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thesis entitled

PATTERNS OF STAMINATE FLOWER EXPRESSION
IN HERMAPHRODITIC PICKLING CUCUMBER
INDUCED BY SILVER NITRATE

presented by

Mary Holt Hunsperger

has been accepted towards fulfillment
of the requirements for

M.S. degree in Horticulture

Diana Hulst

Major professor

Date May 7, 1981

PATTERNS OF STAMINATE FLOWER EXPRESSION IN HERMAPHRODITIC PICKLING CUCUMBER INDUCED BY SILVER NITRATE

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1981

ABSTRACT

PATTERNS OF STAMINATE FLOWER EXPRESSION IN
HERMAPHRODITIC PICKLING CUCUMBER INDUCED BY SILVER NITRATE

By

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A series of experiments was conducted to examine the effect of silver nitrate ($AgNO_3$) on conversion to staminate flowering in two hermaphroditic pickling cucumber lines used in the production of gynoecious x hermaphrodite F_1 hybrids. The stage of plant development at which treatments were initiated strongly influenced the effectiveness of both silver concentration and application number upon the responses investigated. Based upon days to conversion, duration of conversion, and total number of staminate flowers, the optimal conversion for hybrid seed production was achieved by three applications of 200 mg/l $AgNO_3$ at four day intervals beginning at the first true leaf stage. Evidence for supernumerary bud development is presented.

ACKNOWLEDGMENTS

I would like to thank Dr. Larry Baker for suggesting the research problem and for his guidance through the initial stages during his time at Michigan State University.

My sincere appreciation is extended to Dr. Diana Helsel, not only for her help and encouragement during the writing of this manuscript, but also for her professional example.

I would like to thank Dr. Wayne Adams and Dr. Jon Fobes for serving on my committee and for their review of this manuscript.

I also appreciate all those who helped me throughout the course of my studies, especially Dr. John Gill for his excellent instruction in statistics and his help in the design of Experiment III.

A special thank you is extended to my fellow students, Anand Nandgoankar and Neil Cowen, for their assistance in the execution of my research.

Above all I am indebted to my husband, John, for encouraging me to pursue my education and seeing me through with counsel and constructive criticism.

To the Reader:

This thesis is prepared in journal-style format.

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INTRODUCTION

Single-cross hybrids involving gynoecious (G) x hermaphroditic (H) parents have been used to maximize pistillate expression in hybrids used for pickling cucumber (Cucumis sativus L.) production (7,10). Alternatively, an acceptable level of female expression occurs in three-way crosses utilizing (G x H) F_1 seed parents and monoecious (M) pollen parents (9). Both hybrid schemes fall short of their potential seed yield in the G x H cross as well as in H stock seed increases. Although the exact basis has not been determined, we speculate that the floral structure of the hermaphrodite inhibits bee visitation or the efficiency of pollen transfer. Conversion of the H line to staminate flowering in G x H seed production would equal the effectiveness of the M phenotype as a pollen parent, yet retain the greater contribution of the H genotype to female flower expression in the resulting F_1.

Silver nitrate ($AgNO_3$) has been successfully used on G lines to induce staminate flowering for stock seed increases (4,6,12). We conducted several preliminary experiments to determine an effective, nontoxic concentration range and application number for staminate flower induction in the hermaphrodite based on developmental stages recommended for G lines. A more comprehensive experiment was then performed to evaluate the effect of developmental stage on concentration and application number in two unrelated H lines.

MATERIALS AND METHODS

Three experiments were conducted in the greenhouse during the winter and spring of 1980. Each experiment utilized the H line MSU 7152H. A second H line, MSU 669H, was included in Experiment III to evaluate the effect of $AgNO_3$ on conversion in different genetic backgrounds. Plants were spaced 30 cm by 80 cm and trained to upright supports in raised benches containing an artificial soil medium (VSP Peat Lite Mix, Michigan Peat products, Co., Houston, TX). Temperatures were maintained at 25° $\pm$ 2° C in the day and 15° $\pm$ 2° C at night. Due to the low winter light levels prevailing during Experiments I and II, supplemental cool white fluorescent lighting was provided for 16 hours each day.

Treatment factors and experimental designs are presented in Table 1. Plant developmental stages were defined in the following manner: stage 0 was attained when the cotyledons were fully expanded, while stages 1,2,3, and 4 were reached when the first through fourth true leaves, respectively, had attained a 4 cm diameter. Silver nitrate was applied as a foliar spray to the entire plant until run-off. To avoid the possibility of toxicity due to surfactants, none was included. First applications were made when plants had grown to the desired developmental stage. Subsequent applications were made when each succeeding leaf had attained a 4 cm size; this corresponded to an interval of approximately four days for the greenhouse conditions in these experiments. The

Table 1. Summary of treatment factors and experimental designs used to assess the efficiency of staminate flower induction in hermaphroditic pickling cucumber.

