal of cucumber cotyledons. During the course of study, information has ascertained the effects of this stress upon sex expression in cucumber. After removal of cotyledons, Cantliffe and Omran (1981) noticed increased male flower and delayed female flower formation on predominantly pistillate lines grown under sub-optimal temperature and photoperiod. These authors attributed the phenomenon to changes in metabolite production due to reduced photosynthetic area and restricted intake of CO$_2$. Employing this technique, Takahashi and Suge (1980) reported reduced growth and increased feminization in monoecious cultivars whereas gynoecious lines were unaffected by treatment.
In general, environmental conditions which promote growth were found to enhance female flowering (Tiedjens, 1928; Hall, 1949; Brantley and Warren, 1960; Hopp, 1962, Cantlliffe, 1981; Cantlliffe and Omran, 1981; Lower, Smith and Ghaderl, 1983). However, the trend was species dependent and varied with sex type and variety. Ultimate control of sex expression was said to result from hormonal balance (Brantley and Warren, 1960) and/or from effects of additional metabolites (Takahashi, Saito and Suge, 1983). Environmental effects were shown to influence growth rates, the duration of phases and perhaps the physiology of the meristematic region.

Environmental influences upon sex expression in buffalo gourd have yet to be examined. Nevertheless, correspondence with colleagues has revealed a total inhibition of flowering in buffalo gourds grown in tropical climates (A.C. Gathman, personal communication). For lack of appropriate data, this phenomenon has been tentatively attributed to an adverse reaction to short daylength.

**Genetic Control of Sex Expression**

Shortly after Mendel's laws of inheritance were rediscovered, Correns demonstrated the genetic basis for sex determination using crosses among and within two cucurbit species of differing sex expression, *Bryonia dioica* (dioecious) and *Bryonia alba* (monoecious) (Challakhyan, 1979). Since then, inheritance of qualitative sex expression in di- or polymorphic cucurbits has been studied in relatively few species.
Cucumis sativus

Genetic aspects of sex determination in cucumber have been examined in greater detail than in other cucurbits and have been summarized by Galun (1961) and Frankel and Galun (1977). Pangalo obtained 15 different sex forms in the F2 resulting from a monoecious x andromonoecious cross and estimated involvement of at least ten loci controlling qualitative and quantitative differences (Frankel and Galun, 1977). However, more simplified sex inheritance schemes have been proposed with few major loci controlling dillinous/monoclinous flower formation and/or radically modifying flowering phase duration. These genes have been described to be epistatic over those responsible for flower ontogeny (Frankel and Galun, 1977) and over polygenic control of quantitative sex expression (Shiffriss, 1961a).

Rosa (1928) observed the F1 population of an andromonoecious X monoecious cross to exhibit the phenotype of the pollen parent; the F2 population segregated with 11 plants bearing pistillate flowers and five plants producing hermaphroditic flowers. Expanding earlier studies, Shiffriss (1961) proposed the following genetic scheme for the control of four cucumber sex types:

\[
\begin{align*}
\text{Gynoecious varieties} &= Acr, g^1,2 \\
\text{Hermaphroditic varieties} &= Acr, g g \\
\text{Monoecious varieties} &= acr acr, g \\
\text{Andromonoecious varieties} &= acr acr, g g
\end{align*}
\]

1 alternate gene symbols (st/m; st+/m) proposed by Galun (1961) alternate gene symbols (acrF/m; acr+/m) proposed by Kubicki (1968)
The $g$ allele favors the formation of pistillate over hermaphroditic flowers ($g$) whereas an accelerator of female tendency ($Ac^r$) radically alters polygenic control of the flowering progression eliminating the development of the staminate phase.

Subsequent experimentation over the following decade led to more complete analyses of the $Ac^r$ locus and additional loci affecting the expression of sex types. Kubicki (1965a) discovered additional increases in feminization resulting from dosage effects of the $Ac^r$ ($ac^r_F$) locus in autotetraploids of gynoecious X monoecious $F_1$s. Also, chromatin duplication in autotetraploid monoecious cucumbers increased the total number of female flowers and reduced node number in all but one genotype. Through a series of crosses, he later uncovered the action of an allelic series at the $Ac^r$ locus ($ac^r_F > ac^r_1 > ac^r^+ > ac^r^m$) which in their homozygous states resulted in sex ratios of $0, 1, 10$ and $100$ respectively (Kubicki, 1968; 1969a,b). Phenotypes produced by the $ac^r_F$ allelic series were as follows:

- gynoecious varieties = $ac^r_F ac^r_F$
- predominantly pistillate hybrid varieties = $ac^r_F ac^r^+$
- monoecious lines with pistillate phase = $ac^r_1 ac^r_1$
- monoecious lines with mixed phase = $ac^r^+ ac^r^+$

An additional gene, $E$ (independent of the $Ac^r$ locus) was shown to intensify femaleness in the above monoecious genotypes and to prevent staminate floral induction on gynoecious plants normally possible through the use of plant growth regulants (see discussion below) (Kubicki, 1969c).

Shiffliss, George and Quinones (1964) found expression at the $Ac^r$ locus to be highly modified by background genotype. These authors
used the effects of background genotype advantageously, creating gynodioecious populations of heterozygous predominantly pistillate and heterozygous gynoecious lines.

A recessive gene, \( \text{de} \), which prevents indeterminate growth and precludes lateral branching was employed by George (1970) to produce androecious individuals (\( \text{acr acr}, \text{de de} \)). Drastic reduction in plant growth inhibited the progression of flowering phases, eliminating pistillate flower production. Kubicki (1969d) presented evidence of another recessive gene, \( \text{a} \), (Independent of the \( \text{De} \) locus) which in the presence of \( \text{acr}^+ \text{acr}^+ \) resulted in androecious phenotypes. The \( \text{a} \) allele was characterized in a series of dihybrid gynoecious X androecious crosses (Scott and Baker, 1975). Sex types of \( F_2 \) progeny included monoecious plants with exclusively pistillate phases, monoecious plants with mixed phases and androecious plants. The \( F_2 \) and backcross populations exhibited segregation ratios indicating the \( \text{Ac} \) and \( \text{A} \) loci to be epistatically related. A subsequent study (Scott and Baker, 1976) revealed \( F_1 \) populations generated from gynoecious X androecious crosses to be "as highly female, or more so than hybrids derived from monoecious pollen parents".

Trimonoeccous sex types were found to be conditioned by a dominant gene, \( \text{Tr} \), (Independent from the \( \text{Ac} \) or \( \text{G} \) loci) which resulted in the formation of hypogynous or perigynous (rather than eplgynous) hermaphroditic flowers (Kubicki, 1969e). The gene was most effective in genetic backgrounds promoting andromonecious, monoecious and hermaphroditic phenotypes.
Quantitative sex expression in cucurbita was thought to be controlled by polygenic effects (Stiffiss, 1961a; Frankel and Galun, 1977), but few studies have been undertaken to more fully characterize parameters. However, Galun (1961a) studied the inheritance of node number in F2 and backcross progeny of divergent inbreds, confirmed it to be polygenic and estimated the trait to be controlled by 5-15 loci. More recently, total number of female flowers produced by gynoecious hybrids was increased by formation of multiple pistillate flowers per node (Uzcategui and Baker, 1979). This characteristic was found to be effected by a single gene (mp), recessive to the wildtype (single flower per node) habit (Nandgaonkar and Baker, 1981). This trait was subsequently reported to be conditioned at the Mp locus by an allelic series exhibiting dominance or incomplete dominance (single pistillate flower/node > twin pistillate flowers/node > multiple pistillate flowers/node) (Fujleda et al., 1982).

**Cucumis melo**

Investigating the inheritance of qualitative sex expression in muskmelon, Rosa (1928) examined F2 and backcross progeny of andromonoecious X monoecious crosses and their reciprocals. Total segregation scored over all F2 populations closely approached a monohybrid (3:1) ratio with the monoecious condition dominant over andromonoecious forms. Backcross (testcross) populations exhibited a perfect 1:1 ratio substantiating claims of monogenic inheritance. The isolation of hermaphroditic muskmelon phenotypes and their subsequent use in monoecious X hermaphroditic and andromonoecious X hermaphroditic crosses led Poole and
Grimball (1939) to propose the following genetic hypothesis:

- monoecious varieties (mostly staminate) = $A \_ , G^1$
- gynomonoecious varieties (mostly pistillate) = $A \_ , g \ g$
- andromonoecious varieties (mostly staminate) = $a \ a , G \_ $
- hermaphroditic varieties = $a \ a , g \ g$

Dominance at the $A$ locus was thought to suppress development of stamens in perfect flowers whereas the $G$ gene was presumed to curtail the development of pistils. (Note: the $G$ locus in muskmelon is not synonymous with the $G$ locus in cucumber although both condition the transformation of monoclinous to diclinous flowers). Gynoecious segregates were also uncovered in this study but were described as 'transitory phenotypes' highly affected by environmental conditions. Kubicki (1969f) substantiated evidence for the genetic scheme proposed by Poole and Grimball and demonstrated interaction with loci controlling ovary position. Contrasting schemes for the inheritance of sex expression in cucumber and muskmelon, this author (1969g) commented on the recessive nature of femaleness and the lack of genes altering the progression of flowering phases in the latter species.

Kubicki (1966) outlined the formation of gynoecious muskmelon lines using a variety of crosses. Gynoecious phenotypes obtained were genotypically identical to gynomonoecious lines ($A \ A , g \ g$) described by Poole and Grimball (1939) but were stable in a variety of environments.

Foster and Bond (1967) recovered an abrachiate (branchless) mutant from muskmelon breeding populations. As muskmelon commonly bears

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1. alternate gene symbols ($M/m; G/g$) proposed by Kubicki (1969f)
pistillate or perfect flowers on branches, this mutant constituted an androecious sex type. The abrachlate condition was found to be controlled by a single recessive gene \( (ab) \).

Research to date has also uncovered three recessive alleles \( (ms-1, ms-2 \text{ and } ms-3) \) effecting male sterility in a variety of muskmelon sex types (McCreight and Elmstrom, 1984). Genetic studies indicated these genes to be associated with independent loci.

**Citrus vulgaris**

Although the monoecious phenotype in watermelon \( (Citrus vulgaris) \) predominate, andromonoecious segregates have been reported by Rosa (1926). Examining \( F_2 \) and backcross segregation patterns resulting from monoecious \( \times \) andromonoecious crosses, he determined the andromonoecious phenotype to be regulated monogenically. As in muskmelon, recessive homozygosity at this locus directed the development of perfect flowers in lieu of pistillate flowers in these segregates.

**Cucurbita spp.**

Members of the genus *Cucurbita* are considered to be almost exclusively monoecious, although the formation of perfect flowers has been reported in a few varieties of *C. moschata* and *C. pepo* (Rosa, 1928; Whitaker, 1931). Kubicki (1970a) also recounted the existence of androecious strains in *C. pepo*. As in cucumbers, the androecious condition proved to be simply inherited, with homozygous recessive alleles retarding the flowering phase progression.

Rare within the genus, buffalo gourd populations frequently exhibit sexual dimorphism. In a population derived from a single
accession (colony of asexually reproduced plants), Curtis and Rebelz (1974) scored 560 second season progeny for sex expression recovering 431 monoecious and 129 gynoecious segregates (approximating a 3:1 ratio). Presuming the original colony to be heterozygous, these authors suggested monogenic control of sex expression with gynoecious types resulting from the homozygously recessive condition.

Yousef (1976) supported this genetic hypothesis using data obtained from 90 second season F1 populations derived from gynoecious X monoecious crosses supplied by Curtis and Rebelz. Presuming the presence of abortive male buds to indicate gynoecy, he reported segregation to approximate a 1:1 testcross ratio. For data analysis, this author assumed (as did Curtis and Rebelz) that monoecious parents were heterozygous for the sex controlling gene. He also uncovered substantial numbers of both first and second season plants which failed to develop female flowers, a trend which was more prevalent in monoecious plants. Rather than constituting an androecious sex type, these plants were believed to be juvenile with female expression likely to occur in subsequent seasons.

Dossey, Bemis and Scheerens (1981) proposed an alternate hypothesis for genetic control of buffalo gourd sex expression. A single monoecious individual was used as the seed parent in a series of monoecious X monoecious crosses and as the pollen parent in an additional series of gynoecious X monoecious crosses. Ignoring plants that remained vegetative, first season F1 populations derived from the monoecious X monoecious crosses failed to produce gynoecious segregates whereas similar populations resulting from the gynoecious X monoecious
crosses segregated in nearly a 1:1 ratio. The authors proposed the following inheritance scheme:

\[
\text{gynoecious plants} = M m \\
\text{monoecious plants} = m M
\]

The abortive male bud phenotype was dominant and maintained in a heterozygous condition. The high frequency of vegetative or juvenile plants hindered analysis of sex expression in this study also.

**Endogenous Hormone Levels In Sex Types**

... Information on the endogenous content of some growth regulators is being accumulated. Unfortunately most of the investigations on which this information is based were performed with either/or tests which are not specific enough (bioassays) or not sensitive enough. We are interested in information on the hormonal content in an exactly defined location -- e.g. the ovarial initial or at least the young embryonal floral bud. Hence, the relation between genetic composition, environmental conditions and hormonal distribution is still very far from being solved (Galun, 1980).

Notwithstanding the inadequacy of hormone assay techniques (which prevent precise determination of hormone levels active in differentiating cells), studies of hormone concentrations in plants of varying sex type have contributed significantly to the understanding of hormonal regulation of sex expression.

Hormone Levels In Plant Tissues

Using bioassay techniques, Galun (1959a) uncovered differences in IAA content extracted from apices of cucumber sex types. Gynoecious plants exhibited higher levels of endogenous IAA than did monoecious plants. In a more detailed study, Galun, Izhar and Atsmon (1965) examined IAA levels in andromonoecious and hermaphroditic cucumbers.
Bioassay results suggested higher levels of IAA in tissues of hermaphrodites than in andromonoeclous strains. Extracts of apices containing floral primordia exhibited higher auxin activity than successive stem sections containing buds at more advanced developmental stages. In addition, floral buds of hermaphroditic types were found to differentiate in closer proximity to apical region. Higher IAA levels in apices and closer proximity of developing buds to high auxin concentrations were proposed to be major effects of the \textit{Acr} gene and the mechanisms by which \textit{Acr} promotes female expression.

Auxin effects on cucurbit sex expression have been shown to be mediated at least in part through its stimulation of ethylene synthesis (see discussion of ethylene synthesis, Chapter 2). Examining ethylene evolution in gynoecious and monoeclous cucumbers, Rudich, Halevy and Kedar (1972a) uncovered the following trends: reduced evolution rates exhibited by seedlings in comparison to levels associated with flower buds or apices with developing flower buds; greater evolution rates from apices of gynoecious plants than from monoeclous plants; and greater evolution rates associated with female buds than with their male counterparts. Studying ethylene evolution in cucumber apices, Rudich et al. (1976) demonstrated higher levels of the hormone associated with gynoecious and hermaphroditic sex types than with monoeclous, andromonoecious or androecious phenotypes. Fujita and Fugleda (1981) reported ethylene evolution of seedling cucumbers to vary with sex type according to alleles present at the \textit{Acr} locus. Ethylene evolution rates decreased with gene expression in the following order: \textit{acr$^F$} > \textit{acr$^I$} > \textit{acr$^+$}. 

Ethylene was also suggested as a natural regulator of sex expression in muskmelon (Byers et al., 1972a). A gynoecious breeding line was compelled to produce staminate flowers when grown under hypobaric conditions promoting ethylene removal from tissues. Ethylene evolution rates in muskmelon were lower than those associated with cucumbers and varied among sex types. Gynoecious, andromonoecious and monoecious lines produced far greater quantities than the hermaphroditic phenotype. Although trends were not as definitive as those exhibited by cucumbers, Byers and coworkers concluded high endogenous ethylene level to promote femaleness in muskmelon through the formation of pistillate rather than perfect flowers.

Bioassays of seedling diffusates and root exudates indicated higher GA activity associated with a monoecious cucumber breeding line than was found in its isogenic gynoecious counterpart (Atsmon, Lang and Light, 1968). IAA/GA balance in differentiating cells of bisexual floral buds was presumed to be responsible for triggering the further development of stamens (at high GA levels) or pistils (at high IAA levels). Variation in endogenous GA levels within sex types thought to result from differences in synthetic rates and/or from interconversion of active GAs to inactive or conjugated forms. Paper chromatographic techniques used during sample preparation suggested a predominance of GA_1 or GA_3 in these breeding lines.

Hayashi et al. (1971) quantified free and bound GA contents in gynoecious and monoecious cucumber breeding lines during a period of 18 days following germination. GA activity in bioassays was always lower in extracts of gynoecious plants. Bound GA contents continued to in-
crease with time in both sex types whereas the level of free GAs peaked in monoeclous plants after six days of growth. Gas-liquid chromatography (GLC) revealed a predominance of GA1 associated with monoeclous phenotypes. The authors suggested GA interconversion as a plausible explanation for differences in endogenous GA forms and those found most effective in altering sex expression by exogenous application (see discussion below).

Hemphill, Baker and Sell (1972) plotted GA activity in three cucumber sex types for an eight week period following germination. Andromonoecious and monoeclous types developed peak activity after one week of growth, declined during the second week of growth and thereafter, remained constant. Gynoecious lines always exhibited lower levels of GA activity than did those producing staminate flowers substantiating results of Hayashi and coworkers. In studies by Friedlander, Atsmon and Galun (1977a) quantitation of GA levels by GLC also substantiated earlier reports based on data from bioassays and suggested endogenous GAs to be promoters of male expression in cucumber.

In contrast to trends in cucumber, Hemphill, Baker and Sell (1972) demonstrated monoeclous and andromonoecious muskmelon cultivars to be deficient in endogenous GA content relative to levels found in hermaphroditic and gynoecious lines. These authors attributed the inconsistency in GA effects associated with the two species to result from differences in genetic mechanisms controlling staminate flower production. Also, Ghosh and Basu (1983a) reported progressive increases in endogenous GA corresponding to increases in female expression of monoe-
cious bitter gourd (Momordica charantia), a commonly cultivated cucurbit of the Indian subcontinent.

The role of endogenous ABA in sex expression of monoecious and gynoecious cucumbers was clarified by Friedlander, Atsmon and Galun, 1977a). By GLC analysis of tissues and exudates, these investigators determined ABA concentrations to be higher in shoot tips of monoecious phenotypes and to decline with increasing plant age in both sex types. Gynoecious heterozygotes (Acr aac) displayed an intermediate level of the hormone. Smaller floral buds of monoecious plants contained ABA at levels nearly double those found in similar buds of gynoecious plants, a trend which dissipated with further floral development. Friedlander and coworkers presumed the exhibition of node number in monoecious cucumbers to be a function of ABA, GA and ethylene concentration with reductions in the former two compounds preceding the onset of the mixed phase. In contrast, Rudich, Halevy and Kedar (1972b) demonstrated higher concentrations of endogenous ABA in leaves of gynoecious plants than in those of monoecious types, a phenomenon reversed by treatments with ethephon (an ethylene releasing compound, see discussion below).

Literature concerning the effect of endogenous cytokinins on cucurbit sex expression is nonexistent. However, Sladky, Holman and Jandova (1980) ascertained cytokinin levels in leaves of monoecious cucumbers to be higher than those associated with gynoecious phenotypes.

Environmental Influence on Endogenous Hormone Levels

Short days and low night temperatures, commonly experienced to favor pistillate flowering (see above discussion), lowered both IAA and
GA contents in stem apices of seven monoecious cucumber cultivars (Saito and Ito, 1963). Node numbers of these varieties were reduced and the total numbers of female flowers were increased under this environmental regime. In contrast, Rudich, Halevy and Kedar (1972b) measured higher levels of IAA and of evolved ethylene from monoecious cucumber grown during short daylengths (female flowering promoted by long days). Recently, Takahashi, Saito and Suge (1983) examined growth habits and endogenous hormone levels in two monoecious cucumber varieties which differed in their response to daylength. Growth rates were optimized, levels of GA were greatest and ethylene evolution was lowest under short day conditions for both cultivars. However, differences between varieties for maximum pistillate flower production with respect to photoperiod indicated a separation of hormonal and photoperiodic effects.

Growth rate and photoperiod may also influence carbohydrate levels in the apical region. Although, the effects of carbohydrate balance on cucurbit sex expression have not been ascertained, Berghoeff and Bruinsma (1980) demonstrated the importance of assimilate levels as affected by photoperiodic conditions for successful development of female buds in *Begonia francois*.

The effects of nutritional status (especially nitrogen) upon sex expression in cucurbits have been frequently noted (see discussion above). Although interactions of plant nutrition and growth substance levels with respect to sex expression have not been clarified, the influence of nutritional balance upon endogenous growth regulator biosynthesis and/or transport has been well documented (Marschner, 1982).
Effects of Endogenous Hormone Transport

Several studies have investigated changes in cucurbit sex expression through translocation of hormones and/or metabolites across a graft union. Hayase (1966) reported the formation of hermaphroditic flowers in flower clusters of androecious cucumber scions when grafted to gynoecious rootstocks of the same species. Grafting monoecious scions on gynoecious stocks reduced node number whereas reciprocal grafts had the opposite effect (Friedlander, Atsmon and Galun, 1977b). A short day monoecious variety grown under non-inductive conditions was most affected by the graft as both floral induction and substantial changes in sex expression occurred. These effects were thought to be the result of an unknown substance(s) presumably originating from the gynoecious stock. Whether induction and changes in floral habits were hormonally related phenomena was not ascertained.

Floral induction and sex expression was studied further using reciprocal intergeneric grafts of *Sicyos angulatus* (a short day feral cucurbit naturalized along Japanese river banks) to various stocks of day neutral cucumber or short day *Luffa cylindrca* (Takahashi, Saito and Suge, 1982). Both cucumber and photoinduced *L. cylindrca* stocks mediated flower induction on *S. angulatus* scions grown under non-inductive conditions indicating free movement of the floral stimulus across the graft union. Also, when photoinduced *S. angulatus* was employed as a stock, markedly reduced node numbers were recorded in cucumber scions suggesting participation of the floral stimulus in sex expression.
The masculinizing effects of monoecious and andromonoecious muskmelon stocks upon gynoeclous scions of the same species have been demonstrated by several authors (Mockaitis and Klivilaan, 1964; Analis, 1971). Grafting of gynoeclous muskmelon scions to andromonoecious Cucurbita pepo also resulted in the development of staminate flowers upon the former.

Genetic Mechanisms and Endogenous Hormones

As stated in the introduction of this section, cellular mechanisms controlling sex expression are virtually unexplored, due in part to inadequacies in analytical technique. Kubicki (1972) viewed sex modification to be the result from the interaction of growth regulants and/or external factors affecting gene expression. Specific gene products affecting sex expression were thought to result from selective induction or repression at discrete loci.

Information concerning this process in cucurbits is virtually unavailable. However, in other studies (Nigam, Varkey and Reuben, 1981), streptovaricin (an inhibitor of m-RNA synthesis) was used to demonstrate the influence of protein synthesis on sex expression in dioecious hemp (Cannabis sativa). At low doses, the inhibition of transcription increased flowering in androecious plants as well as effecting decreased flowering and stimulated production of anther-like structures in female buds on their gynoecious counterparts. At high doses of the inhibitor, converse effects on flowering were observed. Specific mRNAs differentially transcribed with changes in streptovaricin
concentration were presumed to direct formation of specific proteins responsible for flowering and sex expression.

Genetic and hormonal interactions responsible for sex expression have been more fully elucidated in dioecious *Mercurialis annua* than in any other species (Dauphin-Guerin, Teller and Durand, 1980; Champault et al., 1981). The discovery of sex type specific marker proteins has greatly facilitated study of sex expression control; From male and female flower primordia, researchers have isolated three specific isoperoxidases and two specific isoesterases respectively. Exogenous application of IAA, which promotes male flower development on gynoecious plants has been shown to stimulate production of the isoperoxidases whereas applications of 6-benzylaminopurine (6-BA, a synthetic cytokinin) on androecious plants induced the formation of female flowers and the biosynthesis of the isoesterases.

Detailed analysis of endogenous cytokinins in *M. annua* apices revealed compositional and quantitative differences between sex types with free trans-zeatin (10⁶ Ade) predominating in and occurring only in gynoecious plants. This cytokinin was considered the major feminizing hormone. Male plant apices exhibited high levels of 10⁶ ribotide. Differences in cellular concentration of hydrolases (cleaving ribosyl and phosphatide groups from 10⁶ ribotide) and phosphokinases (forming 10⁶ ribotide from 10⁶ Ado) between sex types were noticed, with the former being most prevalent in female plants. The formation of these enzymes which regulate the intracellular level of 10⁶ free base and ribotide were shown to be controlled by two complementary loci with the
accumulation of free base in gynoeclous plants being mediated by the presence of recessive alleles.

