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Effects of Naphthaleneacetic Acid on Fruit Setting and Development In the Apple

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EFFECTS of NAPHTHALENEACETIC ACID on FRUIT SETTING and DEVELOPMENT in the APPLE

FRED O. HARTMAN and FREEMAN S. HOWLETT

INTRODUCTION

The importance of thinning flowers and young fruits of the apple to improve fruit size and quality, and to aid in maintaining annual bearing is well appreciated. The amount of research that has been conducted during the last fifteen years on apple thinning is relatively large. Much time and labor has been expended in an attempt to learn what chemical(s) and concentration, as well as time of application, would give a desirable degree of thinning of fruits of the different apple varieties and have no accompanying adverse effects on the growth of the plant.

Although the use of synthetic growth regulators for thinning sprays is now an accepted practice in most apple growing regions, the results obtained have often been erratic. Much of the variability in response to thinning sprays may be attributed to weather conditions existing prior, during and subsequent to the time of application of the spray. At the time this work was initiated, the authors were of the belief that more basic research was required to afford additional information and a better understanding in regard to how sprays of naphthaleneacetic acid cause fruit abscission; why the time of application gives varying results; and why the difference in response to a given concentration by different varieties. Obtaining information relative to these points should result in more satisfactory and precise results in the future with the use of this chemical. In addition, it was thought that this research should furnish information helpful in understanding the natural differences in fruit-setting behavior of apple varieties.

This paper presents the findings and progress made to date on this predominately basic research project. Further research on this subject is being conducted by the authors.

LITERATURE REVIEW

Naphthaleneacetic Acid (NAA) and Fruit Set

It was two decades ago that Burkholder and McCown (3), in attempting to increase the set of fruit on Starking apple trees with a-naphthaleneacetic acid and amide sprays during bloom, found that on the contrary a reduction in fruit set occurred. Since that time numerous workers have employed synthetic growth regulators during and after bloom to bring about thinning of apple flowers and fruits. The first post-bloom thinning was conducted by Davidson *et al.* (4) who found that NAA was effective as a thinning agent up to about three weeks after petal fall.

An effect of NAA, when used as a thinning agent, that has been reported by a number of investigators (1, 9, 11, 16, 17, 18, 20, 22) is its delaying action upon fruit abscission. This effect was stressed by Struckmeyer and Roberts (20) and interpreted as being the mechanism by which dropping eventually was increased due to competition between fruits. Murneek and Teubner (16) referred to ". . . . . a temporary retardation in separation of cells along the abscission zone of the pedicel." Powell (17) stated that abscission was delayed when NAA was used at petal fall and the delay was less pronounced when used after the first drop. According to Luckwill (11) a temporary inhibiting effect on abscission developed for one to three weeks following application of the spray.

Fruit Size

By its action in causing fruit abscission, NAA results in a reduction in number of fruits and consequently, usually in an increase in fruit size. However, NAA has, at times, been found to cause a reduction in fruit size. Greene (5) found that NAA wax emulsion applied nearly a month after full bloom retarded growth of fruits of Starking apples. Luckwill (11) reported that NAA had a direct retarding effect on fruit growth which was more pronounced the later the spray was applied. Only a slight retardation of growth of fruits 11 days after spraying with NAA was reported by Murneek and Teubner (16). The same investigators reported later (22) that retardation of fruit development was one of the first effects of the thinning spray.

Seed Number and Weight

Powell's (17) work showed that there was no difference in weight of seeds from mature fruits of the Golden Delicious apple taken from NAA sprayed and unsprayed trees. Luckwill (11) made seed counts

at harvest and found that the total number of seeds per fruit was about the same for all treatments, but the fruits from sprayed trees contained a marked increase in the percentage of abortive seeds. More recently, Marsh, Southwick and Weeks (12) reported that, "Mature viable Golden Delicious seeds from some of the less effective thinning treatments were up to 10 percent lighter in fresh weight than similar seeds from hand-thinned trees. However, the Golden Delicious trees which were most heavily thinned with NAA and NAA plus Tween 20 possessed seeds whose average fresh weight was equal to or greater than the fresh weight of seeds from the check trees."

Seed Analysis

A. Hormone Assay

Luckwill (6) and Teubner (21) have isolated a hormone from apple seeds. The latter has indicated the chemical nature of the hormone as an indole compound with an Rf value of 0.82 which is in close agreement with that obtained by Luckwill.

Luckwill (7) developed a method for estimating the hormone content from apple seeds by measuring the amount of growth of unpollinated tomato ovaries to which the hormone extract had been applied. He found (8) that the hormone reached a peak about one month after petal fall which was about the time that the endosperm became cellular and corresponded to the end of the first drop. Later, he reported (10) that another peak was reached 7 to 10 weeks after petal fall which was about the time of completion of embryo growth and the end of the June drop.

B. Cytological Examinations

Murneek (15) reported that NAA affected the seed, endosperm and nucellus, which had collapsed. Luckwill (11) observed aborting seeds from fruits from trees sprayed at petal fall and found that the nucellus was the first tissue affected. This occurred within 14 days after spraying and was followed by a cessation of growth and necrosis of embryo and endosperm. He stated that seeds appeared to be susceptible to damage by NAA only as long as the endosperm was in the free nuclear stage.

Teubner and Murneek (22) found that in later stages of apple embryo development, abortion or inhibition of the embryo could be secured without an accompanying abscission of fruit.

MATERIALS AND METHODS

The experiments were conducted in the orchards, greenhouses and laboratories of the Department of Horticulture at the Ohio Agricul-

tural Experiment Station at Wooster and the Department of Horticulture and Forestry at The Ohio State University at Columbus.

The thinning sprays of the sodium salt of naphthaleneacetic acid (NAA) were applied, as seen in Table I, to individual tree limbs with a small mechanical sprayer having a 15-gallon tank and operating at a pressure of 250 pounds. Usually three or four representative limbs were selected per tree and the flowers counted prior to any dropping. Subsequent to the "June drop" the fruits were counted and the percent set determined.

Fruits from which seed counts were to be made and seed weight determined were harvested when mature and placed in a refrigerated storage until needed. Before removing the seeds, the fruits were distributed into groups of different sizes based on the transverse diameter of the fruit.

Young fruits were collected at weekly intervals after petal fall, the seeds were removed, and hormone assay of the seeds was made following the method used by Luckwill (7). The tomato plants used in the assaying were grown in 12-inch clay pots both inside and outside the greenhouse. Prior to anthesis, all flowers in a given cluster on the

Table I.—Concentration and time of application of naphthaleneacetic acid sprays.

Year	Place	Concentration (ppm)	Time
1953	Wooster, Ohio	30	petal fall
		40	petal fall
		60	8 days after p. f.*
1953	Columbus, Ohio	20	petal fall
		30	petal fall
		40	3 days after p. f.
		50	14 days after p. f.
		80	35 days after p. f.
1954	Columbus, Ohio	20	5 days after p. f.
		30	9 days after p. f.
		50	14 days after p. f.
1955	Columbus, Ohio	20	petal fall
		30	3 days after p. f.
		40	9 days after p. f.
		60	21 days after p. f.
1956	Columbus, Ohio	37	11 days after p. f.

*p. f. means petal fall

tomato plants were removed except for the two largest flowers which were emasculated and the ovularies treated with the apple seed hormone extract. The extract contained the active principle from 500 seeds concentrated in one ml. of water. A control series of tomato ovularies was treated with water and another with 2-naphthoxyacetic acid (BNOA) at 100 ppm.

Flowers and young fruits were collected at one- to two-day intervals after anthesis and placed in a killing and fixing solution of Bellings' Modified Navashin Fluid, dehydrated by using tertiary butyl alcohol, and embedded. The stain employed was Heidenhain's iron hematoxylin. Subsequently, this material was used for a study of the developing embryo and endosperm.

RESULTS

Effects on Fruit Set[1]

Results of naphthaleneacetic acid sprays on percentage of fruit set at Columbus in 1953 are seen in Table II and Figure 1. There was an increase in the amount of thinning as the concentration of NAA was increased from 20 to 40 ppm. A delay of two weeks in the application of the 50 ppm spray gave approximately the same fruit set as the 20 ppm spray applied at petal fall. When the application was delayed to 35 days after petal fall, the 80 ppm spray resulted in no thinning over the control.

It may also be noted from Figure 1 and Table II that there was a temporary delay in the abscission of young fruits. This was evident on the May 20 count in the case of the 20 and 30 ppm treatments and the June 2 count of the 50 ppm treatment.

The extent of thinning of NAA sprays which were applied at the orchards of the Ohio Agricultural Experiment Station are given in Table III. Here the results were similar to those obtained at Columbus, in that an increase in the amount of thinning took place as the concentration of NAA was increased from 30 to 60 ppm.

The results obtained relative to the effect of NAA on reduction of fruit set are presented for 1953 only. However, the results are representative of those obtained in succeeding years and, in general, agree with those obtained by other investigators to the following extent:

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[1]Much of the data presented on fruit set, fruit size, and seed number and weight were obtained by Toshio Murashige (author's advisee) and presented as a thesis in partial fulfillment of the requirements for the Master's Degree, The Ohio State University, 1954.

Table II.—The effect of naphthaleneacetic acid on the abscission of treated flowers and fruits of the Golden Delicious apple. The Ohio State University orchard, 1953.

Treatment	Date sprayed	Percentage fruit set				
		May 9	May 20	June 2	June 18	July 20
Check	———	100.0	77.7	33.6	26.8	26.7
20 ppm at petal fall	May 9	100.0	82.6	19.6	14.8	14.3
30 ppm at petal fall	May 9	100.0	81.7	18.3	13.2	13.0
40 ppm 3 days after petal fall	May 12	100.0	62.4	15.2	9.2	9.4
50 ppm 14 days after petal fall	May 23	100.0	77.7	39.8	14.2	14.5
80 ppm 35 days after petal fall	June 13	100.0	77.7	33.6	27.1	26.8

1. That increased concentrations of NAA resulted in increased thinning when applied about the same time.

2. When sprays were delayed too long after petal fall even high concentrations of NAA failed to result in fruit thinning.

Effects on Fruit Size

The data which show the size of mature fruits which were measured after harvesting are given in Table IV and Figure 2 for the 1953 season at Columbus. The 30 and 40 ppm sprays resulted in the highest percentage of large fruits ($2\frac{1}{2}$"-3" in diameter). Over 60 percent of the fruits in both treatments were of this size. The 20 and 50 ppm

Table III.—Fruit set of the Golden Delicious apple sprayed with naphthaleneacetic acid. The Ohio Agricultural Experiment Station orchards, 1953.

Treatment	Date of application	Days after petal fall	Fruit set (percent)
Check	—	—	17.5
30 ppm	May 19	petal fall	6.1
40 ppm	May 19	petal fall	5.6
60 ppm	May 27	8	3.7

sprays resulted in somewhat smaller fruits than the former sprays, and although they decreased fruit set to some extent, apparently little or no increase in fruit size occurred when compared to the check.

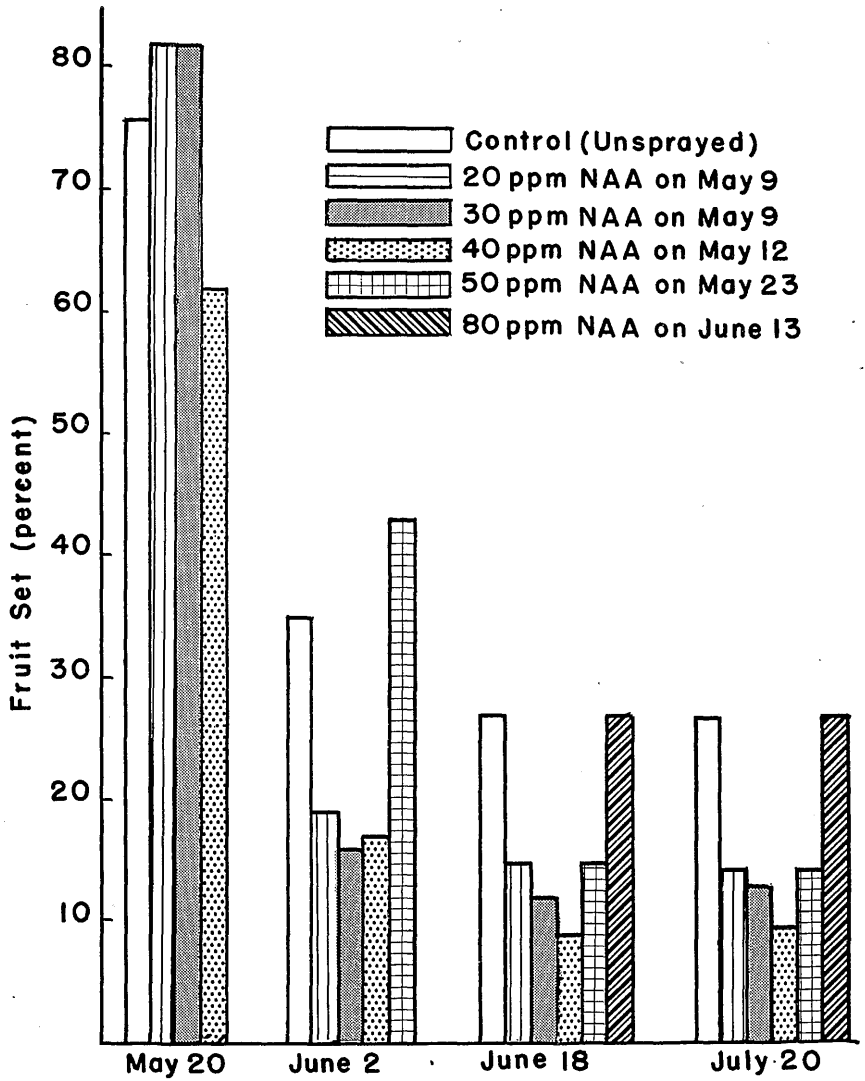


Fig. 1.—Fruit set of the Golden Delicious apple sprayed with naphthaleneacetic acid (NAA). The Ohio State University orchard, 1953.

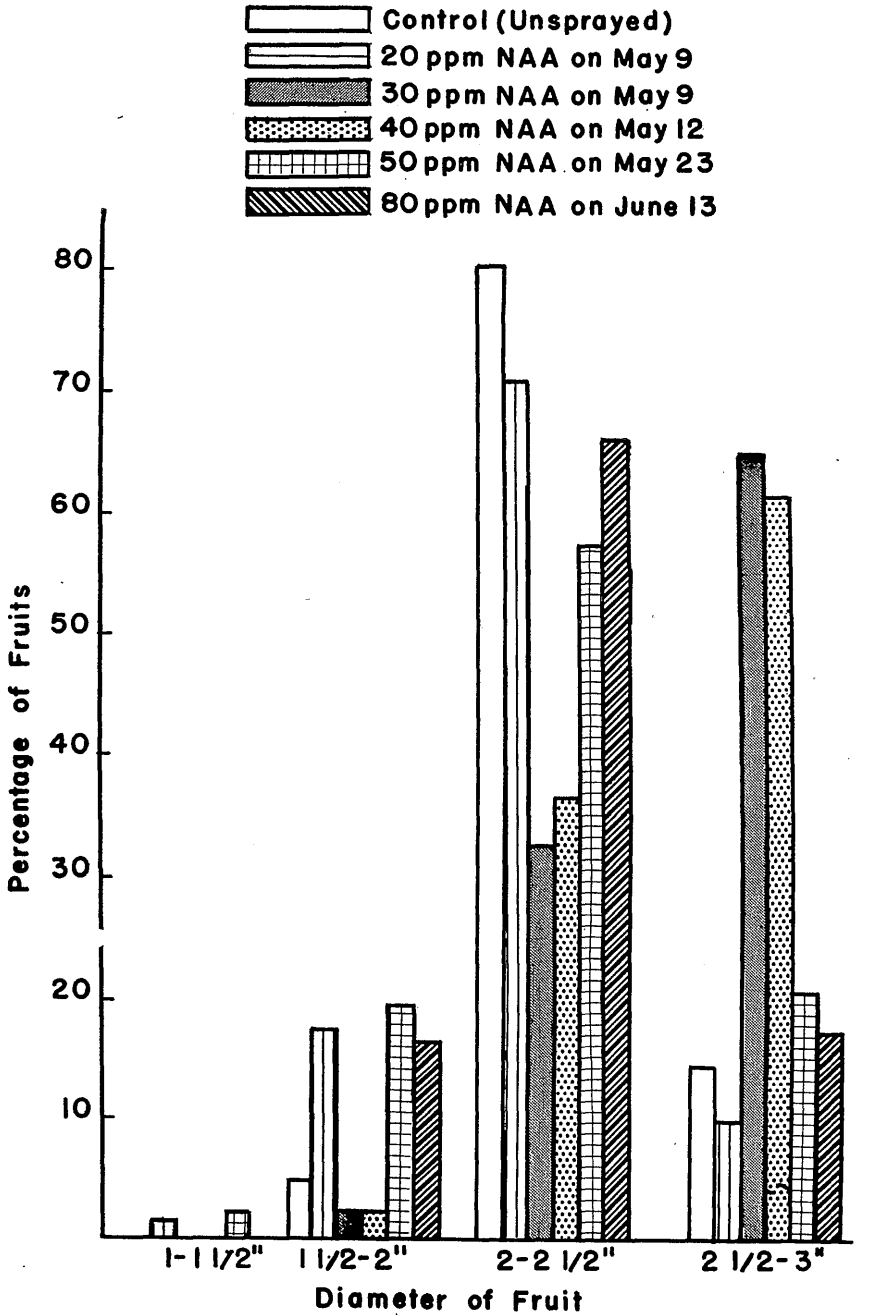


Fig. 2.—Fruit size of Golden Delicious apple sprayed with various concentrations of naphthaleneacetic acid. The Ohio State University orchard, 1953.

