EFFECT OF SEVERAL SEED AND FOLIAR TREATMENTS ON FLOWERING TIME OF INBRED AND HYBRID MAIZE

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INDUCTION OF MALE STERILITY IN INBRED MAIZE WITH GIBBERELLIN SPRAYS

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Paul Milton Nelson

1958

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by

Paul Milton Nelson

AN ABSTRACT

Submitted to the School of Graduate Studies of
Michigan State University of Agriculture and
Applied Science in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

Department of Farm Crops

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Approved 

PAUL MILTON NELSON ABSTRACT

PART I

It is sometimes desirable in the production of corn hybrids to cross inbreds or single crosses which do not shed pollen and silk at the same time. To synchronize floral development, the earlier parent is planted an appropriate number of days after emergence of the later parent. This study investigated the possibilities of using various seed and foliar treatments to delay the early flowering line or hasten that of the later flowering line so both parents could be planted at the same time.

Paraffin, silicone, enamel, and gelatin capsules were tried as seed coatings to delay germination. Gibberellin, in solutions, was used for soaking seed and slurry treatments alone and in combination with the insecticide-fungicide, Delsan A-D, to hasten germination and seedling emergence. The influence of gibberellin in foliar sprays on flowering was also studied. The length of time gibberellin, in combination with Delsan A-D, would retain its activity on seed corn in storage was investigated. R53, a relatively early flowering inbred, and Oh51, a relatively later flowering inbred, and a double-cross hybrid Exp. 54-56 were used to measure the response of the various treatments.

The trials with enamel seed coatings were the most effective delaying treatment. Three coatings, in field trials, delayed flowering nine days for R53 and delayed flowering

five days for Oh51 with only slight reductions in stand.

Enclosing seed in gelatin capsules delayed emergence four to five days and appears to merit further trials.

Seedling emergence from seed soaked in gibberellin was similar to untreated controls in most treatments. Gibberellin seed treatments were largely ineffective in hastening seedling emergence.

Gibberellin-Delsan A-D slurry seed treatments were not effective in delaying or hastening flowering. Seedling growth was increased for the first 18 days after emergence. Stalks were weak and spindly, leaves were narrow, and the plants lodged. These differences soon disappeared and there was no difference in height of mature plants.

Seed treated with gibberellin-Delsan A-D and stored six months still showed stimulation of early seedling growth.

Gibberellin in foliar sprays induced sterile and partially sterile tassels and caused delays of four to five days in pollen shedding on Oh51. The resultant male sterility (Part II) would discourage this treatment to delay or hasten flowering.

PART II

Since the large-scale application of cytoplasmic male sterility in the production of corn hybrid, the possibilities

of chemical induction of male sterility have received less attention. Chemical induction, if reliable, would still be very helpful in seed production until cytoplasmic male sterility could be induced into the appropriate inbreds. The unexpected appearance of male sterility on plants sprayed with gibberellin in the greenhouse led to field experiments designed to study chemical induction of sterility with gibberellin.

Foliar spray applications of gibberellin induced male sterility in two inbred lines, R53 and Oh51. Complete male sterility occurred in Oh51 planted July 8.

Consistent induction of male sterility depended upon the concentration of gibberellin, directing the spray into the leaf whorl, and the stage of plant development. The estimated effective range of gibberellin was 5 to 35 milligrams per plant. The critical stage occurred when plants had developed approximately one inch of immature tassel. There were no "carry-over" effects on pollen production in the succeeding generation.

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INDUCTION OF MALE STERILITY IN INBRED MAIZE
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PART I

EFFECT OF SEVERAL SEED AND FOLIAR TREATMENTS ON FLOWERING TIME OF INBRED AND HYBRID MAIZE

INTRODUCTION

Some outstanding corn hybrids involve crosses of inbreds or single crosses that differ in maturity so that pollen shedding of one parent does not match silking of the other parent. In these cases, the earlier parent, which is usually the pollen parent, is planted an appropriate number of days after emergence of the latter parent.

In Michigan and other northern areas early maturity of corn is a very important characteristic of corn hybrids. Hybrids using two relatively late maturing inbreds and two relatively early maturing inbreds are frequently more productive and equal in maturity to hybrids composed of four relatively early inbreds. The two late lines must possess outstanding combining ability and the two early lines must carry a high degree of genetic dominance for early maturity.

The double-cross hybrids may be produced by crossing $(L_1 \times L_2) \times (E_1 \times E_2)$ or $(L_1 \times E_1) \times (L_2 \times E_2)$ where L_1 and L_2 represent the two late inbreds and E_1 and E_2 represent the two early lines. In the former situation, the producer of the double-cross seed would need to delay planting the single-cross pollen parent. A much larger acreage of delayed plantings would be necessary than in the latter case. Here the producer of single-cross seed would need to delay planting of both pollen parent inbred lines in the

production of seed for the two single crosses. A much smaller acreage of delayed planting would be involved. The double-cross seed producer could plant both parents at the same time.

Theoretically, one might expect more uniformity in maturity of double-cross plants from crosses of $(L_1 \times L_2) \times (E_1 \times E_2)$ than from crosses of $(L_1 \times E_1) \times (L_2 \times E_2)$. However, some hybrids of the latter type are equal in uniformity of maturity when early maturity is highly dominant.

There are several difficulties associated with delayed plantings. Variations in environment make it difficult to estimate the exact length of delay to assure matching. Rain may interfere with timely planting of the delayed parent. Crusting and hardening of the seedbed frequently result in poorer stands in the delayed rows. Weeds may get a head start in these rows and clean cultivation becomes difficult or impossible.

The purpose of this study was to investigate the possibilities of using various seed treatments and gibberellin to delay flowering of the early parent or hasten that of the later parent.

LITERATURE REVIEW

Green (4) delayed flowering in inbred corn by flaming treatments with a four-row flame cultivator. The longest delay (approximately 3 days) occurred when plants two inches tall were flamed and followed by another flaming when the plants had again reached a two inch height. Reductions in yield were significant but not great.

Dungan and Gausman (3) retarded pollen shedding in inbreds and single-cross hybrids with timely pruning of seedlings. Delays of one week resulted when plants approximately 24 inches tall were clipped three inches above the growing point. A 50 per cent reduction in grain yield accompanied this treatment. Plants clipped slightly above growing point did not recover. Plants of single crosses up to 16 inches tall and inbreds up to 12 inches tall gave significant delays to clippings made level with the soil.

Wittwer and Burovac (8) reported that slurry treatments of gibberellin in combination with Delsan A-D on sweet corn did not induce earlier seedling emergence except with concentrations exceeding 1,000 ppm. Then, the seedlings were weak and survival was low. In greenhouse trials preliminary to the present investigation, Bell (1) soaked inbred corn seed in solutions containing 10, 40, 60, 80,

and 160 ppm gibberellin for various lengths of time up to 48 hours. There was no significant effect on time of emergence.

Gibberellin sprays hastened flowering of stocks petunia, larkspur, English daisy, China aster and gerbera(5), tomatoes (2), and induced long-day plants (lettuce, endive, radish, mustard, spinach, and dill) to flower under short-day conditions (9).

MATERIALS AND METHODS

Preliminary work was done in the greenhouse during the winter and spring of 1957. Field trials were conducted on University Farms during the summer of the same year.

Greenhouse Trials

Seed Treatment. Paraffin, silicone, enamel, and gelatin capsules were tried as seed coatings to delay germination. Gibberellin was tried in solutions for soaking seed and in slurry treatments with Delsan A-D to hasten germination and seedling emergence.