Studies were also performed to clarify the hormonal role in gene expression. Kinetic investigations of intracellular cytokinin and auxin binding indicated a higher quantity of cytokinin binding sites in primordia of male plants and greater number of auxin binding sites associated with primordia of female plants. Champault et al. (1981) presumed certain cytokinin binding sites regulating gene expression for specific masculinizing proteins in gynoeclous plants to be auxin dependent. Conversely, certain auxin receptors moderating formation of feminizing proteins in androeclous plants were thought to be free-base cytokinin-dependent. The auxin/cytokinin balance was thought to affect hormone binding and thus differentiation processes.

**Effects of Exogenously Applied Growth Regulants**

Application of chemicals to plants in order to promote or inhibit their flowering has been part of the most popular kind of empirical investigation, properly named 'spray and pray'. . . . There is no obligatory relationship between the effect of externally applied substances and their endogenous levels in plants as there may be no correlation between the endogenous level of one substance and the developmental phenomenon (Bernier, Kinet and Sachs, 1981b).

There are two basic questions about the response of plants having dichlinous (unisexual) flowers to compounds affecting sex expression: (1) Is the change in sex expression realized by suppression of the reproductive members of the floral bud or by a direct promoting activity? (2) Do the growth regulators affect the embryonal floral buds themselves, thus causing them to develop further into either male or female flowers, or do these regulators act at an earlier stage, perhaps indirectly, inhibiting or promoting potential male or female floral buds from incipient development? (Frankel and Galun, 1977).
Of the volume of literature concerning sex expression in plants (and in cucurbits, especially), most pertains to the effects of exogenously applied regulants. While these studies do not specifically enhance our understanding of sex determination in-vivo, the ability of these compounds to modify sexual tendencies indicates potential research topics which may be enlightening and remains of vital interest to the development of hybrid seed stocks (see discussion below). A display of chemical agents formerly used in cucurbit sex expression research can be found in Figure 8. The number of studies reported (well over 50) prohibits individual discussion. However, a summary of effects mediated by exogenously applied growth regulants on sex expression of various cucurbits is offered in Table 6. A discussion of these effects follows below.

Auxins

Of natural compounds considered to be auxins, only IAA and its metabolite, hydroxymethylloxindole (HMO, see Chapter 2 and Figure 1) have been examined as exogenously applied effectors of sex expression (Frankel and Galun, 1977; El-Kholy and Hafez, 1982; Krishnamoorthy and Sandooja, 1982; Ghosh and Basu, 1983a,b). IAA was perhaps the first chemical agent demonstrated to modify sex in cucurbits; it was shown to increase femaleness through promotion of pistillate or hermaphroditic flowering, suppression of staminate flowering or by acceleration of flowering phase shifts. Studies also indicated increased levels of ethylene evolution associated with application of IAA. The results elicited might have been predicted from investigations of endogenous
Figure 8: Exogenously-applied Modifiers of Cucurbit Sex Expression

**Auxins**

A: IAA (indole-3-acetic acid)
B: HydroxyxymethylxIndole
C: NAA (naphthaleneacetic acid)
D: TIBA (2,3,5-trilodobenzoic acid)

**Auxin Antagonists**

E: Chlorflurenol [methyl-2-chloro-9-hydroxy-fluorene (9) carboxylate]
F: Morphactin [ethyl-2-chloro-9-hydroxy-fluorene (9) carboxylate]
G: MH (1,2-dihydro-3,6-pyridazine-dione; maleic hydrazide)
H: SADH (1,1-dimethyl-2-succinyl-hydrazine; B-9; B-995, Alar)

**Ethylene and Ethylene Inducers**

I: Ethylene
J: ACC (1-aminoacyclclopropane-1-carboxylate)
K: Ethephon (2-chloroethylphosphonic acid; CEPHA; Ethrel)

**Ethylene Antagonists**

L: AVG (aminooctethoxyvinylglycine)
M: Silver nitrate
N: MCEB (5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2,1,3-benzothiadiazole)
O: AC 94377 [1-(3-chlorophthalimido)-cyclohexane-carboxamide]
P: AC 99524 [1-(1-cyclohexene-1,2-dicarboximido)-cyclohexanecarboxamide]
Figure 8: Exogenously-applied Modifiers of Cucurbit Sex Expression
Figure 8: (Continued)

**Gibberellins**
- Q: GA$_3$
- R: GA$_4$
- S: GA$_7$
- T: GA$_{13}$

**Gibberellin Antagonists**
- U: CCC (2-chloroethyl-trimethylammonium chloride; cycocel, chlormequat)
- V: AMAB (allyl-trimethylammonium bromide)
- W: Amo-1618 (4-hydroxy-5-isopropyl-2-methylphenyl-trimethylammonium chloride, 1-piperidine carboxylate)

**Cytokinins**
- X: CP (N-3-chlorophenyl-N'-phenylurea)
- Y: 6-BA (6-benzylaminopurine)
- Z: Kinetin (6-furfurylaminopurine)

**Abscisic Acid**
- AA: ABA (abscisic acid)
Figure 8: (Continued)
Figure 8: (Continued)

Steroids
BB: 17-\(\beta\)-Estradiol
CC: Testosterone
DD: Cortisone

Carbon Monoxide, Carbon Dioxide
EE: Carbon monoxide
FF: Carbon dioxide

Potential Modifiers of Cucurbit Sex Expression\(^1\)
GG: Glyphosate (N-phosphonomethylglycine, Roundup)
HH: Cucurbitacin B

\(^1\)See discussion below and information in Chapter 4
Figure 8: (Continued)
Table 6: Alteration of Cucurbit Growth and Sex Expression by Exogenous Application of Growth Regulants

<table>
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<tr>
<th>Compound Class</th>
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Table 6: (Continued)

1. Compound abbreviations are identical to those in Figure 8

2. Sex type abbreviations are as follows:
   - Mon = monoeocious
   - Gyn = gynoeocious
   - Her = hermaphroditic
   - Ann = andromonoecious
   - Pen = predominantly pistillate
   - Gyn = gynoeocious

3. References:
   - Anels, 1971
   - Arora, Pandite and Sihlu, 1982
   - Atsmon, 1968
   - Atsmon and Tabbak, 1979
   - Augustine, Baker and Sell, 1973
   - Beyer, 1976a
   - Bose and Nitsch, 1970
   - Breyer, 1976b
   - Brei and Warren, 1960
   - Byers, Baker, Sell, Heren and Dilley, 1972
   - Cantille and Phetek, 1974
   - Cantille and Robinson, 1971
   - Christopher and Loy, 1962
   - Clark and Kenny, 1969
   - El-Kholy and Hafez, 1962
   - Frieclander, Atsmon and Gelun, 1977c
   - Fuchs, Atsmon and Helevy, 1977
   - Gelun, 1956a,b
   - Gelun, 1961
   - George, 1971
   - Ghosh and Basu, 1962
   - Ghosh and Basu, 1983a
   - Ghosh and Basu, 1983b
   - Globerson and Degen, 1973
   - Grewlenowski, Cheaney and Harch, 1971
   - Helevy and Rudich, 1967
   - Hopping and Hawthorne, 1979
   - Hunsperger, Heisel and Baker, 1983
   - Iwahori, Lyons and Selto, 1970
   - Kerch, 1970
   - Kerch and Govers, 1972
   - Ken et al., 1962
   - Kopcewicz, 1971
   - Krishnamoorthy and Sendoja, 1980
   - Krishnamoorthy and Sendoja, 1982
   - Kubicki, 1970a
   - Leibach and Kribben, 1981 (Frankel and Gelun, 1977)
   - Lipper et al., 1972
   - Loy, 1971
   - Loy, Nettl, Zech and Frills, 1979
   - Mc Murray and Miller, 1969
   - Minina, 1938 (Frankel and Gelun, 1977)
   - Mitchell and Wittner, 1962
   - Nijs and Vissers, 1979
   - Owens, Peterson and Tolle, 1980
   - Owens, Tolle and Peterson, 1980
   - Peterson and Anhder, 1966
   - Phetek and Cantille, 1976
   - Pike and Peterson, 1969
   - Reh, 1952
   - Rodriguez and Lambeth, 1972
   - Rudich and Helevy, 1974
   - Rudich, Helevy and Keder, 1969
   - Rudich, Helevy and Keder, 1972b
   - Shannon, 1976
   - Shannon and de la Guardia, 1969
   - Sherman, Jyrilshi and Agramel, 1980
   - Shibutsuna and Jones, 1972
   - Splittoemser, 1970
   - Takehashi, Salto and Suge, 1982
   - Takehashi and Suga, 1980
   - Takehashi and Suga, 1982
   - Takehashi, Suge and Salto, 1980
   - Tolle and Peterson, 1979
   - Tronickova, 1976
   - Xu and Bukovka, 1983a,b
hormone levels and from studies of ethylene metabolism (see discussion above). An exception to the above trends was demonstrated in *Cucurbita pepo*, where IAA treatment effected a slight increase in sex ratio.

Studies with other species yielded conflicting results. In hemp and spinach, application of IAA to the root medium induced the formation of pistillate flowers on hermaphroditic plants and skewed the expected ratio of sex types in segregating populations, greatly increasing the number of plants which were phenotypically gynoecious (Challakhyan and Khryanin, 1979). Also, IAA applied to corn retarded tassel development (Khryanin and Challakhyan, 1978). However, exogenous treatments of IAA failed to alter sex expression in androecious forms of *Melandrium* spp. (Heslop-Harrison, 1972) or grape (*Vitis vinifera*) (Negi and Olmo, 1966).

Recent evidence for the feminizing effects of HMO applied to monoecious forms of cucumber and bitter gourd have been proffered by Ghosh and Basu (1983a,b). This IAA metabolite had been suggested earlier to exhibit auxin-like qualities (Moyed and Tull, 1968).

Synthetic auxins such as naphthaleneacetic acid (NAA) and 2,3,5-triliodobenzolic acid (TIBA) have been demonstrated to produce similar but more pronounced feminizing effects upon cucurbits than natural compounds in this class (Rehm, 1952; Galun, 1959a,b; Brantley and Warren, 1960; Kubickl, 1956b; Shannon and de la Guardia, 1969; Bose and Nitsch, 1970; Kubickl, 1970a; Friedlander, Atsmon and Galun, 1977c; Sharma, Jyotishi and Agrawal, 1980; El-Kholy and Hafez, 1982; Khan et al., 1982; Krishnamoorthy and Sandooja, 1982). In contrast, Freytag, Lira and Isleib (1970) noted increased masculinity in monoecious cucumbers upon
application of TIBA. These authors attributed this anomaly to peculiarities in background genotype.

Galun (1959a, b) evinced reduced growth rates in conjunction with increased female flowering in plants treated with NAA. The effects of NAA treatments were stimulated by removal of leaves and were reversed by simultaneous application of GA3. Flowering and growth of muskmelons generally were enhanced under long day photoperiods (Brantley and Warren, 1960). Under these conditions, NAA decreased the ratio of staminate to perfect flowers. Under short day conditions, higher concentrations of NAA 'caused delays in floral development', indicating correlative influences of growth rate and the flowering process. Monoeccious strains of Cucurbita pepo responded to NAA treatment with increased feminization (Krishnamoorthy and Sandooja, 1982) whereas similar treatments of androecious types failed to induce pistillate flowering (Kubicki, 1970a).

Similar to results of IAA treatments, both NAA and TIBA applications had no effect on grapes (Negl and Olmo, 1966).

Auxin Antagonists

Antagonists were thought to alter endogenous hormone levels through control of their synthesis, degradation, metabolism, transport and/or compartmentalization or to compete for hormone receptor sites. Chlorflurenol [methyl-2-chloro-9-hydroxy-fluorene (9) carboxylate] and morphactin [ethyl-2-chloro-9-hydroxyfluorene (9) carboxylate] were shown to mediate their effects on plant development through inhibition of IAA transport (Beyer and Quebedeaux, 1973). At low doses (below 10 ppm)
these compounds increased the development of staminate flowers in monoecious and gynoecious cucumbers (Cantliffe, 1974) and in monoecious Luffa acutangula (Bose and Nitsch, 1970). The masculinizing effect of morphactin treatment in Luffa was said to be "very powerful and long lasting". However, higher doses in cucumber severely reduced growth rates, leaf development and masculinity. Reduced pistillate flowering at high application rates was presumed to result from depressed plant growth.

Practical use of these compounds was demonstrated through their ability to enhance parthenocarpic fruit development, increasing yields and improving fruit quality in production fields planted at high densities (Cantliffe, Robinson and Shannon, 1972). Normally elevated levels of IAA in ovarian tissue prior to anthesis and during fruit set (see Chapter 2) were found to be enhanced through lack of auxin transport (Cantliffe, 1974). Similar effects were uncovered in a study of male sterility and parthenocarpy in tomato (Jain and Mukherjee, 1980). In addition, chlorflurenol has been found to interrupt ovule development in muskmelons of various sex types, insuring that fruit developed would be parthenocarpic in origin (Snyder, Carter and Knavel, 1983).

Although SADH (B-9, B-995, Alar, 1,1-dimethyl-2-succinyl hydrazine) and MH (maleic hydrazide, 1,2-dihydro-3,6-pyridazine-dione) are known auxin antagonists, they were demonstrated to promote femaleness in most trials reported (Hillyer and Witwer, 1959; Halevy and Rudich, 1967; Bose and Nitsch, 1970; Iwahori, Lyons and Smith, 1970; Loy, 1971; Rodriguez and Lambeth, 1972; Rudich, Halevy and Kedar, 1972c; El-Kholy and Hafez, 1982; Ghosh and Basu, 1982). These effects were accompanied by reduced plant growth (Halevy and Rudich, 1967) and were enhanced by
concurrent applications of ethephon (an ethylene releasing compound, see discussion below) (Loy, 1971; Rodrigues and Lambeth, 1972).

SADH was thought by several researchers to antagonize auxin action by reducing IAA synthesis or by increasing its destruction and to suppress ethylene evolution. However, the effects of this inhibitor upon cucurbit sex expression closely paralleled results obtained by treatment with auxins. This apparent anomaly was clarified in part by results of Rudich, Halevy and Kedar (1972c). These authors demonstrated reduced endogenous GA levels in andromonoecious muskmelons treated with SADH and attributed its effects on sex expression in this species to an inhibition of GA synthesis (see discussion below). SADH was shown to inhibit action of kaurene synthetase in pea root tips and thus, curtail GA production (Wylie, Ryugo and Sachs, 1970). In addition, several reports indicated high GA levels to 'spare' auxin activity, presumably through inhibition of IAA-degrading enzymes (IAA oxidase and peroxidases, see Chapter 2) (Halevy, 1963). Consequently, reduced endogenous levels of growth promoters may have accounted for sex alterations and reduced growth rates associated with SADH treatments.

Recently, Lee (1982) elucidated evidence of IAA depletion of tobacco tissues treated with dilute concentrations of glyphosate (Roundup, N-phosphonomethylglycine) through increased formation of IAA conjugates and/or accelerated IAA degradation. This regulant, widely used as a broad spectrum herbicide has not as yet been examined for its potential as a sex modifying agent in cucurbits (see Chapter 4). However, Scorza, Welker and Dunn (1984) have evinced the anti-auxin
characteristics of this regulant through increased shoot proliferation reduced root growth in cranberry (Vaccinium macrocarpon) node explants.

Ethylene and Ethylene Inducers

An ethylene-enriched atmosphere lowered node number and enhanced femaleness in monoecious cucumber (Iwahori, Lyons and Smith, 1970) as did application of its biosynthetic precursor, ACC (see Chapter 2) to cucumbers of various sex types (Takahashi and Suge, 1982). In the latter study, ethylene evolution from ACC-treated tissues was found to increase, an effect substantiated during investigations of similarly treated leaf tissue disks (Cameron et al., 1979).

Perhaps ethephon (ethrel, CEPHA, 2-chloroethylphosphonic acid) has been included in more trials than any other growth regulant. This compound is unique in that it degrades upon topical application, releasing ethylene, a natural plant hormone. However, the effects of ethephon upon cucurbit growth and sex expression have been shown to far surpass those of its natural counterpart (Iwahori, Lyons and Smith, 1970). Ethephon has been demonstrated to be a feminizing agent which reduced both sex ratio and node number in monoecious cucumber, muskmelon, Cucurbita pepo, C. maxima, C. moschata and Lagenaria siceraria (Table 6). Similar feminizing effects have been observed in individuals of other sex types and in some instances, flowers atypical to specific phenotypes have been induced (Augustine, Baker and Sell, 1973; Karchi, 1970). Ethephon-treated monoecious and andromonoecious cucumbers manifested sex patterns of plants grown under winter conditions (short day-length, low temperatures) with increased female expression and fewer
bare (blind) nodes or abortive buds (Dax-Fuchs, Atsmon and Halevy, 1978). In addition to increased pistillate flowering, Karchl (1970) also noted ethephon-released ethylene to repress anther development in buds of andromonoecious and hermaphroditic muskmelon cultivars. Direct application of ethephon to developing buds of all types caused them to abort (Karchl and Govers, 1972). The extent of ethephon-induced sex alteration in cucumber and squash cultivars has been shown to be affected by background genotypes (George, 1971; Shannon and Robinson, 1979). In addition to changes in sex expression, ethephon-treated individuals often exhibited reduced growth, shortened internodes or at higher doses, epinasty and chlorosis (Splittstoesser, 1970; Karchl and Govers, 1972).

Treatment of monoecious watermelon with ethephon resulted in increased sex ratios (especially at high concentrations) which constituted a notable exception to the feminizing effects associated with ethephon application in other species (Christopher and Loy, 1982). At low concentrations, increased maleness was realized through promotion of staminate floral development at nodes formerly destined to support pistillate flowering. Shimostuma and Jones (1972) reported sex expression in this species to be unaffected by treatment with ethephon under any daylength regime.

The synergistic or antagonistic effects of this compound applied in combination with other growth regulators suggested possible modes of ethephon action on cucurbit sex expression. Freytag, Lira and Isleib (1970) noted ethephon's ability to override the masculinizing action of TIBA when the two compounds were applied simultaneously. These authors suggested the relative importance of ethylene-controlled biological
sites affecting sex expression. Robinson, Shannon and de la Guardia (1969) proposed ethylene to act as an anti-gibberellin as it antagonized production of effects normally associated with GA application in cucumber. Rudich, Halevy and Kedar (1969) supported this contention with results from ethylene treatment in a number of species. Ethephon was shown to effect manifestations similar to those of SADH (an auxin antagonist, ethylene suppressant and GA synthesis inhibitor) on growth and sex expression in muskmelon (Loy, 1971) and cucumber (Rodriques and Lambeth, 1972). Surprisingly, however, effects of ethephon treatment were more pronounced and long lasting than those of SADH. In cucumber, Iwahori, Lyons and Smith, (1970) reported failure of exogenous GA applications to overcome the effects of ethephon treatments. Additionally, Rodriguez and Lambeth (1972) discussed evidence for the synergistic masculinizing action of combined GA/SADH or GA/ethephon applications.

Effects of ethephon-induced ethylene upon cellular metabolism offered some clarification of possible mechanisms for its control of growth and floral differentiation. Ethephon treatment on monoecious and gynoecious cucumbers effected reductions in endogenous IAA and GA levels, but was found to increase levels of ABA (Rudich, Halevy and Kedar, 1972b), a pattern substantiated in other species. These researchers suggested a high inhibitor/promoter ratio (a condition directly influenced by ethephon action) to be a major cause of female expression in cucurbits (see discussion below). Support for this hypothesis was advanced by Shannon (1976), who demonstrated enhanced levels of IAA oxidase and peroxidases in tissues after treatment with ethephon. Thus, synergistic effects of ethephon and other growth retardants such as
SADH, may have resulted from mutual influences on levels of growth promoting substances.

Ethephon has also been shown to be a potent feminizing agent when applied to other species. For instance, Mohan Ram and Jalswal (1974) reported the induction of pistillate flowers on androecious hemp plants in proportion to ethephon dosages administered. These results suggest ethylene to be a natural regulant of hemp sex expression.

Ethylene Antagonists

The inhibition of ethylene synthesis by rhizobitoxine, a phytotoxin produced by certain strains of *Rhizobium japonicum*, (soybean root nodulation bacterium) was demonstrated to result from blockage in the conversion of methionine to ethylene (Owens, Lieberman and Kunishi, 1971). It and its ethoxy analog, AVG (aminoethoxyvinylglycine), were later shown to inhibit the formation of ACC from S-adenosylmethionine through obstruction of pyridoxyl phosphate, an enzymatic cofactor (see Chapter 2) (Sembdner et al., 1980).

Among other horticultural uses, AVG has been indicated as a potent masculinizing agent in cucumbers (Atsmon and Tabbak, 1979; Owens, Tolla and Peterson, 1980) and muskmelon (Loy et al., 1979; Owens, Peterson and Tolla, 1980). As might have been expected, ethylene evolution was drastically reduced in gynoecious cucumber treated with AVG. Staminate flowers, hermaphroditic flowers and/or flower clusters were induced at normally pistillate nodes. Strength of treatments were dependent on timing and dosage but appeared to be independent of background genotype. AVG treatment enhanced hermaphroditic flower develop-
ment in gynoanemoecious muskmelon without influencing pistillate flowering rates. Also, their formation on gynoecious types was induced by this treatment. In contrast, Christopher and Loy (1982) found AVG treatments on monoecious watermelon to cause transformation of normally unisexual flowers to hermaphroditic flowers. At low AVG doses, the staminate/pistillate ratios on control plants were not statistically different from the staminate/hermaphroditic ratios of treated individuals whereas high doses effected the conversion to bisexuality in both unisexual types. All studies uncovered reduced growth rates and mild chlorosis associated with higher doses of this regulant.

Dissolved silver salts such as silver nitrate and silver thiosulfate have been shown to alter sex expression of cucumber, muskmelon and watermelon in a manner similar to that of AVG (Beyer, 1976a; Atsmon and Tabbak, 1979; Nijs and Visser, 1979; Tolla and Peterson, 1979; Owens, Peterson and Tolla, 1980; Takahashi and Suge, 1980; Hunsperger, Helsel and Baker, 1983). Beyer (1976a) has shown silver nitrate to protect treated plants against ethylene-induced growth inhibition. Unlike AVG, which inhibits ethylene synthesis, Ag⁺ ions have been suspected of interfering with ethylene oxidation at the binding site and consequently to block action of the endogenous hormone (Beyer, 1976b; Kende et al., 1982). According to Atsmon and Tabbak (1979):

This latter finding fits well with the increase in ethylene evolution which we [the authors] found in silver nitrate-treated plants, while the blocking of biosynthesis [by AVG] also caused the expected suppression of ethylene evolution in our experiments. Thus, these two compounds lead to the same basic situation -- the action of endogenous ethylene on the plant tissue is very much reduced. . . . The possible difference in the metabolism of these two compounds may be responsible for some quantitative differences in their morphogenetic effects. . .
Silver nitrate proved to be a more potent masculinizing agent than GA$_{4+7}$ (see discussion below) in gynoecious cucumber (Tolla and Peterson, 1979) and to be more effective than either GA or AVG in gynoecious muskmelon (Owens, Peterson and Tolla, 1980). In the former study, perpetuation of gynoecious breeding lines with highly effective staminate flower-producing silver compounds was thought to drastically reduce possibility of shifts in background genotype toward partially pistillate types. Nijs and Visser (1979) described phytotoxic effects associated with high doses of silver nitrate and silver thiosulfate.

Silver nitrate influenced sex expression of monoecious watermelon in a manner similar to AVG action (i.e. promotion of hermaphroditic flowering and reduced production of staminate buds) (Christopher and Loy, 1982).

Use of both AVG and Ag$^+$ ions has proven to be effective methodology for increasing maleness in other species. Mohan Ram and Sett (1980) effected induction of staminate flowers on gynoecious lines of castor bean with silver nitrate, a technique thought useful for line maintenance, breeding and improvement. Cobalt chloride (Co$^{++}$) was also found to be effective. In later studies, these authors (1982a,b) advanced evidence for staminate flower induction in gynoecious segregates of hemp using silver nitrate, silver thiosulfate and AVG. In androecious types, AVG treatments countermanded effects of pre-applied ethephon.

MCEB (5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2,1,3-benzothiadiazole), an additional synthetic regulant inhibiting ethylene action, was applied to gynoecious sex types of both cucumber (Augustine,
Baker and Sell, 1973) and muskmelon (Byers et al., 1972b), effecting the induction of staminate and hermaphroditic floral buds respectively. However, research with this compound has been terminated due to suspension of its manufacture (Owens, Peterson and Tolla, 1980).

Phthalimides, a recently synthesized group of compounds, have been found to confer effects on plants similar to those associated with exogenous GA treatments. Some interactions of these regulatory compounds and developmental processes have been reviewed by Los et al. (1980) and by Suttle and Schreiner (1982). Phthalimides have been reported to: exhibit auxin or gibberellin-like activity in appropriate bioassays, promote growth in several species, improve tomato and potato yields, cause early bolting and flowering in lettuce, increase axillary bud development in woody ornamentals and increase protein content of corn seedlings. However, this growth promoter failed to demonstrate any cytokinin-like attributes.

Two phthalimides, AC-94377 [1-(3-chlorophthalimido)-cyclohexanecarboxamide] and AC-99524 [1-(1-cyclohexene-1,2-dicarboxylimido)-cyclohexanecarboxamide] were suspected to alter cucumber sex expression by acting as anti-ethylene agents (Xu and Bukovac, 1983a,b). The former compound was most active in gynoeclous strains, effecting induction of staminate flowers whereas the latter compound most substantially increased node number traits of monoecious lines. Interactions of compound effectiveness and seasonal variation were noted and regarded as complex.

