Ralowicz, Andrew Edward

FLOWERING ASPECTS OF THE BUFFALO GOURD CUCURBITA FOETIDISSIMA HBK.

The University of Arizona M.S. 1986

FLOWERING ASPECTS OF THE BUFFALO GOURD

CUCURBITA FOETIDISSIMA HBK.

by

Andrew Edward Ralowicz

A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN AGRONOMY AND PLANT GENETICS

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 8 6

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: A. E. Rakowicz

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

J. E. Endrizzi

J. E. ENDRIZZI
Professor of Plant Sciences

5/8/86
Date

DEDICATION

This manuscript is dedicated in loving memory

of my late father

EDWARD DANIEL RALOWICZ

for it was he who demonstrated the power of knowledge and the initiative to obtain it, and he who fostered my love for the plant kingdom.

and

to my mother

HELEN ELIZABETH McGLAUGHLIN RALOWICZ

for the unending encouragement and financial assistance supplied throughout my academic sojourn.

ACKNOWLEDGMENTS

The author would like to extend gratitude to a number of individuals who aided in this endeavor. A special thank you must be extended to Dr. J. C. Scheerens for help on this thesis and its research experiments, and all moments we have shared during my employment. I would like to thank Dr. J. E. Endrizzi for help in the preparation of this manuscript, and in addition for the opportunity to be under his guidance as a graduate student. Additionally, I would like to thank Drs. H. Muramoto and W. P. Bemis for their help in reviewing this document and for serving as members of my graduate committee.

I would like to thank Drs. J. W. Berry, C. W. Weber and P. G. Bartels for the use of laboratory space and materials. I would like to thank Dr. S. E. Smith for assistance with the statistics, and Dr. A. K. Dobrenz, Dr. D. T. Ray and Holly Reith for the use of their microcomputer facilities.

My gratitude must be extend to Michaela Hasan for help with collecting data and to Terry McGriff for discussions with purpose and meaning, and for his friendship and trust.

I would like to acknowledge the patience of a group of individuals, John Swanson, Walt St. Pierre, Keith Scuito, and Mick Reed, the members of the musical troupe "Last Flite," for when this this thesis is accomplished, indeed, molten rock will flow.

I would like to thank all the individuals whom I have failed to mention who have either helped or provided encouragement and enthusiasm throughout this thesis and my education.

Finally, I must express my thanks and love to all the members of my family: my mother Helen, siblings Leo, Phillip, Stephen, Ravi Inder Kaur and her family, Peter and his family, and John for the cherished support provided throughout this undertaking.

TABLE OF CONTENTS

		Page
	LIST OF TABLES	viii
	LIST OF FIGURES	ix
	ABSTRACT	x
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	5
	Flower Differentiation	5
	Models of Floral Differentiation	5
	Dicliny	7
	Natural Flowering Habits in Cucurbitaceae	7
	Quantitative Sex Expression	12
	Methods of Quantification	12
	Floral Modification in Cucurbits With Respect to Other Species	13
	Effects of Exogenously Applied Growth Regulants Upon Sex Expression in Buffalo Gourd	18
3.	MATERIALS AND METHODS	21
	Histology	21
	Floral Taxonomy	22
	Flower Development	22
	Growth Regulator Experiments	23
4.	RESULTS AND DISCUSSION	25
	Histology	25
	Floral Taxonomy	25
	Flower Development	26
	Effects of Growth Regulants on Growth and Flowering	29
	Morphological Manifestations	29
	Growth Rate	29
	Floral Development	32
	Summary	37

TABLE OF CONTENTS--Continued

Page

APPENDIX A: IMBEDDING SCHEDULE 39

APPENDIX B: STAINING SCHEDULE 40

APPENDIX C: ANALYSIS OF VARIANCE TABLES 41

LITERATURE CITED 54

LIST OF TABLES

Table		Page
1.	Common Types of Sex Expression in Flowering Plants . . .	8
2.	Qualitative Sex Expression in Selected Cucurbits	10
3.	Natural and Synthetic Growth Regulands and Their Effect on Cucurbit Sex Expression	15
4.	Buffalo Gourd Floral Dimension Data	27
5.	Effects of Growth Regulants on Growth Parameters for Monoecious and Gynoecious Sex Types During Experiment Sets I and II	31
6.	Effects of Growth Regulants on Flower Abortion for Monoecious and Gynoecious Sex Types During Experiment Sets I and II	33
7.	Effects of Growth Regulants on Flower Frequencies on Gynoecious Plants During Experiment Sets I and II	35
8.	Effects of Growth Regulants on Flower Frequencies on Monoecious Plants During Experiment Sets I and II	36

LIST OF FIGURES

Figure		Page
1	Frequency Distributions of Days to Anthesis	28

ABSTRACT

Four investigations were conducted to reinforce and expand the basic knowledge data base of growth and flowering in monoecious and gynoecious buffalo gourd (Cucurbita foetidissima HBK.) sex types. Objectives included enhanced pistillate flower production on both sex types by application of growth regulants, observation of flower maturation periods and floral dimensional relationships, and finally documentation of the morphological bisexual stage characteristics of other immature cucurbit flower buds.

Histological examination of immature staminate and pistillate buds failed to reveal a morphological bisexual stage.

Staminate buds on monoecious segregates achieved anthesis in 19.5 $\pm$ 3.0 days. Pistillate bud maturation periods for monoecious and gynoecious plants were 10.9 $\pm$ 1.0 days and 9.7 $\pm$ 1.5 days, respectively.

Synthetic growth regulants shown to modify sex expression in other cucurbits were investigated for feminizing potential. BA (benzyladenine) failed to modify growth and flowering in both sex types. NAA (naphthaleneacetic acid) reduced staminate flower production and growth, however demonstrated no enhancement of pistillate flower primordia. In contrast, high concentrations of CCC (chlorocholine chloride) advanced evidence for enhanced male flower production; however, no corresponding modification of pistillate flower frequency was reported.

CHAPTER 1

INTRODUCTION

The buffalo gourd, Cucurbita foetidissima HBK 1817, is a feral xerophytic cucurbit indigenous to western North America and northern Mexico. Its current range encompasses an area from Guarajuato in central Mexico northward to the South Dakota border, and from southern California eastward to Missouri and Illinois.

The plant is perennial because of its large fleshy root. Three or four growing seasons can produce roots exceeding 40 kg. Vines are frost sensitive; however, the root crown rests 5 to 7 cm below the soil surface. This buffer, along with an impermeable suberized periderm, allows the root to withstand subfreezing temperatures. Additionally, vines are low, spreading, and the leaves typically hastate, pubescent, and gray-green in color. Any developing node on the vine has the capacity to generate an adventitious root in the proper environment, and at the proper stage of development. Consequently, large clonal populations are produced and maintained asexually. Optimal growing conditions allow virtually unwanton growth. A plant growing wild near an arroyo in New Mexico produced over 350 annual shoots with a total vine length greater than 2,000 meters covering an area twelve meters in diameter (Dittmer and Talley, 1964).

C. foetidissima has been associated with humans for approximately 9,000 years in Mexico (Cutler and Kaplan, 1956). An agrestial, the gourd still grows on dump heaps of Hopi villages. These Indian communities are some of the oldest continuously inhabited settlements in America. Historic utilization included topical or internal medicines, food, rattles, and ladles. Current interest in the plant initially stemmed from the potential use of the seed, which is rich in oil and protein. Berry et al. (1976) reported embryo composition as 37.5% protein and 48.0% oil. Interest now, however, is focussed on the root, as potentially commercial yields of starch have been achieved with high density planting. Nelson et al. (1983) demonstrated starch yields greater than 3,000 kg/ha with 550,000 plants/ha.

The unisexual flowers (pistillate or staminate) can arise singly at a node on the vine. Corolla color ranges from pale yellow to orange, and the petal tips are UV reflective due to flavinoids, displaying a characteristic bull's eye landing spot for bees (Buchman, 1982). Squash and gourd bees of the genera Peponapsis and Xenoglossa are the principle pollinators (Hurd and Linsley, 1964).

Individual plants of the species are either monoecious (possessing staminate and pistillate flowers) or gynoecious (producing only pistillate flowers) resulting in monogynodioecious populations. Gynoecy is the result of a single dominant allele (M), and monoecy is the homozygous recessive condition (Dossey et al., 1981). Although still variable, fruit yields of gynoecious plants have been greater than monoecious plants, a fact with potential agronomic importance (Gathman and Bemis, 1985; Wilkins, 1980).

Antherless staminate flowers of C. foetidissima have been examined histologically. Abortive male flower buds initiated stamens when flowers were smaller than 1 mm. Stamen and flower development were arrested at 3 mm and in many instances flower abortion occurred when less than 5 mm (Yousef, 1976).

There is a plethora of literature substantiating the manipulation of flower ontogeny with exogenous growth hormones in Cucurbitaceae. The buffalo gourd is no exception. Successful production of the monoecious phenotype on gynoecious segregates has been accomplished with aminoethoxyvinylglycine (AVG) and silver nitrate ($Ag\ NO_3$).

During in vitro experiments, indirect evidence for ethylene-mediated control of male flowering was advanced after staminate proliferation in buds of gynoecious explants treated with silver nitrate ($Ag\ NO_3$) and by formation of stamenless buds on monoecious explants treated with ethephon. However, low levels of floral induction under culture conditions employed rendered these results inconclusive (Scheerens, 1985).