Silicone is a water soluble concentrate chemically related to glass or quartz. When applied to glass and plastic surfaces, it gives a coating that is physiologically inert and under normal conditions is moisture repellent and unaffected by heat and most common chemicals. Gelatin capsules were the kind used in pharmacy. The enamel was a water-proof type material used to water-proof paper, wood surfaces, etc. Clear and flat black colors were tried. Potassium salt of gibberellic acid was used in all gibberellin seed treatments.

Seed of a relatively early flowering inbred line, R53, a relatively later flowering inbred line, Oh51, and a double-cross hybrid, 54-56 (Wf9 x SSS2873) (Oh43 x MS214),

were used to measure the response of the various treatments. Seed, except those treated with paraffin and silicone, was treated with the combination insecticide-fungicide, Delsan A-D, before the various coating materials were applied.

Paraffin coated seed was germinated in 6-inch pots containing sand. Enamel coated and gibberellin treated seed were tested in flats containing soil and also in the germinator. Silicone coated seed were tested in the germinator. Daily emergence was observed and recorded.

Paraffin seed coatings were applied by quickly dipping the seed with a table fork into and out of melted paraffin (140-145° F).

Silicone seed coatings were applied according to the directions given for applying this moisture repellent compound to a plastic surface. Seed was soaked in various solutions (5, 10, 15, 25, 50, and 100 per cent) of the silicone concentrate for various lengths of time (5, 15, 30, and 60 minutes). Excess silicone was gently rinsed off the seed with water. The remaining silicone coating was allowed to harden for 24 hours.

The water-proof enamel was sprayed on seed with the hand dispensing ("bomb") container. One to six coatings were applied. Seed was sprayed on a wide mesh (3/16" x 3/16") screen to facilitate quick and uniform drying. This method was satisfactory for small amounts of seed, but would not be practical for treating large quantities of

seed. The seed could be stored without sticking together when the enamel hardened before the seed was mixed.

Solutions containing 1,000, 2,000, 5,000, 10,000, 15,000, and 20,000 ppm of gibberellin were used to soak seed for 6 and 24 hours.

Gibberellin-Delsan A-D slurry seed treatments were prepared by adding one gram of Delsan A-D to five milliliters of gibberellin solution. Gibberellin solutions ranged from 1,000 to 5,000 ppm. The combination slurry was mixed with seed at the rate of one tablespoon per pound of seed.

Foliar Spray Treatments. The influence of gibberellin foliar sprays on flowering dates of R53 and Oh51 inbred lines were studied. Plants were grown in soil in 12-inch pots. Nutrient solution was added when needed. Plants were sprayed at various stages of plant development starting when the immature male inflorescence (immature tassel) was approximately one-fourth of an inch in length and continuing up to the time the tassel emerged from the leaf whorl. Concentrations of 100, 500, and 1,000 ppm gibberellic acid and Tween 20 (a wetting agent) were used. The spraying apparatus was an ordinary insect spray gun. Stage of plant maturity was determined by defoliating test plants to measure the length of the immature male inflorscence.

Storage of Gibberellin-Treated Seed. Gibberellin in solution loses its potency after about two weeks. An experiment was conducted to determine how long gibberellin, in combination with Delsan A-D, would retain its effect on stored seed corn. Double-cross seed of the hybrid experimental 54-56 treated with the combination slurry was stored at the Farm Crops Department seed storage with average temperature of 65°F. A similar treated lot was stored at 35-40° F. At the end of six months (July-December) the seed was tested in the greenhouse in flats containing soil. Rate of emergence and average height, 15 and 19 days after emergence, were recorded.

Field Trials

Experiment 1. Four replications of a split plot experiment using R53 and Oh51 inbred lines as main plots and 12 foliar and seed treatments as subplots were planted on June 1. The seven foliar treatments were: 100, 500, and 1,000 ppm gibberellin, each sprayed 48 and 56 days after planting and a control. The seed treatments were: one, three, and six seed coatings of clear enamel, seed soaked 12 hours in 10,000 ppm gibberellin, and in tap water as a soaking control.

Experiment 2. Double-cross hybrid seed of Experimental 54-56 with 12 different seed treatments were planted June 1 in a randomized block designed with four replications. Concentrations of 1,000, 5,000, 10,000, 15,000, and 20,000 ppm gibberellin were each applied alone

and in combination with Delsan A-D as slurry seed treatments. Controls were Delsan A-D alone and no seed treatment.

Experiment 3. R53 was planted on July 1 in a randomized block design with four replications of five treatments--control, gibberellin foliar sprays of 1,000 ppm applied 39 and 46 days after planting, a foliar spray of 2,500 ppm gibberellin applied 39 days after planting, three coats of clear enamel on seed, and two coats of black enamel on seed.

The potassium salt of gibberellic acid was used throughout. Tween 20 at 0.1 per cent was used as a wetting agent. Spraying apparatus was a "Funnel-Top" 6-quart sprayer. Emergence, flowering dates, and plant height was recorded.

Gelatin Capsules. Two seeds (R53) were inserted into each of 10 No. 00 gelatin capsules and planted in the field in early August. Rate of emergence was recorded.

RESULTS

Greenhouse Trials

Paraffin and silicone were unsuccessful as seed coating materials to delay germination. Paraffin seed coatings seriously reduced germination and only slight delays in germination occurred with silicone coatings. No data are presented.

Enamel Seed Coatings. Water-proof enamel seed coatings showed promise as a method for delaying germination and dispersing seedling emergence over a longer period. Table 1 shows the rate of seedling emergence for R53 inbred as influenced by various numbers of enamel seed coats. Three coats delayed emergence one to five days and dispersed emergence over a six day period while the control emerged in two days. Four and six coats dispersed emergence through a six day period with a slight decrease in total emergence. Six coats delayed emergence three to seven days.

Gibberellin Seed Soaking Treatment. Figures 1, 2, 3, and 4, show seedlings of Oh51 inbred and Experimental hybrid 54-56 from seed soaked 6 and 24 hours in various concentrations (1,000 to 20,000 ppm) of gibberellin. Rate of emergence is given in Table 2.

TABLE 1. Daily and total per cent emergence in greenhouse trials of R53 seed with various numbers of water-proof enamel seed coats.

No. of Seed Coats	Days from Planting to Emergence									Total Emergence
	7	8	9	10	11	12	13	14	15	
Control	90	10								100
1/2*	40	50								90
2-1/2**	50	40	10							100
1	10	90								100
2		20	60	20						100
3		10	40	20	10	10	10			100
4		10	0	30	10	20	20			90
6				40	0	10	30	0	10	90

* Seed was coated on one side only.

** Seed had two coats on one side.

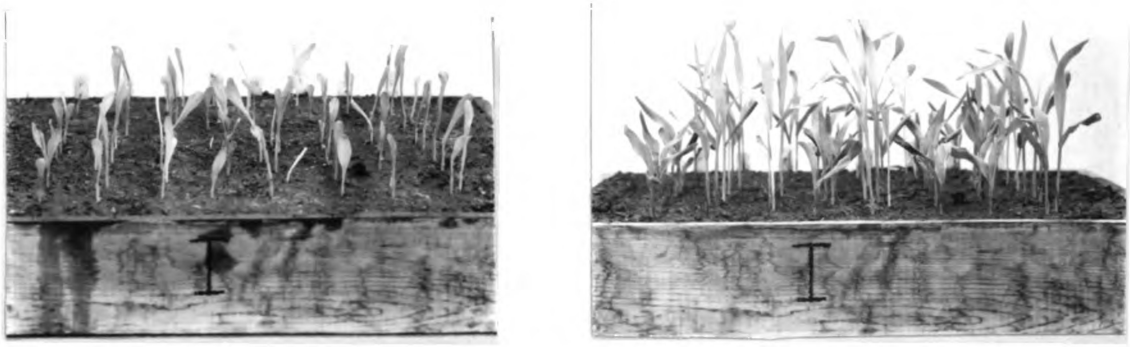

Figure 1. Seedlings from Oh51 inbred seed soaked 6 hours in gibberellin. Left, 8 days after planting; right, 13 days after planting. Treatments were, left to right, control (no soaking), 15,000 ppm, 10,000 ppm, water-soaked control, 20,000 ppm, 1,000 ppm, 2,000 ppm, and 5,000 ppm.