In monoecious cucumber, AC-99524 overcame the normal action of ethephon (Xu and Bukovac, 1983b). However, like ethephon, it also sup-
pressed the formation of all flowers on early nodes. The phthalimide response may have been influenced by endogenous GA levels but evidence for this interaction was not uncovered in these studies.

**Gibberellins**

Exogenous application of naturally-occurring hormones was first shown to effect cucumber sex expression with the auxin studies of Laibach and Kribben. Almost a decade later, exogenously applied GA$_3$ was demonstrated to influence cucumber sex expression by Wittwer and Bukovak (Frankel and Galun, 1977). The first in-depth study of GA$_3$ effects was summarized by Galun (1959b). In monoecious types, GA treatments increased node number and increased the total number of nodes bearing staminate flowers. These phenomena appeared to be influenced by dosage and were counteracted by concurrent application of NAA. Partially pistillate phenotypes treated with GA$_3$ exhibited increases in node number. GA$_3$ treatments also altered plant growth. The first few internodes (1-5) were elongated by treatment whereas subsequent internodes appeared to be reduced in size. These growth effects were compounded by repeated application of this regulant. Peterson and Anhder (1960) demonstrated exogenous applications of GA$_3$ to be useful in propagation of gynoecious breeding lines. Dosages of 1500-2000 ppm gave best results with repeated treatments increasing the percentage of plants induced and the percentage of nodes bearing staminate flowers. The authors uncovered variability in response to GA$_3$ and suggested possibilities for future genetic and biochemical investigations of these variants, perhaps clarifying the role of GAs in cucurbit sex expression.
addition to the masculinizing effects of GA$_3$, Mitchell and Wittwer (1962) and Kubicki (1965b) proffered evidence for its inhibitory influence on pistillate flower development.

A mixture of GA$_{4+7}$ was also shown to be a potent masculinizing agent in gynoecious and monoecious cucumbers (Atsmon, 1968). However, in this study, pistillate flower inhibition was noted only in gynoecious types. GA treatment reduced genetically controlled differences in growth rate, branching habit and internode length between sex types. Staminate flower induction potential of GA$_{4+7}$ mixtures proved to surpass those of GA$_3$ with the former treatment exhibiting desired results at one-tenth the optimum concentration of the latter (Pike and Peterson, 1969). This trend was confirmed by Clark and Kenney (1969), who also demonstrated the comparative inactivity of GA$_{13}$ (a C-20 GA lacking lactone functionality) in altering sex expression in this species.

Hayashi et al. (1971) discussed possibilities for in-vivo interconversion of various exogenously applied GAs to forms ultimately responsible for expression of masculinity (perhaps GA$_1$, see both Figure 5 and discussion above concerning endogenous GAs in monoecious cucumbers). Atsmon and Tabbak (1979) found the effects of GA$_{4+7}$ on monoecious cucumber to be manifested more quickly and to be longer in duration than those of AVG or silver nitrate.

The consequences of GA action on individual cucumber buds has been studied in detail (Galun, 1961b; Fuchs, Atsmon and Halevy, 1977; Dax-Fuchs, Atsmon and Halevy, 1978). Using GA treatments on monoecious cucumber lines, Galun (1961b) determined the time frame between physiological bisexuality of buds (i.e. buds not yet physiologically commit-
ment to staminate or pistillate development) and the loss of morphological bisexuality (i.e., buds exhibiting unisexual characteristics) to be approximately one week. GA treatments applied more than one week prior to staminodila degradation presented the possibility for transformation of the bud from female to male sex expression. Time estimates varied among genotypes and with plant ontogeny. Close scrutiny of bud sex 'conversion kinetics' in gynoeclous sex types treated with GA$_{4+7}$ led to somewhat different conclusions about the plasticity of morphologically bisexual buds (Fuchs, Atsmon and Halevy, 1977). As noted above, treatments inhibited the development of pistillate buds and ultimately resulted in their abortion, sometimes before buds became visible. Treatments also induced formation of adventitious staminate buds next to the aborting pistillate tissues. The ontogeny of these adventitious buds was accelerated by treatment which caused significant development at three days after induction, a time period far shorter than that reported to be necessary for conversion of a morphologically bisexual bud. Treatment effects were reduced with application at higher node numbers and were proportional to distance (number of nodes) from treatment site. In untreated plants, dormant staminate initials were thought to be maintained through a lack of proper hormone stimulus.

Dax-Fuchs, Atsmon and Halevy (1978) discussed the influence of environment, endogenous hormone levels and plant ontogeny upon the development of GA-treated buds. GA treatment precipitated male bud abortion in monoecious plants under summer conditions, presumably due to supraoptimal GA accumulation. This 'supraoptimal hormone concentration'
phenomenon seemed to extend also to increased abortion of ethephon-treated pistillate buds on plants grown under winter conditions.

Although GA treatments increased masculinity in cucumbers and squashes under most conditions (Table 6), their effects on sex expression in other cucurbits were varied. Anais (1971) noted pistillate bud suppression following GA application to monoecious and hermaphroditic varieties of muskmelon, but was unable to obtain staminate flower induction on gynoecious types by similar treatment. These results were duplicated by Owens, Peterson and Tolla (1980), who uncovered no statistical differences between GA-treated gynoecious muskmelons and controls. In contrast to earlier studies, EI-Kholy and Hafez (1982) found GA treatments to reduce the sex ratio in monoecious muskmelon. Feminizing effects of GA applications were also reported in investigations with Luffa acutangula and Momordica charantia with the latter species exhibiting its highest level of endogenous GA during the onset of the female flowering phase (Bose and Nitsch, 1970; Ghosh and Basu, 1983a).

The mechanism of GA action on sex expression in cucurbits is still under investigation. As the effects of GA and ethephon seemed to be antithetical, original theories suggested GA suppression of ethylene synthesis as a possible mode of sex regulation. However, evidence conflicting with this early hypothesis gradually accumulated, including: reported lack of interaction in ethephon and GA treatments (Iwahori, Lyons and Smith, 1970) and/or the synergistic action of these two compounds on masculinization of cucumber (Rodriques and Lambeth, 1972); the apparent lack of GA influence on ethylene evolution rates in-vivo; and the differences found in the action of GA and anti-ethylene agents.
on pistillate buds (Atsmon and Tabbak, 1979). Iwahori, Lyons and Smith (1970) concluded that GA and ethephon have different sites of action. In addition, Atsmon and Tabbak (1979) suggested GA to influence staminate flower production by an unknown mechanism which differed from that stimulated by ethephon. Conversely, the feminizing influence of GA treatments on Momordica charantia sex expression could not be attributed to altered ethylene evolution (Ghosh and Basu, 1983a).

The effects of GA applications on sex expression in other common crops were also varied. After treatment with GA₃, segregating populations of both spinach and hemp exhibited more androecious phenotypes than similar control groups (Challakhyan and Khryanln, 1978; Challakhyan, 1979) and application of GA₄+7, GA₇ and GA₉ induced staminate buds on gynoecious hemp plants (Mohan Ram and Jaiswal, 1972, Mohan Ram and Jaiswal, 1974). In the latter studies, GA promotion of staminate buds was nullified by concurrent application of ABA or ethephon. These authors suggested sex expression in hemp to be regulated by cellular levels of GA and ethylene in differentiating tissues. GA₃ applied to gynoecious asparagus (Asparagus officinalis) produced sterile staminate structures with collapsed anther walls (Lazarte and Garrison, 1980). In contrast, GA applied to castor beans markedly increased femaleness delayed flowering and modified growth habits (Shifriss, 1961b). Khryanin and Challakhyan (1980) found GA to accelerate the tasselling process in corn whereas Hansen, Bellman and Sacher (1976) outlined procedures for GA-induced tassel sterility for use in various breeding schemes. Male sterility in sunflower (Helianthus annuus) was also facilitated by GA application (Miller and Fick, 1978) and in con-
Gibberellin Antagonists

GA antagonists and their proposed modes of action were reviewed by Halevy (1963), Harada and Lang (1965), Wylie Ryugo and Sachs (1970) and Corcoran (1975). A commonly employed GA antagonist, CCC (cycocel, chloromequat, 2-chloroethyl-trimethyl-ammonium chloride) inhibits GA formation through suppression of kaurene synthetase, an enzyme complex responsible for cyclization of GGPP and formation of ent-kaurene. In addition, this compound enhances the action of IAA-degrading enzymes.

The effects of CCC on quantitative sex expression in monoecious cucurbits could be predicted (for the most part) from results obtained from exogenous application of GAs. Reduction in node number and sex ratio were reported as consequences of CCC treatment in cucumbers and melons (Atsmon, Lang and Light, 1968; El-Kholy and Hafez, 1982). However, Iwahori, Lyons and Smith (1970) failed to note changes in sex expression in monoecious cucumbers following CCC application. Results of CCC treatment on Luffa acutangula and Momordica charantia (species which show increased female expression as the result of added GA) were mixed, with the latter species exhibiting increased masculinization and the former displaying increased feminization (Bose and Nitsch, 1970; Ghosh and Basu, 1982). The surprisingly similar effects of added GA and CCC (its synthesis inhibitor) in sex expression of Luffa might have been mediated through CCC action on other cellular systems. Wylie, Ryugo and Sachs (1970) and Corcoran (1975) both cautioned about assuming only one
cellular function for plant growth inhibitors as their action was apt to be biochemically complex.

A derivative of CCC (AMAB, allyl-trimethyl-ammonium bromide) was also shown to effect increased pistillate flowering in monoecious cucumber (Mitchell and Wittwer, 1962). This compound also reduced internode length and its influence on both sex expression and growth in this species was proportional to the dosage applied.


Cytokinins

The effects of exogenously applied cytokinins on sex expression in cucurbits have been examined in relatively few studies and in all investigations of this type, synthetic cytokinins were employed. Treatment with either 6-BA (6-benzylaminopurine), kinetin, or CP (N-3-chlorophenyl-N'-phenylurea) has been reported to result predominantly in increased female expression (Bose and Nitsch, 1970; Krishnamoorthy and Bhatia, 1976; Takahashi, Suge and Salto, 1980; El-Kholy and Hafez, 1982). Direct application of 6-BA to staminate inflorescences of Luffa cylindrica caused the formation of pistillate flowers at the floral apex, hermaphroditic flowers adjacent to the apex and a proliferation of shoot growth presumably due to decreased apical dominance. Application
to the stem at the second true leaf stage suppressed the formation of all flower types, a phenomenon also noted in *Luffa acutangula* as a result of applied cytokinins (Krishnamoorthy and Bhatia, 1976). Christopher and Loy (1982) reported 6-BA to be ineffective in altering sex of monoecious watermelon.

Sex modification through cytokinin application was first studied in grapes (Negl and Olmo, 1966). In this pioneer investigation, formation of hermaphroditic flowers on androecious plants was noted following application of PBA [6-benzyamino-9-(tetrahydro-2-pyryl)-purine], a synthetic cytokinin. Pollination of these flowers resulted in normal seed development. Moore (1970) obtained similar results in androecious grapes with treatments of 6-BA. He hypothesized male and female inflorescence development in grapes to be regulated by the ratio of two endogenous inhibitors. Hermaphroditic flower induction by cytokinin was thought to result from the compound's ability to suppress the action of both endogenous inhibitors simultaneously. Negl and Olmo (1972) examined the effects of PBA on embryological and biochemical aspects of sex conversion in grape. Application of this cytokinin enhanced meiotic activity in megaspore mother cells and mitotic divisions in pistillate tissue. Protein synthesis was also shown to be increased. In addition, the dominant gene *Su* was thought to curtail endogenous production of cytokinins in developing floral meristems of androecious grapes, a condition rectified by exogenous cytokinin application. Naturally-occurring cytokinins, especially zeatin and dihydrozeatin, were implicated as regulators of sex expression in a publication by Hashizume and Izuka (1971).
Asparagus varieties, homozygous and heterozygous for the androecious condition, responded to treatments of PBA (Lazarte and Garrison, 1980). In heterozygotes, treatment effected the formation of hermaphroditic flowers which developed into parthenocarpic fruit; in homozygotes, pistillate structures appeared, but were devoid of embryo sacs. Cytokinins also promoted feminine expression in corn (Khryanin and Challakhyan, 1978), hemp (Challakhyan and Khryanin, 1978; Challakhyan, 1979), and spinach (Challakhyan, 1979).

Abscisic Acid

Rudich and coworkers (Rudich, Halevy and Kedar, 1972b; Rudich and Halevy, 1974) were first to monitor the effects of exogenous ABA applications on the sexual development of cucumbers. These studies treatments resulted in marked increases in feminization of gynoecious plants through reduced node number and increased numbers of pistillate flowers. However, ABA treatments applied to monoecious plants appeared to have no effect on sex expression. Similar results with monoecious lines of strong male tendency were obtained earlier by Iwahori, Lyons and Smith (1970). The authors of the latter studies proposed sex control in cucumber to be moderated by ethylene, auxin, GA and ABA and commented on the probable importance of promoter/inhibitor ratios in the vicinity of the differentiating bud. They also conceded that evidence for direct interaction of ABA and other hormones (i.e. the level of one hormone affecting the concentration of another) was still unavailable.

Friedlander, Atsmon and Galun (1977c) substantiated earlier claims of increased feminization of gynoecious cucumbers by exogenous
ABA applications. In addition, this study uncovered the converse effect (increased masculinization) of ABA on monoecious types. The divergence in results prompted this research group to consider ABA as an indirect effector of cucumber sex. Perhaps more importantly, this study revealed ABA to stimulate post-differentiation female floral development in gynoecious lines and to inhibit male-differentiated flower development in their monoecious counterparts. The dual influence of hormone concentration (i.e. affecting both bud differentiation and subsequent floral development) and its importance in sex expression was described. Precise hormone balance at both the time of sexual differentiation and thereafter during bud maturation was said to be upset through exogenous compound application. Supraoptimal concentrations of various hormones were thought to result in bud abortion after differentiation process had occurred (Dax-Fuchs, Atsmon and Halevy, 1978) and thus, substantially affect sex expression.

Influence of exogenous ABA applications upon sex expression in other species has been investigated. Christopher and Loy (1982) found this compound to have no effect on the flowering habits of monoecious watermelon whereas Challakhyan (1979) reported an increase of phenotypically gynoecious spinach plants in a treated segregating population. Challakhyan and Khryanin (1978) disclosed a slight femininizing effect of ABA in segregating hemp populations but Mohan Ram and Jalswal (1972) found no effects associated with treated gynoecious plants. However, the latter authors described the mutual antagonism of simultaneously applied GA and ABA treatments in magnitudes relative to the ratio of promoter/inhibitor concentrations.
Steroids

The Importance of steroids in orchestrating nearly every facet of animal growth and development is without challenge. Endogenous plant steroids (some of which are identical to compounds derived from animal tissues) are also reputed to be ubiquitous and their isolation and use as pharmacological agents predate modern medicine. However, the role of endogenous plant steroids in plant growth and development is less understood than that of their animal counterparts. Geuns (1978) proffered an exhaustive review of endogenous steroid action in plant species as well as outlining results obtained from studies of exogenously applied plant and animal steroids.

The effects of exogenously applied steroids on sex expression in cucurbits and other species has been examined cursorily. Gawlenowski, Cheney and Marsh (1971) evinced a reduction in node number and an increase in female flower production in monoecious cucumbers following treatments with either 17-β-estradiol (an estrogen) or testosterone (an androgen). The influence of estrogen application appeared to be stronger and longer in duration than that of the androgen treatment. In monoecious Echballium elaterium, estrogens increased the total number of flowers and reduced the sex ratio whereas androgens effected an increase in the number of staminate flowers (Kopcewicz, 1971). Treatment with cortisone (a corticosteroid) resulted in increased flowering with no subsequent change in sex ratio. Geuns (1978) summarized studies with spinach and Melandrium dioecum indicating steroid-induced masculinization or feminization following treatment with androgens or estrogens respectively. In certain Melandrium types, rudimentary staminate or
pistillate organs which normally degenerated were conditioned to develop and function through androgen or estrogen treatment.

Kopcewicz and coworkers (Kopcewicz, 1971; Geuns, 1978) examined steroid effects upon the endogenous levels of accepted plant hormones and vice versa. Through a number of investigations, this research group determined the following trends: exogenous applications of estrogens increase endogenous levels of both IAA and GAs in plant tissues; ethephon-released ethylene and applied cytokinin increased endogenous estrogen concentrations in tissues of squash and bean respectively; and application of CCC (a GA synthesis inhibitor) effectively lowered endogenous estrogen levels.

A number of steroids not implicated as effectors of sex expression have been demonstrated to alter biological activity of accepted plant hormones under bioassay conditions. Cucurbitacins, a class of bitter steroid compounds predominantly associated with the squash family, have been shown to exhibit anti-gibberellin activity when applied simultaneously to TN-1 rice seedlings (dwarf rice bioassay strain) (Guha and Sen, 1973). GA-Inhibitory responses were dependent upon interaction with applied GA and varied in intensity among individual cucurbitacins and with cucurbitacin dosage.

This compound class has long been studied by medical researchers for its pharmacological properties and by horticulturists for its obvious effects on vegetable quality. Currently, entomologists are suggesting cucurbitacins as phagostimulants for use in controlled pest management schemes. The effect (if any) of endogenous cucurbitacins on cucurbit sex expression remains to be elucidated.
Carbon Dioxide and Carbon Monoxide

Node numbers and sex ratios of monoecious cucumber and muskmelon lines were increased by CO₂-enriched atmospheres whereas masculinization of gynoecious types was manifested through formation of hermaphroditic flowers. Masculinization of cucubits by exogenous CO₂ enrichment seemed anomalous in view of CO₂'s stimulatory effect on ethylene evolution (See Chapter 2). However, Osborne (1982) cited other examples of opposing physiological responses effected by ethylene and CO₂. "In view of the known CO₂ enhancement of ethylene production from green tissues in the light, CO₂ must be seen as a further agent mediating ethylene and other hormonal controls of cell growth in a way that becomes almost infinitely flexible". Carbon dioxide was said to mediate its effects on cucumber and muskmelon sex expression through suppression or competitive inhibition of ethylene action (Eyers, Baker, Sel, Herner and Dilley, 1972; Rudich, Halevy and Kedar, 1972b).

In a landmark experiment performed by Minina, CO (a predominant component in a flue gas mixture) was administered over a 12 hr period to monoecious cucumbers (Frankel and Galun, 1977). The resultant increase in the ratio of female to male flowers perhaps comprised the first evidence of sex modification in angiosperms through the use of chemical agents.
In conclusion, I hope that these various examples have shown that the technique of culture in vitro, by providing a much more precisely defined chemical environment, has great possibilities for plant science in general, especially plant physiology, biochemistry and genetics (Nitsch, 1972).

The advantage of in vitro culture for the study of flowering is the ability to isolate potential flowering sites and to use such tissue to test the effects of various parameters on flowering avoiding the confounding influences of substances produced in vivo by leaves and roots (Scorza, 1982).

Aspects and advantages of in-vitro culture for studies of the flowering process were reviewed by Nitsch (1969, 1972) and Scorza (1982). Nitsch (1967) envisioned that proliferated use of in-vitro techniques might "open the way to an experimental study of the biochemistry of flowering, a study which has struggled for years with the difficulties caused by the size and complexity of intact plants".

In-vitro studies of flowering are commonly initiated using entire plants, meristems or explants devoid of meristems cultured in liquid or solid media. Scorza (1982) and de Fossard (1974) consider the culture of meristems (devoid of interactions with differentiated tissues) to be the ultimate technique for study of the flowering process. However, in practice, cultures of this type almost always include leaf primordial tissue, and possible physiological or hormonal interactions among tissues must be appraised. As physiologically active compounds are distributed in gradients along plant axes, position of tissue excised for study are thought to affect experimental results both qualitatively and quantitatively (Scorza, 1980). Advantageous to the study of floral induction, in-vitro culture techniques can be utilized to
compare treatment effects in juvenile or adult tissue, often with similar ease. Flowers formed in tissue culture are usually undersized and may be malformed.

A variety of media have been reportedly used with the in-vitro study of floral initiation and development (Nitsch, 1972; Scorza, 1982) and the formulations employed undoubtedly effect developmental processes (de Fossard, 1974). Published accounts of unexpected results such as those reported by Galun, Jung and Lang (1963, see discussion below) often assumed discrepancies between theory and observation to be the consequence of unknown media factors. Media ingredients presumed to be important included inorganic salts, growth regulators, carbohydrates, amino acids, vitamins and other co-factors as well as specific enzymes.

Studies of in-vitro flowering have addressed questions concerning either floral induction or floral development. Both processes (induction and development) have been shown to be moderated by culture conditions and by media constituents (Scorza, 1982).

In some cases, appropriate culture conditions have substituted for pre-induction of tissues from photoperiodic species or from those that require vernalization. Nitsch and coworkers (Nitsch, 1966, 1972) demonstrated floral induction in non-meristematic root explants of chicory (Chicorium intybus), but only in tissues that had been vernalized in-vivo prior to excision. Later work by Plerik (de Fossard, 1974; Scorza, 1982) circumvented the necessity for pre-induction of chicory explants through modifications in media constituents and cold treatment. Pereira reported the successful induction of large Wedgewood Iris (Iridaceae) explants following in-vitro treatment
at 13 C whereas smaller explants failed to be respond under these conditions (de Fossard, 1974).

In-vitro induction of non-meristematic plumbago (*Plumbago indica*) stem segments (Nitsch, 1966, 1967) or of spinach apical buds (Culafic and Neskovic, 1980) have been shown to parallel daylength requirements of these species grown under natural conditions. In plumbago explants, adventitious buds developed in-vitro under long days were vegetative whereas floral buds were produced under the opposite light regime. However, Scorza (1982) summarized studies which failed to induce in-vitro flowering of photoperiodic species under any photoperiod treatment. In-vitro photoinduction appeared to be enhanced by media constituents, most notably, high carbohydrate levels (Nitsch, 1972). Optimal carbohydrate levels have been suggested to affect flowering by stimulation of the pentose-phosphate pathway (Nitsch, 1972; Scorza, 1982).

Other media constituents were found to influence in-vitro floral induction. High nitrogen levels, cytokinins, ABA, as well as various steroids, nucleic acids and amino acids generally enhanced in-vitro flowering whereas IAA and GA most often inhibited this process (Scorza, 1982). IAA and cytokinins were presumed to act through control of DNA transcription. Although GA acts as a deterrent to floral induction in explants, it often exhibited a stimulatory effect upon in-vitro floral development (discussed below).

Culafic and Neskovic (1980) have also revealed combinations of culture conditions (photoperiod and temperature) and media formulations (growth regulants) resulting in variable rates of floral induction of
excised spinach apices. Also, media and culture conditions had dissimilar effects on flowering of male and female apices, altering the sex ratio of the resulting explant population.

A review of media and cultural parameters influencing the in-vitro ontogenesis of flowers or floral parts is perhaps, more germane to topics discussed in Chapter 5. Of importance in studies of this type, are possible correlative effects of developing organs upon one another. Blake reported the successful development of excised floral buds of Viscaria candida through anthesis. This process was found to be dependent on media constituents but relatively unaffected by photoperiod. Calyx and petal elongation was stimulated when GAs were added to the growth medium. Although the response was highly variable, this regulant also enhanced ovary development in a few explants. Added GA had a pronounced stimulatory effect on staminate development, but was considered non-essential due to successful production of pollen in some control cultures. GA enhancement of masculinity under these conditions was in accordance with most known data concerning hormonal regulation of sex expression in cucurbits. Conversely, high temperatures inhibited staminate development in cultured buds as did the inclusion of high kinetin levels in the medium. Although Blake (1969) failed to uncover evidence for mutual influence of proliferating organs, this author cited the work of others indicating developmental interaction among floral parts in many species including those in Cucumis discussed below.

The first successful in-vitro study of cucumber floral development was reported by Galun, Jung and Lang (1962). These authors cul-
tured immature (bisexual) floral buds from monoecious plants on a modified White's media (Henderson, Durrell and Bonner, 1952) supplemented with IAA. Floral buds were excised from potentially male or female nodes. Ovary development exhibited in both potentially male and potentially female buds was attributed to the IAA supplement. This effect was reduced by simultaneous addition of GA$_3$, which also stimulated the development of staminate tissue.

In a subsequent study employing similar techniques (Galun, Jung and Lang, 1963), these authors cultured small (containing underdeveloped stamen primordia) and large (exhibiting well developed stamen primordia) floral buds excised during the male flowering phase. IAA again caused ovary development on small, potentially male buds and had a similar but more pronounced effect on large buds. Developing ovaries appeared to arrest further stamen growth in both bud types, a phenomenon which was overcome through application of GA$_3$ in small buds only. Simultaneous development of both sex organs resulting in hermaphroditic flowers was not observed under any treatment regime. In contrast, GA$_3$ and IAA failed to suppress either staminate or pistillate primordia development respectively in buds isolated from hermaphroditic plants. The authors attributed this lack of response to a stable background genotype lacking the $G$ gene for triggering diclinous floral development under appropriate environmental conditions (see discussion of genotypic effects above). Potentially female buds excised from gynoecious plants and cultured in-vitro were also unaffected by treatment with growth IAA or GA$_3$.