The primary goal of this research is to develop a model for hormonal control of pistillate flowering in the buffalo gourd. To attain this goal, the research consisted of four major areas of analysis. The first was to initiate earlier production of functional pistillate flowers through the use of exogenously applied growth regulants; the second was to monitor and compare flower development periods for staminate and pistillate flowers; the third objective was to measure and

compare flower parts and relationships from a homogeneous agronomic population; and the final objective was to document the early ontogeny of pistillate, and staminate flowers.

CHAPTER 2

REVIEW OF LITERATURE

Flower Differentiation

Floral differentiation has undoubtedly intrigued persons since the earliest dawns, yet precise mechanisms of this passage remain unsolved. During the transition to flowering, vegetative or uncommitted apices are transformed into potential reproductive meristems. These meristems then undergo a determinate schedule of growth, producing, in a series, lateral organs of varying functions and morphologies. The organs are either vegetative or spore producing. The outer floral envelopes are sterile, and the sporophylls differentiate as stamens or carpels. Both stamens and carpels are present in monoclinous flowers; in diclinous flowers, only one class of essential organ is present.

Models of Floral Differentiation

Expanding on earlier investigations, Wardlaw (1957) envisioned the floral meristem as a reaction system passing through an irreversible sequence of phases. Their analysis depicted the floral bud as a shortened, determinate variant of a shoot tip in which the apex developed into a receptacle and the foliar initials into floral members (calyx, corolla, androecium and gynoecium). In their examination of floral ontogeny, Wardlaw partitioned the apex by applying concepts previously

presented by Mather (1948). The "sub distal region" contained the illusive florigen(s), affecting not only the preliminary changes of the shoot system but influencing the entire sequence of events concluding with anthesis.

Heslop-Harrison (1963) postulated that with few exceptions hermaphrodite, monoecious and dioecious species possess the common trait of a basically hermaphroditic or monoclinous plan. Ontogenically, the floral meristem produces sequentially the primordia of lateral organs of various potentialities. He compared the determination of whorls of lateral members in a flowering apex successively in space and time to a sequence of relays, the beginning of each phase being restricted by the termination of the previous one and in turn leading to the next phase. His relay system is founded upon the regulator-gene/operon concept. In the presence of an effector, a succession of gene complexes would be activated in an irreversible fashion, the hiatus between the onset of successive stages depended upon the time needed to establish effective levels of each consecutive inducer. As a final constraint, each specific inducer had short intercellular ranges and effects.

Bernier et al. (1981) state" . . . despite 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 researchers discussed cellular and sub-cellular events generally related to increased meristem metabolic activity. Increased respiratory function, increased transcription

and translation and increased rate and regulation of cell division initiate morphological changes in meristem arrangement and that of the subtending tissue. These authors perceived these modifications, not as a number of events seriatim, but rather as a number of parallel and interacting sequences, each potentially affected by both endogenous and environmental stimuli.

Types of sex expression typical of flowers, plants and plant populations are presented in Table 1.

Dicliny

The majority of unisexual or diclinous flowers start with a primordium which exhibits a hermaphroditic pattern. Unbalanced proliferation in androecium and gynoecium, one developing to the exclusion of the other, results in functional unisexuality. In many instances these flowers visually display the rudiments of the missing sex upon anthesis. In contrast, other diclinous species florally generate only one class of lateral members, stamens or carpels. Rudiments of the missing sex are not visible at maturity as the primordium never entertains a period of potential bisexuality (Heslop-Harrison, 1963).

Natural Flowering Habits in Cucurbitaceae

The Cucurbitaceae excel in sex variability, both within genera and species. True hermaphrodite species are rare, but in some species, Bryonia dioica for example, hermaphrodite plants do occur (Whitaker, 1931). Almost all species have only diclinous flowers (male or female),

Table 1. Common Types of Sex Expression in Flowering Plants [1/]

Type	Description
Individual Flowers	
Monoclinous:	
Hermaphroditic	containing both stamens(male organs) and carpel(s) (female organs)
Diclinous:	
Staminate	possessing only stamens
Pistillate	possessing only carpels
Individual Plants	
Hermaphrodite	only hermaphroditic flowers present
Monoecious	both staminate and pistillate flowers present
Androecious	only staminate flowers present
Gynoecious	only pistillate flowers present
Andromonoecious	having both staminate and hermaphroditic flowers.
Gynomonoecious	having both pistillate and hermaphroditic flowers
Trimonoecious	having staminate, pistillate and hermaphroditic flowers
Plant Populations	
Monomorphic:	
Hermaphrodite	consisting of only hermaphrodite plants
Monoecious	consisting of only monoecious plants
Dimorphic:	
Dioecious	consisting of androecious and gynoecious plants
Androdioecious	containing hermaphrodite and androecious plants
Gynodioecious	containing hermaphrodite and gynoecious plants

1/ Adapted from Frankel and Galun (1977)

or both monoclinous and diclinous flowers. The first group includes dioecious and true monoecious species, and the second group contains a predominance of species with andromonoecious plants (Frankel and Galun, 1977).

Monoecy is the typical form of qualitative sex expression of individual plants. Whitaker (1931) and others (Shifriss, 1961; Poole and Grimball, 1939) reported that the major sex type of the genus Cucumis is monoecious. Moreover, monoecy predominates in the genus Cucurbita. Hermaphroditic exceptions have been noted in C. pepo and C. moschata (Whitaker, 1931). Kubicki (1970) described an androecious anomaly in C. pepo. Cucurbita foetidissima (buffalo gourd) displays monoecy, but gynoecy was first described by Curtis and Rebeiz (1974). In addition, functional hermaphroditic flowers were discovered on a single segregate developed in a breeding program for root shape improvement. Furthermore, observations within buffalo gourd accessions collected in Mexico uncovered staminate flowers containing developed stigmatic and stylar tissue. The pistillate tissue was encased within the coalesced staminate structure. Histological examination of these "psuedo-hermaphroditic" flowers revealed no ovarian tissue (Scheerens, 1985). Both Citrullus and Lagenaria are typically monoecious, although Whitaker (1931) noted the exception of andromonoecy in Citrullus vulgaris (lanatus).

Characteristic qualitative sex expression of individuals and populations in some cucurbit species is displayed in Table 2. Entries

Table 2. Qualitative Sex Expression in Selected Cucurbits

Species	Common Name	Sex Variants	References[1/]
Bryonia alba		monoecious	2
Bryonia dioica		gynoecious	2
Citrullus vulgaris (Ianatus)	watermelon	monoecious	20
		andromonoecious	20
Cucumis anguria	gherkin	monoecious	9, 18
Cucumis melo	muskmelon, cantaloupe	gynoecious (unstable)	19
		gynoecious (stable)	11
		gynomonoecious	19
		hermaphroditic	19
		monoecious	19, 20
		andromonoecious	19, 20
		androecious	5
Cucumis sativus	cucumber	gynoecious	23
		gynoecious (predom. pist.)	12, 13, 14
		gynoecious (multiple pist.)	6, 17, 26
		hermaphroditic	23
		monoecious (with pist. phase)	12, 13, 14
		monoecious (with mixed phase)	12, 13, 14 20, 23
		andromonoecious	20, 23
		androecious	7, 15, 21
		trimonoecious	16
Cucurbita foetidissima	buffalo gourd	gynoecious	3, 4
		monoecious	3, 4
Cucurbita maxima	squash (various cult.)	monoecious	27
Cucurbita moschata	squash (various cult.)	monoecious	27
Cucurbita pepo	squash, pumpkin (various cult.)	monoecious	27

Table 2 (continued)

Species	Common Name	Sex Variants	References 1/
Echballium elaterium	squirting cucumber	monoecious	10
Lagenaria siceraria	bottle gourd kettle gourd	monoecious	22
Luffa acutangula	loofah	monoecious	1
Luffa cylindrica	dishcloth gourd	monoecious	25
Momordica charantia	bitter gourd	monoecious	8
Sichyos angulatus		monoecious	24

1/ References:

1. Bose and Nitsch, 1970
2. Chailakhyan, 1979
3. Curtis and Rebeiz, 1974
4. Dossey, Bemis and scheerens, 1981
5. Foster and Bond, 1967
6. Fugieda et al., 1982
7. George, 1970
8. Ghosh and Basu, 1983
9. Hall, 1949
10. Kopcewicz, 1971
11. Kubicki, 1966
12. Kubicki, 1968
13. Kubicki, 1969a
14. Kubicki, 1969b
15. Kubicki, 1969c
16. Kubicki, 1969d
17. Nandgaonkar and Baker, 1981
18. Nitsch et al., 1952
19. Poole and Grimball, 1939
20. Rosa, 1928
21. Scott and Baker, 1975
22. Sharma, Jyotishi and Agrawal, 1980
23. Shifriss, 1961
24. Takahashi, Saito and Suge, 1982
25. Takahashi, Suge and Saito, 1980
26. Uzcategui and Baker, 1979
27. Whitaker, 1931

represent common cultivated cucurbit species and less common family members for which sex expression data have been generated.