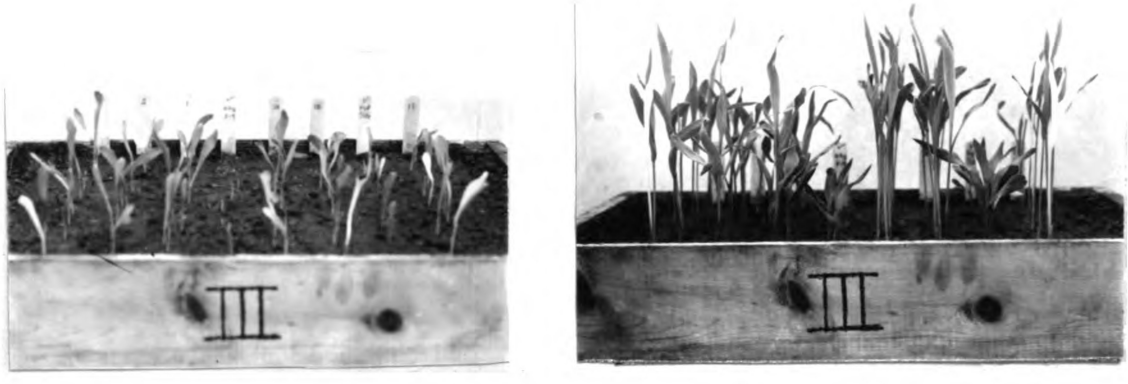

Figure 2. Seedlings from Oh51 inbred seed soaked 24 hours in gibberellin. Left, 7 days after planting; right, 12 days after planting. Treatments were, left to right, 5,000 ppm, 2,000 ppm, 1,000 ppm, control (no soaking), 20,000 ppm, 10,000 ppm, water-soaked control, and 15,000 ppm.

Figure 3. Seedlings from Exp. 54-56 hybrid seed soaked 6 hours in gibberellin. Left, 8 days after planting; right, 13 days after planting. Treatments were, left to right, control (no soaking), 15,000 ppm, 20,000 ppm, water-soaked control, 2,000 ppm, 1,000 ppm, 10,000 ppm, and 5,000 ppm.

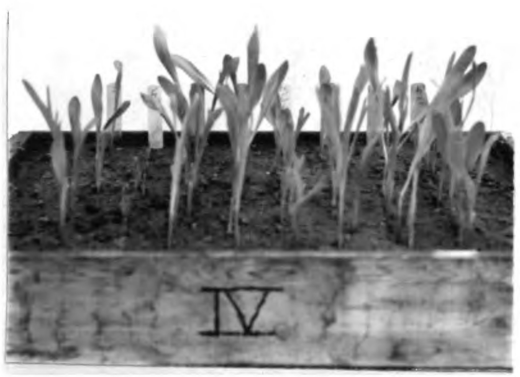

Figure 4. Seedlings from Exp. 54-56 hybrid seed soaked 24 hours in gibberellin, 7 days after planting. Treatments were, left to right, 2,000 ppm, control (no soaking), 5,000 ppm, 10,000 ppm, water-soaked control, 15,000 ppm, 20,000 ppm, and 1,000 ppm.

TABLE 2. Cumulative daily emergence in a greenhouse trial of Oh51 inbred and Experimental 54-56 hybrid seed soaked 6 and 24 hours in several concentrations of gibberellin.

Gibberellin (ppm)	Per Cent Emergence									
	Oh51 Days after Planting					Exp. 54-56 Hybrid Days after Planting				
	4	5	6	7	8	4	5	6	7	8
6 hours soaking										
No soaking	0	0	60	90	90	0	0	70	100	
Water (0)	0	0	60	100		0	20	100		
1,000	0	0	60	90	90	0	40	100		
2,000	0	0	60	90	90	0	40	100		
5,000	0	10	80	100		0	30	100		
10,000	0	10	60	80	90	0	50	100		
15,000	0	0	90	100		0	40	100		
20,000	0	10	70	100		0	50	90	90	90
24 hours soaking										
No soaking	0	0	60	100		0	0	100		
Water (0)	0	60	100			10	100			
1,000	10	80	100			70	100			
2,000	0	90	100			20	90	100		
5,000	0	60	100			50	100			
10,000	10	90	100			70	90	100		
15,000	0	80	100			50	90	90	100	
20,000	0	80	100			70	90	90	100	

Seedlings from soaked seed emerged one to two days, on the average, before seedlings from unsoaked seed. Addition of gibberellin to the soaking solution did not increase appreciably the rate of emergence.

Average seedling heights 13 days (6 hours soaking) and 12 days (24 hours soaking) after planting are presented in Table 3. Addition of gibberellin to the soaking solution markedly increased seedling growth. Seedlings from gibberellin soaked seed were two to three times taller than those from unsoaked seed or seed soaked in water. Increase in seedling height appeared to reach a maximum with a concentration of 10,000 ppm. Soaking seed for 24 hours resulted in more rapid seedling growth than soaking for 6 hours.

Seedlings from gibberellin-soaked seed had narrower stems and leaves than control seedlings. With concentrations above 5,000 ppm, the seedling stalks were weak and lodged readily.

Foliar Spray Treatments. Excessive variability in flowering time of R53 and Oh51 inbred lines under greenhouse conditions made it impossible to determine if gibberellin foliar sprays influenced flowering dates. Male sterile tassels were observed on plants sprayed with 500 and 1,000 ppm gibberellin. Investigation of this phenomenon was conducted in more detail in the field and results are reported in Part II.

TABLE 3. Average seedling height (centimeters) in a greenhouse trial of Oh51 and Experimental 54-56 hybrid seed soaked 6 and 12 hours in several concentrations of gibberellin.

Corn	Con-trol	Concentrations of Gibberellin (ppm) 0	1,000	2,000	5,000	10,000	15,000	20,000
6 hours soaking -- 13 days after planting								
Oh51	9.0	9.5	9.0	11.0	15.5	15.0	15.5	16.0
Exp. 54-56	12.5	16.0	18.5	21.5	23.0	27.5	27.5	29.5
24 hours soaking -- 12 days after planting								
Oh51	6.0	9.0	15.5	15.0	17.5	19.0	18.0	19.0
Exp. 54-56	9.5	17.0	24.0	24.5	29.0	30.5	28.0	29.0

Storage of Gibberellin-Treated Seed. Figures 5 and 6 show seedlings from gibberellin-Delsan A-D treated seed of Experimental 54-56 stored six months. Rate of emergence and average seedling height 15 and 18 days after planting are given in Table 4.