Rute, Butenko and Maurinya (1982) studied the effects of six compounds on sex expression of androecious and gynoecious cucumber shoot
apices cultured in a liquid formulation of Murashige-Skoog medium (Murashige and Skoog, 1962) and grown under long or short day conditions. The process of in-vitro culture influenced sex expression of the gynoecious variety as 90% of the untreated control apices produced male floral buds (i.e. exhibited sex tendencies of a monoecious line). Increased feminization of cultured apices of this variety was evident under all treatment regimes, with the greatest effects exhibited by cultures treated with ethephon and NAA. Feminizing effects were mediated predominantly through a reduction in male flower formation. IAA and kinetin also inhibited the production of male flowers, whereas TIBA and dimethylsulfoxide (DMSO, a heretofore unknown regulator of sex expression) increased femaleness through enhanced production of pistillate buds. Effects of the latter four compounds were not statistically significant. Increased femaleness was noted in cultured androecious apices after treatment with ethephon IAA, DMSO, whereas application of NAA and kinetin produced no modification in sex expression and TIBA induced female floral buds only under short daylength conditions. In general, the effects of applied growth regulants were greater in apices of both sexes under short daylength regimes.

In-vitro techniques were also employed to study the flowering process in other cucurbits. Porath and Galun (1967) and Galun and Porath (1967) reported the use of radiolabelled bases (thymidine and uridine) and autoradiography to follow differentiation in organs of cultured hermaphroditic muskmelon flowers. Nuclear activity was evident throughout the floral bud during its early ontogeny and later was found centered in staminate, ovarian and perianth tissues in succession. This
Pattern of floral development was identical to that found in buds grown under natural conditions. In contrast to results from in-vitro studies with cucumber flowers, these experiments also revealed IAA to be required for successful anther development in small, potentially staminate buds.

In-vitro culture (White's medium) of pistillate Acorn squash buds during their bisexual stage promoted the development of staminate tissue and reduced ovary growth (Pereira, 1968). Both effects were more evident in smaller buds, excised early during their ontogeny. The effects of added growth regulants (IAA and p-chloroenoxyisobutyric acid, a competitive inhibitor for auxin binding sites) were slight, with equal mixtures of the two compounds increasing differentiation in ovary tissue. Growth regulants produced no noticeable change in the development of staminate buds cultured in-vitro.

Modification and Use of Sex Expression for the Production of Hybrid Seed

... wherever technically possible, especially when commercial vegetable seeds are concerned, the trend throughout the world is towards hybrid seed production (Galun, 1973).

As pressure for increased yields and for mechanization increases in the vegetable industry, hybrids offer, in many of the crops, an attractive answer. Hybrids are often superior in many characters, such as yield, uniformity of product and maturity, insect and disease resistance, and consumer appeal. These compensate for the added seed cost (Litzow and Osbun, 1979).
Knowledge of the genetic mechanism and the environmental conditions which affect sex expression is of obvious advantage for good crop management. However, the most significant utilization of this knowledge is for F₁ hybrid seed production. The objective of developing such F₁ cucumber hybrids could be realized only after sufficient information about the genetics and physiology of sex expression in this plant had accumulated (Frankel and Galun, 1977).

The use of genetically controlled and hormonally manipulatable sex expression in cucurbits for the commercial production of F₁ hybrid seed began nearly 15 years ago with the release of "Spartan Dawn" (C.E. Peterson), a hybrid pickling cucumber with a gynoecious seed parent (Litzow and Ozbun, 1979). Although scientific advances have made possible the development of melon and squash hybrids using similar systems, the hybrid cucumber seed industry is considered the most advanced in exploitation of cucumber sex expression.

A detailed scheme for the development of inbred gynoecious seed parents, their maintenance and use in F₁ hybrid seed production was proffered by Galun (1973) and reviewed later by Frankel and Galun (1977). The gynoecious trait was transferred to the desired background genotype through repeated backcrosses to a suitable monoecious recurrent parent. Using exogenous applications of GA₃ or GA₄+7 for the induction of male flowers, the resulting inbreds (heterozygous at the Acr locus) were self-pollinated for two generations to isolate segregates homozygous for the gynoecious trait. Selected gynoecious (Acr Acr) seed parents were maintained in a similar manner. Hybrid seed was produced using monoecious (acr acr) pollen parents resulting in F₁s which were heterozygous or predominantly pistillate. Commercial production fields were established after blending seed of a suitable pollinator variety.
with that of the hybrid at appropriate levels. The above scheme or
modifications of it (e.g. the use of parthenocarpy to eliminate the need
for pollinators in the commercial production field) have been used to
produce the myriad of commercially available cucumber hybrids.

Although widely used, the system of hybrid seed production
described above was found to impart certain negative qualities to the
commercially available seedstock resulting from the use of GAs for
staminate flower induction (Pike and mulkey, 1971). This technique,
necessary for the maintenance of seed parent lines, was found to pas-
sively select for increasingly masculine background genotypes and resul-
ted in the formation of partially pistillate hybrids which increased
their proclivity for male flower production with each cycle of produc-
tion. Pike and mulkey (1971) found the phenomenon particularly detri-
mental in pickling hybrids and recapitulated the effects of masculiniza-
tion upon the quality of the commercial product. Early and concen-
trated fruit set, a hybrid advantage insuring a maximum number of me-
chanically harvestable immature cucumbers of specific and uniform size
was said to be dependent upon expression of strong gynoecious tenden-
cies. In addition, hybrids designed to produce cucumbers parthenocarpl-
cally were found to set seeded (undesirable) fruit when pollen was
available in the commercial field.

Objections to F₁ hybrids produced through the use of growth
regulants were overcome in part after the adoption of silver nitrate and
AVG as masculinizing agents (Tolla and Peterson, 1979). These synthetic
hormones controlling ethylene metabolism were many times more effective
in the induction of staminate flowers, preventing the gradual masculini-
zing shift in background genotypes of seed parents. In addition, Cantliffe and Phatak (1975) and Ells (1983) proposed the use of ethephon and chlorflurenol in pickling cucumber production fields to partially compensate for negative qualities of predominantly pistillate hybrids. Ethephon eliminated staminate flower production on hybrids whereas chlorflurenol promoted the development of parthenocarpic fruit in large numbers suitable for 'once-over' harvest procedures.

The development of \( F_1 \) hybrid cucumbers without the use of growth regulants was first proposed by Kubicki (1964). Hermaphroditic lines \((g g)\) were suggested for use as maintainers of seed parent stock held in heterozygous state \((G g)\). Hybrid seed production fields were projected to require rogueing for removal of hermaphroditic segregates. Kubicki also suggested the use of an allelic series at the \( Acr \) locus for manipulation of flowering and fruiting characteristics of the hybrids produced \((Kubicki, 1965c,d)\) and the maintenance of seed parents through use of complementary hermaphroditic lines \((Kubicki, 1970b)\). Pike and Mulkey (1971) suggested employment of hermaphrodites not as maintenance lines, but as pollen parents of crosses with gynoecious lines for the production of \( F_1 \) hybrids. The resulting progeny were fully gynoecious, eliminating the problem of "erratic proportions of monoecious plants in present pickling cucumber hybrids" (Pike and Mulkey, 1971).

A third method for the production of \( F_1 \) hybrid cucumber seed using monoecious varieties as both seed and pollen parents was outlined by Rudich, Kedar and Halevy (1970). Lines designated to be seed parents were sprayed with a mixture of ethephon and SADH, which inhibited staminate flower production for a period of 2-3 weeks. Fruit harvest was
carefully timed to insure the acquisition of a high proportion of cross-
seeded seed. The advantages of this hybrid production scheme (i.e. the
ability to use lines exhibiting high combining ability without first
backcrossing for inclusion of the \textit{Ac} gene, the high level of female
tendency in hybrids resulting from passive selection through use of
ethephon and SADH, and the elimination of the need for blending a pol-
linator with the hybrid seedstock) far outweighed the disadvantage
associated with possible contamination of the hybrid resulting from sib-
crosses in the seed parent line. These authors also recommended this
method for hybrid seed production in melons and squash.

Nearly two decades ago, Foster (1966) published evidence indicat-
ing the superiority of F\textsubscript{1} hybrid muskmelons in yield capacity, fruit
quality and resistance to disease. In the same year, Kubicki (1966)
outlined a procedure for producing the hybrids which was later reviewed
in detail by Frankel and Galun (1977). Seed parents of hybrids were
chosen from gynomoeneclous varieties (\texttt{A a}) producing mostly pistillate
flowers which could be maintained by crossing with appropriate hermaph-
roditic lines (\texttt{a a}) followed by rogueing of hermaphroditic progeny.
Pollen parents of hybrids were selected from monoecious or andromono-
ecious stock. Frankel and Galun (1977) suggested the use of gynomo-
ecious lines which possessed background genotypes bearing sufficient
number of hermaphroditic flowers for maintenance of the population as
seed parents, eliminating the need for rogueing in the hybrid seed
production field.

Foster (1967, 1968a,b) evaluated a more elaborate scheme for the
production of F\textsubscript{1} hybrid muskmelons using an allele for male sterility,
genetic markers and judicious field placement of seed and pollen parents which optimized the percentage hybrid seed produced. Seed parent populations were composed of two isogenic monoecious lines, one homozygously recessive for a male sterility factor, the other heterozygous at the male sterility locus. Maintenance of this population was accomplished by harvest of seed from male-sterile plants. Both seed parent lines were also homozygously recessive at a locus controlling a glabrous (hairless) seedling marker. To obtain hybrids, seed parents segregating for male sterility were crossed to monoecious or andromonoecious pollen parents which were homozygously male-fertile and homozygously hirsute (hairy, a dominant trait) at the seedling marker locus. Through proper field placement of seed and pollen parents, growers seed was obtained which was comprised of 75 - 80% F1 hybrids. Hybrids could be distinguished from self-cross or sib-cross progeny in the production fields through the use of the seedling marker. A modification of Foster's scheme was suggested by Lee and Janick (1978). The percentage of hybrids formed was found to increase by timely application of ethephon to the seed parent populations which greatly reduced staminate floral development on these plants.

In addition to suggestions by Rudich, Halevy and Kedar (1970), use of exogenously-applied regulants to reduce or eliminate staminate production in potential seed parent lines of Cucurbita was advocated by Hillyer and Wittwer (1959) and by Coyne (1970). The latter author found repeated doses of ethephon to reduce the sex ratio and significantly increase the node number of the first male flower in Butternut squash with total suppression of staminate buds at early nodes. As reported in
other studies, application of this regulant also resulted in shortening of internodes and reduced overall plant size. In the earlier study, similar growth habits and flowering trends were witnessed in Acorn squash treated with MH (Hillyer and Wittwer, 1959; see discussion above). However, the effects of this regulant were more pronounced when applied late in the growing season indicating a correlative influence of shorter daylengths and cooler temperatures.
CHAPTER 4

EFFECTS OF EXOGENOUSLY APPLIED NATURAL AND SYNTHETIC GROWTH REGULANTS UPON SEX EXPRESSION IN BUFFALO GOUD

Modification of qualitative sex expression in buffalo gourd through exogenous application of natural and synthetic growth regulators was explored through the design and implementation of seven field experiments performed during the 1979, 1980, 1981, 1983 and 1984 growing seasons. The rationale for these studies, experimental scope, as well as treatment regimens and application methodology employed during their execution are summarized in Table 7. The primary objective of Experiments I - V was to identify regulators and application methods effecting the induction of male flowers on gynoecious phenotypes. Possible abnormalities in meristem growth and development which could be attributed to exogenously applied compounds were also of interest. Upon the implication of ethylene as a controlling element in staminate floral development, Experiments VI and VII were undertaken to quantify effects of an ethylene synthesis antagonist on gynoecious plants and an ethylene inducing compound on monoecious plants. The influence of these ethylene regulating agents upon meristem growth and pistillate floral initiation was also monitored.
Table 7: Experimental Methodology and Rationale for Exogenous Application of Growth Regulants

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Date</th>
<th>Treatments Applied</th>
<th>No. of Plants Treated</th>
<th>Application Method</th>
<th>Experiment Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>06/08/79</td>
<td>To second-season gynoecious plants in their mixed phase predominantly exhibiting blind nodes or abortive male buds:</td>
<td>Aqueous solns.</td>
<td>Experiment designed to examine effects of exact GA-3 dosage upon sex exp., type I and type II gynoecious plants. Production of viable male buds on gynoecious plants desired.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blind node phenotypes;</td>
<td>0.05% surfactant</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000 ppm GA-3</td>
<td>4  0</td>
<td>applied as 50 ul</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ppm GA-3</td>
<td>4  0</td>
<td>droplet to meristem.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000 ppm GA-3 repeated</td>
<td>4  0</td>
<td>Multiple applications</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ppm GA-3 repeated</td>
<td>4  0</td>
<td>5 day intervals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abortive male bud phenotypes;</td>
<td>0.05% surfactant</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000 ppm GA-3</td>
<td>4  0</td>
<td>repeated 4X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>07/02/79</td>
<td>To seedlings of undetermined sex expression:</td>
<td>Aqueous solns.</td>
<td>Experiment designed to examine ability of growth regulator in gynoecious plants to alter expected 1:1 ratio of population segregation for sex exp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5000 ppm GA-5</td>
<td>501 501</td>
<td>sprayed on meristem</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2500 ppm GA-3</td>
<td>501 501</td>
<td>repeated 4X</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ppm GA-3</td>
<td>501 501</td>
<td>5 day intervals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 ppm GA-4+7</td>
<td>501 501</td>
<td>repeated 4X</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>control</td>
<td>501 501</td>
<td>(approx. 2 ml)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Applications</td>
<td>4  0</td>
<td>repeated 4X</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 day intervals</td>
<td></td>
</tr>
<tr>
<td>III.</td>
<td>06/15/80</td>
<td>To second-season gynoecious and monoecious plants in their mixed phase:</td>
<td>Aqueous solns.</td>
<td>Experiment designed to examine effects of several growth regulators upon sex exp. in both gynoecious and monoecious sex types. Production of viable male buds on gynoecious plants desired. Synergistic action of various masculinizing agents explored. Effects of regulators found to feminize other curculbits also of interest.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ppm GA-3</td>
<td>4  1</td>
<td>sprayed on meristem</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 ppm GA-3</td>
<td>4  1</td>
<td>repeated 2X</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 ppm GA-3</td>
<td>4  1</td>
<td>5 day intervals</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 ppm GA-4+7</td>
<td>4  1</td>
<td>repeated 2X</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ppm GA-3</td>
<td>4  1</td>
<td>GW post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 ppm GA-3</td>
<td>4  1</td>
<td>GW post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 ppm GA-4+7</td>
<td>4  1</td>
<td>GW post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ppm GA-3</td>
<td>4  1</td>
<td>GW post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 ppm GA-3</td>
<td>4  1</td>
<td>GW post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 ppm GA-4+7</td>
<td>4  1</td>
<td>GW post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ppm GA-3</td>
<td>4  1</td>
<td>GW post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>08/10/81</td>
<td>To first-season gynoecious plants in mixed phase:</td>
<td>Spray trts.;</td>
<td>Experiment designed to determine effects of application technique on bioactivity of various growth regulators. DMSO added as a penetrating agent. Lamolina</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spray treatments (aqueous):</td>
<td>Aqueous solns.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ppm GA-3/10% DMSO</td>
<td>3  0</td>
<td>sprayed on</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 ppm GA-3/10% DMSO</td>
<td>3  0</td>
<td>repeated</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 ppm all. nlt./10% DMSO</td>
<td>3  0</td>
<td>GW post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>control</td>
<td>3  0</td>
<td>GW post-treatment</td>
<td></td>
</tr>
</tbody>
</table>

DIA: DIA, DIA agent. DIA Trt(s): DIA treatment(s).
Table 7: (Continued)

<table>
<thead>
<tr>
<th>Experiment Identification</th>
<th>Treatment Description</th>
<th>No. of Plants Treated</th>
<th>Application Method</th>
<th>Experiment Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV. (continued)</td>
<td>Paste treatments (lanolin): 1000 ppm GA-3</td>
<td>3</td>
<td>g</td>
<td>Used to enhance persistence of regulator absorption by plant tissues. Production of viable male buds desired.</td>
</tr>
<tr>
<td></td>
<td>250 ppm GA-3</td>
<td>3</td>
<td>repeated 4X et 3 day intervals.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 ppm sl. nit.</td>
<td>3</td>
<td>Paste trifl To</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000 ppm GA-3/10% DMSO</td>
<td>3</td>
<td>dissolved growth regulators supplied.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500 ppm GA-3/10% DMSO</td>
<td>3</td>
<td>in lanolin and DMSO and applied 1X (by soft brush) to internodes below the first free-standing leaf.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250 ppm sl. nit./10% DMSO</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. 08/04/83</td>
<td>To first-season gynoecious and monocots in their mixed phase: Drench treatments (aqueous): 1000 ppm GA-3</td>
<td>4</td>
<td>to determine effects of application technique on bioactivity of various growth regulators, DMSO added as a penetrating agent. Alcohol used as a solvent and a penetrating agent. Production of viable male buds on gynoecious plants desired.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250 ppm GA-4/7</td>
<td>4</td>
<td>Brush trifl To</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250 ppm sl. nit.</td>
<td>4</td>
<td>at 2 day intervals.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250 ppm IAA</td>
<td>4</td>
<td>Brush trifl, ethaphon, AVG at 2X followed by 1X (by soft brush)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000 ppm phthalimide</td>
<td>4</td>
<td>of growth regulators containing 25% DMSO and 0.05% surfactant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250 ppm glyphosate</td>
<td>4</td>
<td>used as a mutagenic agent, AVG applied as a spray to enhance development. Production of viable male buds on gynoecious plants desired.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 ppm AVG</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5000 ppm bitter root extract</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI. 08/02/84</td>
<td>To first-season gynoecious plants in their mixed phase: Aqueous solutions of growth regulators containing 25% DMSO and 0.05% surfactant used to quantify mass-lining effects of AVG with respect to dosage applied and to observe effects of AVG on meristem development.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500 ppm AVG</td>
<td>10</td>
<td>as a mutagenic agent, AVG applied as a spray to enhance development. Production of viable male buds on gynoecious plants desired.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>250 ppm AVG</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>125 ppm AVG</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 ppm AVG</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 ppm AVG</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII. 09/01/84</td>
<td>To meristems in their male phase on first-season monocots; all trifl. administered to all plants on individual meristems: Aqueous solutions of growth regulators containing 0.05% surfactant applied as a spray to examine effects of ethaphon on male bud (either) development.</td>
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(Continued)
Materials and Methods

Terminology

Terms used below to describe methodology, the sex type of plants and/or sex expression at individual nodes of vines have specific definitions as used in this manuscript. A plant was considered monoecious by virtue of its production of stamen-containing male floral buds during the male floral phase. Plants differentiating female flowers preceded exclusively by the production of antherless male buds or blind nodes were classified as gynoeccious.

Antherless buds were previously described as belonging to one of four discrete classes dependent upon bud size at the time of abortion (Curtis and Rebelz, 1974). However, abortive buds exhibited on Arizona breeding material seemed to defy classification using the above scheme and to range in continuum from those buds barely visible to antherless flowers reaching anthesis (see discussion, Chapter 3). During the course of current experimentation, a bud was considered antherless only if it was large enough to allow visual confirmation of a receptacle devoid of staminate tissue (at least 2 mm in length). A node displaying miniscule buds or no visual bud tissue was said to be blind. Nodes termed male possessed developing male floral tissue, male flowers or the remains of flowers; nodes termed female exhibited developing floral tissue, female flowers, fruit or aborted fruit.

The meristematic region (in reference to treatment application) was defined as the terminal end of a growing shoot containing the apical meristem and including several additional meristems and/or differenti-
ating primordia at developing nodes. Nodes of the meristematic region were observed to be telescopically compressed and surrounded by an envelope of expanding leaf tissue. The node possessing the first free standing leaf (i.e. an expanding leaf not in physical contact with the meristematic region) was often used as a "before and after" reference point when observing treatment effects.

Acquisition of Growth Regulants and Reagents

Growth regulants used in field studies were acquired from the following sources. GA$_3$ (Experiments I - V), ABA (Experiments III and V) IAA (Experiments III and V) and silver nitrate (Experiments III - V) were purchased from Sigma Chemical Co., Inc. (St. Louis, MO). A 21% solution of GA$_4$7 (Experiments II - V), phthalimide AC 94377 (Experiment V) and AVG (Experiment V) were gifts from Abbott Laboratories, Inc. (N. Chicago, IL), A.D. Pavlstra of the American Cyanamid Co. (Princeton, NJ) and P.F. Sorter of Hoffman-La Roche, Inc. (Nutley, NJ) respectively. AVG (Experiment VI) was also procured from Fluka Chemical Co., Inc. (Hauppauge, NY). Testosterone and 17-β-estradiol (Experiment VI) were purchased from Steraloids, Inc. (Wilton, NH). Glyphosate (Roundup, Experiment V) and ethephon (Ethrel, Experiment VII) as manufactured by Monsanto, Inc. (St. Louis, Mo.) and Union Carbide, Inc. (Triangle Park, NC) were obtained from a local vendor. Bitter root material (Experiment V) containing unknown levels of cucurbitacin aglycones and glycosides was extracted from ground whole root with 80% ethanol. Ethanol was removed from solution by rotary evaporation and the resulting aqueous mixture was lyophilized and stored at room temperature. All other
growth regulants were stored under conditions specified by their manufacturers.

The surfactant used in all experiments (polysorbate 80; polyoxyethylene(20)sorbitan monooleate) was purchased from Sigma Chemical Co, Inc. (St. Louis, MO). Agents used to aid epidermal penetration of growth regulants, dimethysulfoxide (DMSO) and lanolin were obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ) and Fisher Chemical Co. (Fair Lawn, NJ), respectively.

Preparation and Storage of Growth Regulant Solutions

All growth regulant treatments exogenously applied to gynoeclous and/or monoeclous plants during Experiments I-VII were prepared at concentrations similar to those employed in published studies of sex expression in various cucurbits (Chapter 3).

Crystalline growth regulants, surfactant and penetrating agents (where applicable) were dissolved in the appropriate solvent, diluted to specified concentrations in accordance with treatment schemes designated in Table 7. Solutions were prepared prior to experimentation and stored under refrigeration until use. Growth regulants obtained as solutions (GA$_{4+7}$, glyphosate and ethephon) were diluted to specified concentrations in appropriate solvents. GA$_{4+7}$ and glyphosate solutions were stored as stated above whereas ethephon solutions (Experiment VII) were prepared in the field, just prior to application. In preparation of paste treatments (Experiment IV), small amounts of growth regulants in concentrated aqueous solutions were blended with lanolin (µg/ml growth regulant per g lanolin) and stored under refrigeration.
Application Techniques

Application techniques employed during each experiment are summarized in Table 7. Techniques were modified from study to study to satisfy experimental objectives or to maximize application effectiveness. All growth regulants were administered to plant tissues in conjunction with a surfactant. Penetrating agents were also included in most formulations prepared for Experiments IV–VII. Growth regulants employed in Experiments V–VII were applied before 10:00 a.m. to retard rapid evaporation of solvents.

During Experiment I, aqueous solutions of GA₃ and polysorbate 80 were applied to meristematic regions in 50 µl droplets after a technique reported by Fuchs, Atsmon and Halevy (1977). The microliter syringe technique was abandoned for the more commonly used foliar spray method (Experiments II, III, IV and VII) as described by a number of research groups (Peterson and Anhder, 1960; Pike and Peterson, 1969; Rudich, Halevy and Kedar, 1972c; Rodrigues and Lambeth, 1972). Treatments were applied as a fine spray 'until run-off' to meristematic regions and subtending developing leaf surfaces. Although absolute dosages applied were less accurate using this technique, the treated surface area (and thus, the probability of regulant penetration to target tissues) was greatly enhanced. Experiment IV also contained ten treatments applied to plants as suspensions in lanolin paste. Pastes with suspended regulants and/or penetrating agents were applied once to the internode below the first free-standing leaf using a soft brush. Paste treatments were employed to retard the drying effects of the environment, thus, enhancing possibility for regulant absorption by plant tissues.
During Experiment V and VI, aqueous solutions of growth regulators were applied as meristem drenches. Terminals of vines were submerged for approximately 15-30 seconds in a vial containing treatment solutions. Solutions were agitated during the drenching period, ensuring liquid contact with the entire meristematic region, including primordia tightly covered and protected by the envelope of developing leaf tissue. This technique, while highly conservative of often costly growth regulators, afforded maximum regulator exposure to meristematic tissues. Regulators applied during Experiment V (some of which were water insoluble) were also administered as 95% ethanolic solutions to internodes below the first free-standing leaf using a soft brush.