Quantitative Sex Expression

Quantitative sex expression can be described as differences in the ratio of flower types and developmental pattern among individuals displaying the same qualitative sex expression. The flowering pattern of the monoecious cucumber can be divided into three phases: exclusively staminate--staminate and pistillate--exclusively pistillate flowering (Shifriss and Galun, 1956). A similar transition from male to female expression has been reported in the buffalo gourd. Yousef (1976) described four phases of flower patterns as: nonflowering phase, male phase, mixed (pistillate and staminate flowers) phase, and vegetative phase. Cucurbita pepo cv. acorn has five phases: underdeveloped male flowers, normal male flowers, male and female flowers, giant female and inhibited male flower buds, and finally parthenocarpic female flowers (Nitsch et al., 1952).

Methods of Quantification

Several methods of quantifying sex expression have been proposed. "Sex tendency" is measured by the percentage of staminate or pistillate flowers along the stem. Because of environmental variations affecting staminate and pistillate flower numbers, this technique has reduced reliability. Shifriss (1961) proposed the use of the number of nodes from the cotyledons to the first pistillate flower as the measurement of sex tendency. The smaller the number of nodes the greater the tendency toward femaleness and vice versa.

Nitsch et al. (1952) suggested the use of the percentage of pistillate flowers per short stem interval along the vine to detect the shift from maleness to femaleness. Plotting the percentage of pistillate flowers per ten nodes against the total number of nodes, these researchers named the resulting angle between the correlation line and the abscissa as the "index of feminization."

Sex ratio can be measured in two ways. The first method calculates the ratio of staminate to pistillate flowers achieving anthesis. The second method relies on the early recognition of male and female flower buds; the sex ratio is then determined by the ratio of male flowering nodes to female flowering nodes, or vice versa. Sex ratio has been shown to vary according to variety and environmental factors (Whitaker, 1931; Nitsch et al., 1952). Additionally, sex ratio can be further distorted by the presence of maturing fruit on the vine which inhibits the later production of pistillate flowers (Scott, 1933).

Floral Modification in Cucurbits With Respect to Other Species

Expanding populations dictate maximum productivity from our land resources. In an effort to keep pace with this expansion, researchers constantly seek methods of enhancing food and fiber production. In many instances the fruit is the commodity of interest; increases in fruiting structures per plant (a potential avenue for satiating the increased food demand) can result from increased production of pistil-bearing flowers. Plant sex expression involves the interaction of genetic information with external and internal stimuli (hormones). The exogenous

application of growth regulants to plants has demonstrated true merit in a variety of agricultural endeavors, including those of flower and fruit production. Furthermore, synthesis, modification and degradation of cellular hormone levels have shown association with applied chemical agents. It is quite possible that more investigations into the effects of growth regulants on flowering and sex expression have been focussed on the Cucurbitaceae than on any other plant family. This is most likely due to the conspicuous flowers and well documented growth patterns, and the additional consequence that the fruits supply relished food and feed. Typical effects of synthetic and natural growth regulants on sex expression in Cucurbits are summarized in Table 3. This section presents evidence substantiating both growth and flower modifications in the Cucurbitaceae as influenced the growth regulants naphthaleneacetic acid (NAA), benzyladenine (BA), and chlorocholine chloride (CCC). The effects of each regulant is discussed below.

Enhancement of pistillate flower formation by auxins on cucumbers (C. sativus) was demonstrated by Laibach and Kribben (1950). NAA treatments increased the proportion and total number of female flowers. Additionally, the ratio of female:male buds in C. pepo increased with the application of 500 ppm NAA (Krishnamoorthy and Sandooja, 1982).

Experiments by Brantley and Warren (1960) on muskmelon (C. melo) examined the effects of three concentrations of NAA (.01, 0.1, 1.0 ppm) by severing and immersing the first leaves in aqueous auxin solutions. Under long days the number of staminate and perfect flowers was depressed

Table 3. Natural and Synthetic Growth Regulants and Their Effect On Cucurbit Sex Expression

Regulants	Typical Effect On Cucurbit Sex Expression
Natural Regulants (hormones)	
Abscisic acid (ABA)	Mixed
Gibberellic acid (GA_3)	Masculinize
Gibberellic acid (GA_{4+7})	Masculinize
Indole-3-acetic acid (IAA)	Feminize
Synthetic Regulants	
Silver nitrate ($AgNO_3$)	Masculinize
Aminoethoxyvinylglycine (AVG)	Masculinize
6-Benzylaminopurine (BA)	Feminize
2-Chlorocholine chloride (CCC)	Feminize/mixed
Estrogen	Mixed
2-Chloroethylphosphonic acid (ETHEPHON)	Feminize
Naphthalene acetic acid (NAA)	Feminize
Phthalimide	Masculinize
1,1-Dimethyl-2-succinyl-hydrazine (SADH)	Feminize
Testosterone	Mixed
2,3,5-Triodobenzoic acid (TIBA)	Feminize

by the lowest dosage. The reduction in staminate flowers was proportionally greater, and decreased the ratio of staminate to perfect flowers. Higher concentrations produced the same effect on perfect flowers, however staminate flowers were further decreased. In contrast 0.01 ppm NAA promoted flowering and increased the proportion of perfect flowers during short days. Additionally, higher dosages decreased both flower types while increasing the ratio of staminate to perfect flowers. Concentrations exceeding 0.01 ppm caused a delay in flower development. Internode length was not affected by NAA, but internode number was reduced for long day auxin treatments.

Galun (1959a) showed that cucumbers (C. sativus) treated with 0.1% NAA began the mixed and female phase closer to the base of the plant. Morphologically, NAA at 10 ppm decreased leaf blade and petiole lengths, increased hypocotyl diameter, but showed no change in hypocotyl length (Galun, 1959b).

Treatments using NAA, BA, and CCC at 100 ppm on the snake cucumber suppressed male floral development, while inducing the formation of pistillate flowers at lower nodes than controls (El-Kholy and Hafez, 1982). As plant sex is directly related to fruit set, and since only female flowers produce fruits, fruit set, size, and yield were also examined. Compared to the control, all three treatments independently demonstrated an increase in marketab[illegible] fruits and fruit weight. Fruit size, defined by length, was increased by NAA and BA, but reduced by CCC. Fruit size, defined by diameter, was less than the control for

all growth regulants. Furthermore, the mean number of branches and the mean axis length were greater for all treatments versus the control treatments. Similar results have been reported in the genus Luffa. Bose and Nitsch (1970) soaked seeds of Luffa acutangula in aqueous solutions of NAA, BA and CCC at 100, 10, and 1000 ppm respectively. As expected, the auxin treatment increased the number of female flowers. CCC also increased the number of female flowers, and furthermore decreased the number of male flowers in the first ten nodes. Benzyladenine displayed the most striking feminizing effect, stimulating pistillate flowers beginning at the third node of 50 percent of the treated plants. Evidence that BA induced female flower production in Luffa during long day photoperiod was also advanced.

A later study in Luffa cylindrica on the effect of BA on sex expression and growth was made by Takahashi, Suge and Saito (1980). Treatments, applied four times in twelve days, of 50 ppm on staminate inflorescences stimulated lateral shoots and further floral development including bisexual and pistillate flowers.

Cucumber seedlings sprayed with NAA (25 - 100 ppm) at the second leaf and three and four leaf stages, resulted in earlier appearance of the first pistillate flower with respect to both number of days and node number. Additionally, a lower ratio of male to female flowers was observed on the treatments compared to the control (Singh and Singh, 1984).

Lagenaria siceraria var. Pusa Summer Prolific Long was treated with four nitrogen rates alone and in combination with growth regulator sprays. NAA at 25 ppm combined with 100 or 150 kg/ha of nitrogen induced earlier formation of pistillate flowers at lower nodes, increased number of female flowers and a higher ratio of female to male flowers resulting in increased fruit yields (Sharma, Jyotishi and Agrawal, 1980).

Effects of Exogenously-Applied Growth Regulants Upon Sex Expression in Buffalo Gourd

Modification of growth and flowering in the buffalo gourd has been monitored in depth by Scheerens and co-workers. Experiments surveying chemical effects and application efficiencies commenced in 1979 and terminated in 1984. For a thorough review, see Scheerens (1985).

A number of the chemicals surveyed failed to modify sex expression in the buffalo gourd; however, abberations in growth and morphology were manifested by these same regulants. Glyphosate (an auxin inhibitor) administered at low concentrations promoted stem and meristem thickening, and suppressed leaf, flower and tendril formation. Application of ethephon to buffalo gourd meristems curtailed growth rates, decreased internode lengths and caused suppression or abortion of floral buds. In conjunction with these responses, ethephon-treated plants exhibited abnormal stem inflections at nodes, "hooked" meristems, tightly curled tendrils and substantially reduced leaf expansion. Developmental abnormalities associated with the exogenous

application of gibberellins to buffalo gourd included internode, petiole and tendril elongation; leaf blade arching and pronounced veins; floral and peduncle elongation; and suppression of anther elongation.

Early investigations into the effects of the ethylene antagonist silver nitrate on monoecious and gynoecious buffalo gourd meristems revealed neither morphological nor floral modifications. However, more recent experiments with silver nitrate at high rates of application (750 and 1500 ppm) have resulted in successful staminate floral induction on gynoecious segregates. Differences in background genotype were attributed to the nonuniform response among replicates.

Application of 80 ppm AVG (another ethylene antagonist) demonstrated the first successful induction of male floral buds on gynoecious buffalo gourds (Scheerens, 1985). The application of AVG failed to affect flower development at existing nodes, and in addition had no effect on pistillate flower formation and production. Staminate flower induction was observed in all replicates treated with AVG at concentrations of 125 ppm and greater; the percentage of induced plants decreased at lower rates of application. The total number of staminate buds produced increased directly with application rate. Growth rates (number of nodes developed per plant) between treatments and controls were not statistically different.