Table IV.—The effect of naphthaleneacetic acid thinning sprays upon size of mature fruits of the Golden Delicious apple. The Ohio State University orchard, 1953.

Treatment	Fruit diameter (inches)			
	1-1 ½	1 ½-2	2-2 ½	2 ½-3
	(percent)	(percent)	(percent)	(percent)
Check	0	5.1	80.3	14.6
20 ppm at petal fall	1.8	17.7	70.8	9.7
30 ppm at petal fall	0	2.3	32.6	65.1
40 ppm 3 days after petal fall	0	2.3	36.4	61.4
50 ppm 14 days after petal fall	2.2	19.6	57.6	20.7
80 ppm 35 days after petal fall	0	16.6	66.3	17.2

Results of thinning sprays on fruit size at Wooster are shown in Table V and Figure 3. All concentrations used resulted in a higher percentage of fruits of 2½-inch diameter or larger in relation to the unsprayed limbs. The 30 ppm spray resulted in approximately 17 percent of 3-inch or larger fruits compared to none for the 40 ppm spray.

Effect on Seed Number and Weight

Seeds from mature fruits from sprayed and unsprayed trees were counted and weighed to ascertain any differences which might have occurred. The average weight per seed and average number of seeds

Table V.—The effect of naphthaleneacetic acid thinning sprays upon size of mature fruits of the Golden Delicious apple. The Ohio Agricultural Experiment Station orchards, 1953.

Treatment	Fruit diameter (inches)			
	1 ½-2	2-2 ½	2 ½-3	3+
	(percent)	(percent)	(percent)	(percent)
Check	2.6	63.6	33.7	0
30 ppm at petal fall	0.7	31.0	51.5	16.8
40 ppm at petal fall	0	44.8	55.2	0
60 ppm 8 days after petal fall	0.7	44.4	51.9	3.0

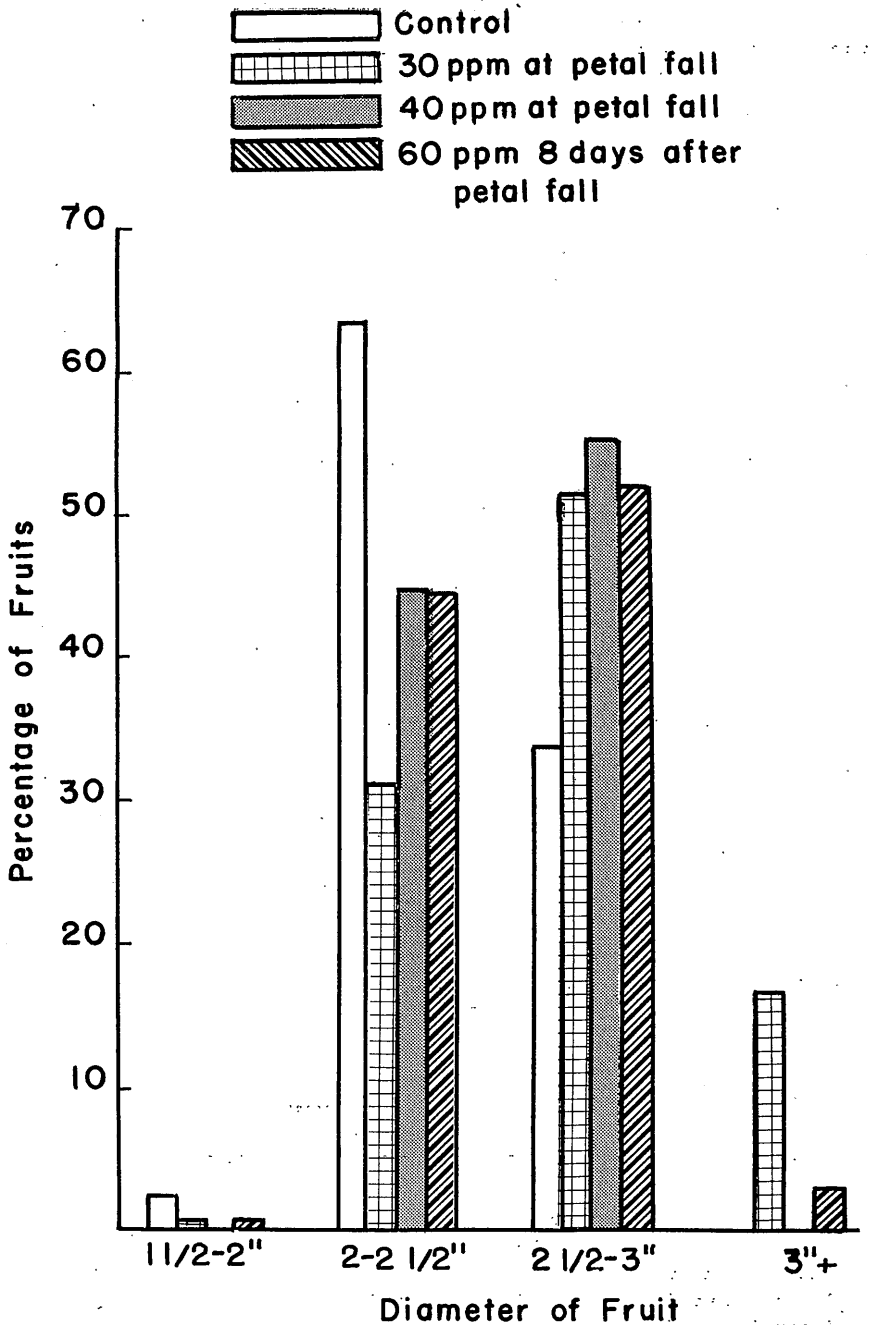


Fig. 3.—Fruit size of Golden Delicious apple sprayed with several concentrations of naphthaleneacetic acid. The Ohio Agricultural Experiment Station orchards, 1953.

per fruit were determined and the average weight of seeds per fruit was derived.

In measurements made at The Ohio State University, the average number of seeds per fruit (Table VI and Figure 4) tended to be less than in the control in all treatments with the exception of the 1½"-2" fruit of the 40 ppm treatment applied 3 days after petal fall (where only one fruit occurred) and the 2½"-3" fruit of the 80 ppm treatment applied 35 days after petal fall.

The effect on individual seed weight was somewhat less evident (Table VI and Figure 5). It may be seen that the 50 ppm spray, applied 14 days after petal fall, was the only spray which reduced the weight of seeds in all three classes of fruit size.

Table VI.—The effect of naphthaleneacetic acid on the seed content of the Golden Delicious apple. The Ohio State University orchard, 1953.

Treatment	Diameter of fruits (inches)	Number of fruits	Average no. seeds per fruit	Average wt. per seed (mg)	Average wt. of seeds per fruit (mg)
Control	1 ½-2	6	9.33	36.0	335.0
Control	2 -2 ½	101	8.65	49.3	427.0
Control	2 ½-3	18	9.17	54.3	497.0
20 ppm at petal fall	1 ½-2	17	7.18	37.3	267.0
"	2 -2 ½	75	7.29	48.1	350.0
"	2 ½-3	10	8.60	54.0	464.0
30 ppm at petal fall	1 ½-2	3	5.00	42.1	210.0
"	2 -2 ½	36	7.33	50.9	373.0
"	2 ½-3	72	8.71	58.3	469.0
40 ppm 3 days after petal fall	1 ½-2	1	10.00	49.3	——
"	2 -2 ½	11	6.91	47.3	326.0
"	2 ½-3	21	8.38	55.9	468.0
50 ppm 14 days after petal fall	1 -1 ½	2	6.50	18.0	117.0
"	1 ½-2	14	7.43	31.6	234.0
"	2 -2 ½	51	8.24	43.3	356.0
"	2 ½-3	18	8.22	50.3	413.0
80 ppm 35 days after petal fall	1 ½-2	26	7.73	29.8	230.0
"	2 -2 ½	100	8.43	51.0	430.0
"	2 ½-3	29	9.48	56.2	532.0

The average weight of seeds per fruit was reduced at all concentrations with the exception of the 80 ppm (Table VI and Figure 6). The 80 ppm spray did cause a reduction in seed weight per fruit in the smallest fruits, but not in those above two inches in diameter.

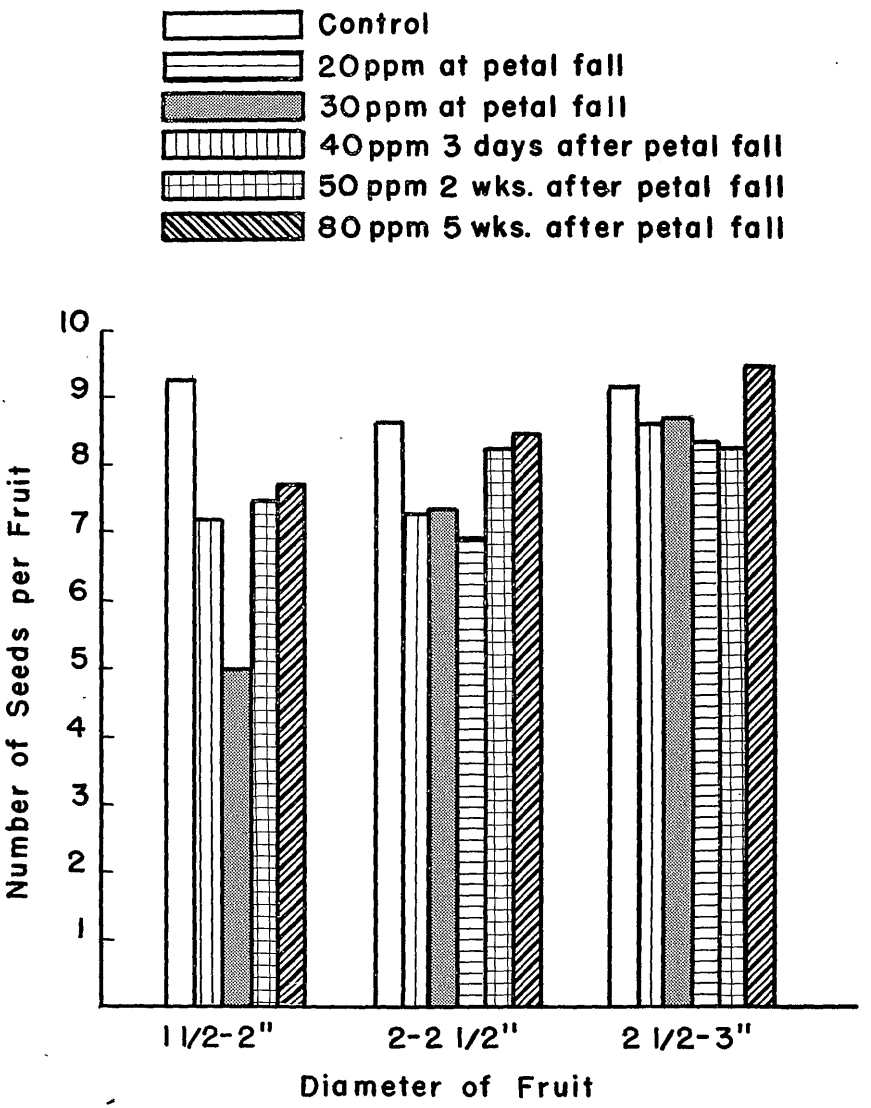


Fig. 4.—The effect of naphthaleneacetic acid on the number of seeds per fruit of the Golden Delicious apple. The Ohio State University orchard, 1953.

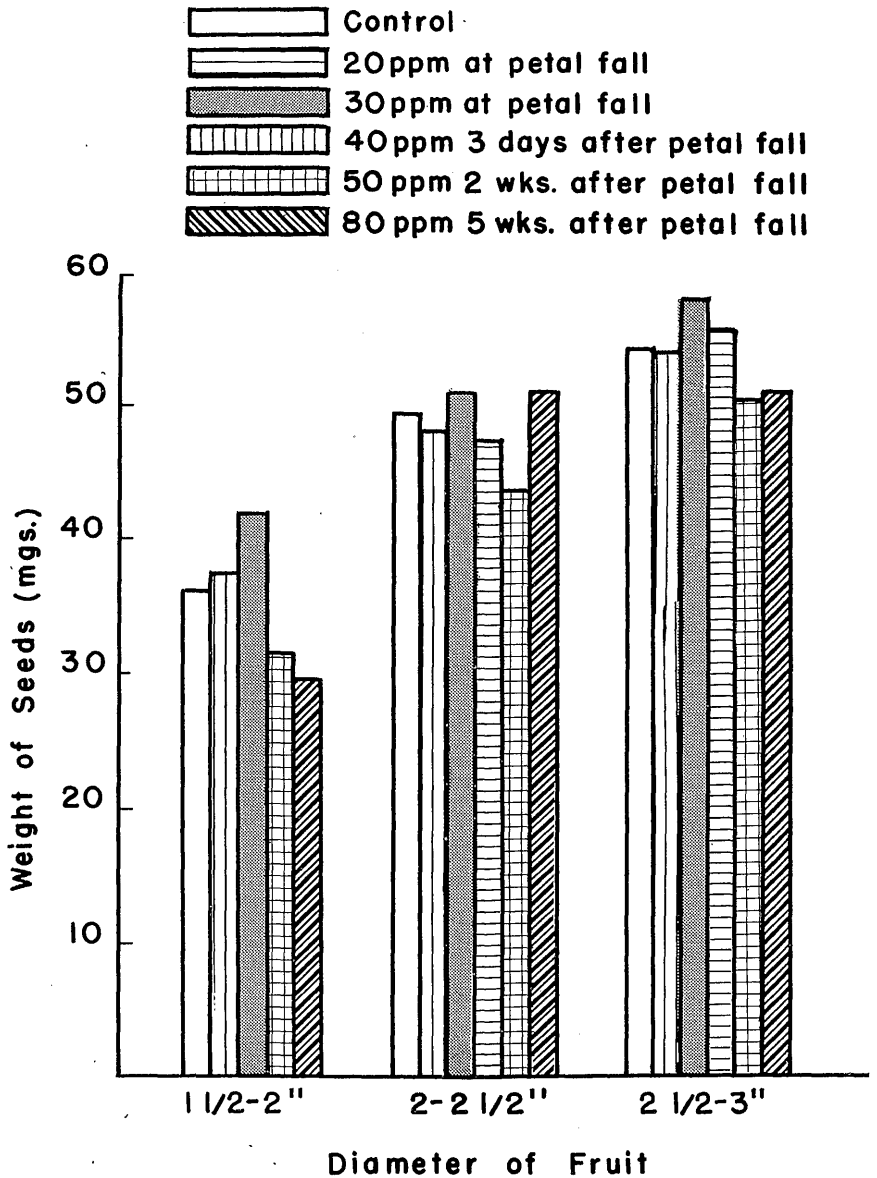


Fig. 5.—The effect of naphthaleneacetic acid on the average weight per seed of the Golden Delicious apple. The Ohio State University orchard, 1953.