Treatment Factors	Experiment		
	I	II	III
	1-14-80[z]	2-4-80	4-7-80
developmental stage at first application[y]	4	3	0 1 2 3
$AgNO_3$ concentration (mg/l)	0 75 150 300	0 75 150 300	0 100 200 300 400
number of applications	1 2 3	1 2 3	1 2 3 4
lines	MSU 7152H	MSU 7152H	MSU 7152H MSU 669H
experimental design	randomized complete block	randomized complete block	randomized incomplete block
replications	5	6	2

z - date of sowing
y - stage 0 is defined as full cotyledonary expansion. stages 1 through 4 are defined as the 1st through 4th true leaves at 4 cm in diameter.

plastochron was chosen as the interval between applications since it is directly related to plant development and can be extrapolated to field growing conditions.

For Experiment I the number of staminate flowers occuring at each node on the main stem was recorded for the entire four week flowering period. Additionally, the height of each plant was determined at the end of the flowering period. An analysis of variance was computed for the number of nodes bearing one or more staminate flowers, total number of staminate flowers, and plant height (Appendix A). For Experiment II the date of anthesis for each staminate flower was also recorded. An analysis of varaince was calculated for the number of nodes bearing staminate flowers, the total number of staminate flowers during each of the four weeks of flowering, the cumulative staminate flower total, and plant height (Appendix B).

In Experiment III the first three weeks of flowering were evaluated. The date of anthesis, nodal position, and sex type were recorded for each flower on the main stem as well as for each staminate flower on the laterals. An analysis of variance was calculated for the following responses: (1) days to first flower, (2) days to first staminate flower, (3) duration of staminate flowering, (4) per cent nodes bearing staminate flowers, (5) total number of staminate flowers, (6) hermaphroditic flowers on the main stem, (7) total number of flowers on the main stem, and (8) per cent staminate flowers on the main stem (Appendix C).

RESULTS AND DISCUSSION

Experiments I and II

The average number of nodes bearing at least one staminate flower varied from 0.2 in the control to 13.5 when 300 mg/l of $AgNO_3$ was applied (Table 2). There was no detectible interaction between $AgNO_3$ concentration and application number on staminate node frequency in either experiment (Appendix A). The number of staminate nodes was more strongly influenced by increasing the silver concentration than by increasing the application number.

The total number of staminate flowers per plant was also examined since our H lines bear a succession of flowers at each node. Significant interaction for this character appeared in both experiments: hence, comparisons were made between levels of one factor within each level of the other factor. Analysis of Experiment I indicated that 75 mg/l did not differ significantly from the control (Figure 1). Totals for the control reflect the tendency of MSU 7152H to produce a few early staminate flowers. No gain in staminate flower number was realized by increasing concentration from 150 to 300 mg/l when a single application was made. With two applications, however, the increase in staminate flowers was substantial. Increasing the application number to three failed to increase staminate flower production, a result possibly due to the approaching termination of vine growth. Results of Experiment II,

Table 2. Average number of staminate nodes per plant as influenced by silver nitrate concentration and application number in hermaphroditic pickling cucumber line MSU 7153H. Data from Experiments I and II.

Treatment Factors	Level	Number of Staminate Nodes per Plant	
		Experiment I	Experiment II
$AgNO_3$ (mg/l)[y]	0	0.9	0.2
	75	5.5	6.8
	150	10.3	9.3
	300	13.5	11.1
	MSD[z]	2.8	1.6
number of applications[x]	1	6.3	5.3
	2	8.4	6.9
	3	8.1	8.4
	MSD[z]	2.1	2.1

z - Scheffe's MSD (5% level)
y - averaged over all application frequencies
x - averaged over all $AgNO_3$ concentrations

Figure 1. Mean number of staminate flowers per plant resulting from $AgNO_3$ applications on hermaphroditic pickling cucumber line MSU 7152H. Data from Experiment I.