The aforementioned work by Scheerens demonstrates the vulnerability of the buffalo gourd to floral modification in the staminate

direction; however, no information exists concerning the susceptibility of this plant to feminizing agents. The majority of the ensuing research was initiated to elucidate the floral and growth responses to three cucurbit feminizing agents NAA, BA and CCC.

CHAPTER 3

MATERIALS AND METHODS

Histology

Terminal sections of shoots, including the meristem and 7-10 cm of the most recently differentiated tissue, were harvested 9 September 1984. All plant materials were collected from a breeding plot of various inbred lines at the Marana Agricultural Center for the histological examination of flower development. Gynoecious and monoecious meristems were immediately fixed in FAA in the field and stored at room temperature in glass vials.

Shoots were rinsed twice in 1:1 ethanol and distilled water, and immature flower buds excised from the nodes. Flowers were automatically embedded with a Tissuematon (Fisher Scientific Co., Pittsburg, PA) over a 24-hr period (Appendix A, Embedding Schedule).

Metal boats were washed with pure ethyl alcohol, and wiped with paraffin oil. Small amounts of liquid paraffin were put into the boats, the flowers were then oriented and covered with paraffin. Blocks were cooled on a Tissue-Tek thermoelectric centre (Lab-Tek Instruments Co., Westmont, IL) for 3 hr, immersed in ice water for 0.5 hr, and stored in a freezer overnight.

Excess paraffin was trimmed off the block prior to sectioning. The microtome (American Optical Co., Buffalo, NY) was adjusted to yield

15 μ sections and the blade angle was 30°. Ribbons were stored in tissue paper in a freezer overnight.

A Tissue-Tek water bath (Lab-Tek Instruments Co.) was washed and filled with distilled water. When the water reached 40°C, 1 g of gelatin (Baker Chemical Co., Phillipsburg, NJ) was added with agitation. Slides were washed in pure ethanol and vigorously wiped with lab tissues. Sections were cut to size and placed onto the slides which were slowly submerged into the water bath, allowing the ribbons to expand. Slides were carefully lifted from the water bath with adhering sections. Slides were dried overnight on a slide warmer (Chicago Surgical and Electrical Co., Melrose Park, IL), and stained over the next 24 hrs (Appendix B, Staining Schedule).

Floral Taxonomy

Fifty staminate and 50 pistillate flowers were harvested at anthesis at the Marana Agriculture Center in early October 1985. Flowers were randomly selected above and below the foliar canopy. Staminate flower measurements included calyx and corolla length, and calyx/corolla ratio. Carpel number, ovary length, width and ratio, corolla length, and stigma length were the measurements taken on pistillate flowers. Mean and coefficient of variation were the statistics evaluated on all these field grown flowers.

Flower Development

Field studies to quantify floral development periods (elapsed time to anthesis) were initiated during late August - early September

1985 using 100 plants of mixed genetic background in their first season of growth at the University of Arizona - West Campus Agricultural Center. Staminate and pistillate flower buds (50 each) present at nodes of the first free-standing leaves (i.e., the first node proximal to the meristematic region of developing shoots) on monoecious plants were tagged and dated, and their subsequent development was observed daily. The number of days required to reach anthesis was recorded for each bud. In addition, development of 50 pistillate buds on gynoecious individuals was monitored in a similar fashion.

Growth Regulator Experiments

Growth regulants (NAA, BA, CCC) used in field experiments were acquired from Sigma Chemical Co., Inc. (St. Louis, MO), and prepared at concentrations similar to those employed in published studies of sex expression in various cucurbits (Chapter 2).

Stock solutions of each growth regulant were prepared prior to experimentation, stored at $5^{o}C$, and fresh dilutions prepared before each application. Experiment Set I stock solutions of NAA and BA were 100 ppm, and CCC was 200 ppm. Stock solution for Experiment Set II was 600 ppm for the three chemicals. Each stock solution contained 6 ml of dimethyl sulfoxide (DMSO) and 3 ml of Polysorbate 80 (Tween 20) per liter. Application rates of 0, 10, 25, and 50 ppm (NAA and BA) and 0, 50, 100, and 200 ppm (CCC) characterized Experiment Set I. Treatments of 0, 200, 600 ppm (NAA and BA) and 0, 400, 600 ppm (CCC) characterized

Experiment Set II. Control solutions of distilled deionized water contailed 0.6% DMSO and 0.3% Tween 20.

Second year buffalo gourd plants of mixed genotypes grown at the University of Arizona West Campus Agricultural Center were selected for study. Fifteen monoecious and fifteen gynoecious plants were randomly assigned the three growth regulants (5 plants/sex type/ regulant). Each regulant was applied at four concentrations, and administered to two runners per concentration. The first treatment day colored yarns were tethered around runners at the first expanded leaf stage to delineate application rates. Treatments for Experiment Set I consisted of submerging apical meristems into glass vials containing growth regulant solutions for 15 seconds with agitation. Control treatments were administered via atomizer sprayers. All treatments for Experiment Set II were accomplished with atomizer sprayers, and applications were made every other day for both sets of experiments. Thirty days after the first application runners were measured and florally mapped for both sets of experiments. Growth and flowering parameters were analyzed with various statistical packages contained within MSUSTAT.

CHAPTER 4

RESULTS AND DISCUSSION

Preliminary studies in the buffalo gourd examining growth, floral development and flowering patterns have been reviewed by Scheerens et al. (1986). Further investigations into growth and flowering were conducted in the present study and aimed at expanding the basic knowledge data base of this species.

Histology

Histological examinations of staminate and pistillate buds from monoecious and gynoecious buffalo gourds did not reveal evidence of a morphological bisexual stage. It is quite possible that the bisexual stage is entertained in younger flowers more tightly contained in the envelope of developing leaves than in the buds examined. Rare functional hermaphroditic flowers have been reported in this species (Scheerens, 1985), and such bisexual stages are characteristic during the early development of unisexual cucumber flowers (Heimlich, 1927; Judson, 1929; Galun, 1961). Both Yousef (1976) and Pereira (1968) failed to uncover bisexual stages in buffalo gourd and *C. pepo*, respectively.

Floral Taxonomy

In an effort to further typify floral relationships in this species, selected measurements (calyx and corolla lengths and their

ratio for male flowers, and equatorial and axial ovary widths and their ratio and corolla and stigma lengths for female flowers) were performed on flowers upon anthesis. Flower part relationships are presented in Table 4. In Table 4 it can be seen that corolla measurements of pistillate and staminate flowers compare closely. The average staminate flower calyx length was 16.6 mm. Current staminate flower measurements given in Table 4 are similar to other such measurements on the buffalo gourd reported by Bailey (1943). The buffalo gourd ovary is almost spherical, and is similar in dimension to C. pepo (Nitsch et al., 1952). Style length of pistillate flowers was 1/3 the corolla length of pistillate flowers. Finally, 80 percent of the pistillate flowers sampled possessed tricarpellate fruit, and the remainder were tetracarpellate.

Flower Development

For purposes of controlled hybridization, it is of benefit to predict the period of time required for floral bud maturation. To estimate the duration of this requirement, potential staminate and pistillate flowers in the axils of the first free standing leaves on shoots were tagged and observed until anthesis. These results are presented in Figure 1. The average time period for staminate bud maturation on monoecious plants was 19.5 $\pm$ 3.0 days (Figure 1a), almost twice that for pistillate buds on the same sex type (10.9 $\pm$ 1.0 days, Fig. 1b) and pistillate buds on gynoecious individuals (9.7 $\pm$ 1.5 days, Fig. 1c). Variability in maturation period was greatest in staminate flowers and it differed as much as 11 days time to anthesis.

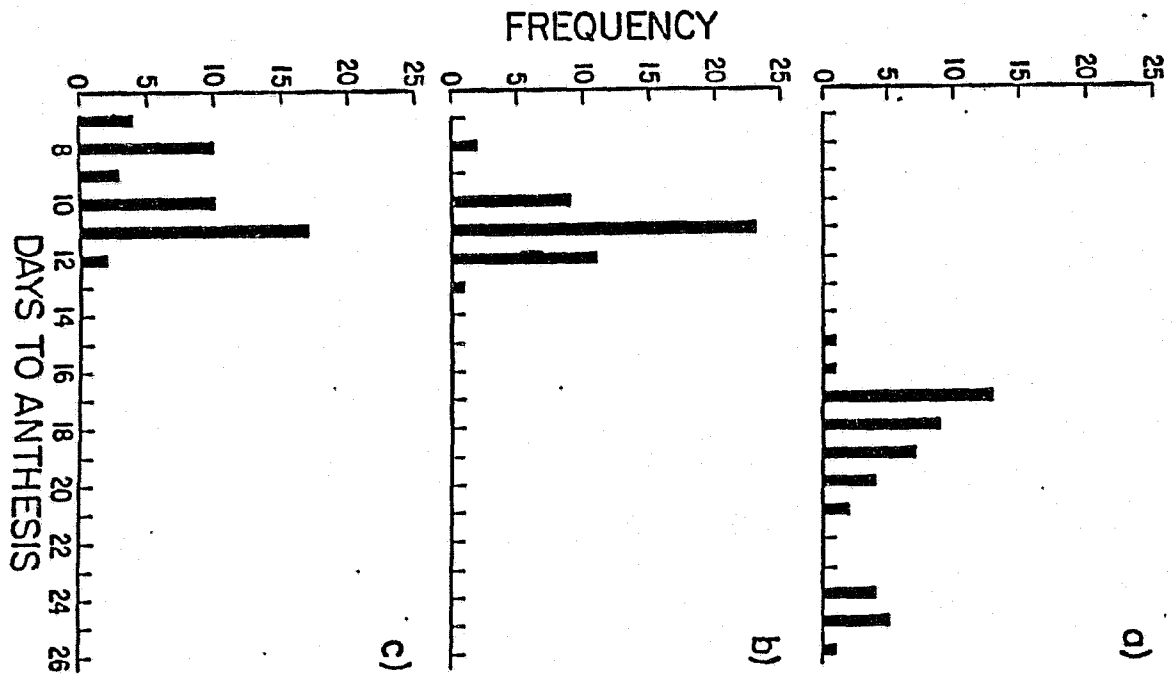
Table 4. Buffalo Gourd Floral Dimension Data