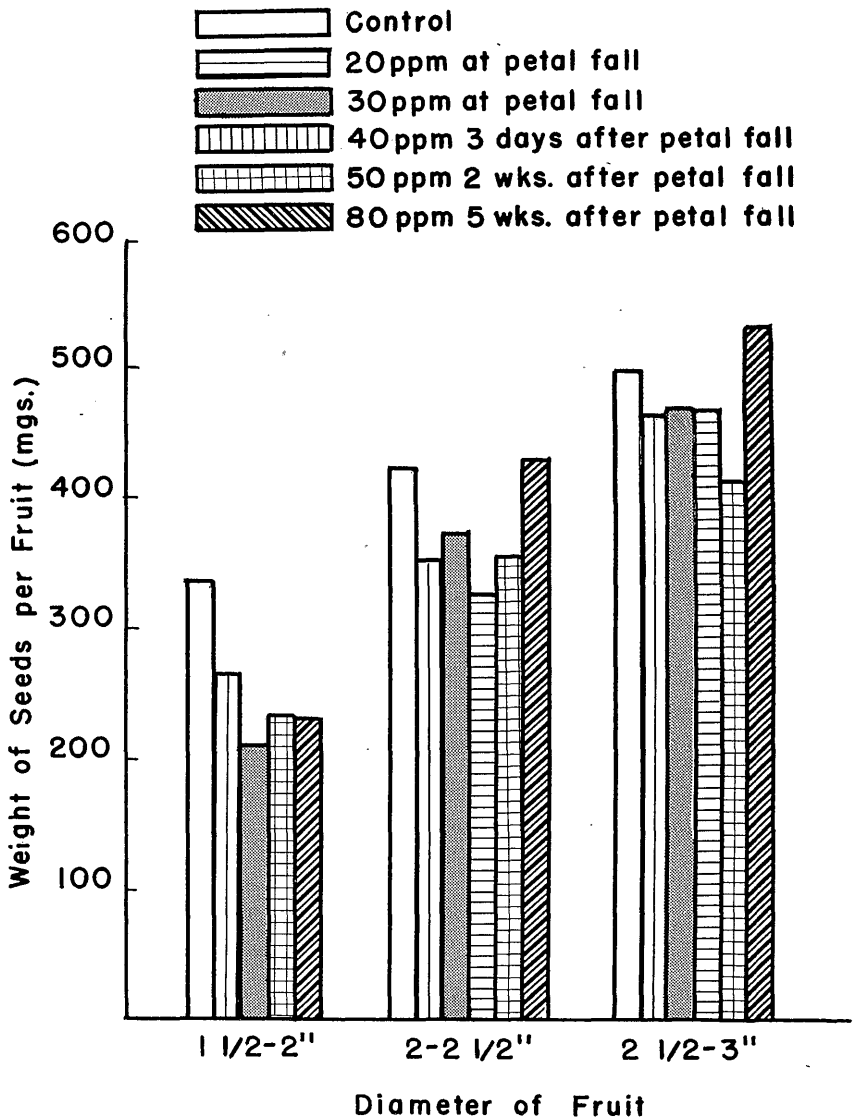


Fig. 6.—The effect of naphthaleneacetic acid on the average weight of seeds per fruit of the Golden Delicious apple. The Ohio State University orchard, 1953.

Measurements made at the Ohio Agricultural Experiment Station indicated that the 30 and 40 ppm sprays reduced seed number per fruit slightly when compared to the check and 60 ppm spray application (Table VII and Figure 7). However, when the average individual seed weight was considered, only the 60 ppm treatment resulted in a noticeable reduction (Table VII and Figure 8). Finally, when these considerations were combined to obtain the fresh weight of seeds per fruit, all treatments, including the 30, 40, and 60 ppm concentrations, resulted in a reduction when compared to the check (Table VII and Figure 9). The reduction in weight of seeds per fruit appeared to increase as the concentration of the spray increased.

In a thinning experiment conducted at the Belmont County Experimental Farm, Delicious apple trees were sprayed with NAA at 15 ppm on May 6, 1954, and given a second application of 10 ppm a week later. This resulted in many small (pygmy) fruits remaining on the trees at harvest. The fruits were separated according to size and the average weight per seed and weight of seeds per fruit was determined for each group (Table VIII).

It may be seen from Table VIII that as the fruit diameter increased the seed (or aborted seed) weight increased from .53 to 45.0 mg., and the weight of seeds per fruit from 2.3 to 353 mg.

Table VII.—The effect of naphthaleneacetic acid on the seed content of the Golden Delicious apple. The Ohio Agricultural Experiment Station orchards, 1953.

Treatment	Fruit diameter (inches)	Number of fruits	Average no. of seeds per fruit	Average wt. per seed (mg.)	Average wt. of seeds per fruit (mg.)
Control	2 -2½	100	8.42	55.6	468
Control	2½-3	71	9.35	58.7	549
30 ppm at petal fall	2-2½	78	8.14	51.9	422
"	2½-3	96	8.51	58.3	496
40 ppm petal fall	2 -2½	96	7.65	55.4	424
"	2½-3	100	8.18	59.0	482
60 ppm 8 days after petal fall	2 -2½	60	8.65	40.5	350
"	2½-3	70	8.87	48.6	431

Seed Analysis

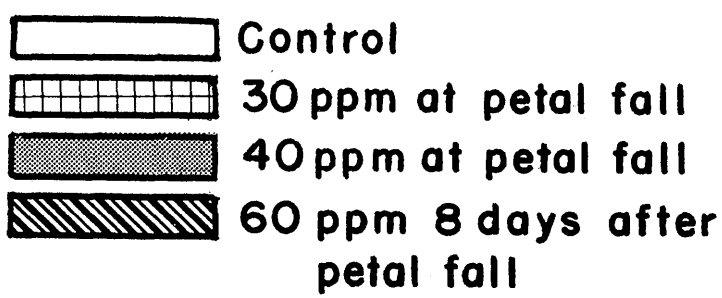
A. Hormone Assay

Hormone assay of apple seeds was begun in 1953. Collections of seeds from Golden Delicious and Delicious apples were made at eleven sampling dates from May 12 to August 13. The apple seed extracts were applied to tomato ovularies and after a period of approximately two weeks the diameter of the ovularies was measured. With the exception of the second sampling date on May 22, it appeared that no response was obtained from the extracts. Table IX shows the diameter of the tomato ovularies from the second sampling date. Only small differences were observed in this first year's work.

Table VIII.—The effect of two naphthaleneacetic acid sprays on the seed content of the Delicious apple. Belmont County, 1954.

Fruit diameter	Average weight per seed (mg)	Average weight of seeds per fruit (mg)
Less than 2.5 cm.	0.53	2.3
2.5-3.0 cm.	0.52	3.2
3.0-3.5 cm.	1.04	6.5
3.5-4.0 cm.	0.83	5.8
4.0-5.5 cm.	2.81	17.9
5.5-7.0 cm. or 2 -2 1/4 in.	13.0	82.0
2 1/4 -2 1/2 in	33.0	186.0
2 1/2 -2 3/4 in.	40.0	270.0
2 3/4 -3 in.	45.0	353.0

Key for Figs. 7, 8 and 9.



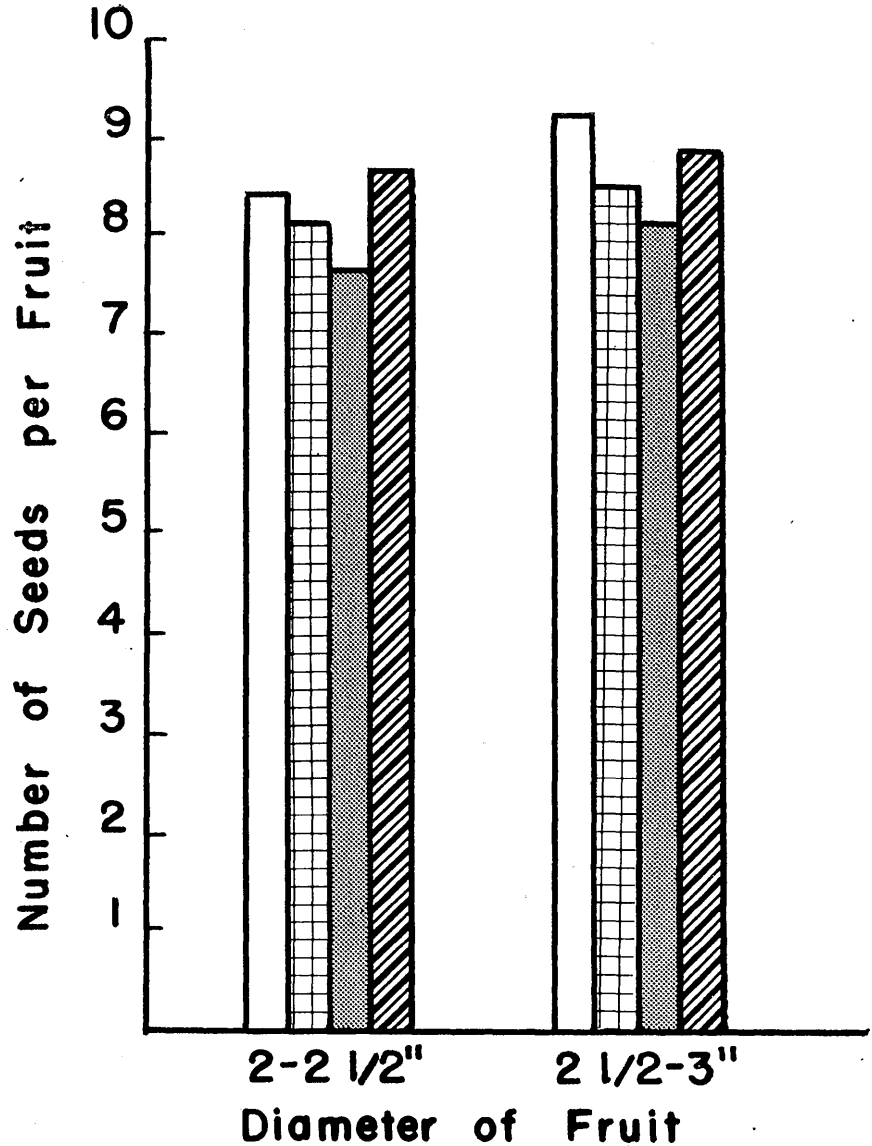


Fig.7.—The effect of naphthaleneacetic acid on the number of seeds per fruit of the Golden Delicious apple. The Ohio Agricultural Experiment Station orchards, 1953.

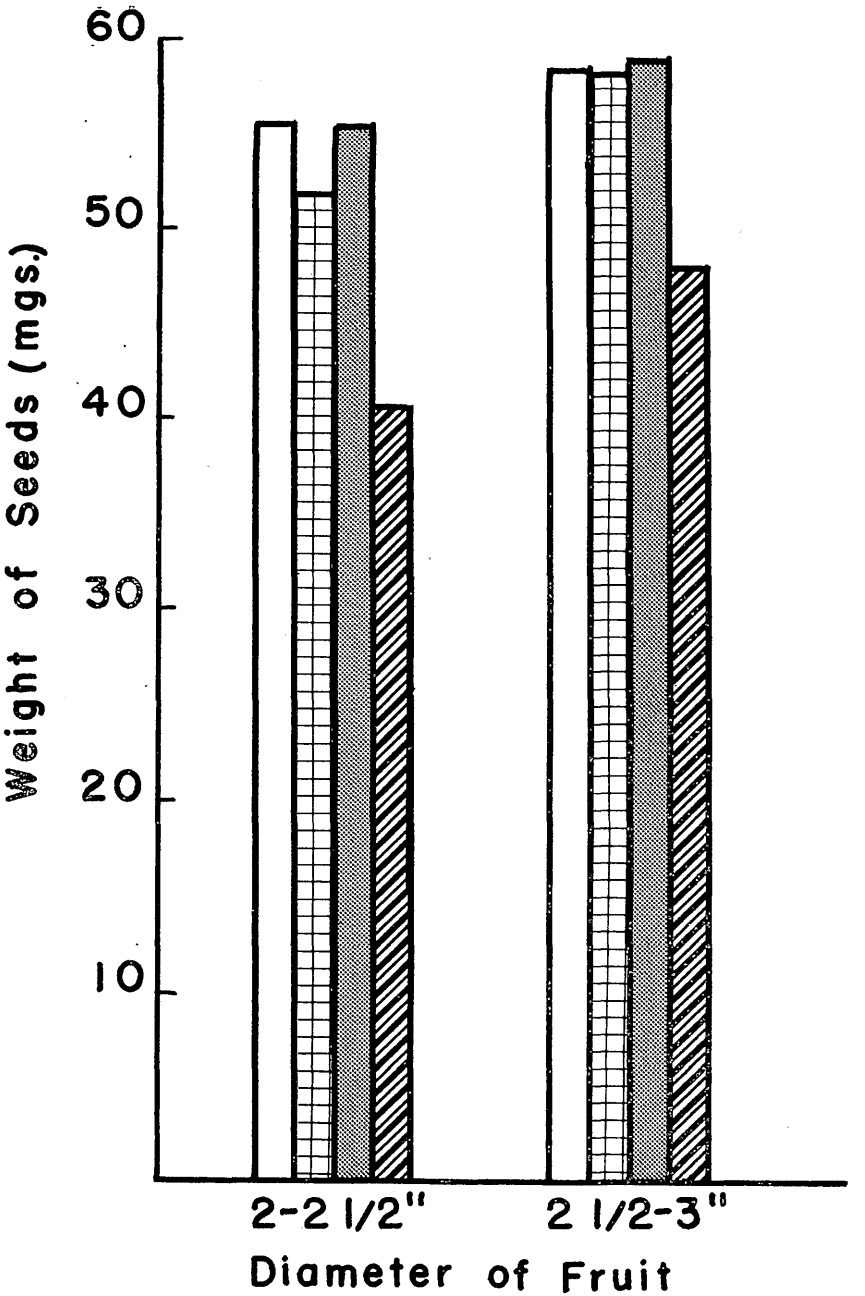


Fig. 8.—The effect of naphthaleneacetic acid on the average weight per seed of the Golden Delicious apple. The Ohio Agricultural Experiment Station orchards, 1953.

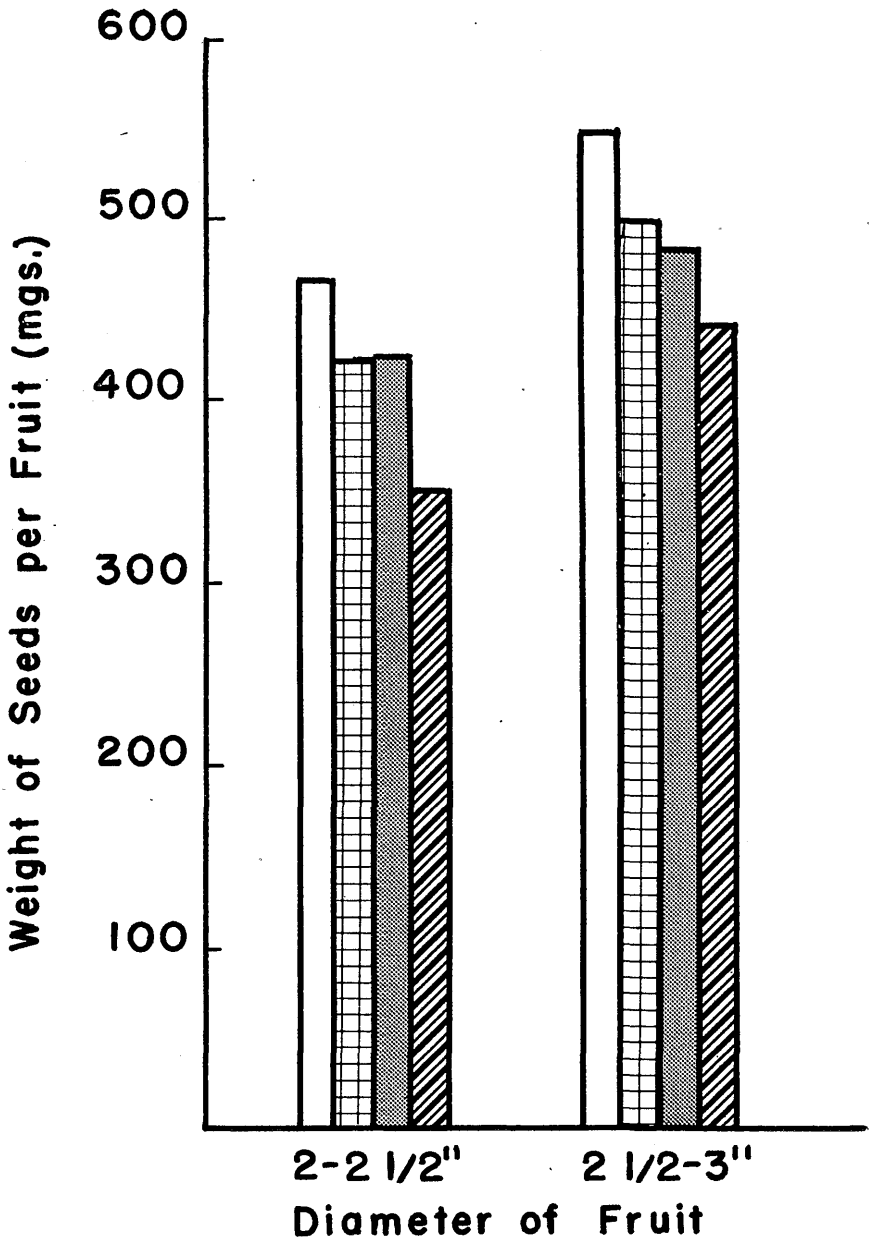


Fig.9.—The effect of naphthaleneacetic acid on the average weight of seeds per fruit of the Golden Delicious apple. The Ohio Agricultural Experiment Station orchards, 1953.