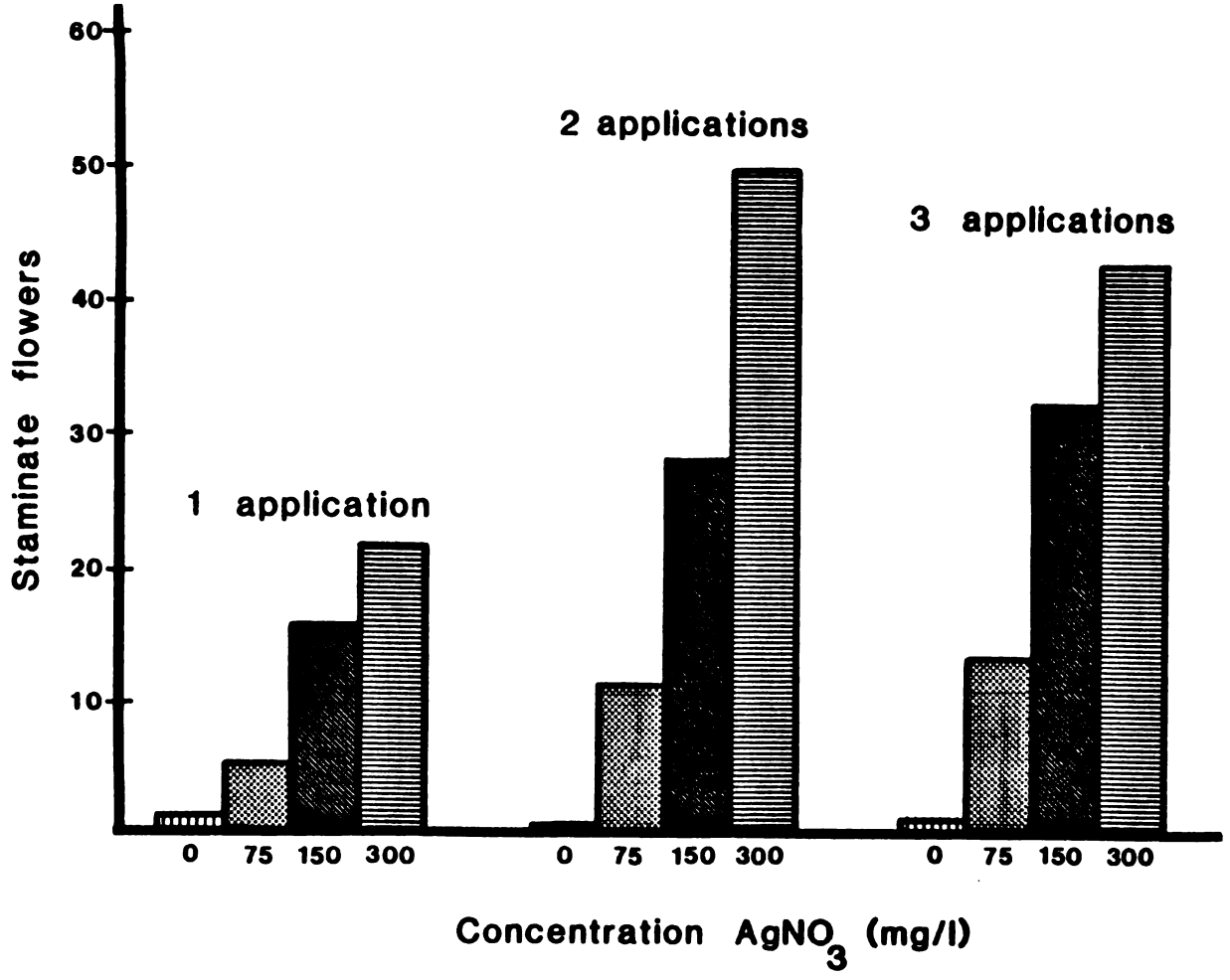

60
50
40
30
20
10
Staminate flowers
1 application
2 applications
3 applications
0 75 150 300
0 75 150 300
0 75 150 300
Concentration AgNO3 (mg/l)

Figure 1.

in which applications were made at stage 3, corresponded well with those of Experiment I.

Examination of the weekly totals for Experiment II revealed that conversion to staminate flowering did not begin until the second week of flowering (Figure 2). Plants treated with a single application demonstrated increasing numbers of staminate flowers with increasing concentration. All other application frequencies were indistinguishable, regardless of the $AgNO_3$ concentrations used. The peak of flowering occured during the third week at which time the greatest treatment differences were seen. Maximum numbers of staminate flowers were induced by three treatment combinations: three 150 mg/l applications, two 300 mg/l applications, or three 300 mg/l applications. Plants treated once or twice with 75 mg/l or once with 150 mg/l were reverting to the hermaphroditic sex type by the third week. This evidence suggests that higher $AgNO_3$ concentrations remain active longer in the plant. By the fourth week of flowering, conversion had ended for all treatment combinations with the exception of plants treated with three 300 mg/l applications.

Serious $AgNO_3$ toxicity on G lines has been noted by other researchers when applications were made before stages 3 or 4 (4,12). Under the winter conditions of Experiment I moderate, but not excessive, necrosis and crinkling of treated leaves was observed. The only treatment combination that resulted in reduced plant size was that of three 300 mg/l applications. Stunting was also accompanied by a reduction of staminate nodes; however, the total number of staminate flowers was not unduly affected (Table 2 and Figure 1). No stunting and only mild necrosis were noted under the more favorable environmental conditions of

Figure 2. Mean weekly per plant totals of staminate flowers resulting from $AgNO_3$ applications on hermaphroditic pickling cucumber line MSU 7152H. Results from Experiment II.