	$\bar{X}$ Length (mm)	CV
Staminate Flowers		
calyx	16.6	19.4
corolla	77.1	15.1
ratio 1/	0.22	24.1
Pistillate Flowers 2/		
corolla	73.2	17.1
ovary (equatorial)	18.3	12.0
ovary (axial)	22.4	12.9
ratio 3/	1.2	10.1
style	27.1	14.0

1/ ratio of calyx to corolla

2/ calyx measurements were not recorded

3/ ratio of equatorial to axial ovary measurements



(a) Staminate flowers
(b) Pistillate flowers (monoecious plants)
(c) Pistillate flowers (gynoecious plants)

Figure 1. Frequency Distributions of Days to Anthesis

Similar studies of floral development in other Cucurbita were not available for comparison. However, staminate buds induced on gynoecious buffalo gourd segregates via the application of AVG achieved anthesis in 21 - 26 days after treatment of the meristems (Scheerens, 1985). A staminate bud induced through chemical treatment may take 5 - 6 days to reach the developmental stage normally found in the buds at the axil of the first free-standing leaves. Considering this initial developmental period, the maturation requirements for induced staminate buds mirrors those observed on their naturally-initiated counterparts.

Effects of Growth Regulants on Growth and Flowering

Morphological Manifestations

Morphological manifestations attributed to the action of DMSO in this study included necrosis of leaf margin, and deformation of leaf shape (a decrease in midrib length but no apparent reduction of leaf width). Scheerens (1985) reported similar results along with corolla damage of pistillate flowers which were advanced in the treated meristematic region.

Growth Rate

Analysis of variance for growth parameters which are given in Table 5 were computed using the statistical program "AVFT" contained within the statistical package MSUSTAT (Montana State University, 1983), and are presented in Appendix C. Effects of regulants on growth

parameters are presented in Table 5. Differences in growth between sex types were observed on BA and CCC replications. These differences are not attributed to chemical effects as interation terms (sex x treatment) are not significant. Growth differences in this instance could have been generated by the small sample size of these studies; the general variability inherent in this still feral species (background genotype); and divergent genetic history of each individual studied.

Differences in growth parameters attributed to treatments were observed in NAA and CCC replications of Experiment Set I (Table 5). NAA significantly decreased internode lengths with increasing concentrations. Moreover, depression of nodes advanced per day was a trend observed as concentrations exceeded 50 ppm. NAA data have been omitted from Experiment Set II (Table 5) as growth and flowering terminated shortly after the first application of NAA. Leaf abberations and reduced growth have been demonstrated by these chemicals in cucumber and bitter gourd (Galun, 1959b; Mangal, Pandita and Singh, 1981).

In Experiment Sets I and II (Table 5) application of BA did not significantly affect plant growth. Increasing concentrations of BA (Experiment Set I) appear to be accompanied by increasing internode lengths. Experiment Set II displays an opposite phenomenon; internode lengths decreased as BA concentration increased. Moreover, nodes per days increased with increasing concentrations of CCC. BA application (50 ppm) to staminate inflorescences of Luffa cylindrica stimulated

Table 5. Effects of Growth Regulants on Growth Parameters for Monoecious and Gynoecious Sex Types During Experiment Sets I and II [1/]

Exp. Set	Regulant	Conc. (ppm)	Growth Parameter: nodes/day	Growth Parameter: $\bar{X}$ Internode Length (mm)
I	NAA	0	0.71 ab	108.8 b
		10	0.74 ab	104.9 b
		25	0.77 b	101.1 ab
		50	0.61 a	88.0 a
	BA	0	0.75 a	98.7 a
		10	0.78 a	108.5 a
		25	0.77 a	109.9 a
		50	0.78 a	111.1 a
	CCC	0	0.78 b	110.1 a
		50	0.69 a	111.9 a
		100	0.73 ab	102.1 a
		200	0.74 ab	110.3 a
II	NAA [2/]			
	BA	0	0.69 a	104.7 a
		200	0.77 a	102.0 a
		600	0.76 a	92.7 a
	CCC	0	0.56 a	83.0 a
		400	0.62 a	86.5 a
		600	0.62 a	82.8 a

1/ Means within treatments bearing similar superscripts are not significantly different at the P = 0.05 level.

2/ NAA applications for Exp. Set II resulted in cessation of growth.

lateral shoot growth, (Takahashi, Suge and Saito, 1980). Stunted growth and leaf yellowing were morphological abnormalities manifested by BA at concentrations less than 100 ppm on Momordica charantia (Ghosh and Basu, 1982).

The sex type vs. treatment interaction for CCC in Experiment Set II (Appendix C #10) investigating internode lengths proved to be significant. Although raw data have been omitted from the text, increasing CCC concentrations decreased internode lengths on monoecious plants while increasing internode lengths on gynoecious plants. A possible explanation of the differential response between sex types to this GA antagonist could be linked to varying endogenous GA levels between sex types. These growth differences do not parallel other published growth studies in the buffalo gourd (Scheerens et al., 1986). Growth parameters (nodes/day and $\bar{x}$ internode length) showed no association with sex type in two separate experiments observing up to 20 plants per sex type.

Floral Development

Effects of growth regulants on flower abortion are expressed in Table 6. In Experiment Set I (Table 6) it can be seen that CCC was the only chemical to significantly affect flower abortion. A difference was observed between the control (17.33% abortion) and application of 50 ppm (3.333% abortion), however, higher rates were not different from controls.

Table 6. Effects of Growth Regulants on Flower Abortion For Monoecious and Gynoecious Sex Types During Experiment Sets I and II [1/]

Exp. Set	Regulant	Conc. (ppm)	% Flower Abortion
I	NAA	0	21.0 [a]
		10	17.4 [a]
		25	21.6 [a]
		50	32.5 [a]
	BA	0	12.5 [a]
		10	9.5 [a]
		25	16.1 [a]
		50	5.7 [a]
	CCC	0	17.3 [b]
		50	3.3 [a]
		100	13.8 [ab]
		200	16.2 [b]
II	NAA [2/]		
	BA	0	16.6 [a]
		200	16.4 [a]
		600	13.0 [a]
	CCC	0	11.2 [a]
		400	7.5 [a]
		600	6.8 [a]

1/ Means within treatments bearing similar superscripts are not significantly different at the P = 0.05 level.

2/ NAA applications for Exp. Set II resulted in the cessation of growth and consequently flowering.

Endogenous GA levels may have caused this puzzling result. Treatment values were averaged over both sex types as there were no differences observed between monoecious and gynoecious individuals. There were no differences in the percentage of flowers aborted for the different concentrations of NAA and BA (Table 6). In Experiment Set II (Table 6) increasing concentrations of BA and CCC appeared to suppress flower abortion. NAA data have been omitted from Experiment Set II (Table 6) as growth and flowering were terminated following application of NAA.

Flower frequency data for Experiment Sets I and II were analyzed using the stastical program "av2w" contained within the statistical package MSUSTAT. The effects of growth regulants on flower frequencies of monoecious and gynoecious plants are presented in Tables 7 and 8.

In Table 7 (Experiment Set I) increasing concentrations of NAA applied to gynoecious meristems appeared to suppress floral primordial development. Moreover, an increase in blind node frequency accompanied the increase in NAA concentration. Data for NAA Experiment Set II have been omitted due to the cessation of growth and flowering after applications of NAA. CCC failed to modify floral frequencies in Experiment Set I, and BA failed to modify floral frequencies in Experiment Sets I and II (Table 7).

As CCC concentrations increased in Experiment Set II gynoecious plants appeared to produce more antherless flowers at the expense of blind nodes (Table 7).