Gibberellin in combination with Delsan A-D appeared to retain, at least, part of its potency on seed stored six months in the Farm Crops seed building (average 65° F) and in refrigerated storage (35-40° F). There was no way to determine if gibberellin had lost any of its activity because seed was not tested before storage. There was no

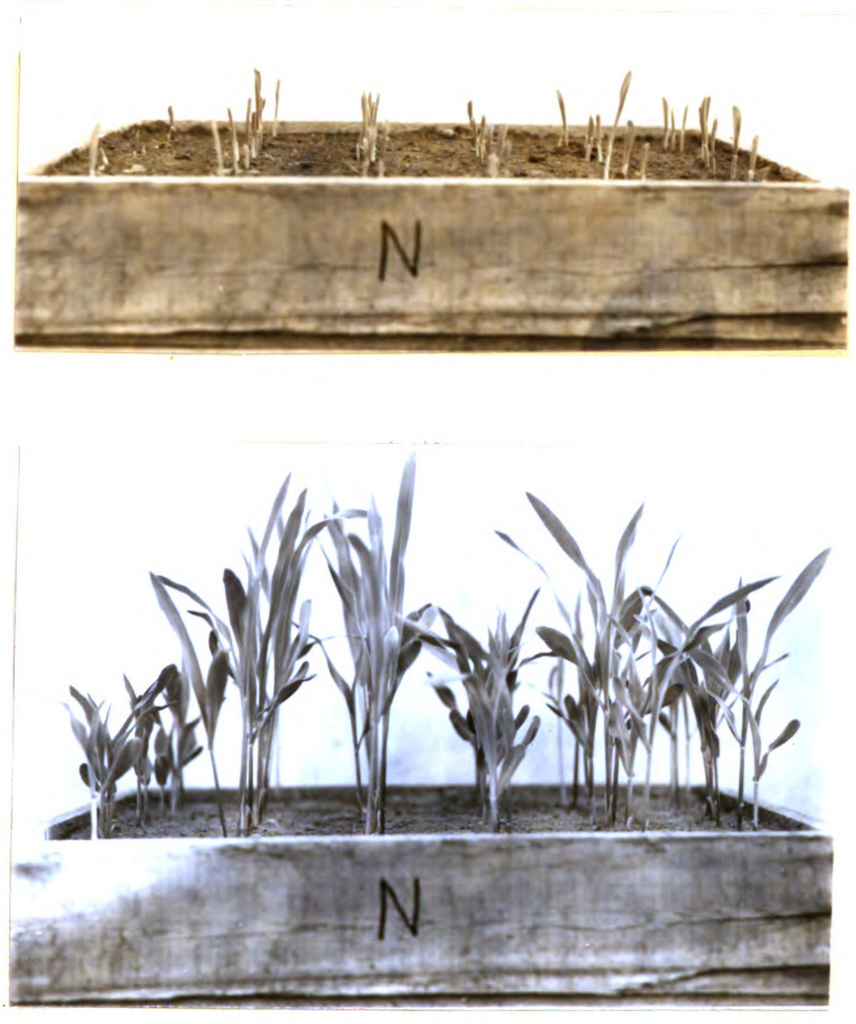

Figure 5. Seedlings from Exp. 54-56 hybrid seed treated with several concentrations of gibberellin in combination with Delsan A-D and stored 6 months at Farm Crops Department seed building. Top, 13 days after planting, bottom, 19 days after planting. Left to right, control (Delsan only), 10,000 ppm, 15,000 ppm, control (Delsan only), 5,000 ppm, and 1,000 ppm.

Figure 6. Seedlings from Exp. 54-56 hybrid seed treated with several concentrations of gibberellin in combination with Delsan A-D and stored 6 months in refrigerated storage (35-40° F). Top, 13 days after planting, bottom, 19 days after planting. Left to right, control (Delsan A-D only), 10,000 ppm, 15,000 ppm, control (Delsan A-D only), 5,000 ppm and 1,000 ppm.

TABLE 4. Cumulative daily emergence and average seedling height 15 and 18 days after planting from Experiment 54-56 hybrid seed treated with several concentrations of gibberellin in combination with Delsan A-D and then stored six months.

Gibberellin (ppm)	Per Cent Emergence Days after Planting				Average Height(cm.) Days after Planting	
	11	12	13	14	15	18
Normal Storage (65°F)						
Deslan only	0	20	70	100	4.0	12.0
Deslan only	0	40	100		6.5	17.5
1,000	40	70	100		7.5	20.0
5,000	20	90	100		9.0	21.0
10,000	30	90	90	100	10.5	25.0
15,000	40	90	90	90	10.5	24.5
Refrigerator Storage (35-40°F)						
Delsan only	0	40	90	100	6.5	14.0
Delsan only	0	70	100		6.5	17.0
1,000	70	100			9.0	20.0
5,000	90	90	100		10.5	22.5
10,000	30	100			10.5	23.5
15,000	70	90	100		11.5	25.5

appreciable difference in response between the two storage locations.

Field Trials

Experiment 1. Data are presented in Table 5. Analyses of variance for dates of pollen shedding and silking are given in Table 6. Since birds destroyed a number of plants during emergence, rate of emergence could not be determined accurately and no data are presented.

Gibberellin in foliar sprays did not significantly influence flowering dates of R53 inbred except when 1,000 ppm gibberellin was applied 56 days after planting. Silking was delayed three days with this treatment.

Gibberellin sprays of 500 and 1,000 ppm, applied 48 days after planting, on Oh51 significantly delayed pollen shedding four to five days. Silking was significantly delayed three days when 500 and 1,000 ppm gibberellin were applied 56 days after planting.

Gibberellin in foliar sprays induced male sterility. Data on male sterility from this experiment are reported in Part II.

Seed coatings with water-proof enamel were the most effective in delaying flowering time. Three coats on the seed resulted in nine days delay in pollen shedding and silking of R53 and five days delay for Oh51. One coat produced a significant delay of three days in pollen

TABLE 5. Flowering time of R53 and Oh51 inbred lines as influenced by various seed and foliar treatments. Experiment 1.

Seed and Foliar Treatments	No. of Enamel Seed Coatings	Gibberellin (ppm)	Days to Spraying	Days to Flowering	
				Pollen	Silk
R53 Inbred					
Control	0	0	0	61.1	62.5
Spraying	-	100	48	61.3	62.8
Spraying	-	100	56	62.1	63.4
Spraying	-	500	48	62.9	64.6
Spraying	-	500	56	62.4	64.2
Spraying	-	1,000	48	61.1	63.0
Spraying	-	1,000	56	62.0	65.7*
Seed coating	1	-	-	64.3**	65.6*
Seed coating	3	-	-	70.3**	71.9**
Seed coating	6	-	-	67.5**	69.3**
12 hr.seed soaking	-	0	-	62.5	64.1
12 hr.seed soaking	-	10,000	-	62.1	63.5
Oh51 Inbred					
Control	0	0	0	72.1	73.3
Spraying	-	100	48 1/	74.7*	73.8
Spraying	-	100	56	73.0	74.4
Spraying	-	500	48 1/	76.3**	73.8
Spraying	-	500	56	74.2	76.1*
Spraying	-	1,000	48 1/	77.1**	73.7
Spraying	-	1,000	56	72.7	76.4*
Seed coating	1	-	-	72.9	73.6
Seed coating	3	-	-	76.9**	78.3**
Seed coating	6	-	-	76.8**	74.4
12 hr. seed soaking	-	0	-	73.7	74.4
12 hr. seed soaking	-	10,000	-	72.2	71.9

* Significant at 5% level. Silking LSD = 2.7
Pollen LSD = 2.4

** Significant at 1% level. Silking LSD = 3.6
Pollen LSD = 3.2

1/ Immature tassels were approximately one inch in length.