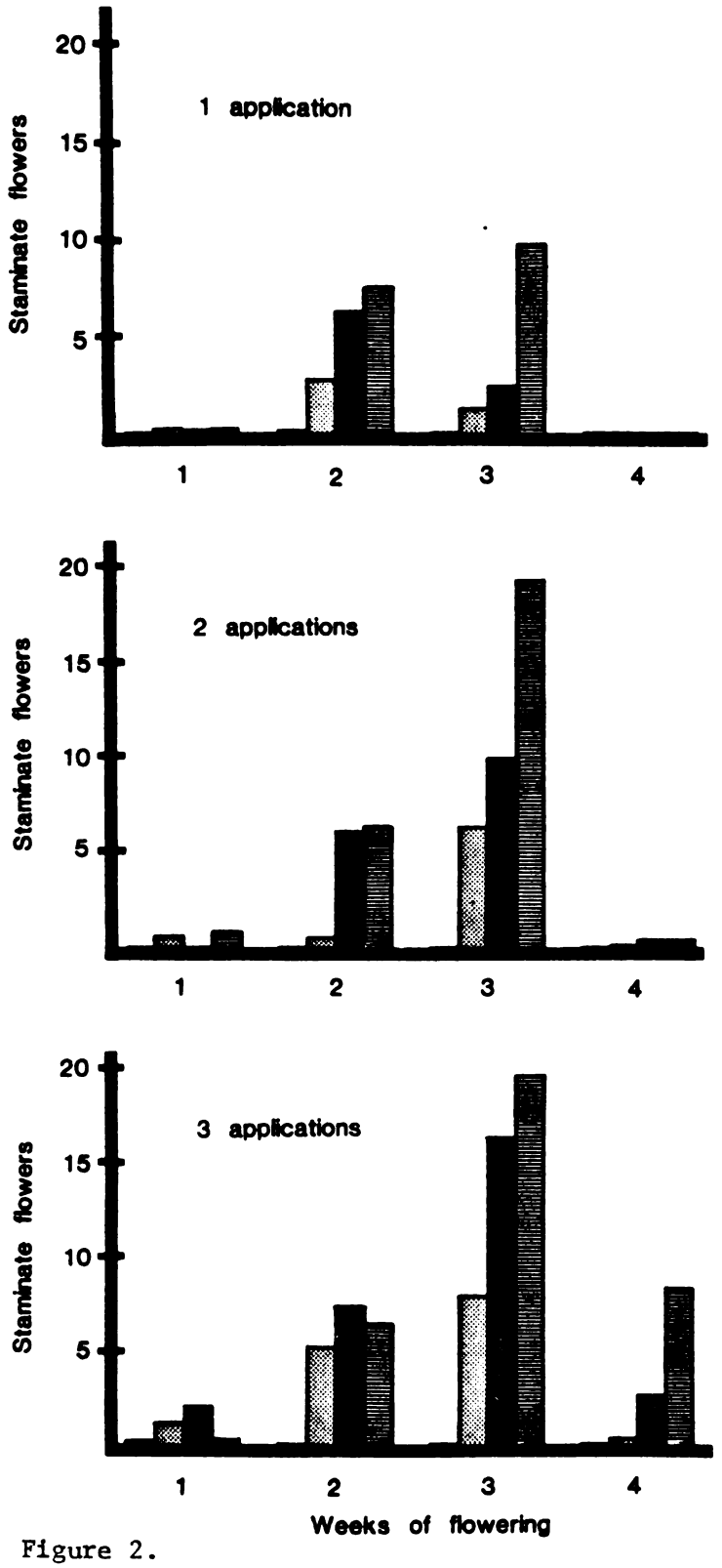

20
15
10
5
Staminate flowers
1 application
1
2
3
4
20
15
10
5
Staminate flowers
2 applications
1
2
3
4
20
15
10
5
Staminate flowers
3 applications
1
2
3
4
Weeks of flowering

Figure 2.

Experiment II.