Table 7. Effects of Growth Regulants on Flower Frequencies on Gynoecious Plants During Experiment Sets I and II[1/]

Exp Set	Regulant	Conc. (ppm)	Floral Frequency		
			Antherless	Pistillate	Blind node
I	NAA	0	46.25 [a]	22.50 [a]	31.25 [a]
		10	35.00 [a]	17.50 [a]	47.50 [b]
		25	26.25 [a]	21.25 [a]	52.50 [b]
		50	32.50 [a]	14.25 [a]	53.25 [b]
	BA	0	13.00 [a]	19.60 [a]	67.40 [a]
		10	12.40 [a]	19.00 [a]	68.60 [a]
		25	11.80 [a]	24.60 [a]	63.60 [a]
		50	13.20 [a]	21.20 [a]	65.60 [a]
	CCC	0	18.00 [a]	22.00 [a]	60.00 [a]
		50	20.67 [a]	14.33 [a]	65.00 [a]
		100	13.67 [a]	22.33 [a]	64.00 [a]
		200	21.00 [a]	16.67 [a]	62.33 [a]
II	NAA [2/]				
	BA	0	14.25 [a]	24.33 [a]	63.25 [a]
		200	14.00 [a]	25.33 [a]	63.50 [a]
		600	8.75 [a]	25.00 [a]	69.75 [a]
	CCC	0	24.33 [a]	25.33 [a]	50.33 [b]
		400	33.33 [a]	24.67 [a]	42.00 [a]
		600	29.00 [a]	24.33 [a]	46.67 [ab]

1/ Means within treatments bearing similar superscripts are not significantly different at the P = 0.05 level.

2/ NAA applications for Exp. Set II resulted in cessation of growth and consequently flowering.

Table 8. Effects of Growth Regulants on Flower Frequencies on Monoecious Plants During Experiment Sets I and II[1/]

Exp Set	Regulant	Conc. (ppm)	Floral Frequency		
I	NAA	0	76.00 ^{b}	20.25 ^{a}	3.75 ^{a}
		10	79.75 ^{b}	14.00 ^{a}	6.25 ^{a}
		25	55.75 ^{a}	31.50 ^{a}	12.75 ^{a}
		50	55.75 ^{a}	33.75 ^{a}	10.50 ^{a}
	BA	0	73.80 ^{a}	11.40 ^{a}	14.60 ^{a}
		10	68.40 ^{a}	14.80 ^{a}	16.80 ^{a}
		25	58.40 ^{a}	13.00 ^{a}	28.60 ^{a}
		50	65.20 ^{a}	11.30 ^{a}	23.80 ^{a}
	CCC	0	93.33 ^{a}	6.667^{a}	00.00 ^{a}
		50	87.00 ^{a}	10.33 ^{a}	1.333^{ab}
		100	84.33 ^{a}	11.33 ^{a}	4.333^{b}
		200	89.67 ^{a}	10.33 ^{a}	00.00 ^{a}
II	NAA [2/]				
	BA	0	65.00 ^{a}	6.00 ^{a}	29.00 ^{a}
		200	57.00 ^{a}	15.00 ^{a}	28.00 ^{a}
		600	72.00 ^{a}	6.00 ^{a}	22.00 ^{a}
	CCC	0	74.33 ^{a}	14.00 ^{a}	11.67 ^{a}
		400	89.67 ^{ab}	1.333^{a}	6.333^{a}
		600	92.00 ^{b}	6.667^{a}	1.333^{a}

1/ Means within treatments bearing similar superscripts are not significantly different at the P = 0.05 level.

2/ NAA applications for Exp. Set II resulted in cessation of growth and consequently flowering.

Data in Table 8 (Experiment Set I) indicate that NAA was a demasculinizing agent on monoecious buffalo gourds. Concentrations exceeding 10 ppm reduced staminate flower production, but did not alter pistillate flower or blind node frequencies. Higher application rates of NAA (Experiment Set II) terminated growth and flowering and therefore these data have been omitted from Table 8. BA had no affect on floral frequencies on monoecious plants (Table 8). In Experiment Set I (Table 8) CCC applications appeared to curtail the development of staminate flowers which is reinforced by a corresponding increase in blind node frequency. In Experiment Set II (Table 8) CCC applications exhibited an increase in masculinity while producing trends of decreased female flower and blind node production on monoecious segregates.

The physiology of floral primordia initiation is perhaps different in buffalo gourd compared to other cucurbits. Taxonomically, the buffalo gourd shows loose affinity to the cultivated and more common cucurbits. The effects of BA noted in Table 8 do not parallel feminizing responses typical of other cucurbits, yet growth and flowering responses of the buffalo gourd to CCC and NAA coincide with results manifested on other cucurbits.

Summary

Histological examination of immature staminate and pistillate flower buds of the buffalo gourd failed to reveal evidence for a morphological bisexual stage typical of other cucurbits. Values for

calyx and corolla measurements on staminate flowers were 16.6 and 77.1 mm, respectively. Corolla and style lengths were 73.2 and 27.1 mm respectively for pistillate flowers. Immature ovaries were almost spherical exhibiting an equatorial/axial length ratio of 1.2. Tricarpellate ovaries comprised 80 percent of a sampled population, the remaining flowers possessed four carpel ovaries. The average time period for staminate bud maturation on monoecious plants was 19.5 $\pm$ 3.0 days, almost twice the requirement for pistillate buds on the same sex type (10.9 $\pm$ 1.0 days) and pistillate flowers on gynoecious individuals (9.7 $\pm$ 1.5 days). The growth regulant NAA demonstrated suppression of staminate floral primordia, and reduced growth. High concentrations of CCC advanced evidence for masculinizing effects. BA failed to modify the growth and reproductive parameters investigated.

APPENDIX A

IMBEDDING SCHEDULE

Solution	Time
55% Alcohol	2 h
70% Alcohol	6 h
85% Alcohol	2 h
95% Alcohol	2 h
100% Alcohol	2 h
1:1 Toluene:TBA	2 h
100% Toluene	2 h
100% Toluene	2 h
Paraffin	2 h
Paraffin	2 h

APPENDIX B

STAINING SCHEDULE 1/

Solution	Time
Xylene	15 m
Xylene	15 m
95% Alcohol	3-5 m
75% Alcohol	3-5 m
50% Alcohol	3-5 m
30% Alcohol	3-5 m
Distilled Water	3-5 m
Aqueous Safranin	17 h
Tap Water	Rinse
Tap Water	Rinse
30% Alcohol	3-5 m
50% Alcohol	3-5 m
70% Alcohol	3-5 m
95% Alcohol	.1 m
95% Alcohol	.1 m
Fast Green 95% Alcohol	.5 m
95% Alcohol	Rinse
Absolute ETOH:Clove Oil:Xylene (1:1:1)	2 m
Absolute ETOH:Xylene (1:1)	2 m
Xylene	15 m
Xylene	15 m
Xylene	15 m

1 ammended from Yousef (1976)

APPENDIX C

ANALYSIS OF VARIANCE TABLES

Analysis of Variance: NAA NODES/DAY EXP. I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	1255.	418.2		
Sex Type	1	16.53	16.53	.7343E -1	.7849
Treatment	3	1158.	385.9	1.714	.1939
Interaction	3	156.6	52.20	.2319	.8735
Residual	21	4728.	225.1		

Analysis of Variance: BA NODES/DAY EXP. I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	4	4601.	1150.		
Sex Type	1	748.2	748.2	6.210	.1793E -1
Treatment	3	68.68	22.89	.1900	.9021
Interaction	3	175.5	58.49	.4855	.6988
Residual	28	3374.	120.5		

Analysis of Variance: CCC NODES/DAY EXP. I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	328.7	191.4		
Sex Type	1	13.50	13.50	.2813	.6095
Treatment	3	256.3	85.44	1.780	.1964
Residual	14	671.9	47.99		

Analysis of Variance: BA NODES/DAY EXP. II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	1035.	344.9		
Sex Type	1	.6667	.6667	.5100E -2	.9423
Treatment	2	281.3	140.7	1.076	.3671
Residual	15	1961.	130.7		

Analysis of Variance: CCC NODES/DAY EXP. II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	1111.	555.4		
Sex Type	1	144.5	144.5	.8005	.3956
Treatment	2	142.1	71.06	.3936	.6887
Interaction	2	73.00	36.50	.2022	.8211
Residual	10	1805.	180.5	180.5	

Analysis of Variance: NAA INTERNODE LENGTH EXP. I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	1544.	514.8		
Sex Type	1	170.7	170.7	.9798	.3349
Treatment	3	1949.	649.8	3.730	.2665E -1
Interaction	3	653.1	217.7	1.250	.3168
Residual	21	3658.	174.2		

Analysis of Variance: BA INTERNODE LENGTH EXP. I

Source	DF	S.S	M.S.	F-Value	P-Value
Blocks	3	3497.	1166.		
Sex Type	1	3378.	3378.	9.089	.6602E -2
Treatment	3	774.2	258.1	.6943	.5687
Interaction	3	588.6	196.2	.5278	.6717
Residual	21	7806.	371.7		

Analysis of Variance: CCC INTERNODE LENGTH EXP. I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	5673.	2836.		
Sex Type	1	.1149E	5.1149E	569.39	.0000
Treatment	3	352.0	117.3	.7081	.5656
Interaction	3	324.6	108.2	.6531	.5970
Residual	14	2320.	165.7		

Analysis of Variance: BA INTERNODE LENGTH EXP. II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	5239.	1746.		
Sex Type	1	354.2	354.2	1.489	.2399
Treatment	2	633.9	317.0	1.332	.2932
Interaction	2	446.4	223.2	.9384	.4155
Residual	15	3568.	237.9		

Analysis of Variance: CCC INTERNODE LENGTH EXP. II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	587.1	293.5		
Sex Type	1	39.90	39.90	.2880	.6081
Treatment	2	53.50	26.75	.1931	.8282
Interaction	2	1242.	620.8	4.481	.4018E -1
Residual	10	1385.	138.5		