TABLE 6. Analyses of variance for dates of pollen shedding and silking. Experiment 1.

Source of Variation	Degrees of Freedom	Pollen Shedding		Silking	
		MS	F	MS	F
Total	89[1]				
Main Plots					
Lines	1	2942.6		2144.2	
Replications	3	42.3		25.4	
Error 1	3	30.6		81.4	
Sub Plots					
Treatments	11	34.6	6.4**	33.7	5.0**
T x L	11	11.2	2.1*	8.8	
Error 2	60[1]	5.4		6.7	
Treatments					
Control vs Treatments	1	43.4	8.0**	29.9	4.5*
Spraying vs Other	1	56.2	10.4**	41.7	6.2*
Within Sprayings	5	6.3	1.2	9.2	1.4
Within Others					
Seed coating vs Soaking	1	139.4	25.8**	130.8	19.5**
Within Seed Coatings	2	53.0	9.8**	62.1	9.3**
Within Soakings	1	3.5		9.6	1.4

* Significant at 5% level.

** Significant at 1% level.

1/ Degrees of freedom minus 6 missing plots.

shedding and silking of R53, but did not influence flowering of Oh51. Six coats significantly delayed pollen shedding six days and delayed silking seven days for R53; and significantly delayed pollen shedding four days for Oh51.

Soaking seed for 12 hours in 10,000 ppm gibberellin did not significantly influence flowering time.

Experiment 2. Data from gibberellin and gibberellin-Delsan A-D slurry treatments on hybrid seed of Experimental 54-56 are presented in Table 7. Analyses of variance for plant height 18 days after planting, number of suckers, and suckers with tassels are given in Table 8. Bird damage altered seedling population so that rate of emergence is not reported. Observation indicated that high concentrations of gibberellin (5,000 to 20,000 ppm) hastened emergence approximately 12 to 24 hours.

Plants from hybrid seed treated with 5,000 ppm or more of gibberellin were significantly taller for 18 days following planting than those from seed receiving no gibberellin (Table 7, Figures 7 and 8). There were no significant differences 25 days after planting. Seedlings from treated seed were taller, more slender, and weak stalked. Three weeks after planting, seedlings from treated seed began to straighten and new leaves were normal in length and width.

Gibberellin in seed slurry treatments produced no significant effects on flowering dates or yield of

TABLE 7. Plant height, flowering time, number of suckers, and yield of Exp. 54-56 hybrid seed treated with several concentrations of gibberellin alone and with Delsan A-D planted June 1. Experiment 2.

Gibberellin (ppm)	Average Height in Inches Days after Planting		Days to Flowering		Suckers Per Plant		Yield (Bu.per Acre)
	18	25	Pollen	Silk	Total	With Tassel	
Gibberellin with Delsan A-D							
Delsan A-D	34.1	54.0	64.8	66.7	0.70	0.32	88.4
1,000	34.1	52.8	64.9	66.7	1.00	0.74*	81.1
5,000	38.7*	52.5	63.1	64.6	1.25*	0.86*	105.0
10,000	42.0*	52.1	65.1	66.0	0.97	0.55*	84.2
15,000	41.9*	54.1	63.9	65.1	1.00	0.72*	83.8
20,000	44.2*	55.3	64.1	65.5	0.72	0.42	82.3
Gibberellin Alone							
0	33.0	51.4	64.7	66.1	0.64	0.33	90.3
1,000	33.2	51.0	64.9	66.5	0.89	0.54	85.7
5,000	36.4*	53.4	64.3	65.9	0.99*	0.73*	86.3
10,000	39.4*	52.2	64.2	66.2	0.90	0.50	78.1
15,000	41.7*	52.2	64.3	65.8	0.83	0.49	81.6
20,000	40.9*	52.4	64.1	65.8	0.77	0.52	79.8

* Significant at the 5% level. Height LSD = 2.43
Total suckers LSD = 0.29
Suckers with tassels LSD = 0.20

TABLE 8. Analyses of variance for plant height 18 days after planting, number of suckers, and suckers with tassels for Exp. 54-56 hybrid in seed treatment. Experiment 2.

Source of Variation	DF	Plant Height 18 Days after Planting		Average Number of Suckers Per Plant		Average Number of Suckers Per Plant with Tassels	
		MS	F	MS	F	MS	F
Total	47						
Repl.	3	59.5	21.3**	0.67	16.8**	0.70	38.9**
Treat.	11	61.2	21.9**	0.13	3.3**	0.21	11.7**
Error	33	2.8		0.04		0.018	

** Significant at 1% level.

Figure 7. Seedlings 14 days after planting Exp. 54-56 hybrid seed treated with 0, 5,000, 10,000, and 20,000 ppm gibberellin alone.

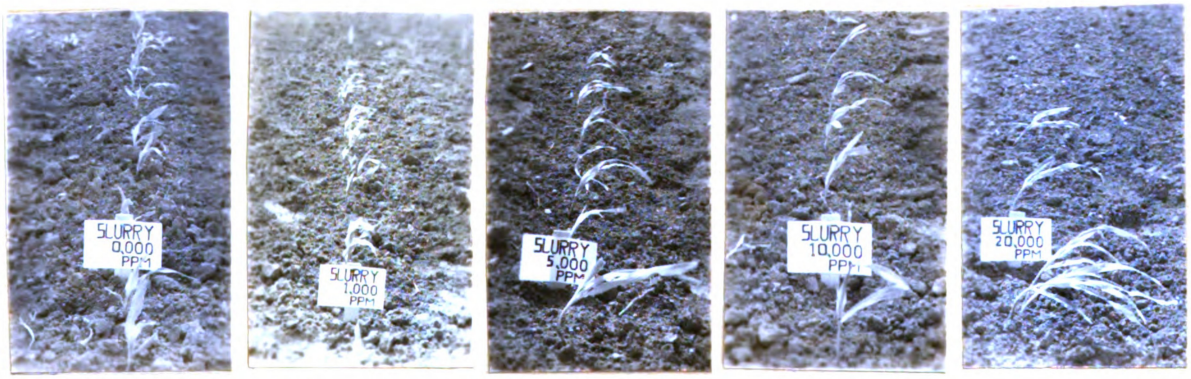

Figure 8. Seedlings 14 days after planting Exp. 54-56 hybrid seed treated with 0, 1,000, 5,000, 10,000, and 20,000 ppm gibberellin and Delsan A-D.

Exp. 54-56 hybrid. It did, however, stimulate early growth of seedlings but they were spindly and lodged. It was equally effective used alone or in combination with Delsan A-D.

Experiment 3. Enamel seed coatings delayed pollen shedding about two days and silking about three days (Table 9). The three day delay in silking was statistically significant. The two day delay in pollen shedding was statistically significant when the foliar spray treatments were removed from the statistical analysis. Analyses of variance for dates of pollen shedding and silking are given in Table 10.

Gibberellin in foliar sprays did not significantly influence flowering time, but did induce male sterility as reported in Part II.

Gelatin Capsules. Enclosing seed in gelatin capsules appeared promising as a technique to delay emergence (and possibly flowering) in a very preliminary field trial. Twenty seeds, two to a capsule, were planted in the field. Emergence was delayed four to six days when compared with the controls. All of the seeds germinated.

This was a very limited trial and the method should be studied in more detail. A delay of one week in emergence may be reduced to only one or two days at flowering. The chore of inserting seeds into capsules is obviously a tedious one unless done mechanically.

TABLE 9. Flowering time of R53 inbred as influenced by enamel seed coatings and foliar applications of gibberellin, planted July 1. Experiment 3.