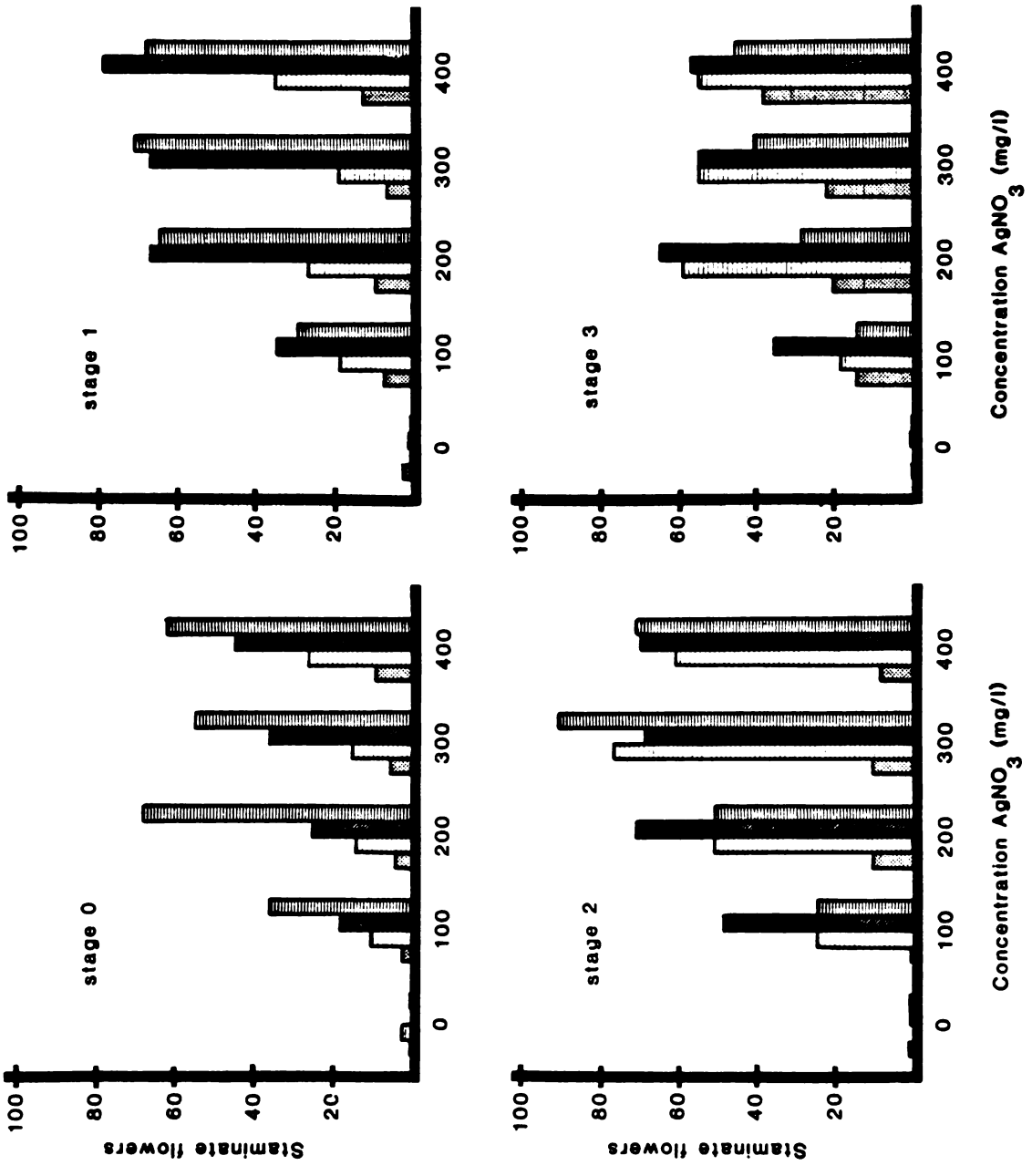
Experiment III

Since the primary goal of these experiments was to provide information useful to the producer of G x H seed, the most definitive criterion for assessing effective conversion was determined to be maximum staminate flower production during the period of most concentrated fruit set. The plant responses most strongly influencing this criterion are days to conversion, duration of staminate flowering, and number of staminate flowers. Evaluation of each response is necessary to determine the optimal treatment combination for effective conversion.

Significant interaction among stage, concentration, and application number appeared for all responses examined in Experiment III except days to first flower and days to first staminate flower. Toxicity was inconsequential at any stage or concentration.

With regard to the total number of staminate flowers produced along the main stem and laterals during the first three weeks of flowering, single applications at any concentration as well as multiple applications at 100 mg/l were in general indistinguishable from the control (Figure 3). These results were similiar to those for Experiments I and II. Treatments initiated at stage 0 resulted in fewer number of staminate flowers than similiar treatments begun at later stages. Stage 2 proved to be the most responsive since it required but two applications of 200 to 400 mg/l to produce as many staminate flowers as were produced by three applications at stage 1. Had flowering been recorded over a longer period, plants treated at stage 3 would have produced more staminate flowers since treatments at higher

Figure 3. Mean number of staminate flowers per plant resulting from $AgNO_3$ applications on hermaphroditic pickling cucumber lines MSU 7152 H and MSU 669H. Data from Experiment III.


stage 0
stage 1
stage 2
stage 3
Staminate flowers
Staminate flowers
Concentration $AgNO_3$ (mg/l)
Concentration $AgNO_3$ (mg/l)
100
80
60
40
20
0
100
200
300
400

rates were continuing to produce staminate flowers at the end of the experimental period. However, early conversion during the first three weeks of flowering is more desirable for G x H seed production since this is the period of most concentrated fruit set (3).

Treatments initiated at stage 0 resulted in staminate flowers at the onset of flowering (Figure 4). Treatments initiated at stage 1 began staminate flowering approximately one day later. The lag time from treatment to staminate flowering depended strongly upon the H line and varied up to eleven days. MSU 7152H first flowered approximately one day earlier than MSU 669H. For treatments initiated at stages 2 and 3, MSU 7152H also entered the staminate flowering phase earlier. Although MSU 669H required a longer period to express staminate flowering, it produced more staminate flowers than did MSU 7152H. Examination of the proportion of staminate flowers on the main stem revealed a significantly higher conversion rate in MSU 669H compared to MSU 7152H for treatments initiated at stages 0 and 1 (Table 3). In addition, at silver nitrate levels of 200 to 400 mg/l, MSU 669H produced a greater total number of flowers on the main stem than did its control (Table 4). MSU 7152H showed a similiar increase in total flower number at 300 mg/l. It therefore appears that silver nitrate can stimulate supernumerary flower bud development as was noted by others working with gibberellic acid (1,5).

The maximum period of conversion occured for treatments initiated at stages 0 and 1 (Figure 5). Additional applications contributed progressively less as the time of initial treatment was delayed. Concurrently, the period of staminate flowering diminished as initial treatment times were delayed. Decreased sensitivity of the plants at

Figure 4. Duration of staminate conversion as influenced by genotype and plant developmental stage at first application. Data from Experiment III on staminate flower induction by $AgNO_3$ on hermaphroditic cucumber lines MSU 7152H and MSU 669H.

▲ Day of first treatment.