Analysis of Variance: NAA FLOWER ABORTION EXP. I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	4836.	1612.		
Sex Type	1	21.12	21.12	.7732E -1	.7799
Treatment	3	1031.	343.5	1.257	.3143
Interaction	3	1563.	520.9	1.907	.1586
Residual	21	5737.	273.2		

Analysis of Variance: BA FLOWER ABORTION EXP. I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	4	1587.	396.7		
Sex Type	1	1392.	1392.	4.424	.4216E -1
Treatment	3	585.9	195.3	.6205	.6112
Interaction	3	616.0	205.3	.6524	.5915
Residual	28	8813.	314.7		

Analysis of Variance: CCC FLOWER ABORTION EXP. I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	694.3	347.2		
Sex Type	1	352.7	352.7	3.603	.7566E -1
Treatment	3	735.0	245.0	2.503	.1011
Interaction	3	489.0	163.0	1.665	.2192
Residual	14	1370.	97.88		

Analysis of Variance: BA FLOWER ABORTION EXP. II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	3343.	1114		
Sex Type	1	1908.	1908.	4.455	.4965E -1
Treatment	2	65.58	32.79	.7656E -1	.9262
Interaction	2	424.1	212.0	.4951	.6240
Residual	15	6425.	428.3		

Analysis of Variance: CCC FLOWER ABORTION EXP. II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	1108.	554.2		
Sex Type	1	29.39	29.39	.4743	.5124
Treatment	2	65.33	32.67	.5272	.6101
Interaction	2	133.8	66.89	1.079	.3778
Residual	10	619.7	61.97		

Analysis of Variance: NAA MONOECIOUS PLANTS STAMINATE FLOWER FREQUENCY EXP. I

Source	DF	S.S	M.S	F-Value	P-Value
Blocks	3	1379.			
Treatments	3	1986.	662.1	7.513	.8360E -2
Error	9	793.1	88.12		
Total	15	4158.			

Analysis of Variance: NAA MONOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	2067.			
Treatments	3	919.2	306.4	1.546	.2684
Error	9	1783.	198.1		
Total	15	4770.			

Analysis of Variance: NAA MONOECIOUS PLANTS BLIND NODE FREQUENCY EXP I

Source	DF	S.S	M.S.	F-Value	P-Value
Blocks	3	1462.			
Treatments	3	1006.	335.3	4.358	.3703E -1
Error	9	692.5	76.94		
Total	15	3161.			

Analysis of Variance: NAA GYNOECIOUS PLANTS ANTHERLESS FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	.1402E	5		
Treatments	3	837.5	279.2	1.144	.3837
Error	9	2197.	244.1		
Total	15	.1706E	5		

Analysis of Variance: NAA GYNOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	975.3			
Treatments	3	168.2	56.08	1.896	.2004
Error	9	266.2	29.58		
Total	15	1410.			

Analysis of Variance: NAA GYNOECIOUS PLANTS BLIND NODE FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	6337.			
Treatments	3	3877.	1292.	3.384	.6742E -1
Error	9	3437.	381.8		
Total	15	.1365E	5		

Analysis of Variance: BA MONOECIOUS PLANTS STAMINATE FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	4	8907.			
Treatments	3	621.0	207.0	.5912	.6355
Error	12	4201.	350.1		
Total	19	.1372E	5		

Analysis of Variance: BA MONOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	4	1175.			
Treatments	3	44.95	14.98	.5851	.6392
Error	12	307.3	25.61		
Total	19	1527.			

Analysis of Variance: BA MONOECIOUS PLANTS BLIND NODE FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	4	9434.			
Treatments	3	620.9	207.0	.5639	.6522
Error	12	-404.	367.0		
Total	19	.1445E	5		

Analysis of Variance: BA GYNOECIOUS PLANTS ANTHERLESS FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	4	3676.			
Treatments	3	171.6	57.20	.4297	.7382
Error	12	1597.	133.1		
Total	19	5445.			

Analysis of Variance: BA GYNOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	4	875.3			
Treatments	3	94.60	31.53	.6734	.5873
Error	12	561.9	46.82		
Total	19	1532.			

Analysis of Variance: BA GYNOECIOUS PLANTS BLIND NODE FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	4	6768.			
Treatments	3	71.40	23.80	.2447	.8639
Error	12	1167.	97.26		
Total	19	8006.			

Analysis of Variance: CCC MONOECIOUS PLANTS STAMINATE FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	128.2			
Treatments	3	132.9	44.30	1.584	.2884
Error	6	167.8	27.97		
Total	11	428.9			

Analysis of Variance: CCC MONOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	87.50			
Treatments	3	47.33	15.78	.9947	.4580
Error	6	95.17	15.86		
Total	11	230.0			

Analysis of Variance: CCC MONOECIOUS PLANTS BLIND NODE FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	18.7			
Treatments	3	37.58	12.53	2.987	.1179
Error	6	25.17	4.194		
Total	11	80.92			

Analysis of Variance: CCC GYNOECIOUS PLANTS ANTHERLESS FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	2141.			
Treatments	3	103.3	34.44	.7619E -1	.9703
Error	6	27.13.	452.1		
Total	11	4957.			

Analysis of Variance: CCC GYNOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	750.2			
Treatments	3	141.7	47.22	1.970	.2197
Error	6	143.8	23.97		
Total	11	1036.			

Analysis of Variance: CCC GYNOECIOUS PLANTS BLIND NODE FREQUENCY EXP I

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	3290.			
Treatments	3	24.25	8.083	.1923E -1	.9958
Error	6	2523.	420.4		
Total	11	5837.			

Analysis of Variance: BA MONOECIOUS PLANTS STAMINATE FLOWER FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	.1029E	f		
Treatments	2	450.7	225.3	.3094	.7471
Error	6	4369.	728.2		
Total	11	.1511E	5		

Analysis of Variance: BA MONOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	358.7			
Treatments	2	216.0	108.0	3.251	.1103
Error	6	199.3	33.22		
Total	11	774.0			

Analysis of Variance: BA MONOECIOUS PLANTS BLIND NODE FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	9213.			
Treatments	2	114.7	57.33	.6168E -1	.9407
Error	6	5577.	929.6		
Total	11	.1490E	5		

Analysis of Variance: BA GYNOECIOUS PLANTS ANTHERLESS FLOWER FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	3095.			
Treatments	2	77.17	38.58	.2872	.7620
Error	6	806.2	134.4		
Total	11	3979.			

Analysis of Variance: BA GYNOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	790.2			
Treatments	2	1.556	.7778	.2612E -1	.9757
Error	4	119.1	29.78		
Total	8	910.9			

Analysis of Variance: BA GYNOECIOUS PLANTS BLIND NODE FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	3	3190.			
Treatments	2	108.5	54.25	.5127	.6263
Error	6	634.8	105.8		
Total	11	3933.			

Analysis of Variance: CCC MONOECIOUS PLANTS STAMINATE FLOWER FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	514.7			
Treatments	2	552.7	276.3	4.593	.9287E -1
Error	4	240.7	60.17		
Total	8	1308.			

Analysis of Variance: CCC MONOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	220.7			
Treatments	2	242.7	121.3	.9077	.4750
Error	4	534.7	133.7		
Total	8	998.0			

Analysis of Variance: CCC MONOECIOUS PLANTS BLIND NODE FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	436.2			
Treatments	2	366.9	183.4	2.782	.1752
Error	4	263.8	65.94		
Total	8	1067.			

Analysis of Variance: CCC GYNOECIOUS PLANTS ANTHERLESS FLOWER FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	.1502E	5		
Treatments	2	121.6	60.78	1.000	.4461
Error	4	243.1	60.78		
Total	8	.1538E	5		

Analysis of Variance: CCC GYNOECIOUS PLANTS PISTILLATE FLOWER FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	1260.			
Treatments	2	1.556	.7778	.1252E -1	.9889
Error	4	248.4	62.11		
Total	8	1510.			

Analysis of Variance: CCC GYNOECIOUS PLANT BLIND NODE FREQUENCY EXP II

Source	DF	S.S.	M.S.	F-Value	P-Value
Blocks	2	7731.			
Treatments	2	104.7	52.33	9.230	.3327E -1
Error	4	22.68	5.670		
Total	8	7858.			

LITERATURE CITED

Bailey, L. H. 1943. Species of Cucurbita. Gentes Herb. 6:265-321.

Bernier, G., J. M. Kinet and R. M. Sachs. 1981. Physiology of flowering (Vol. II). CRC Press, Boca Raton.

Berry, J. W., C. W. Weber, M. L. Dreher and W. P. Bemis. 1976. Chemical Composition of Buffalo Gourd, a Potential Food Source. J. Food Sci. 41:465-466.

Bose, T. K. and J. P. Nitsch. 1970. Chemical alteration of sex expression in Luffa acutangula. Physiol. Plant. 23:1206-1211.

Brantley, B. B. and G. F. Warren. 1960. Sex expression and growth in muskmelon. Plant Physiol. 35:741-745.

Buchman, S. 1982. On the ecology of buffalo gourd (Cucurbita foetidissima) in Arizona. Paper presented to the annual meeting of the Entomological Society of America, Tucson, AZ, June 23, 1982.

Chailakhyan, M. Kh. 1979. Genetic and hormonal regulation of growth, flowering and sex expression in plants. Am. J. Bot. 66: 717-736.

Curtis, L. C. and N. Rebeiz. 1974. The domestication of a wild, perennial, xerophytic gourd, Cucurbita foetidissima, the buffalo gourd. Report of the Arid Lands Agricultural Development Program, The Ford Foundation.