Assays of extractions made during 1954 gave no indications of a hormone material being present until the latter part of June when fresh rather than oven-dried apple seeds were used. The response at this date (June 28), which was approximately 60 days after petal fall, was obtained from seeds of the Kendall variety (Table X, Figures 10 and 11).

Table IX.—The effect of apple seed hormone on tomato ovulary growth. The Ohio State University, 1953.

Treatment	Average diameter of ovularies (mm)
Water	2.50
Delicious - 5	2.76
Delicious - 50	2.93*
Delicious - 500	3.42*
Golden Delicious - 5	2.63
Golden Delicious - 50	3.60*
Golden Delicious - 500	3.88*
BNOA - 100 ppm	4.20*
BNOA - 500 ppm	4.37*

500 - Extract from 500 apple seeds concentrated to 1 cc.
50 - The 500-apple-seed concentrate diluted 10 times.
5 - The 500-apple-seed concentrate diluted 100 times.
*Means statistically significant at .05 from control (water).

Table X.—The effect of apple seed hormone on tomato ovulary growth. The Ohio State University, 1954. Kendall variety.

Treatment	Average diameter of ovularies (mm)
Water (check)	3.65 a
Dried-seed extract	4.20 a
Fresh-seed extract (diluted)	4.92 a
Fresh-seed extract (undiluted)	14.64 b
BNOA - 100 ppm	41.50 c

Means followed by different letters statistically significant at .05.

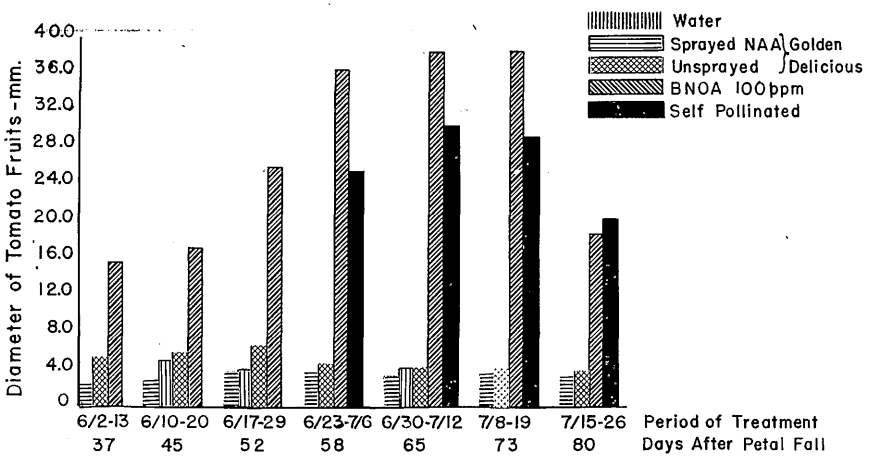


Fig. 12.—Response of tomato ovularies to apple seed hormone extract. The Ohio State University, 1955.

Using the information obtained in 1954, during the 1955 season fresh apple seeds were placed directly in water and boiled without first drying them in an oven. Growth response of tomato ovularies is shown by data presented in Table XI and Figures 12 and 13 (a-f). The greatest apparent growth response of tomato ovularies occurred from apple seed extract obtained from the first through the third sampling dates of the season which was from 37 to 52 days after petal fall. Table XI may not give an accurate picture since no account has been made for variability of environmental factors, such as temperature, which generally increased with succeeding sampling dates. To partly compensate for this, the results are expressed as a percentage of the growth response of BNOA (Table XII). In this case the actual peak response occurred from the first sampling (June 2) which was 37 days after petal fall.

Extracts from seeds taken from fruits from unsprayed trees resulted in a somewhat greater, although not significantly, increase in tomato ovulary diameter when compared to comparable samples from trees sprayed with NAA.

When a second application was made to enlarging tomato ovularies, growth continued but the carpels were hollow and lacked gelatinous pulp (Figure 13 f).

Table XI.—The effect of hormone from seeds of the Golden Delicious apple upon tomato ovulary growth. The Ohio State University, 1955.

Treatment	Diameter of ovularies (mm)							
	Date of hormone extraction and corresponding days after petal fall							
	June 2 (37)	June 10 (45)	June 17 (52)	June 23 (58)	June 30 (65)	July 8 (73)	July 15 (80)	Mean
Water	2.4 a	2.90 a	3.9 a	3.95 a	3.38 a	3.47 a	3.25 a	3.26
Extract from seeds of unsprayed trees	5.4 b	5.71 b	6.5 a	4.67 b	4.18 b	4.06 b	3.85 a	4.95
Extract from seeds of NAA sprayed trees		5.00 b	4.2 a	*	4.19 b			4.52
BNOA 100 ppm	15.66 c	17.21 c	25.7 b	36.25 c	38.33 c	30.17 c	18.46 b	25.22

*Flowers all abscissed.

Means on same date followed by different letter statistically significant at .05.

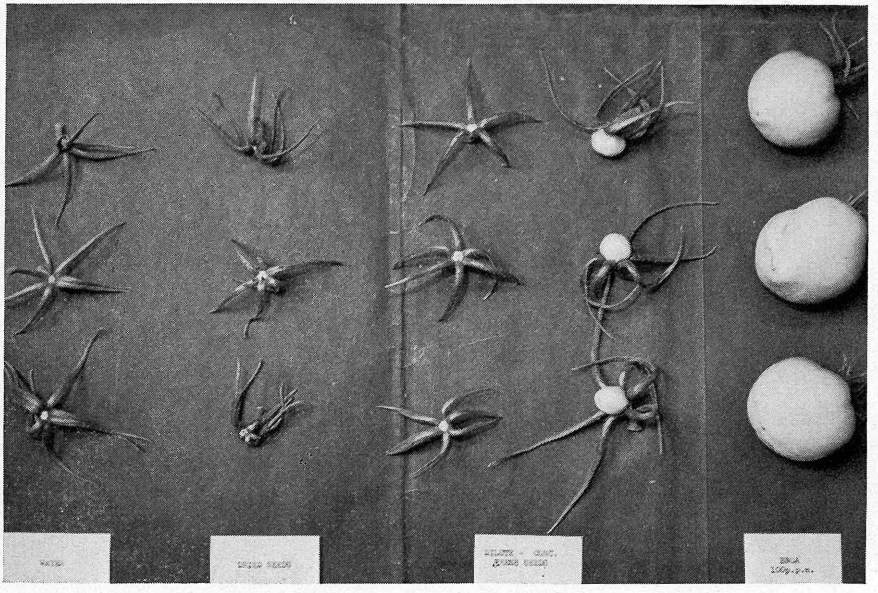


Fig. 10.—Response of tomato ovularies to apple seed hormone. The Ohio State University, 1954. Left to right: water, dried-seed extract, fresh-seed extract (diluted), fresh-seed extract (undiluted), and BNOA.

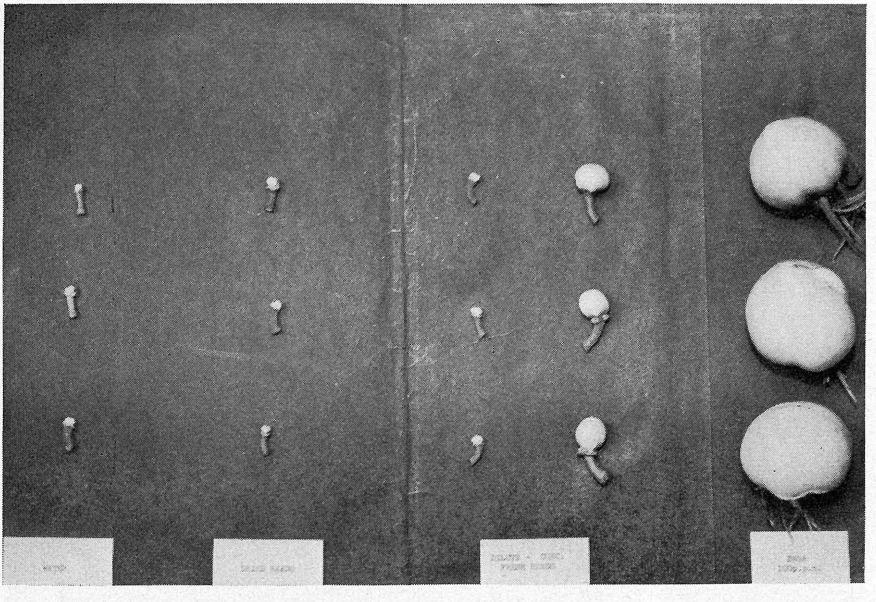


Fig. 11.—Response of tomato ovularies to apple seed hormone. The Ohio State University, 1954. Same as Fig. 10, but sepals have been removed.

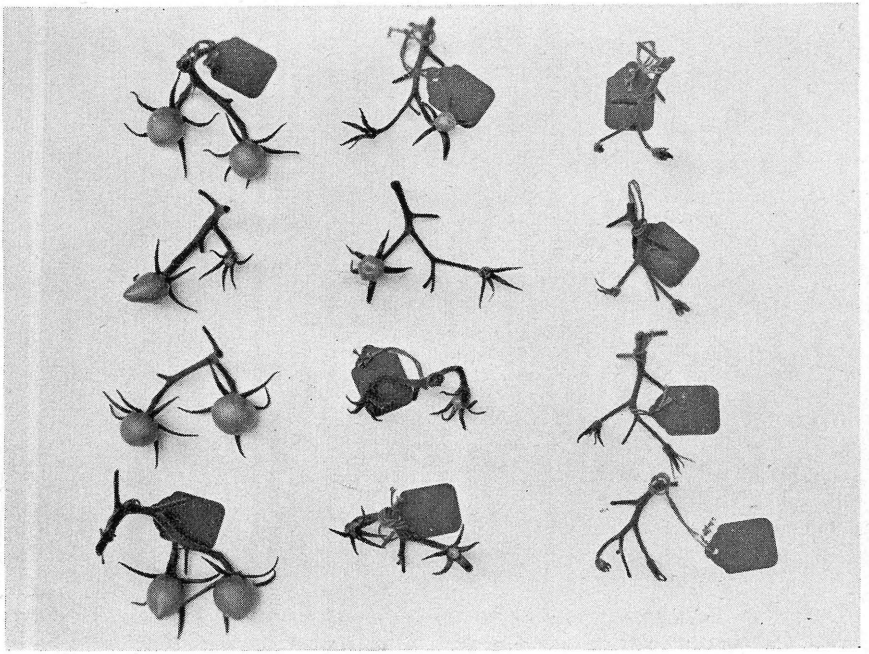


Fig. 13a.—Response of tomato ovularies to apple seed hormone. The Ohio State University, 1955. First collection (June 2). Left to right: Treated with BNOA-100 ppm, Extract from Golden Delicious seeds, and water.

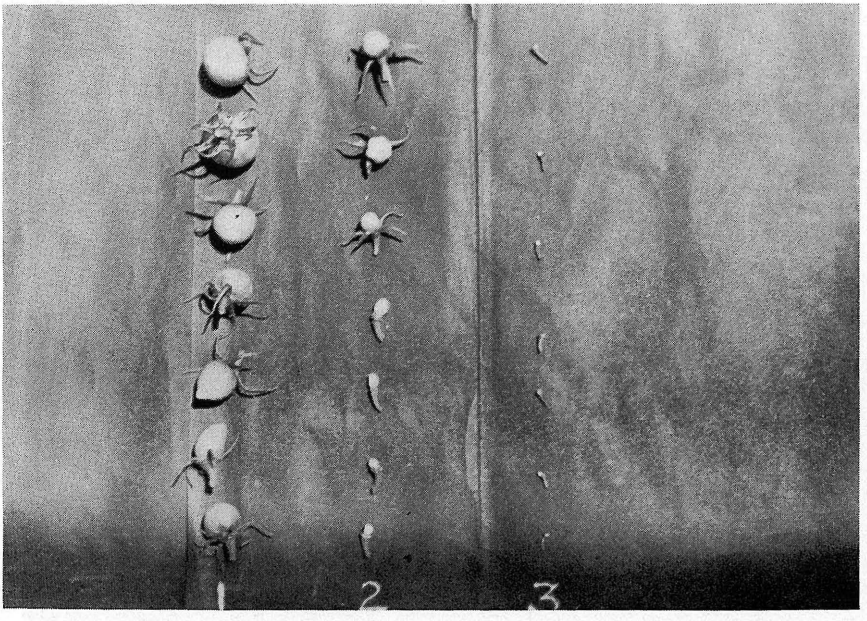


Fig. 13b.—Response of tomato ovularies to apple seed hormone. The Ohio State University, 1955. Same as Fig. 13a with fruits removed from clusters.

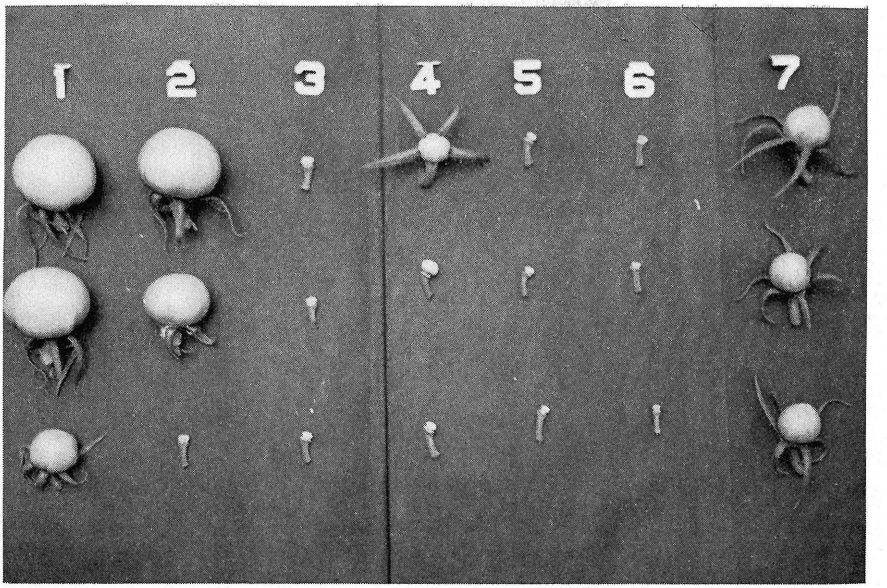


Fig. 13c.—Response of tomato ovularies to apple seed hormone. The Ohio State University, 1955. Third collection (June 17). 1. BNOA-100 ppm, 2. BNOA-50 ppm, 3. BNOA-25 ppm, 4. Extract from Golden Delicious seeds (unsprayed trees), 5. Extract from Golden Delicious seeds (trees sprayed NAA), 6. Water, 7. Self-pollinated.

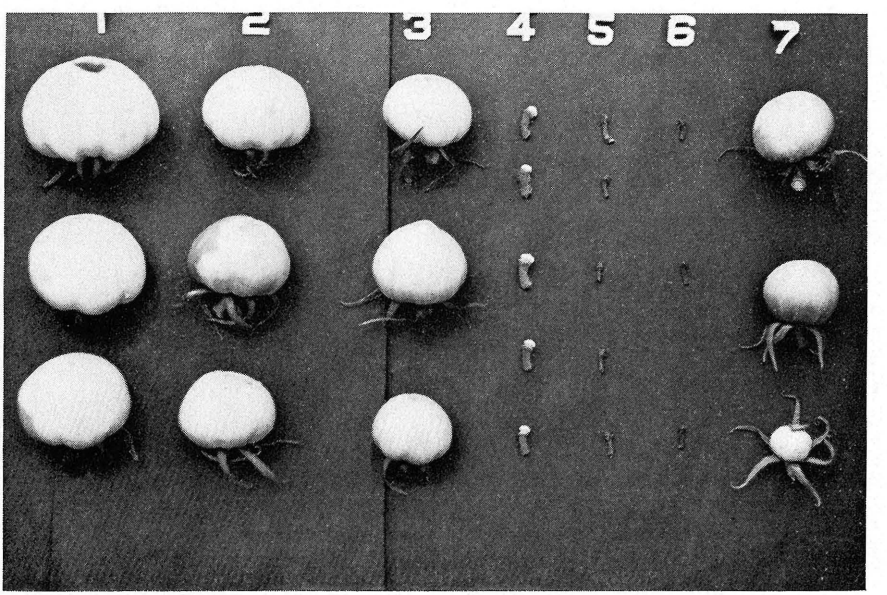


Fig. 13d.—Response of tomato ovularies to apple seed hormone. The Ohio State University, 1955. Fourth collection (June 23). 1. BNOA-100 ppm, 2. BNOA-50 ppm, 3. BNOA-25 ppm, 4. Extract from Golden Delicious seeds, 5. Water, 6. No treatment, 7. Self-pollinated.