Treatments	No. of Enamel Coats or Conc. of Gib. (ppm)	Time of Gib. Application (Days after Planting)	Days to Flowering	
			Pollen	Silk
Control	none	-	60.9	61.7
Gib. spray	1000 each	39 & 46	63.1	63.0
Seed coating	3 clear enamel	-	63.3	64.5*
Gib. spray	2500	39	60.1	62.3
Seed coating	2 black enamel	-	62.9	64.5*

* Significant at 5 per cent level.

TABLE 10. Analyses of variance for dates of pollen shedding and silking of R53 inbred with enamel seed coat treatments and foliar sprays of gibberellin, planted July 1. Experiment 3.

Source of Variation	Dates of Pollen Shedding			Dates of Silking		
	DF[1/]	MS	F	DF	MS	F
Total	12			19		
Replications	3			3		
Treatments	2	6.4	14.9**	4	6.2	6.6**
Error	6	0.43		12	0.93	

** Significant at 1 per cent level.

1/ Foliar spray treatments removed from statistical analysis.

DISCUSSION

The two inbreds, R53 and Oh51, in this study are used in the seed parent single-cross Oh51 x R53 for the production of double-cross seed of Michigan 250 (Oh51 x R53) (W10 x MS206). In producing the single crosses Oh51 x R53 and W10 x MS206, the pollen parent inbreds R53 and MS206 flower earlier than the female parents, thus their planting is delayed. Seedlings of Oh51 should average two inches tall before R53 is planted and W10 should average four inches tall before MS206 is planted.

A given number of seed coatings or a specific concentration of a chemical seed treatment that would consistently delay seedling emergence a given length of time under specific conditions of soil moisture and temperature would permit simultaneous planting of both parents. Since the environment following planting and emergence exerts considerable effect on matching of pollen shedding of one parent with silking of the other parent, a specific delay that matches in one year may not match well another year with a different environment. Thus, a mixture of different numbers of coatings or several concentrations of chemical treatment applied to seed of the delayed parent would provide a spread in emergence and flowering that would more nearly assure matching of the two parents.

Paraffin and silicone coating treatments as used in this study were not effective delay treatments. Germination of paraffin treated seed was low. Temperature of the melted paraffin may have been too high and injured germination or the coating may have been too impervious to air and moisture for germination. Only slight delays in germination occurred with silicone treated seed. The material was water soluble, and rinsing the seed after the silicone treatment may have removed too much of the coating.

The preliminary trials with enamel seed coatings were the most effective delaying treatments. In the field, pollen shedding and silking of R53 were delayed about three, nine, and six days when the seed was treated with one,three, and six coats, respectively. Corresponding delays in flowering of Oh51 were zero, five, and four days for one, three, and six coats, respectively.

Gibberellin-Delsan A-D slurry seed treatment did not effect flowering time. Seedlings from treated seed grew two and three times faster than those from untreated seed during the first 18 days after emergence. Stalks were weak and spindly, leaves were narrow, and the plants lodged. These differences soon disappeared and there was essentially no difference in height of mature plants.

Foliar sprays of gibberellin applied 48 and 56 days after planting were largely ineffective in delaying flowering of R53. Delays of four to five days in pollen

shedding of Oh51 occurred with treatments of 500 and 1,000 ppm 48 days after planting. The development of male sterile tassels (discussed in Part II) as a result of foliar sprays would eliminate them as treatments to delay or hasten flowering.

Enclosing seed in gelatin capsules delayed emergence four to six days. Further studies should be made comparing enamel seed coatings, gelatin capsules, and clipping treatments for their effects on flowering time.

SUMMARY

Several seed and foliar spray treatments were investigated for their effects on emergence and flowering time of inbred and hybrid corn.

Seed Treatments

Paraffin and silicone seed coatings as used in these experiments were not effective treatments to delay emergence and consequently flowering time. The paraffin coat reduced germination.

One to six enamel seed coatings delayed and only slightly decreased germination in greenhouse trials. Three enamel coatings, in field trials, delayed flowering nine days and five days for the two inbreds R53 and Oh51, respectively. There was a slight reduction in stand.

Gelatin capsules in preliminary trials showed promise for delaying germination without decreasing germination. Capsuling seed was more convenient than applying enamel seed coatings with comparable delays in germination.

Seedling emergence from seed soaked in gibberellin was similar to untreated controls in most cases. Gibberellin seed treatments were largely ineffective for delaying or hastening seedling emergence.

Gibberellin in slurry treatments increased early seedling growth of Exp. 54-56 for about 18 days. Plants were spindly and lodged easily. There was no difference in mature plant height. Gibberellin was equally effective alone or in combination with Delsan A-D.

Hybrid seed of Exp. 54-56 treated with gibberellin and Delsan A-D and stored six months still showed stimulation of early seedling growth.

Foliar Spray Treatments

Gibberellin in foliar sprays effected some significant delays in flowering of Oh51. The resultant male sterility (Part II) would discourage foliar sprays aimed to delay or to hasten flowering.

PART II

INDUCTION OF MALE STERILITY IN INBRED MAIZE WITH GIBBERELLIN SPRAYS

INTRODUCTION

Prior to the practical use of cytoplasmic male sterility in hybrid seed corn production, there was intense interest in the possibilities for chemical induction of male sterility to eliminate the tedious task of detasseling seed fields. Cytoplasmic sterility must be introduced into one of the inbreds of the single cross seed parent through a backcrossing program of five or more generations. A few inbreds are difficult or impossible to "sterilize" in this manner. Thus, chemical induction of male sterility, if reliable, would still be very helpful in seed production until cytoplasmic male sterility could be introduced into the appropriate inbreds. Observation of sterile and partially sterile tassels on inbred corn sprayed with gibberellin in the greenhouse (Part I), led to field experiments designed to investigate the possibility of using this chemical to induce male sterility in seed fields.

LITERATURE REVIEW

Moore (6) reported that 600 ppm of maleic hydrazide applied to corn at a critical stage of plant development induced sterile tassels. The critical stage of plant development was not indicated. Naylor (7) reported induction of male sterility in corn with 250 ppm of maleic hydrazide applied when plants were one meter tall with no visible male inflorescence, and suggested the use of maleic hydrazide as a means of inducing pollen sterility for production of hybrid seed corn.

MATERIALS AND METHODS

The effects of gibberellin on flowering of inbred corn were studied in the greenhouse during the winter of 1957. Gibberellin sprays were applied at various stages of plant development starting when the immature male inflorescence (immature tassel) was approximately one-fourth of an inch in length and continuing up to the time the tassel emerged from the leaf whorl. Plants sprayed with gibberellin at 500 and 1,000 ppm, when the immature male inflorescence was approximately one inch in length, developed sterile and partially sterile tassels (Part I). Possibilities of chemical induction of male sterility in corn were investigated with two inbred lines of corn in the field in 1957.

A relatively early flowering inbred line, R53, was planted June 1 and July 1 and a relatively later flowering inbred line, Oh51, was planted June 1 and July 8. The potassium salt of gibberellic acid in concentrations of 100, 1,000, 2,000, and 2,500 ppm and a wetting agent (Tween 20 at 0.1 per cent) was used as a foliar spray. Gibberellin sprays were applied at several stages of plant development ranging from approximately one to three inches of immature male inflorescence. Estimated amount of gibberellin per plant ranged from 1.0 to 10 mg for the planting on June 1,

and 12 to 35 mg for the two later plantings. The increase of gibberellin per plant in the later two plantings was caused by directing a greater amount of spray into the leaf whorl and a higher concentration of gibberellin. A "Funnel-top" six quart sprayer was used for spraying.