Plant Materials, Experimental Design and Methods of Evaluation

Plant populations used in Experiments I - VII all exhibited high levels of phenotypic diversity for most traits. Therefore, quantitative (statistical) evaluation of growth regulator effects upon sex expression was attempted only in those experiments employing a high level of replication per treatment. Experiments involving a large number of treatments replicated at more modest levels were implemented to determine qualitative effects of growth regulator application. Application techniques, experimental designs and methods of evaluation (as discussed below) were modified from study to study to meet specific objectives and to enhance probability of sex modification through exogenous treatment.

Experiment 1. Forty-eight plants used in Experiment I were second-season F1 progeny from controlled crosses among 29 accession segregates. Plants were grown at the Campus Agricultural Center, chosen
during their mixed floral phase for expression of the gynoecious trait, grouped relative to presence or absence of abortive antherless buds and trimmed to four main vines per plant. Two vines on each plant were tagged between the tenth and eleventh differentiated node prior to the meristematic region. Treatments were assigned randomly to plants within groups and both tagged vines on each plant were treated identically with single or multiple applications of GA_3 according to the scheme listed in Table 7. Treatments were applied using the micro-drop technique. Runners were allowed to develop for two weeks after the last application of growth regulant. Floral expression was examined at consecutive nodes of treated vines beginning with the node following the runner tag and continuing for 10 nodes past the final application of growth regulants.

**Experiment II.** This study was performed using 500 seedlings of Arizona Hybrid 81 (a hybrid derived from two open-pollinated populations) grown at the Marana Agricultural Center. Rows of emerging seedlings were thinned to approximately 25 cm between plants and treated at approximately the fourth true-leaf stage with various levels of GA_3 and GA_4+7 (Table 7). These plants of unknown sex expression were presumed to be segregating in a 1:1 gynoecious:monoeclous ratio according to genotype. Treatments (as listed in Table 7) were randomly assigned to rows. Plants (100 per treatment) were sprayed 'until run-off' with growth regulant solutions. Treated plants were allowed to develop for two weeks following the final application of growth regulants, before scoring for sex expression. The criteria for classifying a segregate as monoeclous was identical to those discussed above. However, plants were considered to be gynoecious upon the absence of staminate floral devel-
opment alone. Most plants employed in this study were classified prior to the onset of the mixed phase. Segregation ratios obtained after treatment were compared with expected segregation ratios using the chi-square statistic modified by the Yates correction for continuity as suggested for two cell (single degree of freedom) comparisons (Steel and Torrie, 1960).

**Experiment III.** Arizona Hybrid #1 plants in their second season of growth at the Marana Agricultural Center were selected for their respective sex types (68 gynoecious and 17 monoecious). One vine per plant was chosen and tagged (as in Experiment I) prior to application of growth regulants. Individual treatments (comprised of a variety of natural and synthetic growth regulants known to affect cucurbit sex expression) were assigned randomly to four gynoecious plants and one monoecious plant. Growth regulants in aqueous solution containing surfactant were applied to meristematic regions as foliar sprays (as in Experiment II) in accordance with the scheme outlined in Table 7. Plants were allowed to grow two weeks after the final application of chemical agents prior to observation for modification of meristem development and sex expression (as in Experiment I).

**Experiment IV.** Using methods described above, 45 gynoecious plants were chosen from a first-season population of SYN-1 seedstock (a synthetic population derived from open-pollination of seven hybrids and three monoecious varieties) grown at the Marana Agricultural Center. All surrounding plants were rogued. On remaining plants, individual vines were selected for treatment and tagged as in experiments performed during previous seasons. Treatments were again assigned to plants
randomly. Growth regulators were applied to meristematic regions as foliar sprays or to internodes preceding meristems as lanolin paste suspensions following the scheme presented in Table 7. Treatment effect upon meristem development and sex expression were judged two weeks after final application of growth regulators.

Experiment V. Eighty gynoecious and 20 monoecious first-season SYN-1 plants grown at the Marana Agricultural Center were identified and prepared for treatment as in Experiment IV. As in previous years, treatments were assigned randomly to plants within sex types. Regulators in aqueous solution were administered to plants as meristem drenches whereas regulators dissolved in ethanol were applied to internodes below meristems with a soft brush (Table 7). Two weeks following the final application of treatment, effects on development and sex expression were monitored as described above (Experiment IV).

In conjunction with Experiment V, a gynoecious self-pollination and two gynoecious X gynoecious cross-pollinations were made following the development of male flowers on three plants of this sex type. Progeny of these controlled crosses were grown during the 1984 season to substantiate or refute evidence for monogenic control of gynoecy (Dossey, Bemis and Scheerens, 1978) and to initiate the development of true-breeding gynoecious lines. The observed sex ratio among self and cross progeny were compared by chi-square analysis (Yates correction factor applied) to the 3:1 gynoecious:monoecious segregation pattern expected from the current genetic hypothesis of sex expression control.

Experiment VI. Sixty gynoecious first-season SYN-1 plants grown at the Marana Agricultural Center were chosen to quantitatively examine
the masculinizing effects of AVG, an ethylene synthesis inhibitor. Plants were prepared for experimentation as in previous years except that vines to be treated were tagged at the internode between the meristem region and the first free-standing leaf. Treatments listed in Table 7 were assigned to plants using a completely random statistical design (Steel and Torrie, 1960). Various concentrations of the inhibitor dissolved in an aqueous solution containing surfactant and 2% DMSO were applied to designated runners as meristem drenches. Treated vines were allowed to grow for two weeks following the last application of AVG before the number of female, male and antherless buds as well as blind nodes were scored as described previously. However, adverse field conditions favoring a disease epidemic forced an early analysis of many treated vines partially confounding statistical treatment of the data (see Chapter 4, Results and Discussion). Statistical evaluation of meristem growth rate, induction of staminate flowers and development of pistillate flowers included analyses of variance using a format for completely random designs and tests for differences among means (Duncan's New Multiple Range Tests) (Steel and Torrie, 1960).

To further explore stamen development, AVG-Induced male floral buds developing at 1-9 nodes before the meristematic region were collected from seven gynoecious plants and fixed in FAA (formaldehyde-acetate-alcohol) solution as suggested by Berlyn and Milsche (1976). Male buds from corresponding nodes on seven monoecious vines taken at random from a neighboring plot were also obtained and fixed accordingly. Longitudinal sections of staminate tissue were made to allow comparison of stamen development in AVG-Induced and 'natural' male buds collected
at similar nodes. Bud lengths and widths as well as stamen lengths and widths were measured to the nearest 0.1 mm using a vernier calliper. Selected tissues were photographed.

Experiment VII. To study the effects of exogenously applied ethylene on the development of male floral buds, 10 first-season monoeccious plants grown at the West Campus Agricultural Center were selected from breeding stock of mixed parentage and treated with ethephon, an ethylene releasing compound. Each treatment level was administered as a foliar spray to the meristematic region and first and second free-standing leaves of tagged duplicate shoots on each plant (see Table 7). Runners were allowed to develop for two weeks following the first treatment before examination for floral differentiation, growth rates and morphological abnormalities.

Treatment effects upon growth rate (nodes per day and average internode length) were analyzed statistically as suggested for a randomized complete block design with subsampling (Steel and Torrie, 1960). Genotypes were considered as blocks. Significance of main effects (both considered to be random effects) was tested using the experimental error (individual X concentration) mean square. Means were compared as in Experiment VI. Treatment effects upon bud abortion, antherless bud formation, staminate and pistillate floral development and the number of blind nodes were determined statistically as above after adjusting raw data for significant differences in growth rate (number of nodes observed per treatment, see Results and Discussion).
Evaluation of Exogenously-applied Hormones for use in Breeding Programs, Genetic Studies and for Commercial Production of Hybrid Seed

In addition to experiments V and VI described above, the effects of AVG on floral development was also monitored in treated gynoecious phenotypes used in studies examining heritability of unrelated traits. The suitability of this technique for use in breeding programs was empirically assessed; its potential for use in commercial seed production programs was speculated upon.

Results and Discussion

Like all other physiological events in plant development, the dynamics of the flowering process from induction to senescence are directed by the interaction of hormones at various concentrations (Chapter 2). Hormonal balance at a specific site may be altered through differential rates of hormone synthesis, degradation, storage (conjugation) and transport as well as by changes in membrane permeability affecting hormones or their precursors compartmentalized within the cell. The hormonal balance ultimately influences expression of the genome mediated by hormone interaction with soluble or membrane-bound hormone-protein receptor complexes. The resultant hormonally induced changes in the cellular enzyme complement can control metabolic processes or further alter hormonal balance and thus, direct the development of specific tissues or organs.

Whether initial fluctuation in the level of a given hormone triggers a predesigned response in primordial tissue or controls (in concert with other hormones) a less predetermined developmental pattern
Is unclear (Zeroni and Hall, 1980). Both mechanisms could be active at various stages of floral ontogeny. Current theories of floral development (Heslop-Harrison, 1963; Frankel and Galun, 1977) as proposed earlier by Wardlaw (1957) suggest initial changes in meristematic tissue triggered by hormonal influx. As organ primordia differentiate in a programmed sequence, their regulation is affected by hormones and metabolites produced in the surrounding developing tissues. Diversity in sex expression of individual flowers (i.e., suppression of stamen or pistil primordia) is thought to arise through hormonal differences as mediated by the genome or by the environment (Frankel and Galun, 1977).

Genetic and environmental influence upon endogenous hormonal levels is well documented (Chapter 3). However, as shown by a myriad of additional studies, various genetic and/or environmental conditions and their influence on endogenous hormone levels can be mimicked through the exogenous application of various natural and synthetic growth regulators. Only speculative inference concerning endogenous floral regulation can be drawn from these investigations as results mirror a distorted representation of natural conditions (Frankel and Galun, 1977; Bernier, Kinet and Sachs, 1981b). However, the relative significance of naturally-occurring hormone groups to the flowering process can be deduced from the action of their exogenously-applied counterparts. More importantly, studies of this type are essential to the exploitation of sex expression for the cost-effective production of F₁ hybrid seed (Frankel and Galun, 1977). The effects of exogenously-applied growth regulators upon sex expression in buffalo gourd are discussed below.
Effectiveness of Application Techniques

Application techniques were judged to be effective if treated meristems exhibited morphological evidence of hormonal changes (i.e., changes in growth, organ development or flowering patterns). This assessment was understood to measure indirectly the successful penetration of active materials to developing tissues. Two techniques proved to be superior: application of regulants in aqueous solution with polysorbate 80 (surfactant) and DMSO (penetrating agent) as foliar sprays (Experiments II-IV, VII); and application of the above materials in aqueous solution as meristem drenches (Experiments V-VI). Spray applications maximized treated surface area, were mechanically simple and required little time to administer whereas greater control over application was achieved by the meristem drench technique. Although requiring greater manipulative skill to apply, the meristem drench technique rendered the most efficient use of costly regulants. Application of GA₃ by the microliter syringe technique (Experiment I) failed to produce morphological changes in developing meristems. Ethanolic solutions of growth regulants brushed on internodes adjacent to meristematic regions resulted in less abnormal tissue development than similar aqueous treatments administered as meristem drenches (Experiment V). Shoots treated in this manner became brittle. Lanolin paste suspensions (Experiment IV) effected substantial levels of meristem necrosis, perhaps due to retention of heat from solar radiation.
Developmental Abnormalities Effected by Growth Regulants

Developmental abnormalities (i.e. elongation or deformation of plant parts, chlorosis, bud abortion and tissue necrosis) were evident under most treatments administered in Experiments II-VII. However, assignment of effects was not considered valid unless abnormalities were prevalent in most replicates of a given treatment. Conclusive evidence for growth regulant-induced developmental changes was uncovered following applications of AVG (Experiment VI), glyphosate (Experiment V), ethephon (Experiment VII) and gibberellins (Experiments II-V).

Chlorosis of meristem and leaf tissue was witnessed in most buffalo gourd replicates treated with high doses of AVG (dosage >250 ppm, Figure 9). Unlike similar but transient effects reported in cucumber (Owens, Tolla and Peterson, 1980), muskmelon (Owens, Peterson and Tolla, 1980) and watermelon (Christopher and Loy, 1982), AVG-induced chlorosis of buffalo gourd leaves persisted at leaf margins throughout the course of the experiment. Damage to margins of developing leaves in the meristematic region (also evident in Figure 9) was attributed to the action of DMSO, as this effect was universally noted in all treatments containing the penetrating agent. Whether DMSO promoted the chlorotic condition of AVG treated leaves was not determined. DMSO was also shown to damage corollas of pistillate flowers developing in the meristematic region.

Application of glyphosate at low concentrations drastically altered development of all meristematic tissues in buffalo gourd (Figure 9). Stem and meristem thickening was noted as well as suppression of leaf, flower and tendril development. Scorza, Welker and Dunn (1984)
Figure 9: Developmental Abnormalities Associated with Exogenous Application of Aminoethoxyvinylglycine or Glyphosate

A: Chlorosis associated with application of aminoethoxyvinylglycine at 250 ppm (Experiment VI, deformation of leaf margins due to DMSO added as a penetrating agent)

B: Deformation of meristem and lack of development at nodes of shoots treated with glyphosate (Experiment V)

Blue dot indicates shoot treated with 250 ppm glyphosate
Red dot indicates untreated shoots
Figure 9: Developmental Abnormalities Associated with Exogenous Application of Aminoethoxyvinylglycine or Glyphosate
also reported basal swelling, reduced leaf size and leaf deformity in cranberry node ex-plants treated with glyphosate employed as an auxin antagonist. However, these authors also witnessed a 'cytokinin-like' promotion of axillary bud and root development. This effect (although predictable in view of the anti-auxin activity of this regulant) was not evinced in In treated buffalo gourds.

Application of ethephon to buffalo gourd meristems resulted in reduced growth rates, shortening of internodes and suppression or abortion of floral buds (Figure 10). These classic effects (discussed quantitatively below) were similar to those described in ethephon-treated cucumber (Iwahori, Lyons and Smith, 1970; Cantliffe and Robinson, 1971; George, 1971; Karchi and Govers, 1972; Dax-Fuchs, Atsmon and Halevy, 1978), muskmelon (Karchi, 1970), watermelon (Christopher and Loy, 1982) and squash (Splittstoesser, 1970). In addition to these predictable responses, ethephon-treated plants displayed abnormal stem inflection at nodes, 'hooked' meristems, tightly curled tendrils and drastically reduced leaf expansion.

Gibberellins, exogenously-applied to buffalo gourd meristems, also mediated classic developmental responses (Figure 11). Elongation of internodes, leaves and floral parts were also noted as 'side effects' of GA application on cucumber (Peterson and Anhder, 1960; Pike and Peterson, 1968; Rodrigues and Lambeth, 1972) and muskmelon (Anals, 1971; Rudich, Halevy and Kedar, 1972c). In general, the effects of GA$_{4+7}$ applications were more evident than those associated with applied GA$_3$. Arched leaves with pronounced veins were also found on GA-treated plants perhaps due to differential expansion in various tissues. At high doses
Figure 10: Developmental Abnormalities Associated with Exogenous Application of Ethephon

A: Formation of hooked structure at meristematic region with corresponding structural irregularities at nodes (Experiment VII)

Orange dot indicates shoot treated with 500 ppm ethephon
Red dot indicates untreated shoot from the same plant

B: Flower, leaf and tendril development at nodes of shoots treated with 250 ppm ethephon (Experiment VII)

Yellow dot indicates normal leaf development at node of first free-standing leaf followed by reduction in leaf expansion at successive nodes
Brown dot indicates normal flower formation at node of first free-standing leaf followed by reduced floral development at successive nodes

C: Aborted staminate flower at node of shoot treated with 500 ppm ethephon (Experiment VII)
Figure 10: Developmental Abnormalities Associated with Exogenous Application of Ethephon
Figure 11: Developmental Abnormalities Associated with Exogenous Application of Gibberellins

A: Internode, petiole and tendril elongation (Experiment V)

Red dot indicates shoot treated with 250 ppm GA$_4$+7
Yellow dot indicates untreated shoot of the same plant

B: Leaf blade arching and pronounced veins of plants treated with 500 ppm GA$_4$+7 (Experiment II)

C: Floral, peduncle, petiole and internode elongation on shoot treated with 250 ppm GA$_4$+7 (Experiment V)

D: Abnormalities in stamen development and peduncle elongation (Experiment II)

Green dot indicates flower excised from shoot treated with 500 ppm GA$_4$+7
Orange dot indicates flower excised from shoot treated with 5000 ppm GA$_3$
Blue dot indicates flower excised from shoot of untreated plant
Figure 11: Developmental Abnormalities Associated with Exogenous Application of Gibberellins
(5000 ppm GA$_3$, 500 ppm GA$_{4+7}$, Experiment II) stamen morphology reflected a decrease in the anther length/filament length ratio.

Exogenous application of other growth regulators failed to elicit consistent morphological responses when applied to buffalo gourd meristems.

Influence of Ethylene Antagonists upon Growth and Floral Development

Ample evidence has accrued to suggest the dominant role of ethylene in promotion of femaleness in cucurbits (Chapter 3, Table 6). Conversely, many studies have indicated the usefulness of ethylene synthesis inhibitors (e.g. AVG) and suppressors of ethylene action (e.g. silver nitrate and perhaps, phthalimides) for induction of staminate floral buds on gynoecious or predominately female genotypes in several species.

**Aminoethoxyvinylglycine.** Application of 80 ppm AVG resulted in the first successful induction of male floral buds on gynoecious buffalo gourds (Experiment V). The pattern of floral development in four replicates before and after treatment is depicted in Appendix A. Of the four plants treated, only three formed staminate buds; effects of treatment were noticed at the third to fifth developed node following the original application of AVG and persisted for as many as 12 nodes. Shoots of AVG-induced plants produced from 5-8 male flowers each. The failure of staminate induction on one replicate may have intimated possible effects of background genotype on endogenous ethylene concentration or may have also resulted from differential penetration of applied regulators among genotypes.
In one replicate, formation of functional male buds was preceded by development of two large antherless buds, a phenomenon noticed in subsequent AVG experiments (discussed below in detail). The application of AVG failed to affect floral development at existing differentiated nodes and also appeared to have no effect on the formation of pistillate flowers.

Raw data summarizing the effects of AVG dosage upon buffalo gourd growth rate and floral development (Experiment VI) are presented in Table 8. Staminate floral induction was obtained on all replicates treated with AVG concentrations of 125 ppm and above. Thereafter, the percentage of induced plants decreased in proportion to application rates. These results again intimated possible effects of background genotype on endogenous ethylene synthesis/action. Owens, Tolla and Peterson (1980) failed to demonstrate a significant genotype X AVG concentration interaction among three gynoeclous cucumber breeding lines. However, as most experimentation examining sex expression is accomplished employing inbred varieties or breeding stock, the existence of a genotype/dosage interactions among divergent germplasm sources in other cucurbits has yet to be ascertained. In the present study, no staminate flowers were found on shoots treated with a control solution.

In addition to experimental use elucidating aspects of sex expression, AVG techniques were employed to obtain gynoeclous self pollinations in two separate heritability studies. Solutions containing 125 ppm AVG applied four times at four day intervals effected staminate induction in 100% of gynoeclous plants treated in one study (A.E. Ralowicz, personal communication), and approximately 30% of gynoeclous
Table 8: Raw Data Summary for Growth Rate and Floral Development in Gynoeclous Plants Treated with Aminoethoxyvinylglycine at Various Concentrations

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<td>132</td>
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phenotypes involved in the other study (H.M. Scheerens, personal communication). The poor success rate obtained in the latter program may have been stress-induced. Heavy rainfall during the treatment period resulted in saturation of poorly drained soils affecting the physiological condition of the treated plants. Staminate induction rate appeared to be highest in the areas where the soil was comparatively well-drained.

The average number of nodes/plant developed (observed) following treatment (growth rate) varied little among application rates as did the total number of pistillate flowers recorded per treatment (Table 8). However, the total number of staminate flowers induced per treatment appeared to be directly influenced by AVG concentration.

Statistical analyses of shoot growth rate (nodes differentiated/day), average level of staminate induction (staminate bearing nodes/replicate) and average level of pistillate flower formation (pistillate bearing nodes/replicate) are summarized in Table 9. Growth rates were expressed on a per day basis to account for early and staggered termination of shoot development. Wet weather exacerbated a heretofore unencountered pathological epidemic (a stem rot) forcing the collection of data before treatment effects were completely expressed (Appendix B, note especially replicates receiving higher dosages of AVG). Although plants succumbed to disease at random intervals (i.e. independent of treatment) allowing for statistical analysis of the data, these analyses contain development period as a confounding element. It was assumed that further shoot development would have accentuated statistical differences in staminate flower induction among AVG concentrations.
Table 9: Analysis of Variance Summaries for Growth Rate and Floral Development in Gynoeclous Plants Treated with Aminoethoxyvinylglycine at Various Concentrations

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<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sums of Squares</th>
<th>Mean Squares</th>
<th>F-ratio</th>
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<tr>
<td><strong>Growth Rate of Treated Meristems (nodes/day)</strong></td>
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<tr>
<td>Among AVG Concentrations</td>
<td>5</td>
<td>0.25</td>
<td>0.050</td>
<td>1.52 ns</td>
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<tr>
<td>Within AVG Concentrations</td>
<td>52</td>
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<tr>
<td><strong>Total</strong></td>
<td>57</td>
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<td><strong>Staminate Floral Induction (no./plant)</strong></td>
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<tr>
<td>Among AVG Concentrations</td>
<td>5</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among AVG Concentrations</td>
<td>5</td>
<td>7.5</td>
<td>1.50</td>
<td>1.05 ns</td>
</tr>
<tr>
<td>Within AVG Concentrations</td>
<td>52</td>
<td>74.5</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>57</td>
<td>82.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns: F-ratio not significant at the 0.05 level

**: F-ratio significant at the 0.01 level.
Parameter means and coefficients of variation are compared in Table 10. Mean growth rates (nodes/day) varied little among treatments and modestly among replicates within treatments. Visual observation also suggested internode lengths to be independent of treatment effects. In contrast, transient to severe and persistent stunting of shoot growth has been noted in AVG-treated cucumber (Owens, Tolla and Peterson, 1980), muskmelon (Loy et al., 1979; Owens, Peterson and Tolla, 1980) and watermelon (Christopher and Loy, 1982).

Evidence has accumulated suggesting genetic and environmental manipulation of sex expression to be mediated through hormonal changes in conjunction with altered growth patterns (Chapter 3). However, results obtained herein intimate the expression of the antherless trait to be independent of plant growth rate. Further evidence for the independence of the two phenomena was procured in a separate study of growth rate among and within sex types of buffalo gourd in their first season of growth (Gathman and Scheerens, unpublished data). During a 34 day period, total growth of main shoots averaged 10.2 cm/day in populations of both sex types. The mean number of differentiated nodes (0.8 nodes/day) was also identical among gynoecious and monoecious groups. In addition, numerous attempts to determine sex type based on differences in early growth patterns have been unsuccessful.

As suggested by analyses of variance, only means for staminate flower induction/plant were compared statistically. Higher dosages of AVG (500/250 ppm) resulted in significantly increased staminate flower induction when compared with the number initiated on replicates receiving lower dose rates (50/25 ppm). After treatment of gynoecious
Table 10: Means and Coefficients of Variation for Growth Rate and Floral Development in Gynoeclous Plants Treated with Aminoethoxyvinylglycine at Various Concentrations

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Growth Rate of Treated Meristem (nodes/day)</th>
<th>Staminate Floral Induction (no./plant)</th>
<th>Pistillate Floral Development (no./plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>C.V. (%)</td>
<td>Mean</td>
</tr>
<tr>
<td>500 ppm</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.6</td>
<td>7.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>250 ppm</td>
<td>0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.1</td>
<td>7.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>125 ppm</td>
<td>0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.1</td>
<td>5.3&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 ppm</td>
<td>0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.6</td>
<td>3.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 ppm</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.4</td>
<td>2.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.5</td>
<td>0.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means not statistically compared as a consequence of non-significant F-ratio

<sup>b</sup>,<sup>c</sup>,<sup>d</sup>Means designated with similar superscripts are not significantly different at the 0.05 level
cucumber lines, Owens, Tolla and Peterson (1980) reported similar trends in the relationship of staminate floral induction and AVG concentration. In contrast, these authors (Owens, Peterson and Tolla, 1980) determined staminate floral induction to be independent of AVG dosage in a treated gynoecious muskmelon breeding line, whereas Christopher and Loy (1982) demonstrated an inverse relationship of AVG dosage and staminate floral development in a monoecious watermelon variety.

Variability in the number of staminate buds produced among replicates within treatments was substantial (Table 10, Appendix B). Coefficients of variability for means of AVG treatments ranged from 45.4-85.6%. Examination of flowering patterns in treated plants ( Appendix B) revealed considerable variation in the 'lag period' preceding evidence of treatment effects (i.e. number of nodes adjacent to those containing male floral buds or well developed antherless buds). This 'lag period' may have reflected physiological differences among replicates for the absorption, transport and metabolism of AVG. However, it may have resulted more directly from variation in the number of developing primordia in meristematic regions and/or from differences in primordia development at the time of treatment. The induction of staminate buds on lateral shoots indicated the presence of developing shoot floral primordia within the terminal meristem or suggested the acropetal transport of this material into shoot meristematic regions.