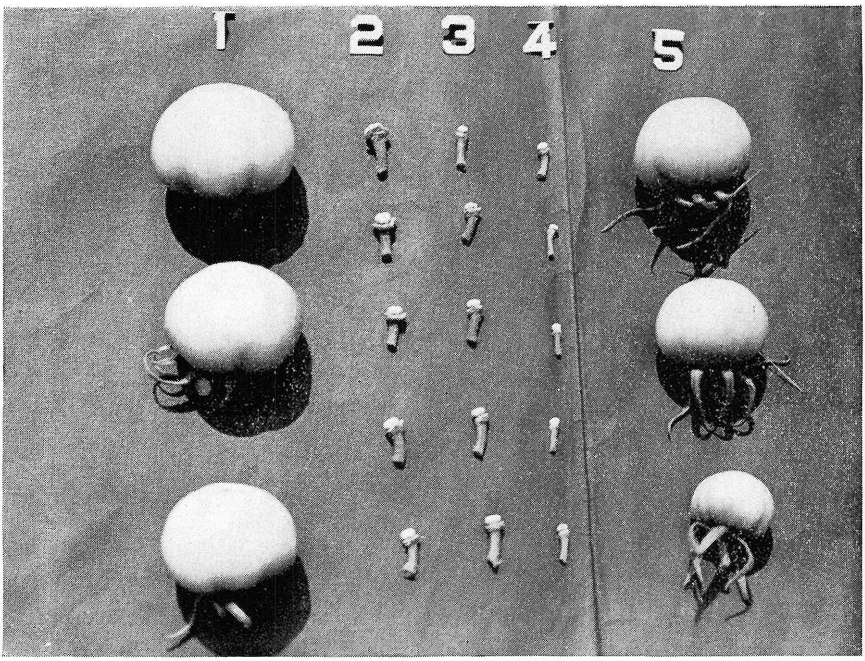


Fig. 13e.—Response of tomato ovularies to apple seed hormone. The Ohio State University, 1955. Fifth collection (June 30). 1. BNOA-100 ppm, 2. Extract from Golden Delicious seeds (unsprayed trees), 3. Extract from Golden Delicious seeds (trees sprayed NAA), 4. Water, 5. Self-pollinated.

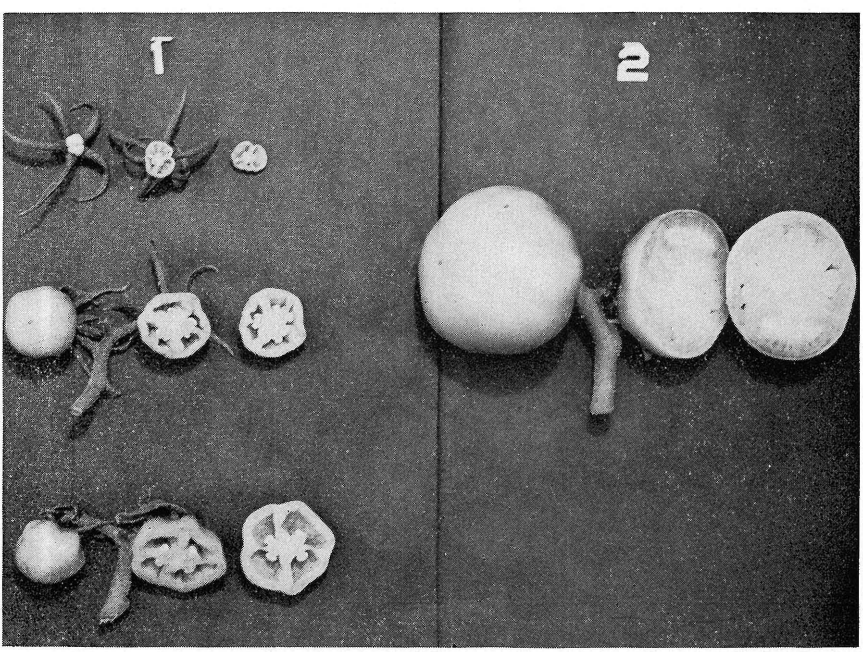


Fig. 13f.—Response of tomato ovularies to two applications of apple seed hormone. The Ohio State University, 1955. 1. Extract from Golden Delicious seeds, 2. BNOA-100 ppm.

The results of the hormone assay conducted at the Ohio Agricultural Experiment Station during 1955 on four apple varieties are presented in Table XIII and Figure 14. The apparent peak response of ovulary growth was obtained from extracts of seeds collected approximately 53 days after petal fall. This was likewise the result when the response was expressed as a percentage of the BNOA treatment with the exception of the Rome Beauty extract. Here the maximum response was earlier, 46 days after petal fall (Table XIV).

Table XII.—The effect of hormone from seeds of the Golden Delicious apple upon tomato ovulary growth. The Ohio State University, 1955.

	Diameter of ovularies as percentage of BNOA growth response						
	Date of hormone extraction and corresponding days after petal fall						
Treatment	June 2 (37)	June 10 (45)	June 17 (52)	June 23 (58)	June 30 (65)	July 8 (73)	July 15 (80)
Water	15	17	15	11	9	12	18
Extract from seeds of unsprayed trees	35	33	25	13	11	14	21
Extract from seeds of NAA sprayed trees		29	16	*	11		

*Flowers all abscissed.

At any given sampling date there was variation between varieties, but the overall pattern was similar except that Rome Beauty apparently reached its peak a little before the other varieties.

During the 1956 season at Columbus the maximum size increase of ovularies treated with the hormone extracts was obtained 44 days after petal fall (Table XV and Figures 15 and 16). The results when expressed as a percentage of the growth response of BNOA are seen in Table XVI. Although the number of assays was limited this year, the results appeared to substantiate those of the previous year.

The hormone determinations conducted at Wooster during 1956 were with the same varieties used the previous year with the exception of the substitution of Richared for Starking. However, the extractions were begun about two weeks earlier, in relation to petal fall, which was about as soon as the young seeds could satisfactorily be removed from the fruits. The peak response as measured by ovulary growth occurred on the last sampling date which was 52 days after petal fall

Table XIII.—The effect of hormone from seeds of four apple varieties upon tomato ovulary growth. The Ohio Agricultural Experiment Station, 1955.

Treatment	Diameter of ovularies (mm)					
	Date of hormone extraction and corresponding days after petal fall					
	June 9 (39)	June 16 (46)	June 23 (53)	June 30* (60)	July 12 (72)	Mean
Water	2.96 a	2.95 a	3.70 a	3.14	2.81 a	3.10
Yellow Transparent seed extracts	3.33 a	3.70 b	7.62 b	3.93	4.07 b	4.52
Rome Beauty seed extracts	3.78 a	4.61 b	6.26 b	4.09	3.19 a b	4.56
Stayman Winesap seed extracts	3.70 a	3.92 a b	9.93 b	6.15	2.86 a	5.19
Starking seed extracts	3.55 a	3.28 a b	9.87 b	3.42	3.59 b	4.79
BNOA 100 ppm	14.42 b	14.43 c	27.99 c	26.23	23.28 c	20.45

Means on same date followed by different letter statistically significant at .05.
*Original data lost for this collection date.

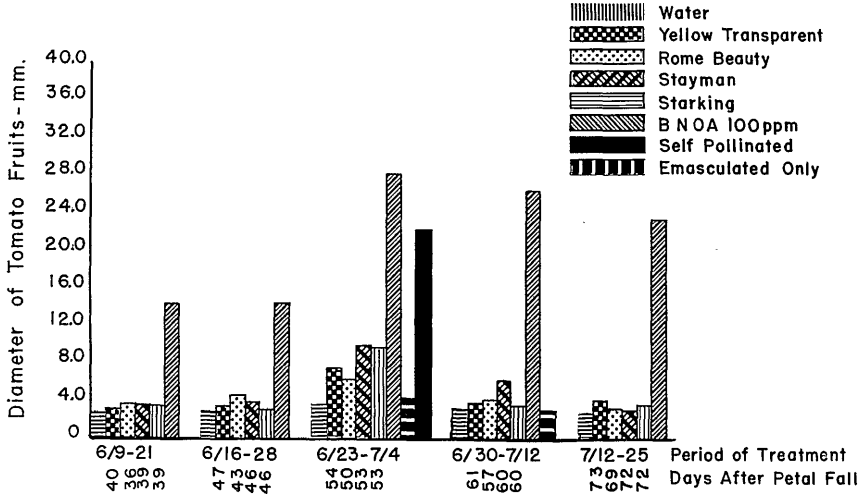


Fig. 14.—Response of tomato ovularies to apple seed hormone extract. The Ohio Agricultural Experiment Station, 1955.

Table XIV.—The effect of hormone from seeds of four apple varieties upon tomato ovulary growth. The Ohio Agricultural Experiment Station, 1955.

Treatment	Diameter of ovularies as percentage of BNOA growth response Date of hormone extraction and corresponding days after petal fall June 9 (39)	June 16 (46)	June 23 (53)	June 30 (60)	July 12 (72)	Mean
Water	20.5	20.4	13.2	12.0	12.1	15.2
Yellow Transparent seed extracts	23.1	25.6	27.2	15.0	17.5	22.1
Rome Beauty seed extracts	26.2	31.9	22.4	15.6	13.7	22.3
Stayman Winesap seed extracts	25.7	27.2	35.5	23.4	12.3	25.4
Starking seed extracts	24.6	22.7	35.3	13.0	15.4	23.4

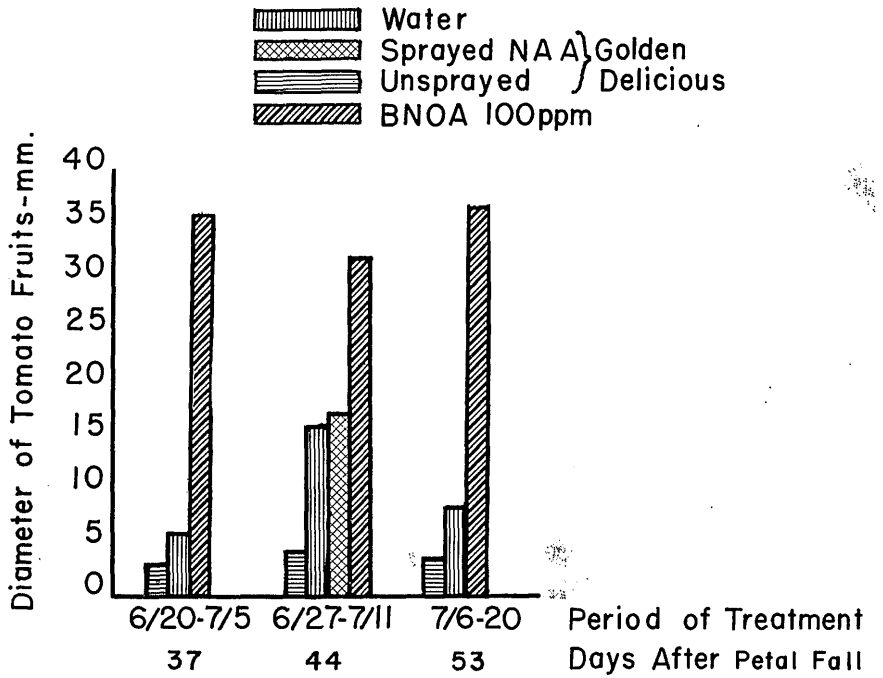


Fig. 15.—Response of tomato ovularies to apple seed hormone extract. The Ohio State University, 1956.

Table XV.—The effect of hormone from seeds of the Golden Delicious apple upon tomato ovulary growth. The Ohio State University, 1956.

Treatment	Diameter of ovularies (mm)		
	Date of hormone extraction and corresponding days after petal fall		
	June 20 (37)	June 27 (44)	July 6 (53)
Water	2.9 a	4.2 a	3.5 a
Seed extract	6.1 b	15.9 b	8.1 a
BNOA - 100 ppm	35.7 c	31.8 c	36.8 b

Means on same date followed by different letter statistically significant at .05.

(Table XVII and Figure 17). This corresponded to the peak the previous year. However, only the Rome Beauty response was signifi-

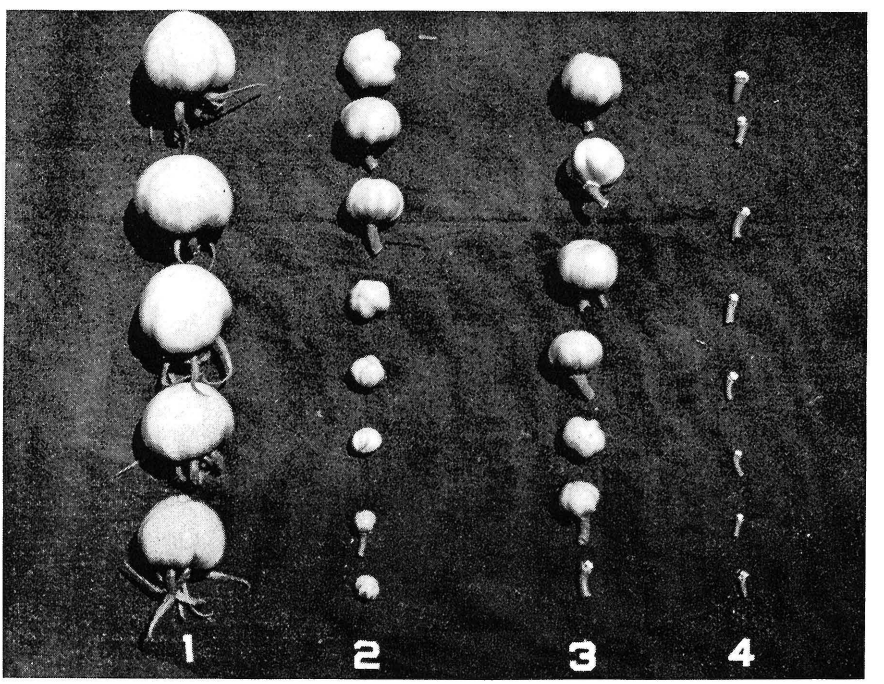


Fig. 16.—Response of tomato ovularies to apple seed hormone. The Ohio State University, 1956. Second collection (June 27). 1. Treated with BNOA-100 ppm, 2. Extract from Golden Delicious seeds (unsprayed trees), 3. Extract from Golden Delicious seeds (trees sprayed with NAA), 4. Water.

Table XVI.—The effect of hormone from seeds of the Golden Delicious apple upon tomato ovulary growth. The Ohio State University, 1956.

Treatment	Diameter of ovularies as percentage of BNOA growth response		
	Date of hormone extraction and corresponding days after petal fall		
	June 20 (37)	June 27 (44)	July 6 (53)
Seed extract	17	50	20

cantly different from the water treatment. The larger than normal size shown for the water treatment on July 9 is believed to have been the result of unsatisfactory or late emasculation. The four ovularies which enlarged to a considerably greater extent were from two clusters. If these were discarded, the average diameter would be approximately 4.0 mm.

When expressed as a percentage of the BNOA response, the peak also occurred 52 days after petal fall (Table XVIII). Noteworthy was the fact that Rome Beauty again showed a somewhat earlier increased

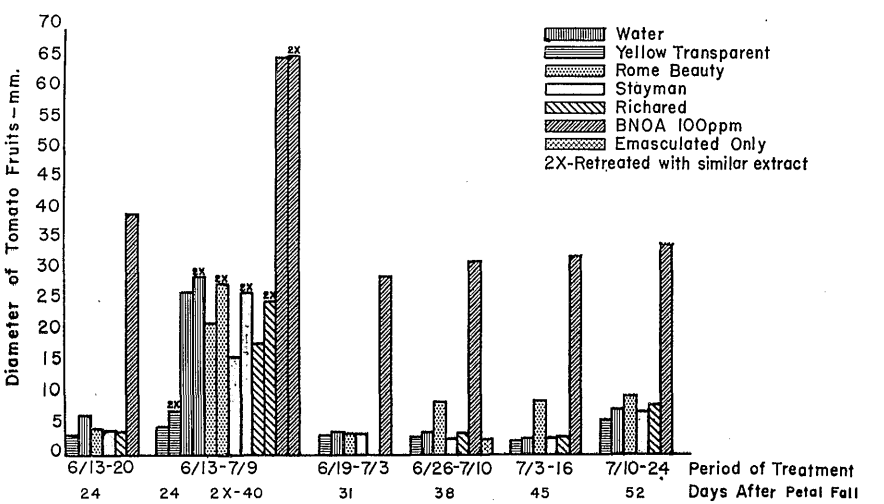


Fig. 17.—Response of tomato ovularies to apple seed hormone extract. The Ohio Agricultural Experiment Station, 1956.

Table XVII.—The effect of hormone from seeds of four apple varieties upon tomato ovulary growth. The Ohio Agricultural Experiment Station, 1956.