Stage of plant development was determined by measuring the length of the immature male inflorescence (immature tassel). Plants were defoliated (with a razor blade) to expose the immature tassel for measurement. Test plants from each planting were used to estimate the stage of plant maturity for the remaining plants.

The number of sterile and partially sterile tassels, plant height at maturity, and flowering dates were recorded.

RESULTS

Male Sterility

Gibberellin-induced male sterility ranged from tassels barren of all floral parts to tassels which approached normal pollen shedding. Most sterile tassels developed all floral parts except stamens (pollen and pollen sacs). On partially fertile tassels the upper portion of the central spike or the terminal portions of the lateral spikes, or both, developed staminate spikelets and shed pollen. The number of anthers extruding from staminate spikelets during flowering were recorded as trace, light, moderate, and normal as a measure of relative fertility (Table 11). Figure 9 shows fertile (normal), partially fertile, and sterile tassels.

More sterile tassels were induced by gibberellin at all concentrations applied 48, 39, and 36 days after planting (first spraying for the three dates of planting). At the time of treatment plants were about knee high and had developed approximately one inch of immature male inflorescence. It was therefore concluded that the critical stage of plant development for effective induction of male sterility occurred when the immature tassel was approximately one inch in length. Variability in maturity among plants at the time of spraying was a factor influencing

TABLE 11. Sterility, flowering time, and average plant height at maturity of the relatively early (R53) and relatively later (Oh51) flowering inbred lines as influenced by gibberellin in foliar spray application.

Time of Spraying[2]	Gib. (ppm)	% Male Sterile Tassels	% Partial Fertile Tassels[1]			Normal Tassels	Flowering Time[2]		Average Plant Height (inches)
			Trace	Light	Mod.		Silk	Pollen	
Oh51 Planted June 1									
--[3]	0	0	0	0	0	100	73.3	72.1	67.0
48[3]	500	32	13	32	23	0	73.8	76.0*	66.8
48[3]	1000	46	18	15	21	0	73.7	77.1*	62.2*
56	500	15	10	20	10	45	76.1*	74.2	71.2*
56	1000	25	3	7	15	50	76.4*	72.7	72.0*
R53 Planted July 1									
--[3]	0	0	0	0	0	100	61.7	60.9	45.6
39[3] & 46	1000 each	87	5	6	2	0	62.9	63.1	61.9*
39[3]	2500	87	10	3	0	0	62.3	60.1	55.0*
Oh51 Planted July 8									
--[3]	0	0	0	0	0	100	70.9	70.9	62.9
36[3] & 43	1000 each	100	0	0	0	0	70.0	MS	65.3
36[3]	2000	100	0	0	0	0	68.8*	MS	63.0
43	1000	33	13	35	19	0	71.0	70.9	64.4

1/ Trace: From one extruded anther to one inch of extruded anthers on any part of the central spike or one lateral spile. Light: More than one inch of extruded anthers on any part of the central spike or one lateral spike, or a trace on two or more spikes,

central or lateral. Moderate: More than one inch of extruded anthers on two or more spikes, central or lateral.

2/ Data represents days after planting.

3/ Immature tassels were approximately one inch in length.

MS Male sterile

* Significant at 5 per cent level.

TABLE 12. Analyses of variance for dates of pollen shedding and silking and average plant height of R53 and Oh51 as influenced by gibberellin in foliar spray applications.

Source	DF	SS	MS	F
Oh51, June 1 Planting, Days to Pollen Shedding				
Total	19	59.7		
Repl.	3	7.4	2.5	
Treat.	4	33.4	8.4	5.3*
Error	12	18.9	1.6	
Oh51, June 1 Planting, Days to Silking				
Total	19	161.2		
Repl.	3	59.0	19.7	
Treat.	4	76.9	19.2	9.1**
Error	12	25.3	2.1	
Oh51, June 1 Planting, Plant Height at Maturity				
Total	19	308.0		
Repl.	3	11.0	3.7	
Treat.	4	250.0	62.5	16.0**
Error	12	47.0	3.9	
R53, July 1 Planting, Plant Height at Maturity				
Total	11	661.7		
Repl.	3	83.1	27.7	
Treat.	2	532.5	266.2	34.6**
Error	6	46.1	7.7	
Oh51, July 8 Planting, Days to Silking				
Total	15	22.9		
Repl.	3	1.5	0.5	
Treat.	3	13.0	4.3	4.66*
Error	9	8.4	0.93	

* Significant at 5% level.
** Significant at 1% level.

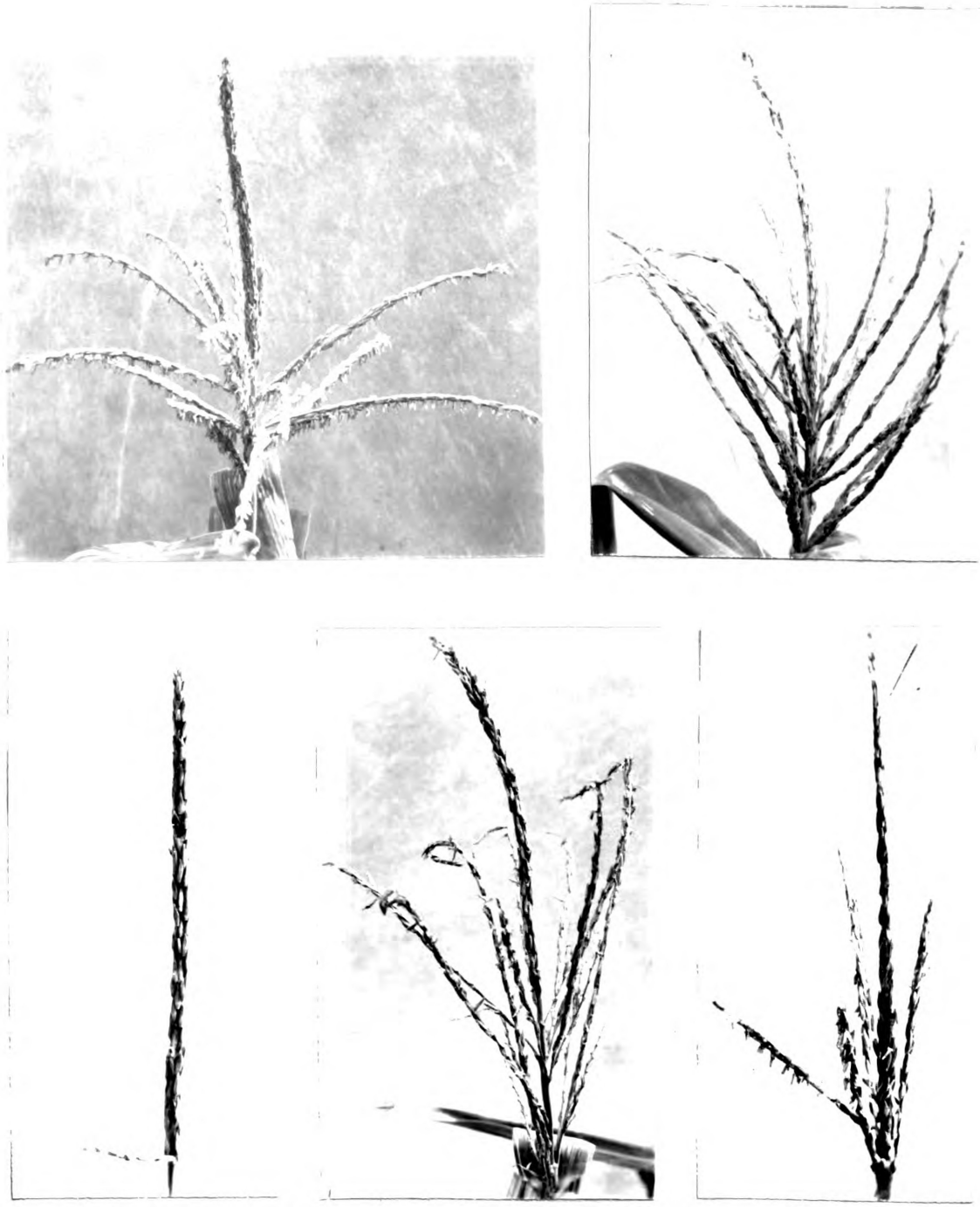

Figure 9. Top: left, normal (fertile) tassel on untreated plant; right, sterile tassel from treated plant. Bottom: Partially fertile tassels on treated plants. Left to right, "trace," "light," and "moderate" fertility (see footnote of Table 11).

the amount and degree of sterility induced in the tassel.