As in Experiment V, the differentiation of well-developed antherless buds in many replicates ( Appendix B), signalled either the onset of treatment effect or its termination. Although phenotypic variability exists for expression of this trait (Chapter 3), antherless
buds produced on untreated gynoecious plants usually abort during early developmental stages. Sustained development of antherless buds through application of an ethylene antagonist suggested low levels of endogenous ethylene to favor corolla development. Failure of the treatment to stimulate anther development in buds at nodes preceding the onset of male flowers may have reflected the timing of application. Atsmon and Galun (1960) suggested that differentiation of anther primordia precedes corolla development in potentially staminate cucumber buds; a similar situation may exist for the sequence of primordia development in staminate buffalo gourd flowers. Failure of stamen development in large antherless buds following AVG-induced staminate nodes may have resulted from reduced AVG concentration or effectiveness.

The developmental progress of AVG-induced staminate buds was evaluated at ten sequential nodes preceding the meristematic region. Lengths and widths of these buds as well as those of their developing stamens were compared to lengths and widths of buds and stamens of their 'naturally-formed' counterparts. Only seven plants of each sex type were surveyed and, as flowers were not produced at all nodes on either sex type, the number of floral buds representing development at any given node was insufficient to warrant statistical treatment of the data. Lengths and widths of buds and stamens are displayed graphically in Figures 12 and 13. Substantial variability in bud lengths and widths was evident in staminate buds excised from either monoecious or gynoecious plants, but at all nodes, the range of bud lengths and widths for both sex types overlapped (Figure 12). A similar relationship among sex types existed for stamen lengths and widths. However, stamen elongation
Figure 12: Means and Ranges for Lengths and Widths of Staminate Buds Excised from Ten Nodes Adjacent to the Meristematic Region of Single Shoots from Seven Monoecious and Seven Gynoecious Plants
Figure 12: (Continued)
Figure 13: Means and Ranges for Lengths and Widths of Stamens in Developing Buds Excised from Ten Nodes Adjacent to the Meristematic Region of Single Shoots from Seven Monoecious and Seven Gynoecious Plants
Figure 13: (Continued)
In AVG-induced buds at nodes eight and nine may have been suppressed slightly. This effect is noticeable in the photograph displayed as Figure 14. The developmental period required to mature induced staminate buds (i.e. from initial treatment of meristem to anthesis) was reported to range from 21-26 days (Experiment V; A.E. Ralowicz, personal communication; H.M. Scheerens, personal communication).

Mean number of pistillate flowers initiated on AVG-treated shoots varied little (Table 10); variation among replicates within treatments was substantial. An examination of flowering patterns (Appendix B) on treated plants again suggested the independence of pistillate flower formation and AVG presence or concentration. Many replicates developed female flowers at regular intervals at nodes interspersed among those bearing induced male buds (e.g. 500 ppm, replicate 7; 125 ppm replicate 6; 50 ppm, replicate 1). A few plants (most notably 500 ppm, replicates 3 and 4) were characterized by the lack of pistillate floral initiation following the onset of staminate buds. However, the predominance of data intimated the action of separate hormonal or metabolic elements controlling the stimulation and sustained development of staminate and pistillate tissues.

Further evidence for the complexity of stamen and pistil development control was realized upon the maturation of a hermaphroditic flower on an AVG-treated shoot (Figure 15; A.E. Ralowicz, personal communication). This flower may have resulted from the timely application of AVG. Action of this regulant could have stimulated stamen development (perhaps by the scheme proposed below) in a floral primordium previously triggered physiologically to proliferate pistillate
Figure 14: Staminate Floral Development at Ten Nodes Adjacent to the Meristematic Region of Single Shoots from Two Monoecious Plants Compared with Staminate Floral Development at Corresponding Nodes of Single Shoots from Three Gynoecious Plants Treated with Aminoethoxyvinylglycine
Figure 15: Hermaphroditic Floral Development at a Single Node of a Gynoecious Plant Treated with Aminoethoxyvinylglycine

A: Intact hermaphroditic flower excised from shoot treated with 125 ppm AVG (personal communication, A. Ralowicz)

B: Hermaphroditic flower with corolla removed

Red dots and corresponding arrows point to staminate tissue
Yellow dots and corresponding arrow points to pistillate tissue

C: Longitudinal section of selected floral parts

Red dot and corresponding arrows point to staminate tissue
Yellow dots and corresponding arrows point to pistillate tissue
issue. If differences in primordia development were conditioned solely by endogenous ethylene levels, the simultaneous differentiation of both staminate and pistillate tissues might not be possible.

The formation of hermaphroditic flowers is common in cucurbits treated with AVG. Atsmon and Tabbak (1979) reported the formation of perfect flowers on treated gynoecious cucumbers "as a transition to the induced, typical staminate buds or upon reversion to the natural female condition". Hermaphroditic flowers were formed at normally pistillate nodes of monoecious watermelon (Christopher and Loy, 1982) and gynoecious muskmelon (Owens, Peterson and Tolla, 1980) treated with AVG, whereas the proportion of perfect to pistillate flowers was increased in gyno monoecious muskmelon without subsequent reductions in the latter flower type (Loy et al., 1979). In Experiment VI, the extent of hermaphroditic development at nodes presumed to sustain pistillate buds was not determined due to existing pathological conditions.

**Other Ethylene Antagonists.** Two additional ethylene antagonists, silver nitrate (Experiments III-V) and AC-99524 (a phthalimide, Experiment V) were evaluated as potential modifiers of buffalo gourd sex expression. Like AVG, these compounds have been demonstrated to promote development of staminate flowers in cucumber and muskmelon through suppression of ethylene action (Atsmon and Tabbak, 1979; Nijs and Visser, 1979; Tolla and Peterson, 1979; Owens, Peterson and Tolla, 1980; Takahashi and Suge, 1980; Hunsperger Helsel and Baker 1983; Xu and Bukovac, 1983a,b). However, application of these compounds to gynoecious and monoecious buffalo gourd meristems failed to elicit significant changes in morphological development or sex expression. The lack
of response may have indicated poor epidermal penetration by these compounds, especially that of AC-99524 which was applied as a wettable powder. However, the lack of staminate floral induction might also have resulted from differences in their mode of action with that of AVG (Chapter 3). Comparative effects of AVG and silver nitrate applications on growth and sex expression have been noted in cucumber (Atsmon and Tabbak, 1979) and muskmelon (Owens, Peterson and Tolla, 1980). Further attempts to induce stamen development in buffalo gourd using silver nitrate are discussed in Chapter 5.

**Proposed Mechanism for Regulation of Staminate and Antherless Bud Development.** In view of successful staminate induction on AVG-treated gynoecious plants and information previously discussed (Chapters 2 and 3), it is tempting to speculate concerning the endogenous regulation of staminate and antherless bud morphogenesis. Control of sex expression in individual primordia by a dynamic balance of promoting and/or inhibiting substances has been intimated by innumerable investigations. Models of regulation have suggested sub- and supra-optimal levels of individual hormones (native or applied) to effect opposing changes in sex expression, especially when interacting with other regulants present or when studied in different environments (Rodriques and Lambeth, 1972; Karchi and Govers, 1972; Friedlander, Atsmon and Galun, 1977c; Dax-Fuchs, Atsmon and Halevy, 1978). Also, several studies have indicated natural and synthetic regulants to promote or inhibit the accumulation of other hormones (Halevy and Rudich, 1967; Rudich, Halevy and Kedar, 1972b,c; Shannon, 1976).
Evolution of endogenous ethylene from gynoecious cucumber meristems was reported to be higher than that emanating from monoecious meristems and female buds were shown to release greater concentrations of ethylene than their male counterparts (Rudich, Halevy and Kedar, 1972a). Ethylene was also indicated as a natural feminizing agent in muskmelon (Byers et al., 1972a). Many studies have demonstrated the feminizing action of ethylene (applied as ethephon) upon sex expression in cucurbits through promotion of pistillate and hermaphroditic flowering and suppression of staminate flowering (Table 6). Application of ethephon to monoecious and gynoecious cucumber has also been shown to promote the accumulation of ABA and to effect reductions in endogenous IAA and GA contents (Rudich Halevy and Kedar, 1972b), perhaps through ethylene-stimulated activity of enzymes responsible for their degradation (Shannon, 1976). In muskmelon flowers, high levels of ethephon-released ethylene resulted in direct suppression of anther formation (Karchl, 1970). In addition, high levels of bud abortion were witnessed upon application of ethephon (Karchl and Govers, 1972) and ABA (Friedlander, Atsmon and Galun, 1977c) to cucumbers of various sex types. The mechanism of ethylene enhanced tissue senescence have been well established by Hanson, Kende and coworkers (Chapter 2, reviewed by Mayak and Halevy, 1980).

In gynoecious buffalo gourd segregates, potentially staminate primordia may be suppressed by a supra-optimal endogenous level of ethylene. High ethylene levels may halt the formation or action of an as yet uncharacterized hormone-protein receptor complex. More likely, ethylene might prevent the formation/action of said complex by modifying
levels of other regulants (such as ABA) through their increased synthesis, metabolism or through changes in compartmentalization. Under more favorable conditions, these complexes (perhaps activated by binding with GA) may interact with the genome, triggering site-specific RNA syntheses which code for enzymes necessary during anther initiation and development (Chapter 2, see Jacobsen, 1977; Venis, 1977; Biswas and Roy, 1978; Stoddart and Venis, 1980). In gynoecious meristems, initially high levels of ethylene surrounding potentially staminate primordia may eventually cause antherless bud abortion through an autocatalytic increase in activity (Mayak and Halevy, 1980) or by interaction with increased concentrations of ABA.

Differences in the timing of antherless bud abortion on untreated gynoecious phenotypes may arise through differences in ethylene levels at the floral primordia or through differential accumulation of ethylene and/or other growth inhibitors within the developing corolla.

In monoecious buffalo gourd meristems, inherently low levels of endogenous ethylene may permit formation of the proposed hormone-protein receptor complex allowing the expression of structural genes coding for stamen development. In addition, the proliferating staminate tissue may also effect an increase in growth promoting substances (especially GAs) and thus, protect the developing bud from suppression or abortion mediated through a low inhibitor/promoter ratio.

Evidence for the masculinizing effects of applied GAs in many cucurbits has been well-documented (Table 6) and high levels of growth promoters have been implicated as being essential for floral development. Concentrations of growth promoters peaked during early floral bud
development in chrysanthemums (Jeffcoat and Cockshull, 1972) and carnation (Jeffcoat and Garrod, 1969). Also, Dathe and Sembdner (1980) uncovered high levels of GA in the developing androecium and perianth of broad bean flowers and high GA levels stimulated corolla expansion in carnation (Jeffcoat and Garrod, 1969).

Supposedly, the application of AVG to gynoecious meristems curtailed endogenous production of ethylene. Lower levels of ethylene may have subsequently permitted stamen primordia differentiation through transcription of genes not normally expressed in this genotype. As proposed for 'naturally-occurring' staminate buds, the AVG-induced staminodium may have increased the level of endogenous promoting substances, thus insuring the continued ontogenesis of the bud. As discussed above, well developed AVG-induced antherless buds may have resulted from the timing of application, or from its concentration or effectiveness at the primordia sites. Although continued corolla and stamen development may both require relatively low levels of endogenous ethylene (and perhaps, ABA), their sensitivity to inhibiting substances may differ. Levels of ethylene which halt stamen initiation may have been tolerated in corolla tissue, allowing for their continued expansion.

Hypotheses discussed above are inherently speculative and are offered as a possible explanation for sexual dimorphism under natural conditions and for experimental results obtained herein. However, alternative hypotheses may also be valid and further evidence substantiating and refuting the proposed scheme is presented below. Elucidation of control mechanisms for buffalo gourd sex expression will require further studies with exogenously-applied regulants, complete charac-
terization of differences in endogenous hormone levels among sex types and ultimately, will depend upon examination of control at the cellular level.

**Influence of an Ethylene Releasing Compound upon Growth and Floral Development**

The feminizing effects of endogenous ethylene and ethephon (an ethylene releasing compound) upon most cucurbits and the possible role of this hormone in regulating buffalo gourd floral differentiation has been reviewed in Chapter 3, Table 6 and in the previous section. Successful Induction of staminate flowers on gynoeclous plants with AVG (an ethylene antagonist) suggested high levels of ethylene to suppress stamen development in primordia of this phenotype. Attempts to substantiate the role of ethylene in staminate inhibition prompted a study of ethephon applied to monoecious plants. Upon design of Experiment VII, it was hypothesized that applied ethylene might curtail normal development of male buds and increase the number of antherless buds formed in this sex type (i.e. mimic the gynoeclous phenotype). Treatments (three dosages and control) were each applied to ten monoecious segregates; treatment effects on shoot growth and floral expression were monitored, analyzed and are reported below.

Ethephon influenced shoot growth as indicated by the simultaneous reduction in number of nodes/shoot and average length/shoot (Table 11, Appendix C). Values for both traits declined as concentrations of applied ethephon increased, with plants treated at higher dosages (i.e. >125 ppm) exhibiting similar levels of growth inhibition. For each treatment, growth rate (nodes added/day) and average internode
<table>
<thead>
<tr>
<th>Concentration</th>
<th>No. Individuals Observed</th>
<th>No. Shoots per Ind. Observed</th>
<th>No. Nodes Observed</th>
<th>Shoot Growth (cm)</th>
<th>Nodes per Shoot</th>
<th>Growth per Shoot (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 ppm</td>
<td>10</td>
<td>2</td>
<td>219</td>
<td>1161</td>
<td>11.0</td>
<td>58.1</td>
</tr>
<tr>
<td>125 ppm</td>
<td>10</td>
<td>2</td>
<td>260</td>
<td>1176</td>
<td>13.0</td>
<td>58.8</td>
</tr>
<tr>
<td>50 ppm</td>
<td>10</td>
<td>2</td>
<td>327</td>
<td>2325</td>
<td>16.4</td>
<td>116.3</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>2</td>
<td>338</td>
<td>3196</td>
<td>16.9</td>
<td>159.8</td>
</tr>
</tbody>
</table>
length/shoot were calculated from raw data. Analyses of variance for growth rate and average internode length/shoot uncovered statistical differences for all main effects; differences among individuals were judged significant whereas differences among ethephon concentrations were found to be highly significant (Table 12). Highly significant concentration X individual interactions for both traits were also apparent. Growth rate of shoots exhibited high levels of variability among individuals when treated with higher levels of ethephon (Table 13, Appendix C). Treatment means for both traits were statistically divided into two groups. Growth rate and internode lengths of shoots treated with 50 ppm ethephon displayed means statistically similar to those associated with controls (Table 13). Mean growth rate exhibited by control group in this experiment (1.2 nodes/day) was slightly higher than that displayed by control plants of Experiment VI (1.0 nodes/day), perhaps due to differences in genotype, plant age or environmental effects.

Reductions in growth rate and/or internode length have been commonly witnessed in cucurbits treated with ethephon. In andromonecious muskmelon, reductions in both traits were reported to be influenced by the dosage of ethephon applied (Lippert et al., 1972). Similar results were obtained in studies with squash (Coyne, 1970; Splittstoesser, 1970). Ethephon applications of 2000 ppm resulted in plant heights which were approximately 10% of those found in controls and moreover, significant reduction in internode length was obtained upon repeated treatment with 250 ppm ethephon. Application of this compound to monoecious cucumbers effected reductions in internode length
Table 12: Analysis of Variance Summaries for Growth Rate Parameters of Monoeclous Plants Treated with Ethephon at Various Concentrations

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sums of Squares</th>
<th>Mean Squares</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Rate of Treated Meristems</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among Individuals (Blocks)</td>
<td>9</td>
<td>2.58</td>
<td>0.287</td>
<td>2.99*</td>
</tr>
<tr>
<td>Among Ethephon Concentrations</td>
<td>3</td>
<td>2.32</td>
<td>0.773</td>
<td>8.05**</td>
</tr>
<tr>
<td>Experimental Error (Concentrations X Indlv.)</td>
<td>27</td>
<td>2.60</td>
<td>0.096</td>
<td>2.52**</td>
</tr>
<tr>
<td>Sampling Error (Among Shoots within Indlv.)</td>
<td>40</td>
<td>1.51</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>9.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Average Internode Length**             |                    |                 |              |         |
| of Treated Shoots (cm)                   |                    |                 |              |         |
| Among Individuals (Blocks)               | 9                  | 145.21          | 16.13        | 2.82*   |
| Among Ethephon Concentrations            | 3                  | 324.24          | 108.08       | 18.90** |
| Experimental Error (Concentrations X Indlv.) | 27                | 154.40          | 5.72         | 4.00**  |
| Sampling Error (Among Shoots within Indlv.) | 40                | 57.13           | 1.43         |         |
| Total                                    | 79                 | 688.98          |              |         |

*F-ratio significant at the 0.05 level
**F-ratio significant at the 0.01 level
Table 13: Means and Coefficients of Variation for Growth Rate Parameters of Monoeocious Plants Treated with Ethephon at Various Concentrations

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Growth Rate of Treated Meristem (nodes/day)</th>
<th>Internode Length of Treated Shoots (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>C.V. (%)</td>
</tr>
<tr>
<td>250 ppm</td>
<td>0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.6</td>
</tr>
<tr>
<td>125 ppm</td>
<td>0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.3</td>
</tr>
<tr>
<td>50 ppm</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.6</td>
</tr>
<tr>
<td>Control</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.7</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means designated with similar superscripts are not significantly different at the 0.05 level.
and leaf size (Shannon, 1976). In similar plant material, McMurray and Miller (1969) found a direct relationship between the promotion of female flowering and reduced growth rate among various ethephon treatments. Rodrigues and Lambeth (1972) demonstrated a reduction in internode lengths of treated monoecious cucumbers without accompanying changes in the number of nodes/plant whereas Karchi and Govers (1972) reported growth reductions and an increased number of nodes/plant in gynoecious and monoecious cucumbers treated at the onset of flowering. In contrast, Loy et al. (1979) failed to obtain significant changes in total growth of ethephon-treated watermelon.

When casually examined, the data summary for floral development in plants treated with ethephon revealed few well-defined trends (Table 14). The number of aborted buds, either antherless, staminate or pistillate, appeared to be more prevalent in plants treated with high ethephon doses. Applications of the compound also seemed to reduce both staminate and pistillate flowering. In contrast to expected results, substantial increases in well-formed antherless buds on treated plants were not realized. Antithetically, the lowest frequency of antherless buds was associated with the highest treatment level.

However, as ethephon significantly reduced the number of nodes/shoot, it also simultaneously reduced the opportunity for floral development. Therefore, in order to examine the effects of ethephon upon flowering patterns independent of its influence on growth rate, it was necessary to express the frequency of floral types as a function of the number of nodes/shoot. Analyses of variance for the percentage of blind nodes/shoot and aborted, antherless, staminate or pistillate
Table 14: Raw Data Summary for Floral Development in Monoeccious Plants Treated with Ethephon at Various Concentrations

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>250 ppm</td>
<td>20</td>
<td>219</td>
<td>86</td>
<td>107</td>
<td>2</td>
<td>96</td>
<td>14</td>
</tr>
<tr>
<td>125 ppm</td>
<td>20</td>
<td>260</td>
<td>97</td>
<td>139</td>
<td>4</td>
<td>108</td>
<td>9</td>
</tr>
<tr>
<td>50 ppm</td>
<td>20</td>
<td>327</td>
<td>63</td>
<td>179</td>
<td>5</td>
<td>117</td>
<td>26</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>338</td>
<td>23</td>
<td>116</td>
<td>5</td>
<td>175</td>
<td>42</td>
</tr>
</tbody>
</table>

\(^{1}\)Abortive buds scored secondarily as either antherless, staminate or pistillate
buds/shoot were performed as above for growth parameters (Table 15).

Highly significant differences in concentration effects associated with the percent aborted buds and blind nodes reflected ethephon inhibition of all floral development. Analyses of variance also uncovered a highly significant effect of individuals upon the percentage of blind nodes. The highly significant concentration X individual interaction for the percentage of aborted buds resulted from high levels of variability for this trait in shoots treated as controls (see discussion below).

Significant differences among means for bud development parameters confirmed ethephon suppression of flowering (Table 16). Shoots treated with higher concentrations of the regulant (250, 125 ppm) held developing floral buds at only 7-8% of the nodes surveyed whereas nearly 60% of the nodes on control shoots displayed potentially mature flowers. The frequency of bud abortion was low in control shoots (7.5%) although variability among individuals of this group was substantial. The high coefficient of variability (145.4%) resulted from the bimodal nature of this population (e.g. 9/23 aborted buds occurred on control shoots of one plant while 1/2 of control shoots exhibited no aborted buds; see Table 14 and Appendix C).

The phenomenon of bud abortion as affected by ethephon, other hormones and environmental conditions was most fully characterized by Friedlander, Atsmon and Galun (1977c) and Dax-Fuchs Atsmon and Halevy (1978). Results were conflicting but in general, ethephon enhanced bud abortion under 'winter conditions' (short days) and GA enhanced bud abortion under 'summer conditions' (long days). Bud abortion was
Table 15: Analysis of Variance Summaries for Floral Development in Monoecious Plants Treated with Ethephon at Various Concentrations

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sums of Squares</th>
<th>Mean Squares</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bud Abortion on Treated Shoots (% expressed as decimal fraction)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among Individuals (Blocks)</td>
<td>9</td>
<td>0.519</td>
<td>0.058</td>
<td>1.45ns</td>
</tr>
<tr>
<td>Among Ethephon Concentrations</td>
<td>3</td>
<td>1.607</td>
<td>0.536</td>
<td>8.05**</td>
</tr>
<tr>
<td>Experimental Error (Concentrations X Indiv.)</td>
<td>27</td>
<td>1.073</td>
<td>0.040</td>
<td>2.35**</td>
</tr>
<tr>
<td>Sampling Error (Among Shoots within Indiv.)</td>
<td>40</td>
<td>0.674</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>3.873</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Blind Nodes on Treated Shoots (% expressed as decimal fraction) | | | | |
| Among Individuals (Blocks) | 9 | 1.203 | 0.134 | 4.19** |
| Among Ethephon Concentrations | 3 | 0.481 | 0.160 | 5.00** |
| Experimental Error (Concentrations X Indiv.) | 27 | 0.863 | 0.032 | 1.28ns |
| Sampling Error (Among Shoots within Indiv.) | 40 | 1.010 | 0.025 | |
| Total | 79 | 3.557 | | |
Table 15: (Continued)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sums of Squares</th>
<th>Mean Squares</th>
<th>F-ratio</th>
</tr>
</thead>
</table>

**Antherless Bud Formation on Treated Shoots (expressed as a decimal fraction)**

- Among Individuals (Blocks) 9 0.011 0.001 1.00
- Among Ethephon Concentrations 3 0.001 <0.001 <1.00
- Experimental Error (Concentrations X Indiv.) 27 0.026 0.001 1.00
- Sampling Error (Among Shoots within Indiv.) 40 0.036 0.001
- Total 79 0.074

**Staminate Nodes on Treated Shoots (expressed as a decimal fraction)**

- Among Individuals (Blocks) 9 1.052 0.117 3.90
- Among Ethephon Concentrations 3 0.240 0.080 2.67
- Experimental Error (Concentrations X Indiv.) 27 0.799 0.030 1.36
- Sampling Error (Among Shoots within Indiv.) 40 0.895 0.022
- Total 79 2.986
Table 15: (Continued)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sums of Squares</th>
<th>Mean Squares</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pistillate Nodes on Treated Shoots (% expressed as decimal fraction)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among Individuals (Blocks)</td>
<td>9</td>
<td>0.109</td>
<td>0.012</td>
<td>2.40*</td>
</tr>
<tr>
<td>Among Ethephon Concentrations</td>
<td>3</td>
<td>0.099</td>
<td>0.033</td>
<td>6.60**</td>
</tr>
<tr>
<td>Experimental Error (Concentrations X Indiv.)</td>
<td>27</td>
<td>0.142</td>
<td>0.005</td>
<td>1.25ns</td>
</tr>
<tr>
<td>Sampling Error (Among Shoots within Indiv.)</td>
<td>40</td>
<td>0.148</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>0.498</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

nsF-ratio not significant at the 0.05 level

*F-ratio significant at the 0.05 level

**F-ratio significant at the 0.01 level
Table 16: Means and Coefficients of Variation for Bud Development Phenomena in Monoeclous Plants Treated with Ethephon at Various Concentrations

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Aborted Buds (% nodes/shoot)</th>
<th>Blind Nodes (% nodes/shoot)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>C.V. (%)</td>
</tr>
<tr>
<td>250 ppm</td>
<td>41.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.8</td>
</tr>
<tr>
<td>125 ppm</td>
<td>40.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.9</td>
</tr>
<tr>
<td>50 ppm</td>
<td>20.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.7</td>
</tr>
<tr>
<td>Control</td>
<td>7.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>145.4</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>Means designated with similar superscripts are not significantly different at the 0.05 level.
thought to be exacerbated by supraoptimal hormone levels resulting from application of compounds presumed to already be at optimum endogenous concentration. ABA effected bud abortion differentially among sex types, promoting the phenomenon in monoecious cultivars and suppressing it in gynoecious phenotypes. Results also suggested certain conditions of hormone treatment and/or environment to be optimal for differentiation of staminate or pistillate flowers while at the same time, supraoptimal or suboptimal for continued floral development.