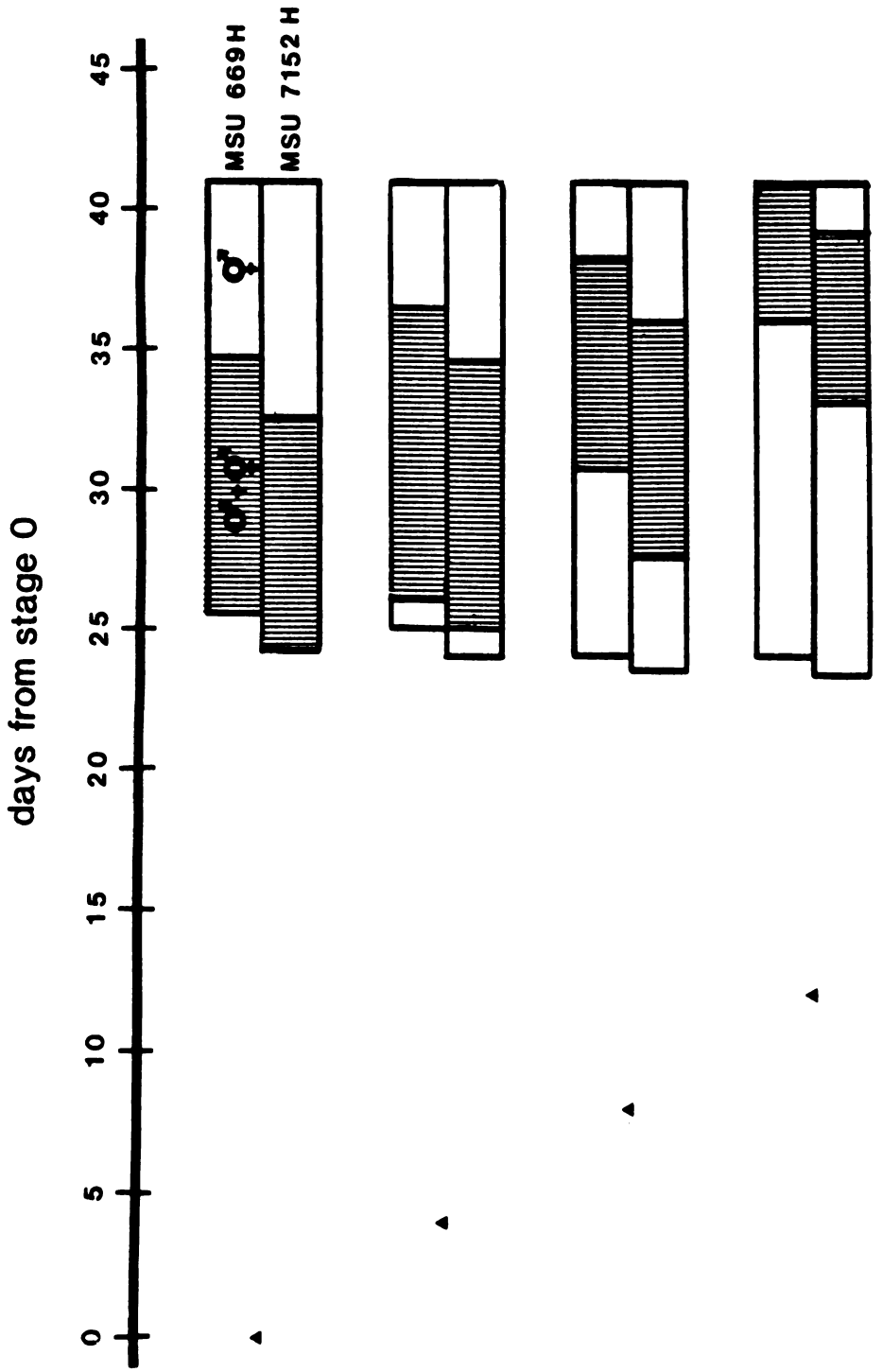


Figure 4.

Table 3. Means for proportion of staminate to total number of flowers per plant (averaged over concentration and application number) resulting from $AgNO_3$ application to two hermaphroditic cucumber lines. Data from experiment III.

Plant Stage	Line MSU 7152H	Line MSU 669H
0	0.44[z]	0.52*
1	0.56	0.63*
2	0.69	0.68
3	0.69	0.75

z - significance within rows at 5% level on arc sin transformations of data presented in table

Table 4. Means for total number of flowers per plant (averaged over stage and application number) resulting from $AgNO_3$ application to two hermaphroditic cucumber lines. Data from Experiment III.

$AgNO_3$ Concentration (mg/l)	Line[z] MSU 7152H	Line[z] MSU 669H
0	53.2	58.4
100	58.2	66.0
200	58.3	81.6
300	65.8	72.6
400	59.4	78.2

z- Scneffe's MSD within columns is 7.1 (5% level)