Cutler, H. C. and L. Kaplan. 1956. Some plant remains from Montezuma Castle and nearby caves. Plateau 28:98-100.

Dittmer, H. J. and B. P. Talley. 1964. Gross morphology of tap roots of desert cucurbits. Botan. Gaz. 125:121-126.

Dossey, B. F., W. P. Bemis and J. C. Scheerens. 1981. Genetic control of gynoecy in the buffalo gourd. J. Heredity 72:355-356.

El-Kholy, E. and H. Hafez. 1982. Response of snake cucumber Cucumis melo var. pubescens) to some plant regulators. J. Agric. Sci. Camb. 99:587-590.

Foster, R. E. and W. T. Bond. 1967. Abrachiate, an androecious mutant muskmelon. J. Heredity 58:13-14.

Frankel, R. and E. Galun. 1977. Pollination mechanisms, reproduction and plant breeding. Springer-Verlag, New York.

Fugieda, K., Y. Fugita, Y. Gunji and K. Takahashi. 1982. The inheritance of plural-pistillate flower in cucumber. J. Jap. Soc. Hort. Sci. 51:172-176, Biol. Abstr. 75:80320.

Galun, E. 1959a. The role of auxins in the sex expression of the cucumber. Physiol. Plant. 12:48-61.

Galun, E. 1959b. Effects of gibberellic acid and naphthaleneacetic acid on sex expression and some morphological characters in the cucumber plant. Phyton 13:1-8.

Galun, E. 1961. Gibberellic acid as a tool for the estimation of the time interval between physiological and morphological bisexuality of cucumber floral buds. Phyton 16:57-62.

Gathman, A. C. and W. P. Bemis. 1985. History, biology and chemistry of the buffalo gourd, Cucurbita foetidissima HBK. In: R. W. Robinson (ed.) Biology and chemistry of the Cucurbitaceae. Cornell Univ. Press, Ithaca.

George, W. L. 1970. Dioecism in cucumbers, Cucumis sativus L. Genetics 64:23-28.

Ghosh, S. and P. S. Basu. 1982. Effect of some growth regulators on sex expression of Momordica charantia. Sci. Hort. (Amsterdam) 17:107-112, Biol. Abstr. 74:68561.

Ghosh, S. and P. S. Basu. 1983. Hormonal regulation of sex expression in Momordica charantia. Physiol. Plant. 57:301-305.

Hall, W. C. 1949. Effects of photoperiod and nitrogen supply on growth and reproduction in the gherkin. Plant Physiol. 24:753-769.

Heimlich, L. F. 1927. The development and anatomy of the staminate flower of the cucumber. Am. J. Bot. 14:227-237.

Heslop-Harrison, J. 1963. Sex expression in flowering plants. Brookhaven Symp. Biol. 16:109-122.

Hurd, P. D. and E. G. Linsley. 1964. The squash and gourd bees - genera Peponapis Robertson and Xenoglossa Smith - inhabiting America north of Mexico (Hymenoptera:Apoidea). Hilgardia 35: 375-477.

Judson, J. E. 1929. The morphology and vascular anatomy of the pistillate flower of the cucumber. Am. J. Bot. 16:69-85.

Kopcewicz, J. 1971. Influence of steroidal hormones on flower sex expression in Echballium elaterium (L.) A. Rich. Z. Pflanzenphysiol. 65:92-94.

Krishnamoorthy, H. N. and J. K. Sandooja. 1982. Effect of auxins and ascorbic acid on growth, flowering and sex expression of Cucurbita pepo L. Haryana Agric. Univ. J. Res. 12:125-127, Plant Growth Reg. Abstr. 9:308.

Kubicki, B. 1966. Genetic basis for obtaining gynoecious muskmelon lines and the possibility of their use for hybrid seed production. Genetica Pol. 7:27-30, Plant Breed. Abstr. 37:7072.

Kubicki, B. 1968. Heterotic hybrids of cucumber. Internat. Z. Landw. 2:176-178, Plant Breed. Abstr. 39:1566.

Kubicki, B. 1969a. Investigations on sex determination in cucumber (Cucumis sativus L.) IV, multiple alleles of locus acr. Genetica Pol. 10:23-68, Plant Breed. Abstr. 40:6275.

Kubicki, B. 1969b. Investigations on sex determination in cucumber (Cucumis sativus L.) III, variability of sex expression in the monoecious and gynoecious lines. Genetica Pol. 10:3-22, Plant Breed. Abstr. 40:6274.

Kubicki, B. 1969c. Investigations on sex determination in cucumber (Cucumis sativus L.) VI, androecism. Genetica Pol. 10:87-99, Plant Breed. Abstr. 40:6277.

Kubicki, B. 1969d. Investigations on sex determination in cucumber (Cucumis sativus L.) VIII, trimonoecism. Genetica Pol. 10: 123-143, Plant Breed. Abstr. 40:6279.

Kubicki, B. 1970. Androecious strains of Cucurbita pepo L. Genetica Pol. 11:45-51, Plant Breed. Abstr. 41:6365.

Laibach, F. and F. J. Kribben. 1950. Der Einfluss von Wuchsstoff auf das Geschlecht der Bluten bei einer monozischen Pflanze. Beitrage Biopl. Pflanzen 28:64-67.

Mangal, J. L., M. L. Pandita and G. R. Singh. 1981. Effects of various chemicals on growth, flowering and yield of bitter gourd. Indian J. Agri. Res. 15:185-188.

Mather, K. 1948. Nucleus and cytoplasm in differentiation. Symp. Soc. Exp. Biol. 2:196-216.

Nandgaonkar, A. K. and L. R. Baker. 1981. Inheritance of multipistillate flowering habit in gynoecious pickling cucumber. J. Am. Soc. Hort. Sci. 106:755-757.

Nelson, J. M., J. C. Scheerens, J. W. Berry and W. P. Bemis. 1983. Effect of plant population and planting data on root and starch production of buffalo gourd grown as an annual. J. Am. Soc. Hort. Sci. 108:198-201.

Nitsch, J. P., E. B. Kurtz, Jr., J. L. Liverman and F. W. Went. 1952. The development of sex expression in cucurbit flowers. Am. J. Bot. 39:32-43.

Pereira, A.S.R. 1968. Sex expression in floral buds of acorn squash. Planta 80:349-358.

Poole, C. F. and P. C. Grimball. 1939. Inheritance of new sex forms in Cucumis melo L. J. Heredity 30:21-25.

Rosa, J. T. 1928. The inheritance of flower types in Cucumis and in Citrullus. Hilgardia 3:233-250.

Scheerens, J. C. 1985. Hormonal control of sex expression in buffalo gourd (Cucurbita foetidissima HBK). Ph.D. dissertation. The University of Arizona.

Scheerens, J. C., Y.M.R. Yousef, A. E. Ralowicz, A. C. Gathman and H. M. Scheerens. 1986 (manuscript in preparation). Buffalo Gourd Sex Expression: Effect of Sex Type on Floral Development, Growth and Flowering Patterns and Growth Rate.

Scott, G. W. 1933. Sex ratio and fruit production studies in bush pumpkin. Proc. Am. Soc. Hort. Sci. 30:520-525.

Scott, J. W. and L. R. Baker. 1975. Inheritance of sex expression from crosses of dioecious cucumber (Cucumis sativus L.). J. Am. Soc. Hort. Sci. 100:457-461.

Sharma, C. P., R. P. Jyotishi and G. P. Agrawal. 1980. Studies on sex expression, sex ratio and fruit set as affected by nitrogen levels and growth regulator sprays in Lagenaria siceraria (Mol.) Standl. JNKUV Res. J. 14:47-52, Plant Growth Reg. Abstr. 9:539.

Shifriss, O. 1961. Sex control in cucumbers. J. Heredity 52:5-12.

Shifriss, O. and E. Galun. 1956. Sex expression in the cucumber. Proc. Am. Soc. Hort. Sci. 67:479-486.

Singh, G. P. and R. K. Singh. 1984. Chemical sex modification and its effect on fruiting in Cucumber (Cucumis sativus). So. Indian Hort. 32(3):127-131. Plant Growth Reg. Abstr. 11:1184.

Takahashi, H., T. Saito and H. Suge. 1982. Intergeneric translocation of floral stimulus across a graft in monoecious Cucurbitaceae with special reference to the sex expression of flowers. Plant Cell Physiol. 23:1-9.

Takahashi, H., H. Suge and T. Saito. 1980. Sex expression as affected by N^6-benzylaminopurine in staminate inflorescence of Luffa cylindrica. Plant Cell Physiol. 21:525-536.

Uzcategui, N. A. and L. R. Baker. 1979. Effects of multiple pistillate flowering on yields of gynoecious pickling cucumbers. J. Am. Soc. Hort. Sci. 104:148-151.

Warlaw, C. W. 1957. The floral meristem as a reaction system. Proc. Soc. Edinburgh Ser. B (Biol.) 66:394-408.

Whitaker, T. W. 1931. Sex ratio and sex expression in the cultivated cucurbits. Am. J. Bot. 18:359-366.

Wilkins, M. A. 1980. Yield studies of Arizona Hybrid #1 buffalo gourd. M. S. thesis. University of Arizona.

Yousef, Y.M.R. 1976. Sex expression in the buffalo gourd Cucurbita foetidissima HBK. Ph.D. dissertation. Univ. of Arizona.