In general, aborted buds occurred most frequently at nodes closest to the point of treatment initiation and nodes proximal to the shoot meristem were more likely to be blind (Appendix C). Aborted buds may have resulted from the eventual termination of developing tissues present in the meristem at the time of first or second treatment; blind nodes may have reflected the inhibition of floral primordia at nodes differentiated while under treatment influence. However, a high percentage of blind nodes was also realized on control shoots. In a previous study, blind nodes were prevalent on monoecious phenotypes late in the growing season following a long sequence of staminate-bearing nodes (Yousef, 1976). Two weeks following termination of Experiment VII, flowering of all plants in the field waned (H.M. Scheerens, personal communication), perhaps due to decreasing daylengths and/or other environmental factors (Chapter 3). If environmental effects were present, they may have resulted in a higher frequency of blind nodes in controls than would have been exhibited during previous months. Environmental conditions adverse to flowering may also have confounded the effects of treatment on the suppression of floral buds or primordia.
Most likely, blind nodes held potentially staminate floral primordia or under different conditions, might have developed antherless buds. Blind nodes were less apt to hold potentially pistillate primordia as pistillate buds were evident in many replicates, even when flanked by a series of blind nodes (Appendix C).

As in buffalo gourd studies, Christopher and Loy (1982) also evinced the inhibition of all flower development and proliferation of blind nodes in ethephon-treated monoecious watermelon, even at low doses (15 and 30 ppm). Delay in the onset of flowering (either male or female) and increased frequency of blind nodes were also found in andro monoecious muskmelon (Loy, 1971), monoecious cucumber (Iwahori, Lyons and Smith, 1970; George, 1971) and in squash (Coyne, 1970; Splitstoesser, 1970) at high application rates or after repeated treatments. George (1971) scored an increase in frequency (25-38%) of blind nodes on several monoecious cucumber varieties after treatment with ethephon. Levels of floral bud suppression were even more pronounced when increased frequencies of aborted buds were considered. This author suggested floral buds of this species to be extremely sensitive to ethephon during the early stages of development.

As was indicated by the raw data, statistical analysis of antherless bud frequency revealed no significant individual or concentration effects (Table 15). Extremely high coefficients of variability for antherless bud formation among treatments could again be attributed to bimodality (Table 17, Appendix C). The existence of antherless buds on control shoots was reminiscent of that reported to occur naturally on monoecious phenotypes (Dossey, Bemis and Scheerens, 1981).
Table 17: Means and Coefficients of Variation for Staminate, Pistillate and Antherless Floral Development in Monoecious Plants Treated with Ethephon at Various Concentrations

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Antherless Buds (% nodes/shoot)</th>
<th>Staminate Nodes (% nodes/shoot)</th>
<th>Pistillate Nodes (% nodes/shoot)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean C.V. (%)</td>
<td>Mean C.V. (%)</td>
<td>Mean C.V. (%)</td>
</tr>
<tr>
<td>250 ppm</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt; 324.4</td>
<td>43.3&lt;sup&gt;a&lt;/sup&gt; 55.3</td>
<td>5.4&lt;sup&gt;bc&lt;/sup&gt; 135.6</td>
</tr>
<tr>
<td>125 ppm</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt; 263.6</td>
<td>43.3&lt;sup&gt;a&lt;/sup&gt; 38.0</td>
<td>2.8&lt;sup&gt;b&lt;/sup&gt; 202.9</td>
</tr>
<tr>
<td>50 ppm</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt; 211.0</td>
<td>35.9&lt;sup&gt;a&lt;/sup&gt; 40.2</td>
<td>7.9&lt;sup&gt;cd&lt;/sup&gt; 91.8</td>
</tr>
<tr>
<td>Control</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt; 221.6</td>
<td>51.3&lt;sup&gt;a&lt;/sup&gt; 38.9</td>
<td>12.3&lt;sup&gt;d&lt;/sup&gt; 68.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means not statistically compared as a consequence of non-significant F-ratio

<sup>b,c,d</sup>Means designated with similar superscripts are not significantly different at the 0.05 level
Individuals differed significantly in their production of staminate-bearing nodes; treatments had no significant effect on the frequency of this flower type (Table 15). Although not compared statistically, the mean percentage of staminate nodes was higher in the control group than that attributed to various treatments. However, ethephon inhibition of floral development might have suppressed a substantial portion of potentially staminate buds in treated shoots and when blind and staminate nodes are considered in conjunction, the trend of increased masculinity with decreased ethephon dose is reversed.

Significant treatment and individual effects on pistillate flowering were apparent (Table 15), with the control group exhibiting a greater mean pistillate frequency than those found on shoots treated at high ethephon levels (Table 17). The inhibitory effect of ethephon on female flowering may have reflected the overall suppression of flower primordia or may have been independent of and/or in addition to its action on primordia suppression.

The apparent reduction in femininity associated with ethephon-treated monoecious buffalo gourd is antithetical to ethephon-induced sex modification of most other cucurbits. Reductions in sex ratio (male/female) are commonly reported for monoecious cucumber (McMurray and Miller, 1969; Rudich Halevy and Kedar, 1969; Freytag, Lira and Isleib, 1970; Iwahori, Lyons and Smith, 1970; Cantiliffe and Robinson, 1971; George, 1971; Karchi and Govers, 1972; Rodrigues and Lambeth, 1972; Shannon, 1976; Friedlander, Atsmon and Galun, 1977c; Dax-Fuchs, Atsmon and Halevy, 1978), and often result from simultaneous promotion of pistillate flowers and inhibition of staminate flowers. Increased
femininity upon treatment is apparently influenced by background genotypes (George, 1971).

Similar results are described for ethephon-treated hermaphroditic, and andromonoecious muskmelon (Karchl, 1970; Anals, 1971; Loy 1971; Lippert et al., 1972; Shimotsuma and Jones, 1972) through conversion of potentially staminate buds to hermaphroditic buds or by the induction of pistillate flowers. Reports also suggest ethephon to increase femaleness in various cultivated species of Cucurbita (Rudich, Halevy and Kedar, 1969; Coyne, 1970; Splittstoesser, 1970; Hopping and Hawthorne, 1979), and Lagararia (Sharma, Jyotlshl and Agrawal, 1980).

However, ethephon-treated gynoeclous cucumbers revealed alternate trends in sex modification. Application of the regulant either reduced pistillate flower formation (Friedlander, Atsmon and Galun, 1977c) or had no effect on floral development (Karchl and Govers, 1972; Augustine, Baker and Sell, 1973). In addition, Christopher and Loy (1982) noted the complete suppression of female development in monoecious watermelon treated with modest doses (60-120 ppm) of ethephon. These authors stated that: "In comparison to other cucurbits in which ethephon levels of 250 to 500 ppm are optimum for promoting pistillate flowering, watermelon appears sensitive to much lower concentrations of applied ethylene, and ethylene affects flowering in an opposite manner".

In summary, results obtained in Experiment VII did not oppose nor lend credence to hypotheses concerning endogenous regulation of staminate flowering in buffalo gourd as discussed above. Increased antherless bud formation was not evinced on monoecious shoots treated
with ethephon, resulting perhaps, from ethephon-induced suppression of all floral differentiation. The levels of applied regulant may have effected internal concentrations of ethylene which were supraoptimal. In addition, high background frequency of blind nodes displayed by control shoots suggested the confounding effects of environment (most likely daylength, see Chapter 3). From results obtained, it was impossible to speculate about the potential antherless/staminate ratio which might have been realized had the experiment been repeated at different dosages or under different environmental conditions. Evidence of ethephon-induced formation of antherless buds on monoecious meristems cultured in-vitro is submitted in Chapter 5.

In Experiment VII, ethephon appeared to suppress female flower differentiation or development. This phenomenon, which is contrary to results obtained with most monoecious cucurbits, may also have been caused by supraoptimal endogenous ethylene levels mediated in part by treatment and environment. Trends may be reversed upon experimentation with lower ethephon doses or under different environmental conditions.

Data gathered from Experiment VII yielded strong (but indirect) evidence for ethylene-enhanced floral bud abortion in this species. Also, drastic reductions in growth rate and internode length of treated monoecious plants in the absence of sex type reversal again suggested the control of sexual dimorphism to be independent of plant growth habits.
Influence of Other Growth Regulants upon Growth and Floral Development

During Experiments III - V, no noticeable changes in sex expression were manifested following applications of IAA, ABA, steroids or glyphosate. Failure of these compounds to affect sex expression may have been precipitated by inadequate concentrations of applied regulators, their lack of absorption through epidermal layers, their inhibited transportation to differentiating primordia or by their inability to effect regulatory changes in primordia tissues. Although applications of GAs often resulted in abnormal elongation of plant parts, they were ineffective in modifying sex expression except perhaps, under the conditions of Experiment II.

Treatment of seedlings with high concentrations of GA$_3$ or GA$_{4+7}$ caused an apparent deviation in the expected 1:1 sex ratio within a segregating population (Table 18). Foliar application of GA$_{4+7}$ at 500 ppm resulted in the greatest deviation from the predicted ratio. Significantly reduced levels of staminate segregates scored following this treatment and after application of GA$_3$ at 5000 ppm may have emanated from direct inhibition of staminate floral buds. However, since plants were scored for sex type prior to the onset of the female flowering phase, changes in the sex ratio may have also reflected a general suppression of all floral tissue. The lack of GA-induced sex modification at similar and lower dosages applied to flowering plants of either sex type (Experiments III - V) substantiated this latter supposition. Dax-Fuchs, Atsmon and Halevy (1978, citing Atsmon and Porath) reported supraoptimal GA concentrations to inhibit male flowering and extend the vegetative phase of monoecious cucumbers. These authors also
Table 18: Chi-square Analysis of Sex Type Ratios Exhibited by Segregating Populations Treated with Gibberellins at Various Concentrations

<table>
<thead>
<tr>
<th>Treatment and Concentration</th>
<th>Monoecious Plants</th>
<th>Gynoecious Plants&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Degrees of Freedom</th>
<th>Probability Range</th>
<th>Chi-square Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 ppm GA&lt;sub&gt;4+7&lt;/sub&gt;</td>
<td>50 16</td>
<td>50 84</td>
<td>1</td>
<td>&lt;0.01</td>
<td>46.24</td>
</tr>
<tr>
<td>5000 ppm GA&lt;sub&gt;3&lt;/sub&gt;</td>
<td>50 39</td>
<td>50 61</td>
<td>1</td>
<td>0.05-0.02</td>
<td>4.84</td>
</tr>
<tr>
<td>2500 ppm GA&lt;sub&gt;3&lt;/sub&gt;</td>
<td>50 41</td>
<td>50 59</td>
<td>1</td>
<td>0.10-0.05</td>
<td>3.24</td>
</tr>
<tr>
<td>1000 ppm GA&lt;sub&gt;3&lt;/sub&gt;</td>
<td>50 42</td>
<td>50 58</td>
<td>1</td>
<td>0.20-0.10</td>
<td>2.56</td>
</tr>
<tr>
<td>Control</td>
<td>50 49</td>
<td>50 51</td>
<td>1</td>
<td>0.50-0.95</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<sup>1</sup>Plants not exhibiting staminate floral development were considered potentially gynoecious (see text)
GA treatments are known to promote male bud formation and development, especially in female genotypes or under female-promoting environmental conditions, but this same hormonal treatment, as well as male-promoting environmental conditions, inhibited floral bud formation when superimposed on plants with pre-existing strong male tendency. In our experiments, CEPA-treatments [ethephon treatments] increased the proportion of aborted female buds in winter-grown gynoecious plants, while GA treatments increased abortion of male buds in summer-grown monoecious plants; both cases may be considered as optimal for differentiation but supraoptimal for floral development.

Although increased female tendency has been reported in GA-treated muskmelon (El-Kholy and Hafez, 1982), Luffa acutangula (Bose and Nitsch, 1970) and Momordica charantia (Ghosh and Basu, 1983a), the evidence for increased masculinization of cucurbits through application of GAs is overwhelming (Chapter 3, Table 6). GA treatment commonly induces staminate buds on cucumber and muskmelon sex types normally devoid of male flowers. Applied GA also increases sex ratio and node number in various species of Cucurbita.

In early studies, staminate primordia differentiation and bud development was thought to be moderated, in part, by interaction of endogenous GA and ethylene; the latter compound was believed to act directly as an anti-gibberellin (Rudich, Halevy and Kedar, 1969). The loss of GA activity and the recovery of high GA inhibitor levels in extracts of ethephon-treated monoecious cucumber substantiated evidence for the anti-gibberellin activity of ethylene (Rudich, Halevy and Kedar, 1972b). However, independent sites of action for the two hormones were indicated by additional studies (see Chapter 3; Iwahori, Lyons and Smith, 1970; Loy, 1971; Rodrigues and Lambeth, 1972; Atsmon and Tabbak, 1979, Ghosh and Basu, 1983a). After comparing the masculinizing action
of GA, and anti-ethylene agents (AVG and silver nitrate), Atsmon and Tabbak (1979) stated:

Since GA did not affect ethylene evolution [as did the other two regulants], we assume that it induces staminate flower formation in gynoecious cucumbers through a basically different, as yet unknown, mechanism. This is reflected also in a different developmental pattern of the GA-treated plants: a) the inhibition of any bud development in the lower nodes, resulting in 'blind' nodes, which have not been observed for the other two chemicals [AVG and silver nitrate], and b) the lack of perfect flowers, as a transition between the natural pistillate and the induced staminate ones. . . . Also, our observations indicate that while GA does not convert predetermined pistillate buds into staminate ones, the anti-ethylene agents seem to convert pistillate buds into perfect or staminate ones.

Despite masculinizing trends found in other species of Cucurbita (Splittstoesser, 1970; Krishnamoorthy and Sandooja, 1980), five attempts (Experiments I - V) to form staminate buds on gynoecious buffalo gourd by exogenous application of GA were unsuccessful. The failure could be interpreted as indicating GA's relatively minor role in the formation of male flowers in this species. However, it may also suggest that high concentrations of ethylene inhibit staminate primordia at metabolic steps preceding those enhanced by optimum GA levels. For example, ethylene may block the formation of essential protein receptors recognized by GA and/or prevent hormone-protein binding. Under this speculative scheme, correcting possible endogenous GA deficiencies through exogenous application would have little influence on the proliferation of staminate tissue.

Potential Use of Exogenously-Applied Hormones in Breeding Programs, Genetic Studies and for Commercial Production of Hybrid Seed

Although the masculinizing effects of AVG in buffalo gourd have been elucidated very recently, their beneficial use in genetic studies
and breeding efforts are currently being realized. During the 1984 season, treatment of gynoeclous plants with this regulant permitted their self-pollination, thus facilitating advances in determination of heritability for seed oil quantity (H.M. Scheerens, personal communication) and increased carpel number (A.E. Ralowicz, personal communication). In addition, sex ratios of progeny from self- and cross-pollinations among gynoeclous phenotypes made during the previous season substantiated the proposed mode of inheritance for the gynoeclous trait (Table 19) (Dossey, Bemis and Scheerens, 1978). All lines exhibited segregation patterns which suggested the probability of inheritance by the proposed monogenic scheme (Chapters 1 and 3); analysis of the composited data yielded a relatively low chi-square value (0.39; $P = 0.75-0.50$). Additional evaluation of selfcrosses and testcrosses made among gynoeclous progeny should indicate segregates which are homozygous at the M locus, providing for initial development of purely gynoeclous lines.

The benefit of AVG-induced staminate flowering for advancement in breeding and genetic studies is unquestionable; however, its potential use for commercial production of $F_1$ hybrid buffalo gourd seed is doubtful within the near future. The retail value of this regulant [approximately $(US)4.00/mg$] reflects, perhaps, its experimental nature and may limit its use on a wide scale for hybrid seed production. This may be especially true for agronomic crops such as buffalo gourd which are cultured at high seeding rates and exhibit a potentially low monetary return/plant.
Table 19: Chi-square Analysis of Sex Type Ratios Exhibited by Progeny of a Self-pollinated Gynoeclous Plant and by Progeny of Gynoeclous X Gynoeclous Crosses

<table>
<thead>
<tr>
<th>Self-/Cross-Pollination</th>
<th>Progeny Number</th>
<th>Monoecious Progeny</th>
<th>Gynoeclous Progeny</th>
<th>Degrees of Freedom</th>
<th>Probability Range</th>
<th>Chi-square Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self # 1</td>
<td>29</td>
<td>7.25</td>
<td>8</td>
<td>21.75</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Cross # 1</td>
<td>15</td>
<td>3.75</td>
<td>2</td>
<td>11.25</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Cross # 2</td>
<td>10</td>
<td>2.50</td>
<td>1</td>
<td>7.50</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Composite</td>
<td>54</td>
<td>13.50</td>
<td>11</td>
<td>40.50</td>
<td>43</td>
<td>1</td>
</tr>
</tbody>
</table>
CHAPTER 5

IN-VITRO FLORAL DEVELOPMENT IN TREATED AND UNTREATED BUFFALO GOURD MERISTEMS

In-vitro culture of meristems permits the close scrutiny of floral development in a chemically precise and well-defined environment which is radically different than that experienced in the field (Nitsch, 1972). Comparison of ontogenetic events in treated and untreated explants and contrast to floral development on similarly treated field-grown meristems may provide useful insights pertaining to hormonal regulation of sex expression. Buffalo gourd floral development in-vitro was monitored during two experiments as summarized in Table 20. The first experiment was designed to indicate growth regulators which would successfully alter floral development in either sex type; the second study was undertaken to quantify the effects of active agents indicated in the preceding study. Main objectives of these investigations included clarification of ethylene's role in staminate floral development and affirmation of GAs' inability to effect staminate induction.

Demonstrating the feasibility of in-vitro techniques for studies of buffalo gourd floral ontogeny constituted an additional objective. Previous studies of in-vitro flowering of cucurbits were summarized in Chapter 3.

Preliminary Investigations of In-vitro Culture

Prior to execution of Experiments VIII and IX, several preliminary investigations were necessary to identify the most appropriate
Table 20: Experimental Methodology and Rationale for Meristem Culture In-vitro

<table>
<thead>
<tr>
<th>Experiment Initiation No.</th>
<th>Date</th>
<th>Treatments Applied</th>
<th>No. Plants Treated</th>
<th>Application Method</th>
<th>Experimental Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIII</td>
<td>06/01/84</td>
<td>To meristems of gynoeclous and monoclous plants which have been excised, trimmed and surface-sterilized with sodium hypochlorite</td>
<td>8 8</td>
<td>Trts. applied originally as a meristem soak (5 min) prior to culturing in media. Second trt. applied dropwise (0.5 ml) to meristems of developing explants on 06/16/84. Run-off gradually absorbed into media</td>
<td>Experiment designed to qualitatively compare the effects of various growth regulators upon floral development in explants vs. their effects on sex expression in field-grown plants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>8 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 ppm GA_4+7</td>
<td>8 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 ppm sili. nitr.</td>
<td>8 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 ppm AVG</td>
<td>8 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 ppm ethephon</td>
<td>8 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>09/17/84</td>
<td>To meristems of both sex types prepared as in experiment VIII.</td>
<td>10 10</td>
<td>Trts. applied as in experiment VIII. Second treatment applied 10/03/84</td>
<td>Experiment designed to quantitatively determine effects of sili. nitr. and ethephon dosages on floral development of meristems cultured in-vitro</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>10 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 ppm sili. nitr.</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 ppm sili. nitr.</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 ppm sili. nitr.</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 ppm ethephon</td>
<td>10</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>50 ppm ethephon</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 ppm ethephon</td>
<td>10</td>
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</tr>
</tbody>
</table>
media and culturing techniques. Culture of buffalo gourd meristems was first attempted using White's medium (Henderson, Durrell and Bonner (1952), but without added growth regulants. Galun, Jung and Lang (1962, 1963) considered this formula to be adequate but not necessarily option­al for growth of cucumber floral buds. However, due to high levels of contamination in cultures (perhaps exacerbated by high levels of added casein and amino acids), buffalo gourd meristems failed to thrive in this medium. More favorable results were obtained when tissues were cultured in Murashige and Skoog medium (Murashige and Skoog, 1962) as suggested by Rute, Butenko and Maurinya (1982). Due to the nature of the experiments performed, the formulation of culture stock was further modified to omit basal levels of growth regulants and to increase phosphorus content by incorporation of major salts as suggested by Miller (1963).

As with the search for an adequate medium for Experiments VIII and IX, the most appropriate tissues for culture were identified by trial and error. First, staminate induction in large antherless buds (>0.5 cm in length) excised from gynoecious individuals and cultured under various growth regulant treatments was attempted without much success. Galun, Jung and Lang (1962, 1963) and Blake (1969) used similar techniques to study floral ontogeny in cucumber and Viscaria re­spectively. Failure to stimulate stamen development was considered to support the hypothesis of irreversible quiescence in staminate primordia when bud growth surpasses a critical morphological stage (Chapter 4). Next, much smaller staminate or antherless buds (<0 mm in length) and subtending stem sections containing nodal tissue were excised from
monoeclous and gynoeclous plants respectively, treated with various
growth regulants and evaluated in culture. In general, these buds
exhibited poor development and gradually senesced. Again, microscopic
examination revealed treatments to have little effect on sex expression
of senescent buds, upholding the theory of irreversible staminate sup­
pression at the primordial stage. Prior to senescence, a few stem
sections developed root and shoot initials or adventitious floral buds. However, adventitious buds failed to develop adequately for examination
of sexual development.

Terminal meristematic regions of the shoot were found to be the
most appropriate buffalo gourd tissue for studies of In-vitro flowering.
In preliminary trials, this material responded well to culture. A high
percentage of explants developed roots, axillary shoots, and leaves in
addition to exhibiting continued growth of the original meristem. Newly
developed tissue often appeared elongated and in some cases, swelling or
callus formation was evident in the basal region of the original shoot.

Materials and Methods

The information obtained in preliminary trials concerning in­
vitro culture of buffalo gourd was employed in the planning and execu­
tion of Experiments VIII and IX discussed below.

Plant Materials

Terminal meristematic regions of buffalo gourd shoots (3-5 cm in
length) were excised from gynoeclous and monoeclous colonies of SYN-1
stock grown in a greenhouse at the Campus Agricultural Center. Colonies
were established from second-season roots previously field-grown and
sexed. Greenhouse-grown meristems were employed as preliminary investigations demonstrated use of these materials to result in fewer contaminated cultures than did explants obtained directly from the field. Shoot tips were placed in polyethylene bags and immediately transported to a tissue culture laboratory on the Main Campus.

In the laboratory, sheared surface of each shoot tip was recut leaving approximately 2 cm of tissue for culture. In addition, tendrils and most developing leaves which envelope the meristematic region were removed. Trimmed tissues awaiting treatment with growth regulators and eventual culture were bathed in deionized H$_2$O to prevent dessication. Meristems prepared for Experiment VIII are pictured in Figure 16.

Trimmed tissues were placed in a laminar-flow hood (designed for execution of sterile techniques) and soaked in a 10% Chlorox solution [10 parts of a 19% sodium hypochlorite solution diluted with 90 parts sterilized (autoclaved) H$_2$O containing a small amount of surfactant (polysorbate 20)] for a 10 minute period. Tissues were then drained, rinsed in sterilized H$_2$O for 10 minutes to remove bleach and treated with various growth regulators following schemes presented in Table 20.

Preparation of Culture Medium

Results of preliminary investigations suggested the use of Murashige and Skoog (1962) media formulations modified through the elimination of added growth regulators and the substitution of major salt components for those providing higher levels of phosphorus (Miller, 1963). Major and minor constituents of the medium used in Experiments VIII and IX are listed in Table 21.
Figure 16: Meristems Prepared for Treatment Prior to In-vitro Culture
Table 21: Tissue Culture Medium Constituents

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Major Salts</td>
<td>NH₄NO₃ 1.0</td>
<td>Minor Salts</td>
<td>H₂BO₃ 6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KN0₃ 1.0</td>
<td></td>
<td>MnSO₄.4H₂O 22.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ca(NO₃)₂.4H₂O 0.5</td>
<td></td>
<td>ZnSO₄.7H₂O 8.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MgSO₄.7H₂O 0.073</td>
<td></td>
<td>Na₂MoO₄.2H₂O 0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KH₂PO₄ 0.3</td>
<td></td>
<td>CuSO₄.5H₂O 0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sucrose 30.0</td>
<td>Vitamins</td>
<td>CoCl₂.6H₂O 0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inositol 1</td>
<td>Thiamine HCl</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacto Agar (Difco) 8.0</td>
<td>Pyridoxine HCl</td>
<td></td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nicotinic acid</td>
<td></td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fe/EDTA</td>
<td>FeSO₄.7H₂O 27.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EDTA 37.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>KI 0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glycine 20.0</td>
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</tbody>
</table>
Medium was prepared in 1.5 l batches. Appropriate amounts of each stock solution and other constituents (except agar) were combined, brought to volume with deionized H₂O and adjusted to pH 7.5 with dilute KOH. Agar was then added and the resulting suspension was autoclaved for approximately 5 minutes to solubilize all components. Approximately 15-20 ml of hot medium was transferred to each of 80 culture tubes (2.5 X 15 cm). Tubes were capped, autoclaved for 20 minutes (121°C) to sterilize medium, cooled and stored at room temperature until used.