Treatment	Diameter of ovularies (mm) Date of hormone extraction and corresponding days after petal fall June 11 (24)	June 18 (31)	June 25 (38)	July 2 (45)	July 9 (52)	Mean
Water	3.37 a b	3.04 a	3.21 a	2.60 a	6.14 a	3.90
Yellow Transparent seed extracts	6.81 a	4.14 b	4.00**	2.89 a	7.82 a b	5.44
Rome Beauty seed extracts	4.78 a b	3.91 a b	8.90 a	9.03 b	9.95 b	7.28
Stayman Winesap seed extracts	4.36 a	3.89 b	3.11 a	3.10 a	7.36 a b	4.66
Richared seed extracts	4.03 b	*	4.00 a	3.16 a	8.44 a b	5.12
BNOA 100 ppm	39.44 c	29.01 c	31.43 b	32.43 c	34.25 c	33.15

*All flowers abscissed.
**Only one flower set fruit.
Means on same date followed by different letter statistically significant at .05.

response than did the other varieties—namely, 38 days after petal fall. Also, the greater response of the Yellow Transparent extract from the first sampling date, 24 days after petal fall, although not significantly different from water treatment, deserves consideration.

Table XVIII.—The effect of hormone from seeds of four apple varieties upon tomato ovulary growth. The Ohio Agricultural Experiment Station, 1956.

Treatment	Diameter of ovularies as percentage of BNOA growth response Date of hormone extraction and corresponding days after petal fall June 11 (24)	June 18 (31)	June 25 (38)	July 2 (45)	July 9 (52)
Yellow Transparent seed extracts	17	14	13**	9	23
Rome Beauty seed extracts	12	14	28	28	29
Stayman Winesap seed extracts	11	13	10	10	22
Richared seed extracts	10	*	13	10	25

*All flowers abscissed.
**Represents only one fruit.

During the 1956 season, some tomato ovularies were given a second application of hormone extract and left on the plants for an additional 12-day period to observe the effect on ovulary enlargement. These results (Table XIX and Figure 18). show that a second application of the seed extracts of each variety increased the amount of ovulary growth. Less variability in response occurred between varieties after the second application.

Table XIX.—The effect of two applications of apple seed hormone upon tomato ovulary growth. The Ohio Agricultural Experiment Station, 1956.

Treatment	Diameter of ovularies (mm)	
	One application (June 13)	Two applications (June 13 and 27)
Water	5.06	7.57
Yellow Transparent seed extracts	26.63	29.00
Rome Beauty seed extracts	21.66	27.93
Stayman Winesap seed extracts	16.13	26.48
Richared seed extracts	18.24	25.07
BNOA - 100 ppm	65.24	65.32

B. Cytological Examinations

Anatomical and cytological examinations of a general nature were made of material collected in 1954 and 1955, and a more critical and thorough examination of the material collected in 1956.

From the 1954 treatments (Table I), cytological examinations were made of the material collected from the 20 ppm spray applied 5 days after petal fall, the 30 ppm spray applied 9 days after petal fall, and the control (unsprayed). The seeds for anatomical and cytological examination were collected from 6 to 11 days after the sprays were applied. As seen in Table XX, the percentage of embryo degeneration or retarded development was 41 and 44 percent, respectively, as compared to 25 percent for the control.

In the 20 ppm treatment, there was some indication that development had been retarded as observed by the presence of fertilized eggs and small embryos (Figure 20). Little degeneration was observed,

but fertilized eggs were seen in the material which was collected from 6 to 10 days after the spray was applied. A comparable seed from the control (unsprayed) is seen in Figure 19 which shows the developing embryo.

A typical example of a degenerating embryo from the 30 ppm spray treatment applied 9 days after petal fall is shown in Figure 22. Few embryos showed signs of degeneration at 6 days after spray was applied, but the percentage was high at 11 days subsequent to spraying. A comparable seed from unsprayed tree is seen in Figure 21.

During 1955, cytological examinations were made of material collected from:

20 ppm spray applied at petal fall;
30 ppm spray applied 3 days after petal fall; and
40 ppm spray applied 9 days after petal fall.

The seeds examined were collected from 5 to 16 days after spray was applied. The percentage of degenerating embryos observed in developing seeds of the above treatments is presented in Table XX.

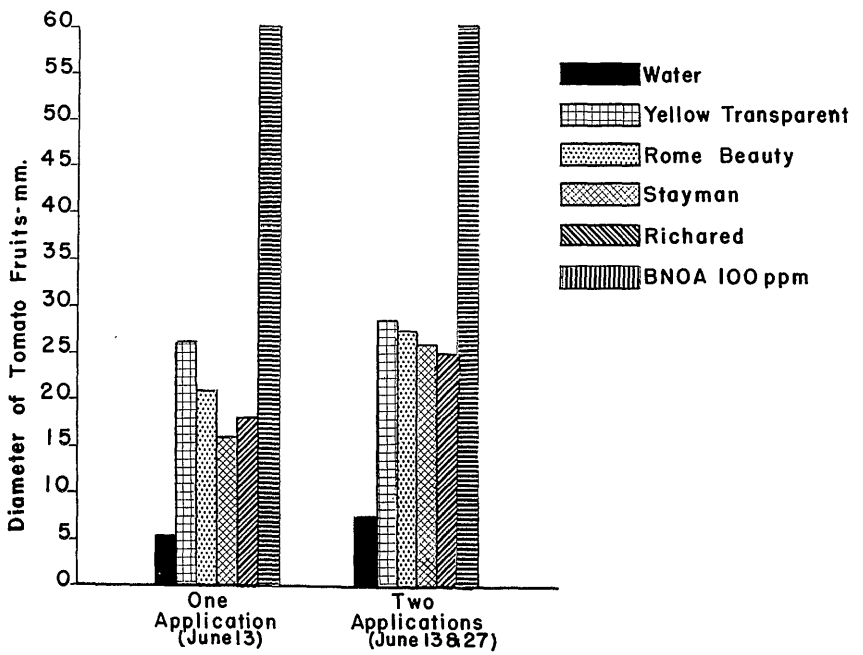


Fig. 18.—Response of tomato ovularies to apple seed hormone extract. The Ohio Agricultural Experiment Station, 1956.

Table XX.—Condition of embryo in developing fruits of Golden Delicious apple trees sprayed with naphthaleneacetic acid. The Ohio State University.

Year	Treatment		Embryo	
	NAA concentration	Sprayed days after petal fall	Normal	Degenerating and/or retarded development
			percent	percent
1954	Check	-	75	25
	20 ppm	5	59	41
	30 ppm	9	56	44
1955	Check	-	81	19
	20 ppm	petal fall	29	71
	30 ppm	3	40	60
	40 ppm	9	27	73

The 20 ppm spray resulted in 71 percent of the young ovules or seeds with retarded development (Figure 24) or showing degeneration (Figure 25) by 15 days after spraying. A comparable seed from unsprayed tree is seen in Figure 23 which shows a well developed embryo and a few endosperm nuclei.

The 30 ppm spray applied 3 days after petal fall contained 60 percent of the ovules or young seeds with degenerated embryos (Figure 26) by 14 days after spraying. Here collapsing of seed was more prevalent.

With the 40 ppm spray applied 9 days after petal fall, degeneration of embryos was observed in 73 percent of the seeds examined (Figure 27) by 7 days after spraying.

In the collection made during 1956, a more critical examination was made of seeds from sprayed fruits (Table XXI) and from unsprayed "sets" and "expected drops" (Table XXII). Seeds examined three days after the spray was applied gave indication, in some cases of degeneration occurring, but this was not too apparent at this time (Figure 29). However, by five days after the spray was applied, degeneration of the embryo was apparent (Figure 31) and the endosperm often appeared to be degenerating (Figure 32). An unsprayed "set" is seen in Figure 30 and an "expected drop" in Figure 33.

At eleven days after spraying, one-third of the embryos observed showed degeneration (Table XXI). However, other seeds contained embryos which apparently were unaffected (Figures 35 and 36), and

developing at a rate comparable to those from unsprayed trees (Figure 34), although the seeds were collapsing.

At any sampling date, condition of embryos was quite variable. Some revealed complete degeneration while others were developing normally (Figure 37). In the examination of seeds of the 1954 and 1955 material, where sprays were applied somewhat earlier, this degree of difference in embryo condition and development was not observed.

DISCUSSION

Effects on Fruit Setting

The effect of NAA on the reduction of fruit set as shown in Tables II and III agrees with the findings of other workers which, in general, have shown an increase in degree of thinning as the concentration of the spray was increased. Southwick and Weeks (19) reported that "at a given time of application after calyx (but prior to the June drop) increases in concentration of the NAA materials usually increased the amount of thinning obtained (but not necessarily in direct proportion to the concentration used) up to a point."

There was also some indication from this work that when the time of application of the spray was delayed, a higher concentration of NAA was required to bring about a comparable amount of thinning. Other investigators (11, 13, 18) have reported findings which show this effect. The date is eventually reached when higher concentrations result in no

Table XXI.—Summary of observations of seeds from sprayed trees. The Ohio State University, 1956.

Collected days after spraying	Seed	Endosperm	Embryo		
			Degenerating	Length	Width
			(percent)	(mm)	(mm)
3	Normal	Some sparse	44	.054	.043
5	Few collapsing	Degenerating	50	.060	.054
8	Collapsing	Sparse, disorganized, degenerating	36	.077	.063
11	Collapsing	Sparse	33	.097	.086
14	Collapsed	Mostly sparse and degenerating. Some normal.	38	.114	.103

fruit thinning. This was the situation when the 80 ppm spray was applied 35 days after petal fall.

Why higher concentrations of NAA are required to bring about comparable thinning with delay in application subsequent to petal fall is believed to be related to the natural auxin produced in the developing seed (as discussed below), which apparently increases in amount until 40-50 days after petal fall.

The results also indicated a temporary delay in abscission of young fruits. This is believed to be due to the action of NAA on the abscission zone of the pedicel (16). This effect is only temporary as in the case of the preharvest sprays. It is a possibility that the reason no delay in abscission of flowers was observed with the 40 ppm spray application was due to the abscission zone having already developed to

Table XXII.—Summary of observations of seeds from unsprayed trees. The Ohio State University, 1956.

Collected days after NAA applied to sprayed trees	Seed	Endosperm	Embryo		
			Degenerating	Length	Width
			(percent)	(mm)	(mm)
	"Sets"				
3	Normal	Normal	20	.051	.043
5	Normal	Normal	20	.066	.054
8	Normal (few collapsed)	Normal (few degenerating)	43	.066	.057
11	Normal (few collapsed)	Normal (few sparse)	31	.071	.063
14	Normal	Normal (few sparse)	20	.100	.083
17	Normal	Normal	0	.114	.106
	"Drops"				
3	Small	Normal	67	.040	.034
5	Small	Normal	67	.054	.043
8	Normal (few collapsing)	Sparse (degenerating)	78	.051	.043
11	Collapsing	Sparse	80	.071	.051
14	Collapsing	Degenerating	54	.097	.077

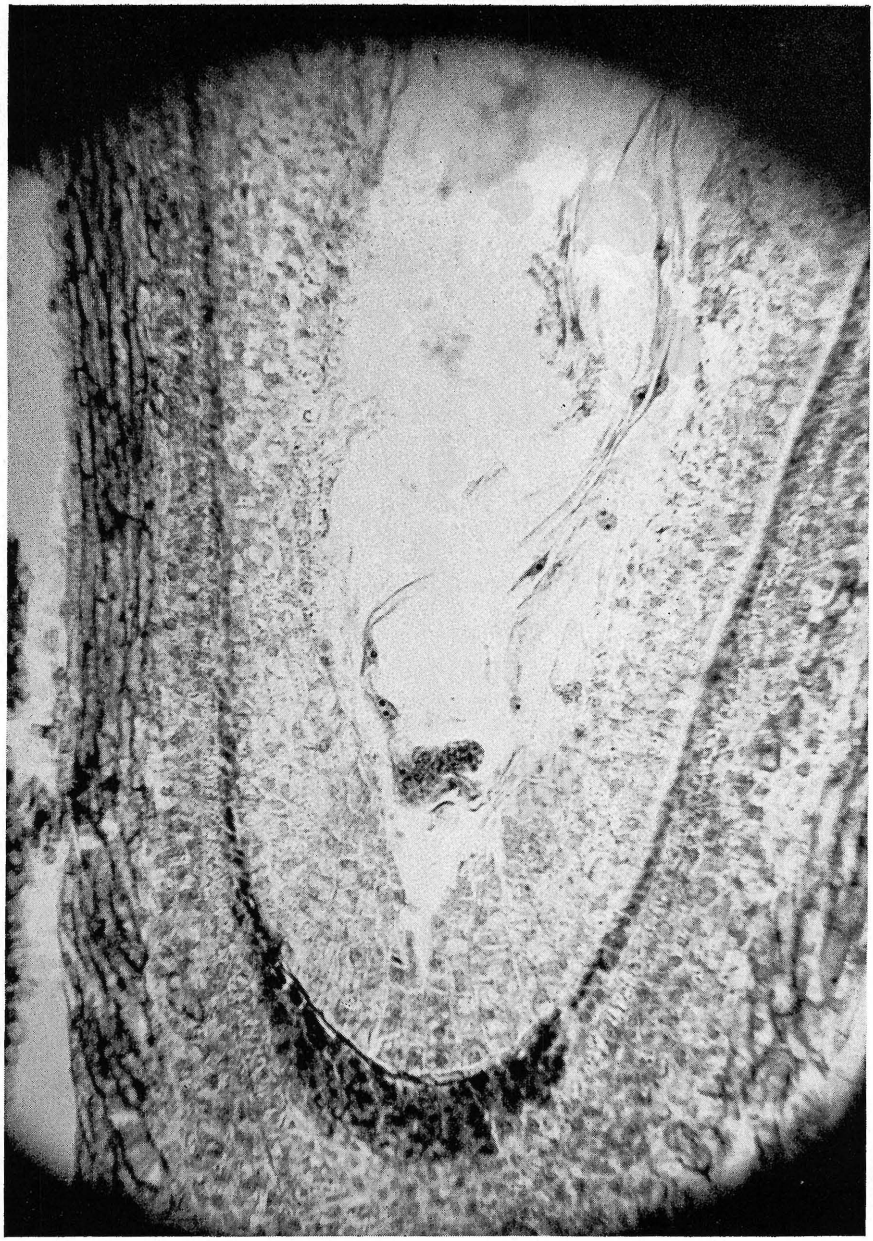


Fig. 19.—Seed from young fruit of control (unsprayed) collected 15 days after petal fall showing embryo and endosperm nuclei. (182X).

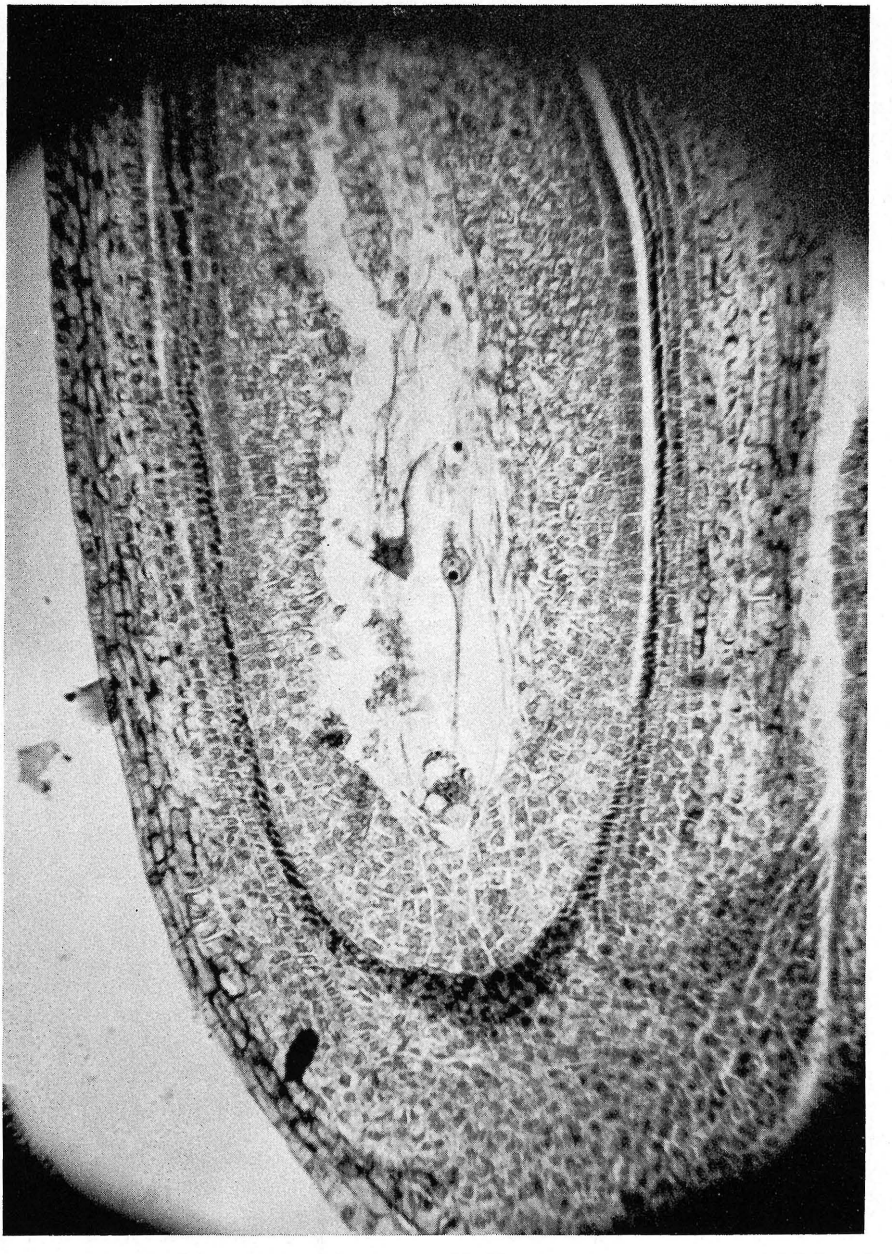


Fig. 20.—Seed from young fruit sprayed with 20 ppm NAA 5 days after petal fall and collected 15 days after petal fall. Development of embryo and endosperm nuclei delayed. (182X).