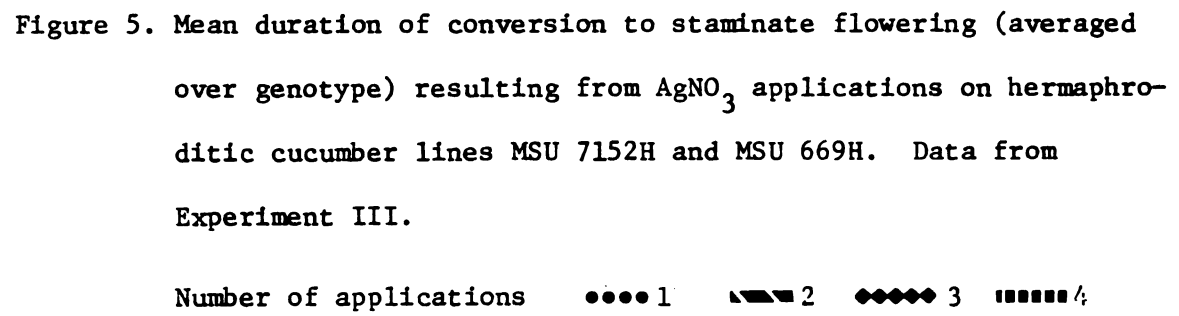


Figure 5. Mean duration of conversion to staminate flowering (averaged over genotype) resulting from $AgNO_3$ applications on hermaphroditic cucumber lines MSU 7152H and MSU 669H. Data from Experiment III.

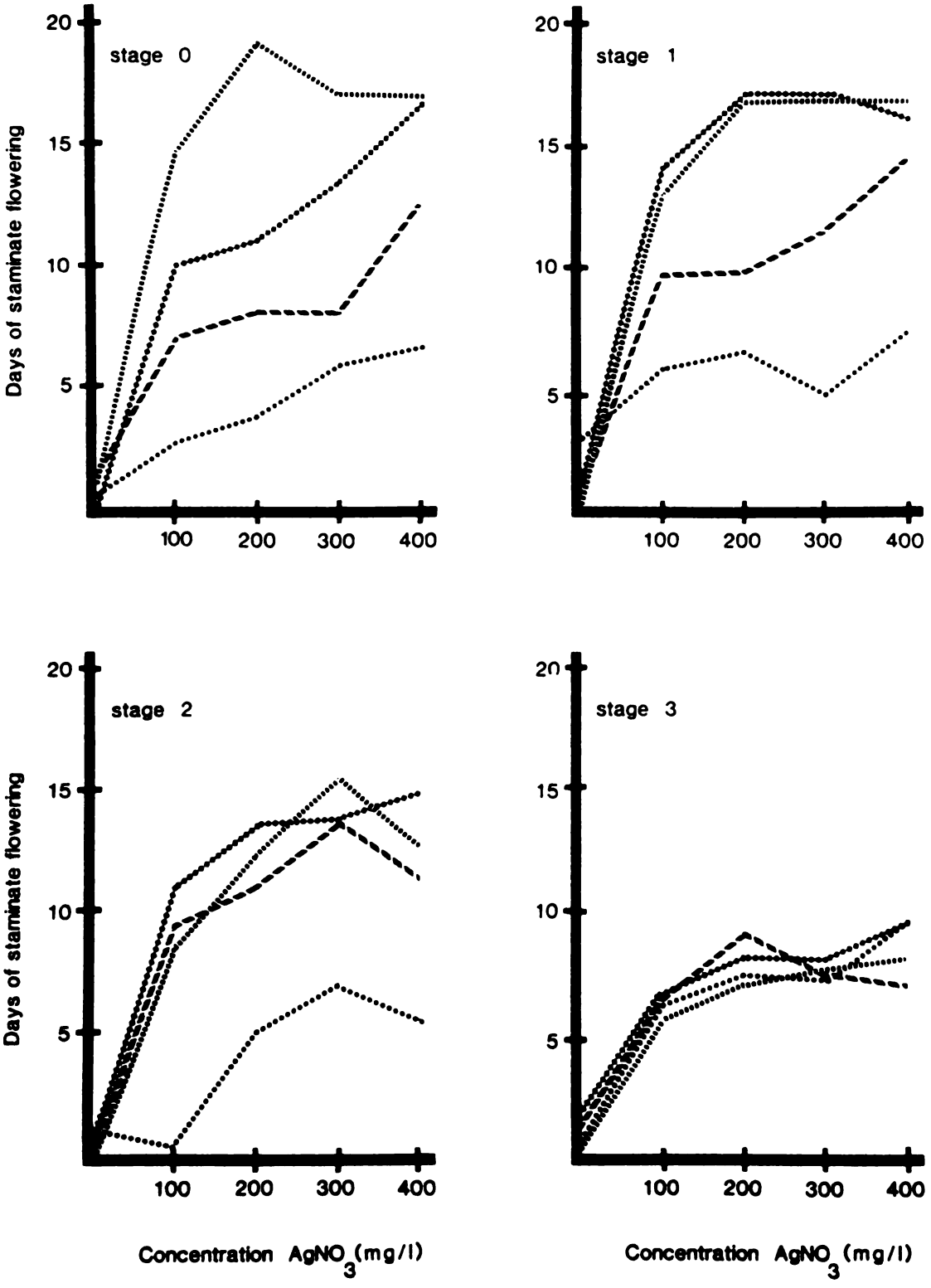

stage 0
stage 1
stage 2
stage 3
Days of staminate flowering
Days of staminate flowering
20
15
10
5
100
200
300
400
Concentration AgNO3 (mg/l)
Concentration AgNO3 (mg/l)

Figure 5.

later stages is supported by the increase in lag time between treatment and conversion noted when treatments were initiated at progressively later stages (Figure 4).