The early flowering line, R53, planted June 1 showed no sterility in any of the treatments and no data are presented. The immature tassels of this earlier flowering line, being considerably more than one inch in length when treated 48 and 56 days after planting, had apparently passed the critical stage of development for chemical induction of male sterility.

The later flowering line, Oh51, planted June 1 showed 32 per cent and 46 per cent totally sterile tassels when treated with 500 and 1,000 ppm, respectively, 48 days after planting (Table 11). Both concentrations of gibberellin (500 and 1,000 ppm) applied 56 days after planting induced fewer sterile tassels on the main stalk, but it was noted that there were more sterile tassels on the "suckers" (lateral shoots) than when the same treatment were applied 48 days after planting. Concentrations of 100 ppm at both times of spraying induced no completely sterile tassels and only a few partially sterile tassels and data were not recorded.

Applying gibberellin closer to the suggested critical stage of plant development and increasing the concentration two and one-half times induced male sterility in the R53 inbred planted July 1. Both treatments (2,500 ppm applied 39 days after planting and 1,000 ppm applied 39 and 46

days after planting) resulted in 87 per cent sterile tassels. Similar treatments on Oh51 planted July 8 induced 100 per cent sterility.

In the two later plantings, flowering occurred in early September when temperature and day length were less favorable for pollen development and shedding. These environmental conditions also may have favored chemical induction of male sterility. It is impossible to separate environmental effects present during the late plantings from the effect of increased concentration.

Flowering Time

Date of pollen shedding for partially fertile tassels of Oh51 planted June 1 was significantly delayed four and five days for the two treatments applied 48 days after planting. There was no significant effect on date of pollen shedding for treatments applied 56 days after planting. Silking was significantly delayed three days for both concentrations (500 and 1,000 ppm) applied 56 days after planting, but the same concentrations applied 48 days after planting had no effect.

Flowering dates for R53 planted July 1 were not significantly influenced by any of the treatments. Silking dates of Oh51 planted July 8 were significantly hastened with gibberellin at 2,000 ppm applied 36 days after planting.

The effects of gibberellin on flowering time were small and inconsistent. The differences, with the accompanying induction of partial male sterility, were not encouraging for the use of gibberellin sprays to delay or hasten flowering time in seed production of hybrids with early and late flowering parents.

Plant Height at Maturity

Oh51 planted June 1 was significantly reduced in height by gibberellin at 1,000 ppm applied 48 days after planting, but significantly increased in height when tested 56 days after planting. Height of Oh51 planted July 8 was not significantly influenced by gibberellin. Both treatments (1,000 ppm at 39 and 46 days, and 2,500 ppm at 39 days) significantly increased plant height of R53 planted July 1.

General Observations

Silks on treated plants appeared functional. Yield was not taken, but size and number of ears of treated and untreated plants were comparable. Lodging of treated plants was increased only in Oh51 planted July 8. No increase in insect and disease susceptibility of treated plants was noted.

DISCUSSION

The present methods of eliminating pollen from the female parent in the production of hybrid seed corn are not 100 per cent efficient. Detasseling by hand is slow and requires going through fields at least once a day and sometimes twice a day to pull out tassels which are shedding or about to shed pollen. At times, the seed producer is unable to do or neglects this frequent detasseling. If the field inspector finds an excess of "shedders" the seed field is rejected. Cytoplasmic male sterility eliminates detasseling but 100 per cent male sterility is not assured. When cytoplasmic male sterile parents are used, the seed producer is advised to rogue the field. For unknown reasons a relatively high per cent of "shedders" are sometimes found. Likewise, chemical induction of male sterility may be of practical value even though it may not be 100 per cent effective. It should be assumed that its practical use would require some roguing of seed fields.

Chemical induction of male sterility, if as reliable as cytoplasmic male sterility, could be used when cytoplasmic male sterile inbreds were not available. If 90 per cent as reliable as detasseling (e.g., left 10 per cent partially fertile tassels), it would (theoretically) reduce detasseling time, number of shedders, and pollen

shed from "shedders," and still be of practical importance to hybrid seed production.

Educating the grower as to how and when to apply the chemical may be a factor in the practical application of chemically induced male sterility. Uniformity of spraying, drift of spray, and other inconsistencies associated with spraying and spraying equipment may discourage the large-scale application of this chemical "sterilization."

Gibberellin effectively induced male sterility in one field trial. Experiments are needed, however, to determine a definite range in milligrams of gibberellin per plant required to assure this induction of male sterility. Sterile tassels occurred with applications of gibberellin at both low (500 ppm) and high (2,500 ppm) concentrations. Gibberellin at low concentrations (500 and 1,000 ppm) may be sufficient to consistently induce sterility if the spray is directed into the leaf whorl and applied at the critical stage of plant development. A second spraying, a few days after the first spraying, may be advisable to "sterilize" both early and late maturing plants and early maturing "suckers."

Greenhouse plantings from open-pollinated seed harvested from male sterile Oh51 plants showed full pollen production during the winter 1957-58. Thus, there would be no need for pollen restoration genes or blending of seed as is necessary in hybrid seed production with cytoplasmic male sterility.

Only two inbred lines, R53 and Oh51, were used in these experiments. With wide differences in response of inbreds and hybrids for many characteristics, it is likely that they may vary also in response to gibberellin for chemical sterilization. Some may be more completely sterilized than others. Dosage requirements may differ for various varieties.

The largest acreage, by far, is devoted to double-cross seed production in which seed is produced in vigorous single-cross plants. Results were obtained from inbred lines used in single-cross seed production. These experiments should be repeated with single-cross hybrids.

Additional field trials are needed to determine the reliability of chemical (gibberellin) induction of male sterility and its influence on the plant in general. Yield, lodging, disease and insect susceptibility, plant abnormalities, and carry-over effects should be studied in greater detail before large-scale application in the production of hybrid seed corn.

SUMMARY

Foliar spray applications of gibberellin induced male sterility in two inbred lines, R53 and Oh51. Complete male sterility occurred in Oh51 planted July 8.

Consistent induction of male sterility depended upon the concentration of gibberellin, directing the spray into the leaf whorl, and the stage of plant development. The estimated effective range of gibberellin was five to 35 milligrams per plant. The critical stage occurred when plants had developed approximately one inch of immature tassel.

Gibberellin effects on flowering time were small and inconsistent. Effects on mature plant height were also inconsistent.

There were no "carry-over" effects on pollen production in the succeeding generation.

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