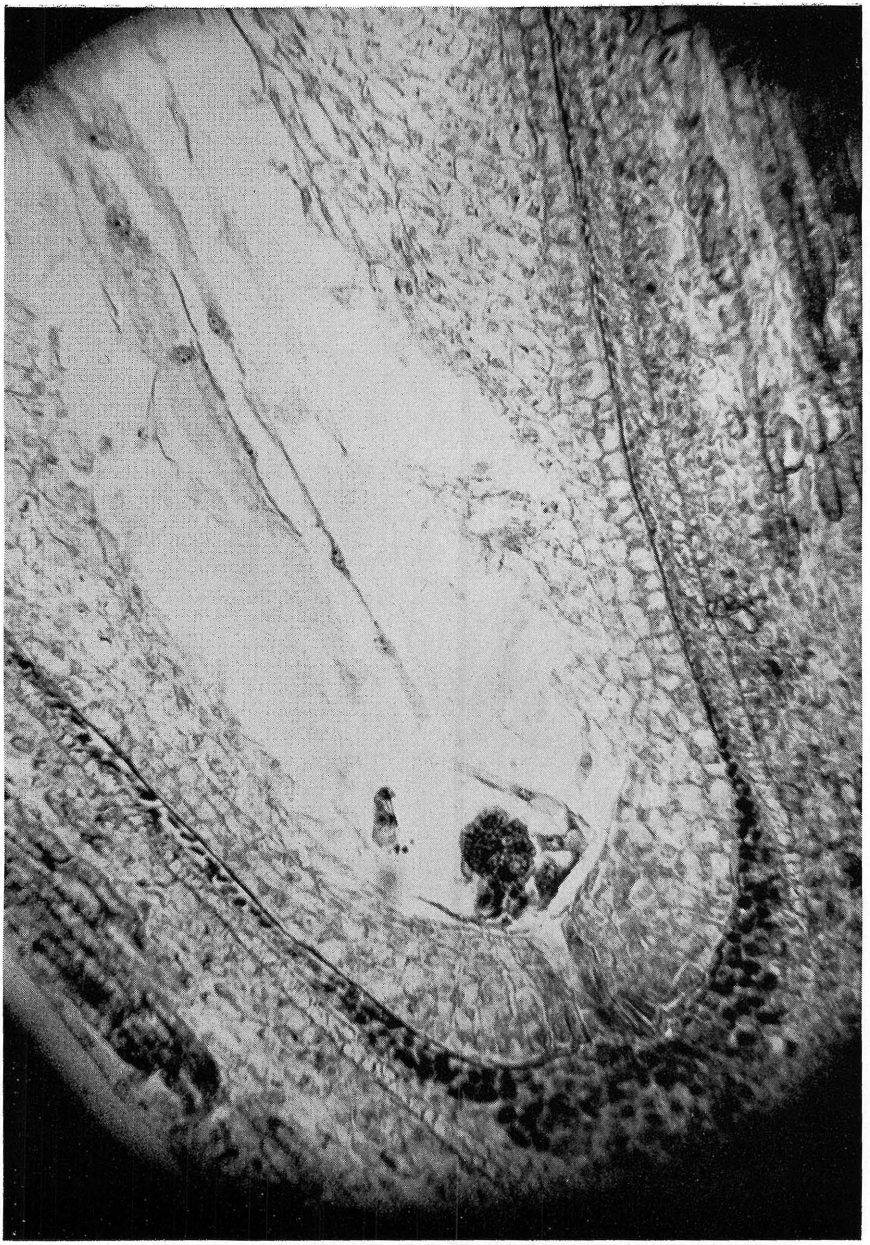


Fig. 21.—Seed from young fruit of control (unsprayed) collected 18 days after petal fall showing embryo and endosperm nuclei. (182X).

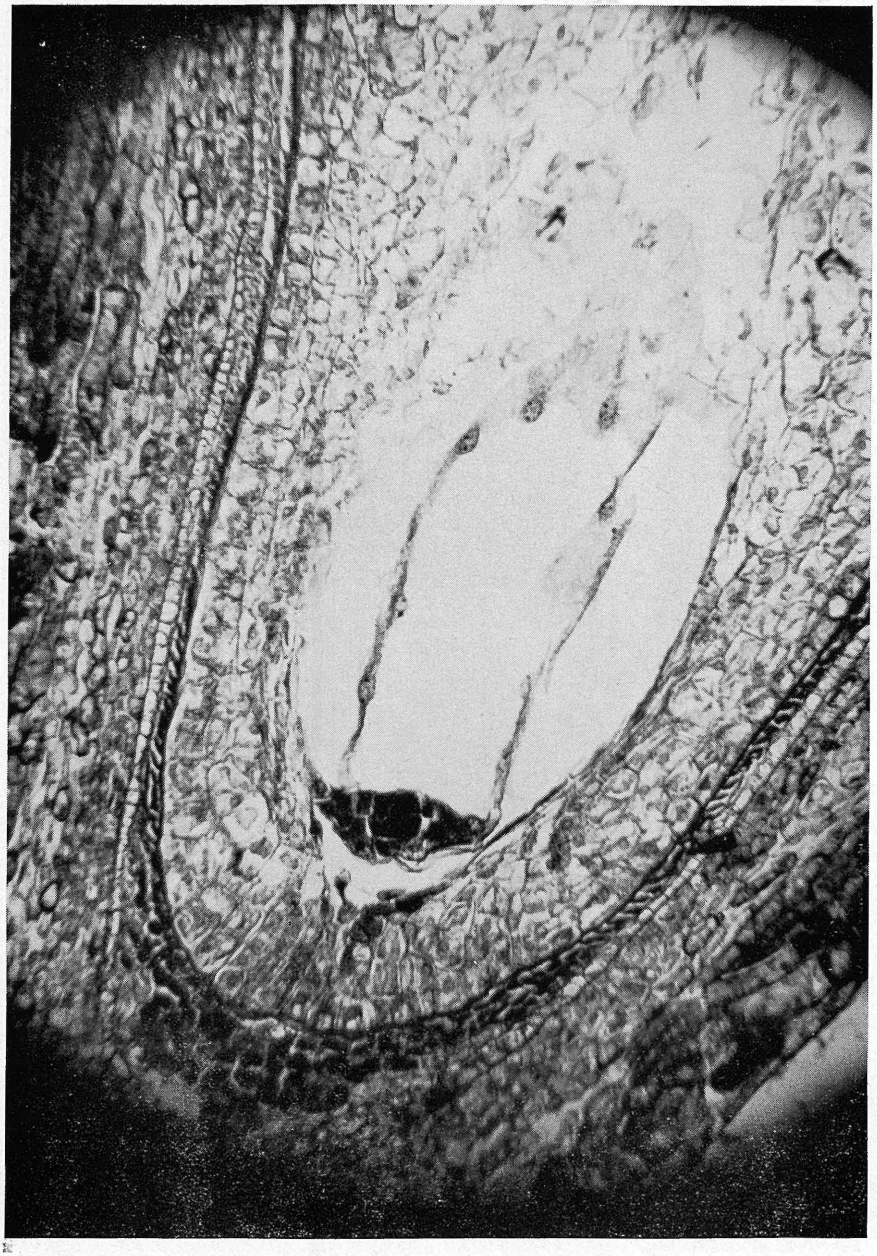


Fig. 22.—Seed from young fruit sprayed with 30 ppm NAA 9 days after petal fall and collected 20 days after petal fall showing a degenerating embryo. (182X).

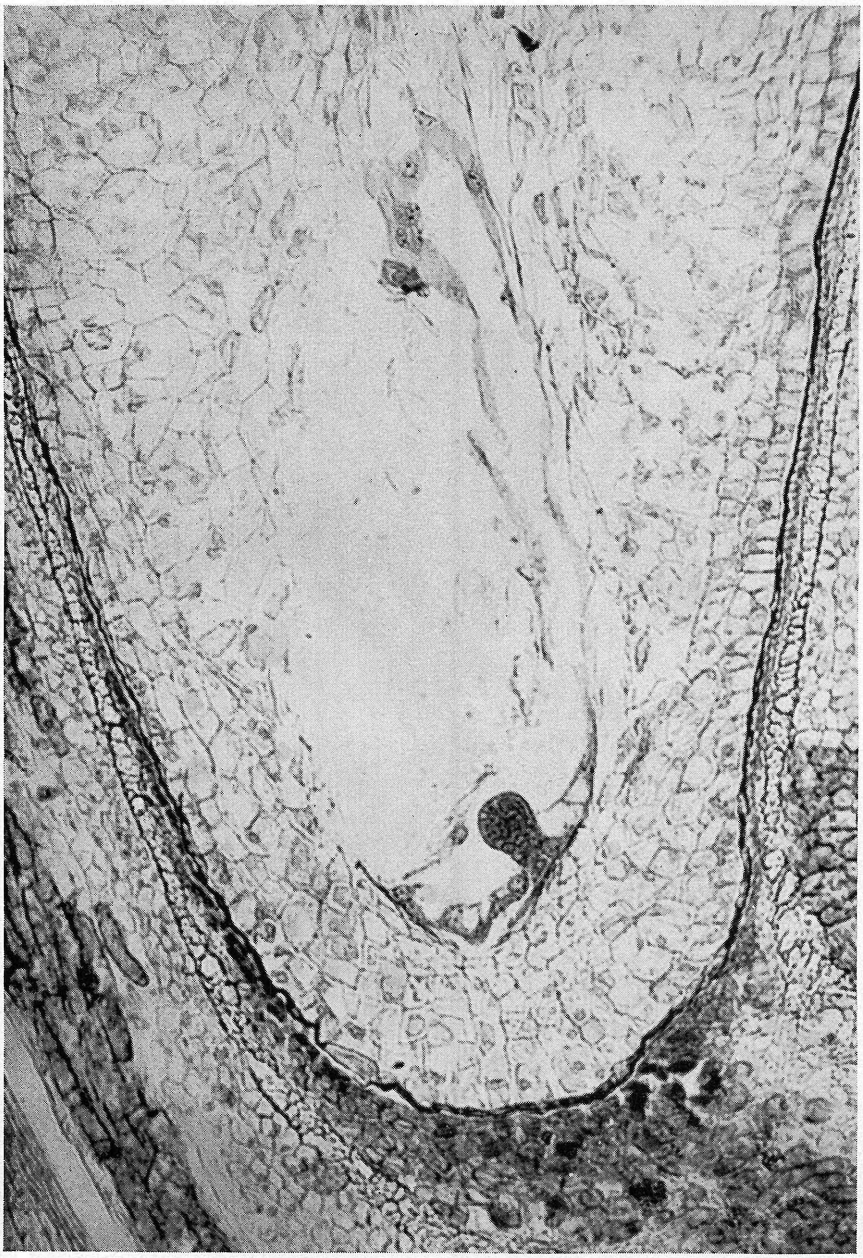


Fig. 23.—Seed of control (unsprayed) collected 14 days after petal fall showing embryo and endosperm nuclei. (173X).

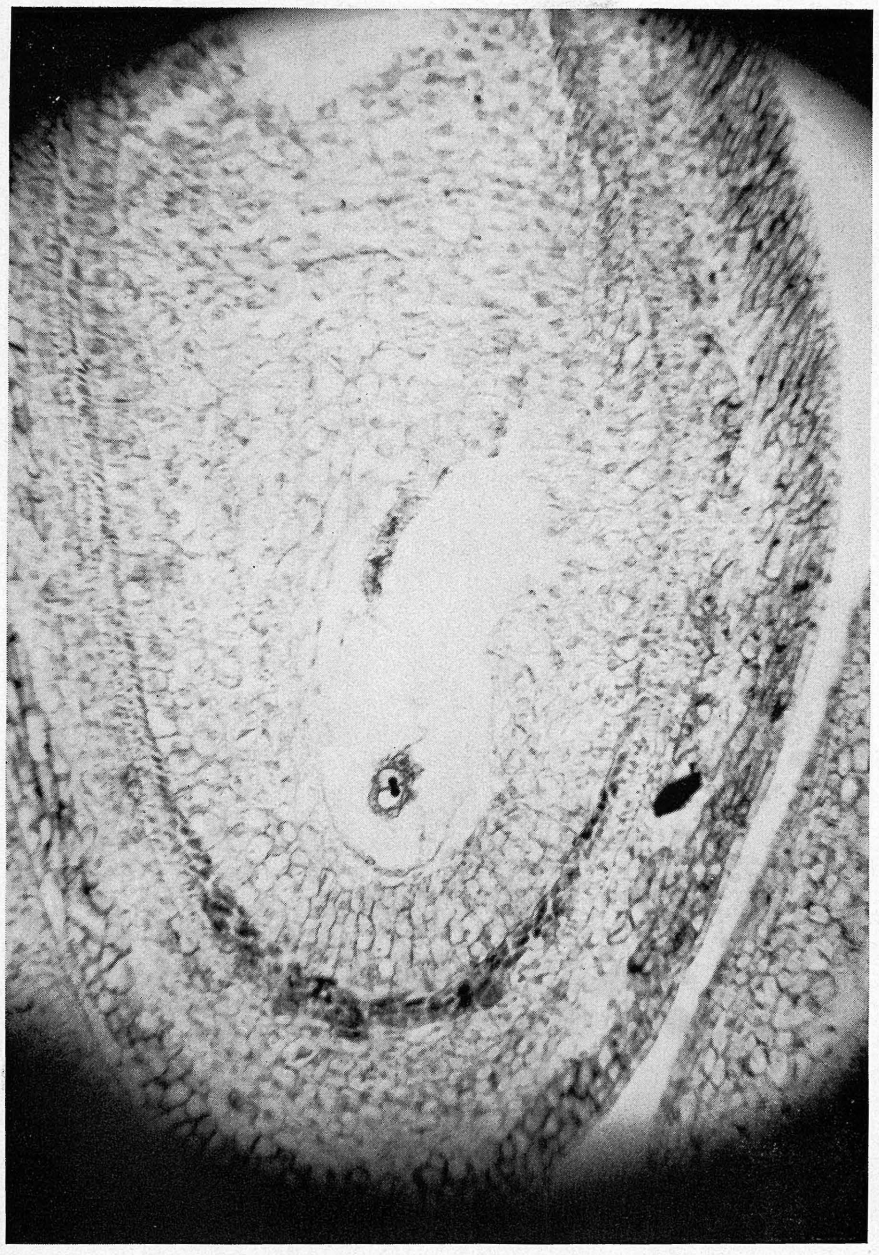


Fig. 24.—Seed from young fruit sprayed with 20 ppm NAA at petal fall and collected 14 days after petal fall showing abnormal or retarded development. (182X).