Other researchers have used the number of staminate nodes as an indication of the effectiveness of treatments on sex expression (1,4,6, 8,11,12). An analysis of variance was calculated for per cent staminate nodes to account for differences in growth rates between MSU 7152H and MSU 669H. The conclusions from this analysis were similiar to those for duration of staminate flowering.

The maximum number of staminate flowers was produced when treatments were initiated at stages 1 and 2. However, since the maximal period of early conversion occured for treatments initiated at stages 0 and 1, stage 1 was determined to be the optimal stage at which to begin treatment. The three responses selected for assessing effective conversion to staminate flowering for G x H seed production were optimized by three to four applications of 200 to 400 mg/l of $AgNO_3$ initiated at stage 1.

A promising treatment combination suggested by these experiments was examined in a preliminary field plot experiment. Excellent conversion through node 20 was observed.

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APPENDICES

APPENDIX A

APPENDIX A

Analysis of variance for Experiment I on staminate flower induction by $AgNO_3$ in hermaphroditic pickling cucumber line MSU 7152H

Sources of variation	df	Staminate Nodes	Total Staminate Flowers	Height (cm)
		MS	MS	MS
concentration	3	458.3**	4127.5**	2222.1**
application number	2	26.3*	901.0**	4585.1**
concentration X application	6	4.1	215.9**	1339.6*
blocks	4	22.8*	296.8**	1160.5**
error	44	6.9	70.0	495.0
total	59			

*significance of F test at 5% level
** significance of F test at 1% level

APPENDIX B

APPENDIX B

Analysis of variance for Experiment II on staminate flower induction by $AgNO_3$ in hermaphroditic cucumber line MSU 7152H.

Sources of variation	df	Staminate Nodes MS	1st week Total MS	2nd week Total MS	3rd week Total MS	4th week Total MS	Cumulative Total MS	Height (cm) MS
concentration	3	407.9**	1.9*	177.1**	870.7**	32.4**	2291.0**	339.4
application number	2	58.6*	6.3**	1.1	364.0**	64.9**	252.2**	1156.3
concentration X application	6	10.8	2.0	9.2	60.0*	28.7**	133.9**	1478.1**
blocks	5	19.6**	0.7	30.6**	115.1**	4.6	302.7**	3259.4**
error	55	5.0	0.7	5.9	23.4	3.4	53.1	373.4
total	71							

* significance of F test at 5% level
** significance of F test at 1% level

APPENDIX C

APPENDIX C

Analysis of variance for Experiment III on staminate flower induction by $AgNO_3$ on two unrelated hermaphroditic cucumber lines, MSU 7152H and MSU 669H. (1) days to first flower, (2) days to first staminate flower, (3) duration of staminate flowering, (4) per cent nodes bearing one or more staminate flowers (arc sin transformation), (5) total number of staminate flowers, (6) per cent staminate flowers on the main stem (arc sin transformation), (7) perfect flowers on the main stem, (8) total number of flowers on the main stem.

APPENDIX C

Sources of variation	d.f.	1 MS	2 MS	3 MS	4 MS	5 MS	6 MS	7 MS	8 MS
stage	3	28.7**	1201.2**	209.5**	0.82**	2932.7**	0.17**	680.4**	4537.5**
concentration	4	4.6	2.1	1285.5**	10.72**	23297.9**	7.82**	11031.3**	2400.2**
stage X concentration	12	4.2	3.6	17.6**	0.11**	464.4**	0.03**	125.7	311.1
application no.	3	3.0	5.5	508.3**	3.98**	18859.1**	3.63**	7065.9**	3171.8**
stage X application no.	9	3.3	4.6	85.6**	0.45**	2263.5**	0.43**	693.4**	568.0**
concentration X application no.	12	0.8	4.0	44.5**	0.33**	1671.9**	0.29**	316.9**	609.1**
stage X concentration X application no.	36	2.5	4.6	10.7**	0.06**	360.7**	0.05**	181.4**	337.2*
line	1	86.1**	289.0**	12.8	0.30	7980.0**	0.22**	465.6**	12300.8**
stage X line	3	2.3	20.6**	11.6	0.03	44.0	0.06**	175.4*	172.7
concentration X line	4	6.3*	1.1	1.6	0.01	1133.0**	0.02	42.1	1060.0**
stage X line X concentration	12	1.8	2.0	2.2	0.03	117.3	0.02	84.4	73.4
stage X line X application no.	9	4.4	6.6	11.8*	0.02	242.7	0.02	49.6	263.2
concentration X line X application no.	12	1.7	7.5	4.5	0.03	265.8	0.02	70.4	283.2
stage X line X concentration X application no.	36	1.9	4.5	5.0	0.03	139.9	0.02	65.5	187.9
replications	1	4.5	0.1	9.1	0.16*	2280.0**	0.20**	348.6*	1224.6**
blocks	6	12.2	10.0	9.4	0.03	955.3**	0.02	75.3	913.9**
error	156	2.3	4.7	5.7	0.03	182.0	0.02	58.5	208.5

* significance of F test at the 5% level; ** significance of F test at the 1% level