Preparation of Growth Regulants

Sources of growth regulants and their storage conditions have been described in Chapter 4. Aqueous solutions of regulants at concentrations listed in Table 20 (0.05% polysorbate 80 added as surfactant) were prepared and sterilized just prior to use. Solutions were sterilized in a laminar-flow hood by passage through a Millipore GS, 0.22μm filter (Millipore Inc., Bedford MA) and collected in sterilized vessels.

Treatment and Culture of Meristems

Growth regulants were exogenously-applied to tissues twice during Experiments VIII and IX. Initially, treatments were accomplished by soaking surface-sterilized meristems in filter-sterilized regulant solutions for 5 min just prior to transferring them to culture tubes. Using sterile techniques, the basal portion of shoot explants was embedded in media far enough to support the treated meristems. Tubes were recapped and placed in a culture room under continuous light (200μE·sec⁻²) at 25°C (culture conditions—personal communication, J.C. Thomas). Two weeks after experiment initiation, a second application of
regulants (0.5 ml) was administered dropwise to shoot tips under a laminar-flow hood using aseptic techniques. Explants were returned to the culture room where solution was gradually absorbed by the media. Explants were allowed to develop 24 days (Experiment VIII) or 34 days (Experiment IX) after administration of the second treatment before developing buds were surveyed for sex expression.

**Determination of Treatment Effects**

Experiments VIII and IX were terminated as cultures began to decline, allowing a maximum time period for bud development. Explants were removed from culture tubes and examined for general vigor, development exhibited in the original meristems and floral buds, proliferation of adventitious tissues (axillary shoots, roots and/or floral buds) and morphological abnormalities. Both newly developed floral buds of adequate size and remaining buds in-situ at experiment initiation were further examined for the differentiation of sex organs under a dissecting microscope at 10-40 X powers.

**Results and Discussion**

Blake (1969), de Fossard (1974) and Scorza (1982) all champion the use of excised meristems or buds essentially devoid of additional (and possibly interacting) tissues for the study of in-vitro floral development. However, due to the architecture of buffalo gourd meristems and the precocious determination of sexual characteristics in floral buds of this species, in-vitro culture was more easily accomplished using the entire meristematic region of shoot apices. Similar techniques were employed by Rute, Butenko and Maurinya (1982) to study
the effects of growth regulants on sexual differentiation in in-situ floral buds of cucumber.

Plant materials were trimmed severely for two reasons. First, as removal of dust and other particulates accumulated on heavily pubescent tissues was difficult, excision of most exterior tissue substantially decreased the probability of bacterial or fungal contaminants in in-vitro cultures. Second, removal of most immature leaves enveloping the meristematic region may have reduced possible interaction of endogenous hormones (produced at high levels in these tissues) with exogenously-applied growth regulants at the time of initial treatment. It was thought that advantages gained through trimming meristems would offset possible confounding effects of increased ethylene production in response to tissue wounding (Abeles, 1973). Latter development in floral buds was undoubtedly influenced by proliferation of root, shoot and leaf tissues evinced in most cultures (described below).

Morphological Development In Explants

In general, morphological characteristics of comparably treated explants were similar in both experiments. Moreover, morphological response to exogenously-applied regulants was similar among sex types. Development of explants was monitored most closely in Experiment VIII. Photographs of plantlets taken upon termination of the experiment are presented in Figure 17.

Development of Adventitious Roots. The prolific initiation of adventitious roots in cultured buffalo gourd explants mirrored the species' propensity for rooting under appropriate field conditions. As
Figure 17: Meristems Excised from Monoecious and Gynoecious Plants, Treated with Various Growth Regulants and Cultured In-vitro

Gynoecious Meristems
A: Control: Note well-formed adventitious roots including those developed aerally. Also, note general vigor of explant.
B: GA_{4+7} (50 ppm): Note elongation of plant parts.
C: AgNO₃ (50 ppm): Note leaf chlorosis.
D: Ethephon (50 ppm): Note meristem necrosis, bud abortion and lack of adventitious root development

Monoecious Meristems
E: Control: Note well-formed adventitious roots (including aerial roots), and general explant vigor.
F: GA_{4+7} (50 ppm): Note shoot and leaf elongation. Note also anthesis of buds in-situ prior to treatment.
G: AgNO₃ (50 ppm): Chlorosis also present in monoecious explants, but not evident in this illustration.
H: AVG (50 ppm): Note lack of adventitious root formation, lack of shoot proliferation and leaf chlorosis.
I: Ethephon (50 ppm): Note meristem necrosis, bud abortion and general lack of explant vigor.
this phenomenon was realized in tissues supported on media devoid of auxins or other added growth regualnts known to enhance root initiation, the high percentage of rooted explants suggested high levels of endogenous IAA or ethylene associated with the shoot meristematic region (see Chapter 2, Table 2). In early phases of preliminary experiments, untreated, cultured explants from gynoecious shoots exhibited a greater capacity for adventitious root initiation (50% rooting) than did explants from monoecious stock (19% rooting). However, this discrepancy lessened somewhat as the preliminary experiment progressed and similar trends were not witnessed in either Experiment VIII or IX. From data obtained in preliminary experiments and extant knowledge of endogenous hormonal levels among cucurbits of various sex types (Chapter 3), it was tempting to speculate concerning differences in IAA content of gynoe­cious and monoecious meristems. Further experimentation including chemical characterization of endogenous regulants will be necessary before validity of the speculation can be confirmed or refuted.

During Experiments VIII and XI, rooting was most prolific in control explants (Figure 17A,E) where root initials developed not only basal regions, but also in aerial portions of the shoot. Roots were conspicuously absent in cultures treated with AVG (Figure 17H) and marginally developed in most ethephon-treated explants (Figure 17D,I).

Development of Shoots. Two types of shoot growth were evident in buffalo gourd cultures: proliferation of existing apical meristems and initiation of lateral shoots, often following the necrosis of terminal buds. Most original shoot apices continued to expand in culture, espe-
cially those within the control and AgNO₃-treated groups (Figure 17A, C, E, G). Senescence of original apices commonly occurred in explants treated with ethephon (Figure 17D, I) whereas lack of development was associated with AVG-treated tissues. These trends were not astonishing considering the reputed role of ethylene in tissue maturation (Chapters 2 and 3) and the widespread horticultural use of ethephon and AVG to enhance or retard senescence in fruits and vegetables respectively. Development of lateral shoots occurred intermittently among explants but was absent from AgNO₃- and AVG-treated groups (Figure 17C, G, H).

Shoots were elongated, especially those treated with GA. Universal, shoots developed leaves which were malformed to various degrees depending upon treatment and/or explant genotype. Chlorosis was evident in leaves of AgNO₃-treated explants which resembled classic symptoms of iron deficiency (Figure 17C). General chlorosis of AVG-treated explants (Figure 17H) might have been expected from field studies using this regulant (Chapter 4) and from previously published information (Owens, Peterson and Tolla, 1980; Owens, Tolla and Peterson, 1980; Christopher and Loy, 1982).

Development of Floral Buds. Floral buds visibly evident at experiment initiation developed and/or gradually senesced. Buds differentiated prior to regulant application remained viable longest on control, AgNO₃ or AVG cultures and aborted soon after treatment in cultures incubated with ethephon. After treatment with GA₄+7, buds in-situ tended to swell and pucker. In preliminary experiments with cul-tured, antherless floral buds, this treatment commonly effected pre-mature anthesis. Blake (1969) noticed enhancement of corolla develop-
ment and cell expansion in *Viscaria* buds after treatment with GA$_3$. In addition, GA hastened anthesis in *Pharbitis nil* (Kalhara and Takimoto, 1983) and induced the process in normally cleistogamous flowers of *Lamium amplexicaule* (Lord and Mayers, 1982).

Shoots developed post-treatment produced floral buds in some instances, which were of interest to the study of staminate induction and ontogeny as affected by added growth regulants. However, these buds were generally minute at the time of experiment termination and in some cases were too underdeveloped and/or undifferentiated for microscopic examination of sex expression (Tables 22, 23). Reduced levels of floral induction occurred during Experiment IX than in the previous experiment reflecting, perhaps, differences in endogenous growth regulants at the time of tissue excision from parent populations. Shoot apices harvested on 06/01/84 (Experiment VIII) exhibited developing floral buds prior to treatment (Figure 16) indicating the presence of a hormone complement conducive to flowering. Conversely, many apices harvested on 09/17/84 (Experiment IX) lacked floral buds in-situ. The lack of developing buds near apical meristems suggested floral induction in these tissues to be inhibited by an unfavorable hormonal environment, perhaps mediated by reduced daylength or light intensity. Empirical evidence for environmental influence upon buffalo gourd flowering was offered in Chapters 3 and 4. Apparently, medium formulation and/or culture environment were inadequate to induce flowering of these apices in-vitro. In-vitro induction has been demonstrated in relatively few species and then, only under exacting culture conditions (Chapter 3).
Sex Expression of Developing Floral Buds

Staminate development (or the lack of it) was observed in all buds large enough for excision and microscopic examination (Tables 22 and 23). In general, fewer buds of adequate size were available on shoots developed during culture than were present in-situ upon experiment initiation. Even though most buds of the latter type gradually senesced, bud tissues remained hydrated, permitting examination of staminate development. All buds of this type were unaffected by treatment (i.e. buds on gynoeclous explants remained antherless whereas those on their monoeclous counterparts continued to proliferate stamens). Similarly, in field-grown plants, continued staminate inhibition was evident in buds developed beyond the primordial stage regardless of exogenously-applied regulants (Chapter 4, Experiments V and VI).

Staminate proliferation in floral buds developed post-treatment appeared to be influenced by both AgNO₃ and ethephon (Table 22), but experimental results were less than conclusive. Although shown to be ineffective for staminate induction in field experiments (Chapter 4), AgNO₃ application [which inhibits the action of ethylene at the binding site (Beyer, 1976a,b; Kende et al., 1982)] resulted in formation of stamens in two of six buds excised from gynoeclous explants. One of these buds contained a well formed staminate structure whereas the other exhibited development of a single stamen initial in addition to the vestigial and possibly aborting staminate tissues also evident. Confirmation of in-vitro staminate induction by AgNO₃ treatment was not evinced in Experiment IX (Table 23), however, only a single bud of sufficient development was available for examination. From these preliminary data,
Table 22: Floral Development on Explants Treated with Various Growth Regulants and Cultured In-vitro During Experiment VIII

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex Type</th>
<th>Surviving Explants</th>
<th>Buds In-situ Prior to Treatment</th>
<th>Buds Developed After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Gyn.</td>
<td>6</td>
<td>All buds examined were stamenless</td>
<td>Buds underdeveloped</td>
</tr>
<tr>
<td></td>
<td>Mon.</td>
<td>5</td>
<td>All buds examined were staminate</td>
<td>Buds underdeveloped</td>
</tr>
<tr>
<td>GA_{4+7}</td>
<td>Gyn.</td>
<td>5</td>
<td>No staminate dev. in 6/6 buds</td>
<td>No staminate dev. in 5/5 buds</td>
</tr>
<tr>
<td></td>
<td>Mon.</td>
<td>4</td>
<td>Normal staminate dev. in 6/6 buds</td>
<td>Buds underdeveloped</td>
</tr>
<tr>
<td>AgNO_{3}</td>
<td>Gyn.</td>
<td>5</td>
<td>No staminate dev. in 12/12 buds</td>
<td>Staminate dev. in 2/6 buds</td>
</tr>
<tr>
<td></td>
<td>Mon.</td>
<td>7</td>
<td>Normal staminate dev. in 6/6 buds</td>
<td>No staminate dev. in 2/4 buds</td>
</tr>
<tr>
<td>AVG</td>
<td>Gyn.</td>
<td>4</td>
<td>No staminate dev. in 9/9 buds</td>
<td>Buds underdeveloped</td>
</tr>
<tr>
<td></td>
<td>Mon.</td>
<td>4</td>
<td>Normal staminate dev. in 8/8 buds</td>
<td>Normal staminate dev. in 7/7 buds</td>
</tr>
<tr>
<td>Ethephon</td>
<td>Gyn.</td>
<td>4</td>
<td>No staminate dev. in 3/3 buds</td>
<td>Buds underdeveloped</td>
</tr>
<tr>
<td></td>
<td>Mon.</td>
<td>6</td>
<td>Normal staminate dev. in 1/1 buds</td>
<td>No staminate dev. in 7/8 buds</td>
</tr>
</tbody>
</table>

1 Treatments administered at 50 ppm concentration

2 Buds formed at newly developed nodes or adventitious buds formed at nodes In-situ prior to treatment

3 One bud exhibited incomplete staminate development

4 Of the 7 stamenless buds, 6 were formed on newly developed tissue, 1 was an adventitious bud formed near the plant base and several were aborting
Table 23: Floral Development on Explants Treated with Various Growth Regulants and Cultured In-vitro During Experiment IX

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>Sex Type</th>
<th>No. Surviving Explants</th>
<th>Buds In-situ Prior to Treatment</th>
<th>Buds Developed After Treatment</th>
<th>Analysis of Bud Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Gyn.</td>
<td>5</td>
<td>All buds examined were stamenless</td>
<td>Buds underdeveloped</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mon.</td>
<td>5</td>
<td>All buds examined were staminate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AgNO₃</td>
<td>Gyn.</td>
<td>3</td>
<td>No staminate dev. in 4/4 buds</td>
<td>Buds underdeveloped</td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>Gyn.</td>
<td>2</td>
<td>No staminate dev. in 3/3 buds</td>
<td>No staminate dev. in 1/1 bud</td>
<td></td>
</tr>
<tr>
<td>100 ppm</td>
<td>Gyn.</td>
<td>5</td>
<td>No staminate dev. in 7/7 buds</td>
<td>Buds underdeveloped</td>
<td></td>
</tr>
<tr>
<td>Ethephon</td>
<td>Mon.</td>
<td>7</td>
<td>Normal staminate dev. in 4/4 buds</td>
<td>Normal staminate dev. in 4/4 buds</td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>Mon.</td>
<td>5</td>
<td>Buds underdeveloped</td>
<td>Normal staminate dev. in 1/1 bud</td>
<td></td>
</tr>
<tr>
<td>50 ppm</td>
<td>Mon.</td>
<td>2</td>
<td>Buds underdeveloped</td>
<td>Buds underdeveloped</td>
<td></td>
</tr>
</tbody>
</table>

¹Buds formed at newly developed nodes or adventitious buds formed at nodes in-situ prior to treatment
only speculation concerning differences in treatment action in-vivo and in-vitro could be advanced. Differences (if they are in fact, real) may have resulted from increased absorption of the regulant in-vitro and/or from its effective transport to the site of stamen initials.

The failure of AVG to effect staminate induction in-vitro may have stemmed from its general inhibition of explant growth.

During Experiment VIII, monoecious explants treated with ethephon (an ethylene releasing compound) evolved antherless floral buds (Table 22). This result, although anticipated from models of hormonal control over stamen production proposed in Chapter 4, was not evinced under field conditions or in the few buds produced on explants during Experiment IX (Table 23). Further field and tissue culture experimentation will be necessary to confirm or refute ethephon-mediated inhibition of stamen development in monoecious phenotypes.

As in field experiments (Chapter 4), application of GAs to in-vitro cultures was ineffectual for staminate floral induction on gynoecious phenotypes. Considering the almost universal masculinization of cucurbits treated with these compounds (Chapter 3, Table 6), the failure of GA3 or GA4+7 applications to effect staminate induction under either in-vivo or in-vitro conditions lent credence to the supposition of staminate primordial inhibition in gynoecious tissues at physiological (biochemical) stages prior to those influenced by or requiring high levels of endogenous GA.
Assessment of In-vitro Techniques for the Study of Sex Expression in Buffalo Gourd

As was noted in spinach explants by Culafic and Neskovic (1982), morphological consequences of added growth regulants to cultured buffalo gourd apices were in accordance with expectations, indicating the effective incorporation of regulants into developing tissues. GA added to buffalo gourd tissues tended to elongate proliferating tissues whereas application of ethephon resulted in high levels of tissue senescence. Application of AVG inhibited tissue growth and prevented tissue decline as might be anticipated by its widespread use as retardant of fruit vegetable ripening. Although regulants were undoubtedly incorporated into developing tissues, the use of In-vitro techniques in elucidation of buffalo gourd sex expression control was less than satisfactory. Definitive conclusions from the data were impossible due to the high level of contamination among cultures, the low level of floral development on post-treatment tissues and the general disagreement between Experiments VIII and IX, potentially caused by variation of endogenous substances in culture material at experiment initiation.

Unlike cucurbits which produce floral buds with a pronounced bisexual stage, buffalo gourd floral differentiation appears to be irreversibly determined at very early ontogenetic stages. This diclinous nature of development is detrimental to the study of sex expression In-vitro as it forces the culture of the entire meristematic region. Subtending tissues associated with original explants or those developed during culture may produce high levels of endogenous hormones confounding effects of added growth regulants. The alternative,
culture of individual excised floral primordia, would require potentially tedious surgical procedures to prepare culture material.

Even if confounding factors of endogenous regulants were discounted, poor floral induction and/or development rates might also hamper the in-vitro study of sex expression in buffalo gourd. Floral development might be enhanced or facilitated by alteration in media formulations. Nitsch (19690 and Scorza (1982) suggest the addition of high levels of sucrose, nitrogen and cytokinins to result in enhanced floral development. Precedent for the inclusion of kinetin and other cytokinins to basal media for the study of sex expression in cucurbits has been established (Galun, Jung and Lang, 1962, 1963; Pereira, 1969).

In summary, in-vitro techniques might become a useful technique for continuing studies elucidating hormonal control of buffalo gourd sex expression only if substantial efforts are expended on preliminary investigations necessary to optimize floral production and minimize contamination in cultures.
CHAPTER 6
CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

To meet the goals of this investigation as outlined in Chapter 1, seven field experiments and two tissue culture studies were initiated a) to examine the effects of natural and synthetic growth regulators upon buffalo gourd sex expression in anticipation of developing techniques for staminate induction on gynoecious phenotypes and b) to elucidate possible schemes for control of flowering by endogenous hormones. A modest level of success in both endeavors was achieved. However, as with most studies of this type, more questions were generated from the data than were answered by it.

The demonstration of AVG-induced staminate flowering on gynoecious segregates was perhaps, the most essential outcome of the investigation (Chapter 4). Response to exogenous application of this compound was linear with administered dose and treatments containing AVG at concentrations greater than or equal to 125 ppm AVG effected staminate induction on all replicates (Experiment VI). The resultant male flowers developed normally and produced adequate pollen for self- and cross-pollinations. AVG application appeared to have little influence on shoot growth rate, pistillate flowering patterns or the ontogeny of antherless buds formed prior to treatment.

Field use of AVG concurrent with experiments reported herein suggested that response to this regulant may be influenced by stressful environments (Chapter 4). Further study of exogenously-applied AVG will
be necessary to assess the effects of environmental stress and potential seasonal influences (e.g. daylength, temperature) on the level of staminate induction, demonstrate possible interaction with background genotypes and to define the limits of effective application (rates and schemes). AVG-induced hermaphroditic flowers developed at potentially pistillate nodes also merits further study as formation of these exceptional flowers might influence the use of this compound in breeding schemes.

The benefit of AVG-facilitated staminate induction to breeding programs is currently being realized. The potential use of AVG for maintenance of seed parent lines in a commercial F₁ hybrid production scheme is less assured due to the high cost of the regulant and to the uncertain value of hybrids produced.

If silver nitrate could be used as a masculinizing agent for this species, the production of F₁ hybrids might be more cost effective. However, only limited success at staminate induction with this compound has been realized, and then only in tissue cultures (Chapter 5). Field-grown plants treated with high doses of silver nitrate (Chapter 4, Experiment III - V) did not exhibit morphological evidence of regulant absorption. Further experimentation focused on appropriate methodology for regulant incorporation is suggested.

Resolving control mechanisms for complex biological processes has proved difficult and time-consuming. Many research groups have labored for decades to compile what is currently known about hormonal regulation of flowering and sex expression (Chapter 3). Moreover, most insights have been derived indirectly from studies employing
exogenously-applied natural and synthetic growth regulators such as those reported herein. Proposed mechanisms have been confirmed by detailed analysis of endogenous compounds and rarely, through demonstration of hormonally-induced genetic activity resulting in alteration of sexual development (Chapter 2).

From this investigation, elucidation of elements controlling sex expression in buffalo gourds was at best, incomplete. Strong but indirect evidence was advanced for inhibited male development at high concentrations of endogenous ethylene presumed to be present in gynoecious shoot apices (Experiments V and VI). However, the application of ethephon-released ethylene to monoecious plants failed to simulate the gynoecious phenotype except on a limited number of explants cultured in vitro (Experiments VII and VIII). Clarification of ethylene's role in staminate flower production will require further field studies with ethephon at different times and/or under different conditions, and will ultimately depend upon characterization of endogenous ethylene levels among sex types.

The failure of applied GAs to elicit male development under any conditions (Chapters 4 and 5) was of importance to the derivation of the proposed control model. In view of their masculinizing effects on other cucurbits and their demonstrated importance to flower development in many species, it was postulated that GA moderation of staminate ontogeny in buffalo gourd (if existent) occurs at developmental steps beyond those influenced by endogenous levels of ethylene. The importance of GAs to staminate flower development remains to be tested, perhaps through
exogenous application of GA synthesis inhibitors (such as CCC or Amino-1618) or through quantitation of endogenous GA levels among sex types.

Finally, models for hormonal control of pistillate flower development need to be advanced as the process has yet to be morphologically or physiologically examined. Evidence reported herein suggests pistillate flowering to be reduced by high (perhaps supra-optimal) levels of ethylene (Experiment VII) and/or to be unaffected by inhibition of ethylene synthesis (Experiment VI). Further studies should be undertaken to monitor the effects of exogenously-applied ethephon, NAA (a synthetic auxin) and 6-BA (a synthetic cytokinin) on the initiation and development of female flowers. As proposed earlier, characterization of endogenous hormone levels may also clarify the nature of floral control.

Some future experiments suggested in this section are underway or are planned for the 1985 growing season. Further research on buffalo gourd sex expression using in-vitro culture is not recommended at this time as considerable effort must first be expended to develop and refine viable techniques (Chapter 5).
APPENDIX A

FLORAL DEVELOPMENT AT NODES PRIOR TO AND FOLLOWING INITIAL TREATMENT OF MERISTEMS ON FOUR GYNOECIOUS PLANTS WITH AMINOETHOXYVINYLGLYCINE DURING EXPERIMENT V

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**LEGEND:**
- • BLIND NODE
- ☂ ANTHESIS MALE BUD
- ◆ MALE BUD
- ♀ FEMALE BUD

240
APPENDIX B

FLORAL DEVELOPMENT AT NODES FOLLOWING INITIAL TREATMENT OF MERISTEMS ON SIXTY GYNOECIOUS PLANTS WITH VARIOUS CONCENTRATIONS OF AMINOETHOXYVINYLGLYCINE DURING EXPERIMENT VI

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>Response to Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 ppm</td>
<td>1</td>
<td>![Diagram 1]</td>
</tr>
<tr>
<td></td>
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<td>![Diagram 2]</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>![Diagram 3]</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>![Diagram 4]</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>![Diagram 5]</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>![Diagram 6]</td>
</tr>
</tbody>
</table>
control

2

3

4

5

6

7

8

9

10
LEGEND:

- • BLIND NODE
- ♣ ANOTHERLESS MALE BUD
- ◆ MALE BUD
- ♀ FEMALE BUD
- ≡ MERISTEM OR VINE NECROSIS
APPENDIX C

FLORAL DEVELOPMENT AT NODES FOLLOWING INITIAL TREATMENT OF MERISTEMS ON TEN MONOECCIOUS PLANTS WITH VARIOUS CONCENTRATIONS OF ETHEPHON DURING EXPERIMENT VII

<table>
<thead>
<tr>
<th>Ethephon Concentration</th>
<th>Replication</th>
<th>Response to Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 ppm</td>
<td>1a</td>
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<td>1b</td>
<td><img src="image2.png" alt="Diagram 1b" /></td>
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<tr>
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<td>2a</td>
<td><img src="image3.png" alt="Diagram 2a" /></td>
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<tr>
<td></td>
<td>2b</td>
<td><img src="image4.png" alt="Diagram 2b" /></td>
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<tr>
<td></td>
<td>3a</td>
<td><img src="image5.png" alt="Diagram 3a" /></td>
</tr>
<tr>
<td></td>
<td>3b</td>
<td><img src="image6.png" alt="Diagram 3b" /></td>
</tr>
</tbody>
</table>

249
LEGEND:

- BLIND NODE
- ANOTHERLESS MALE BUD
- MALE BUD
- FEMALE BUD
- ABORTED BUD
- MERISTEM OR VINE NECROSIS
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