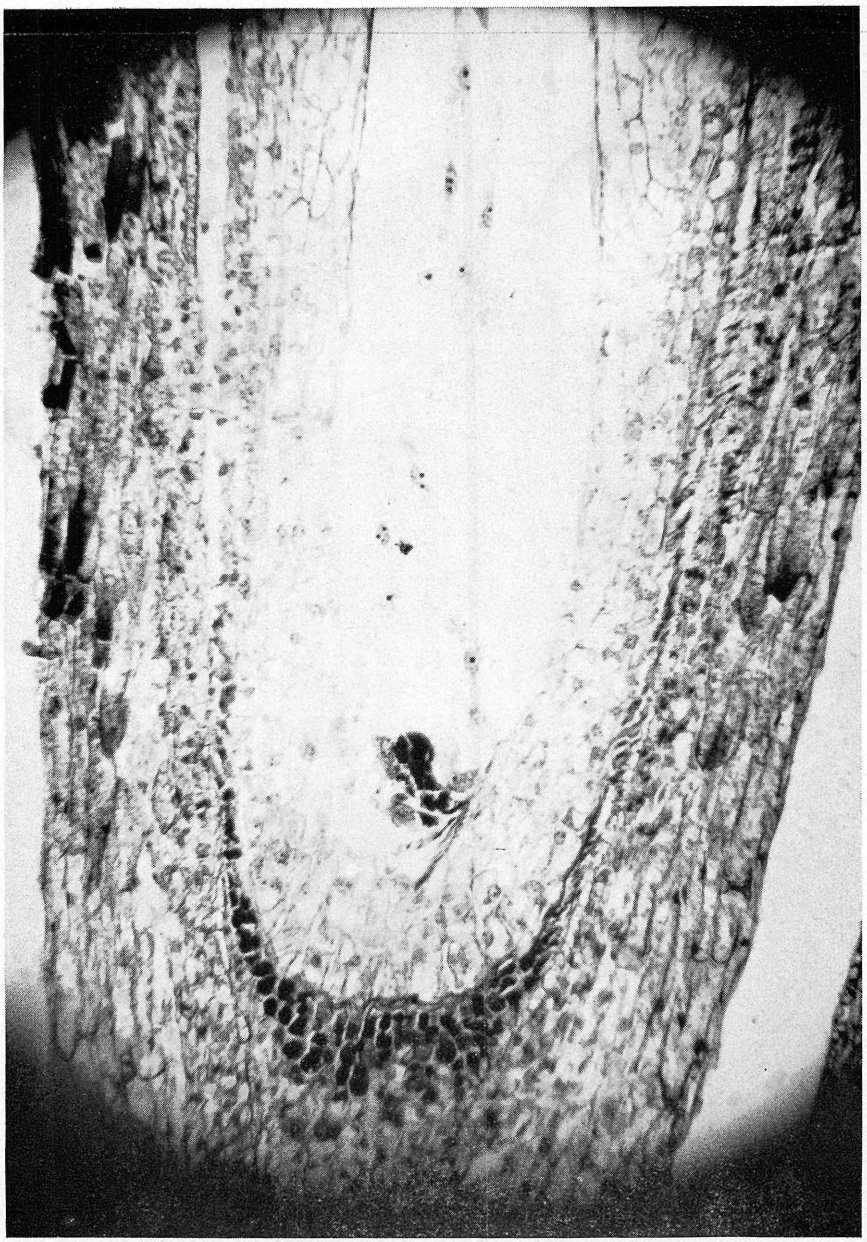


Fig. 25.—Seed from young fruit sprayed with 20 ppm NAA at petal fall and collected 15 days after petal fall showing degenerated embryo. (182X).

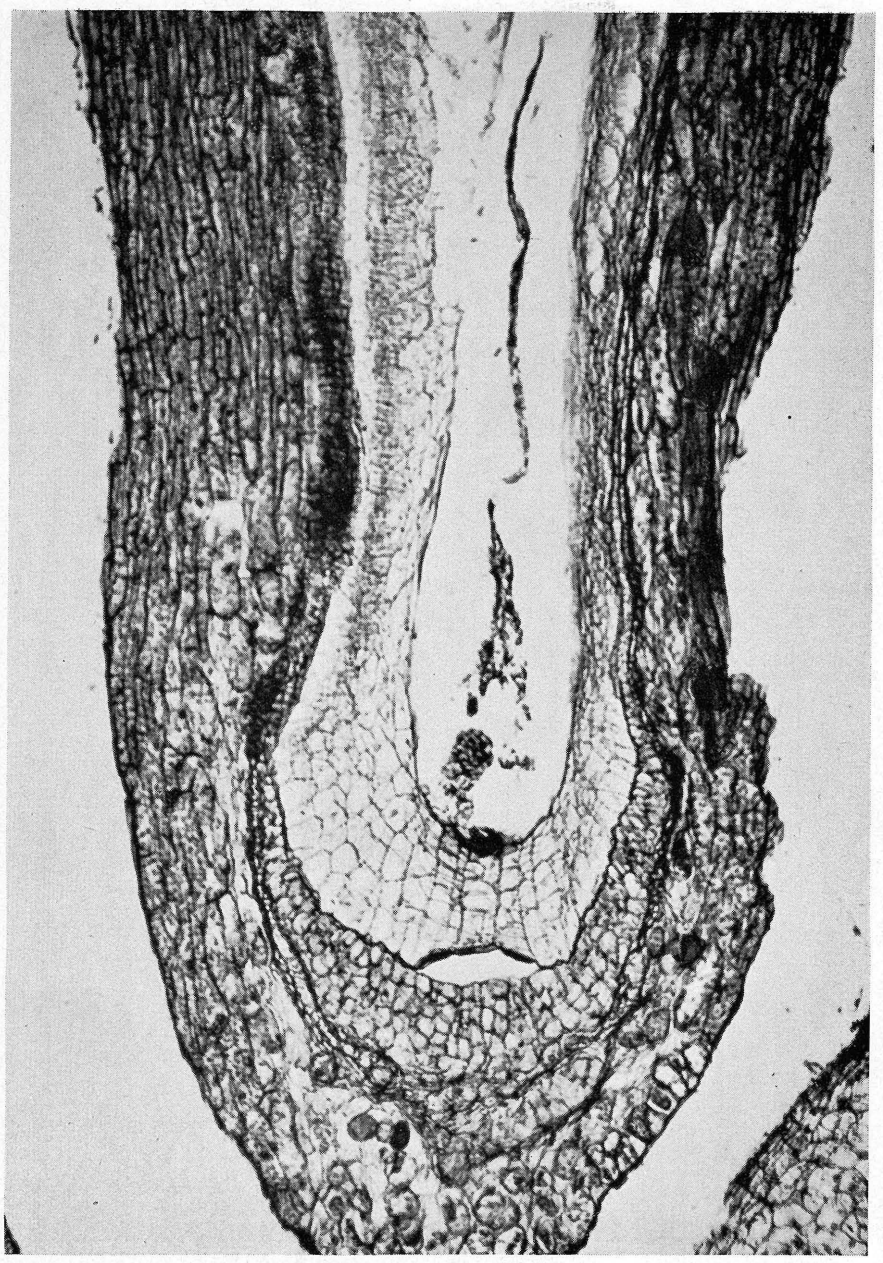


Fig. 26.—Seed from young fruit sprayed with 30 ppm NAA 3 days after petal fall and collected 14 days after petal fall showing degeneration of entire seed. (173X).

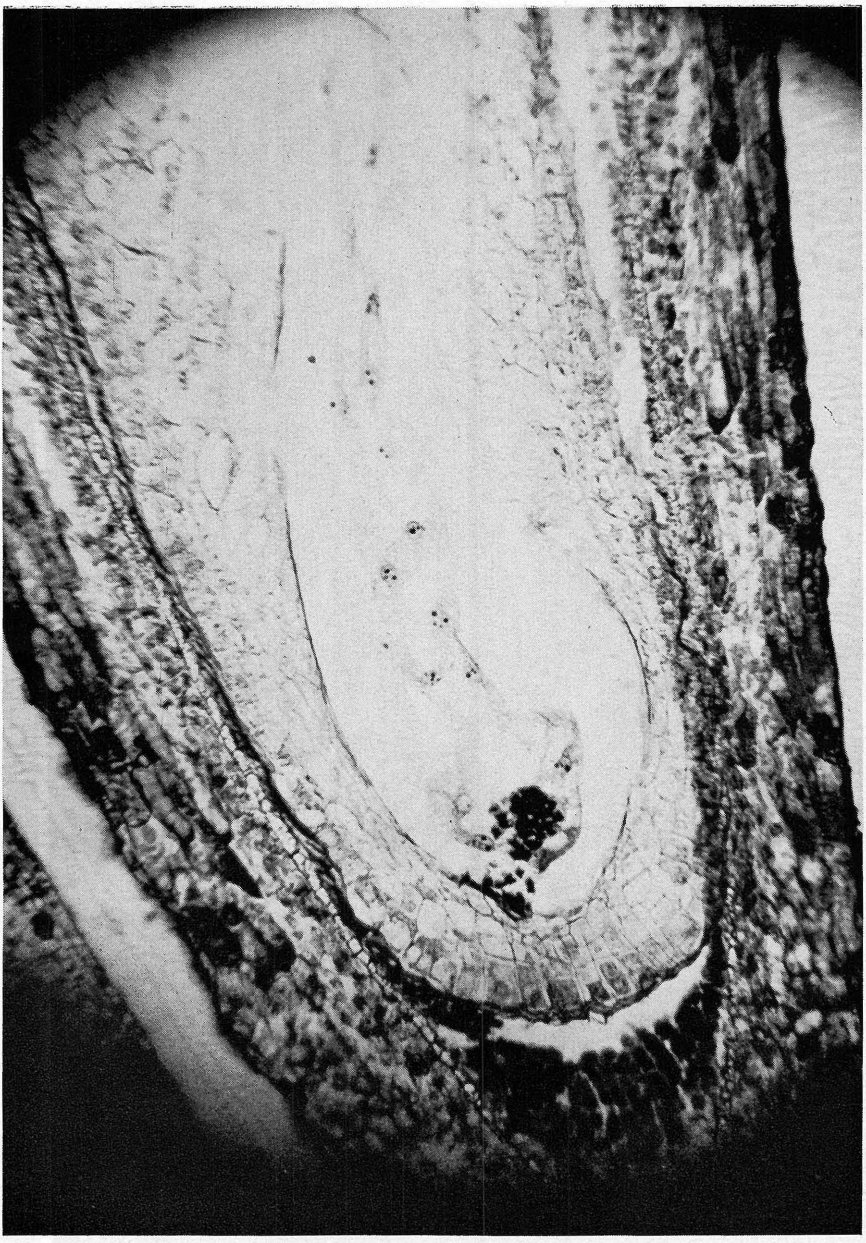


Fig. 27.—Seed from young fruit sprayed with 40 ppm NAA 9 days after petal fall and collected 16 days after petal fall showing degenerated embryo. (182X).

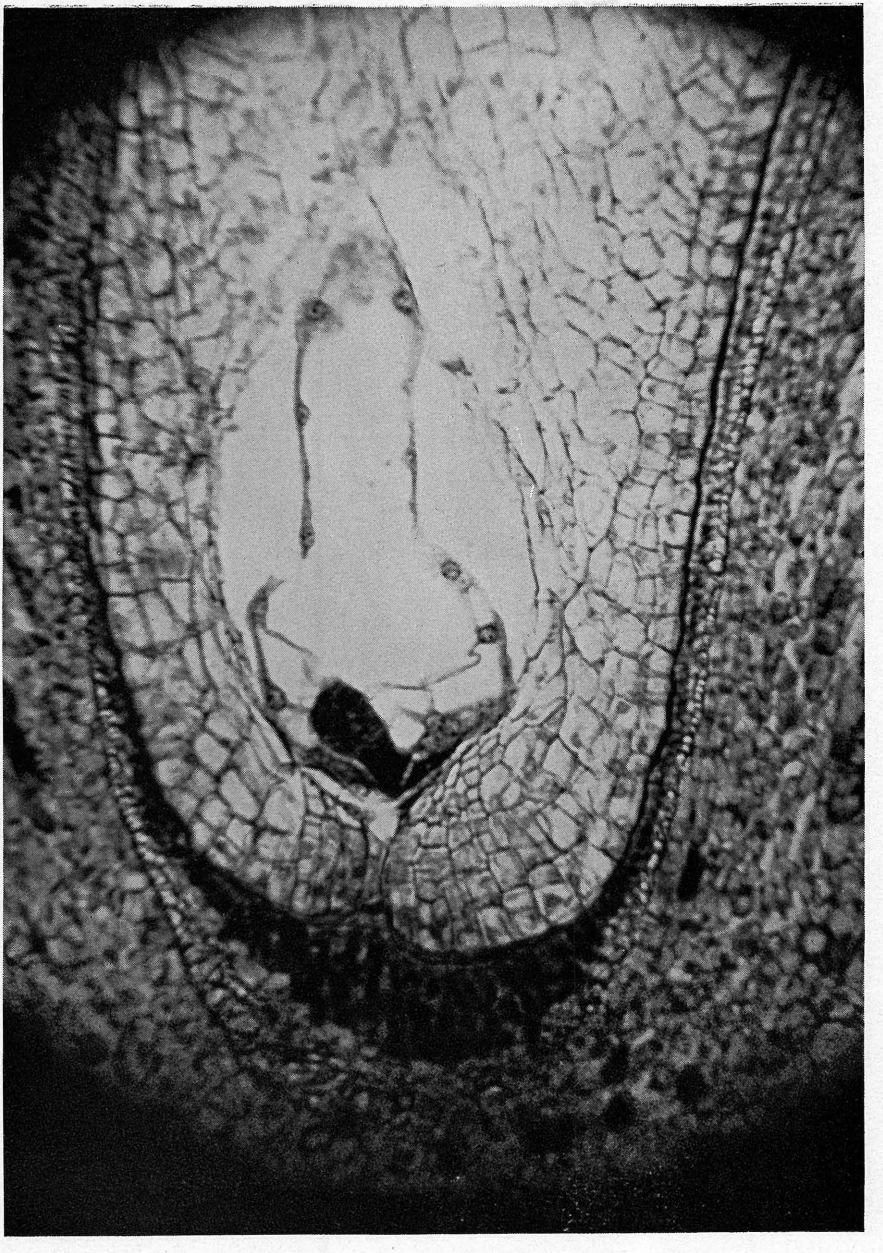


Fig. 28.—Seed from young fruit of control (unsprayed) collected 14 days after petal fall showing embryo and endosperm nuclei. (182X).

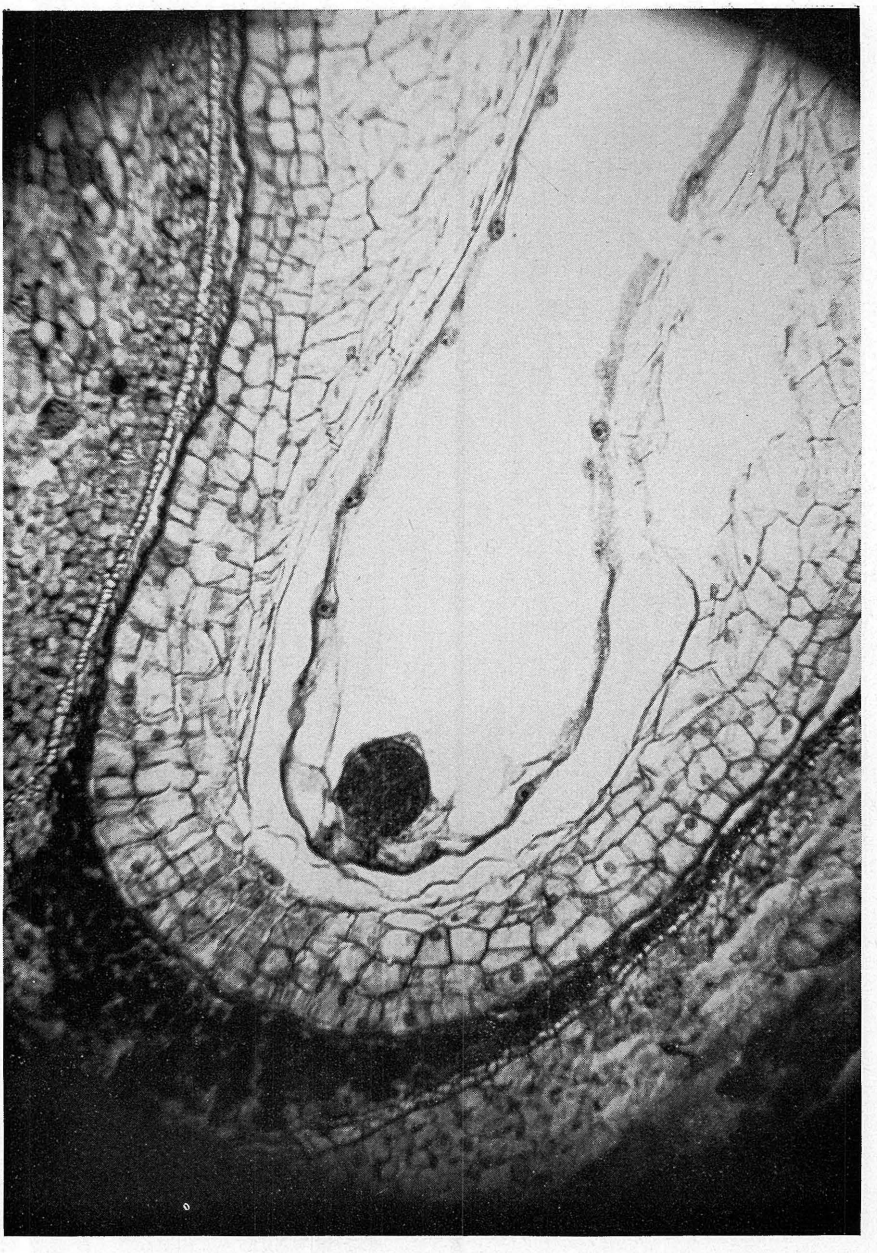


Fig. 29.—Seed from young fruit sprayed with 37 ppm NAA at 11 days after petal fall and collected 14 days after petal fall showing no indication of degeneration this soon. (182X).

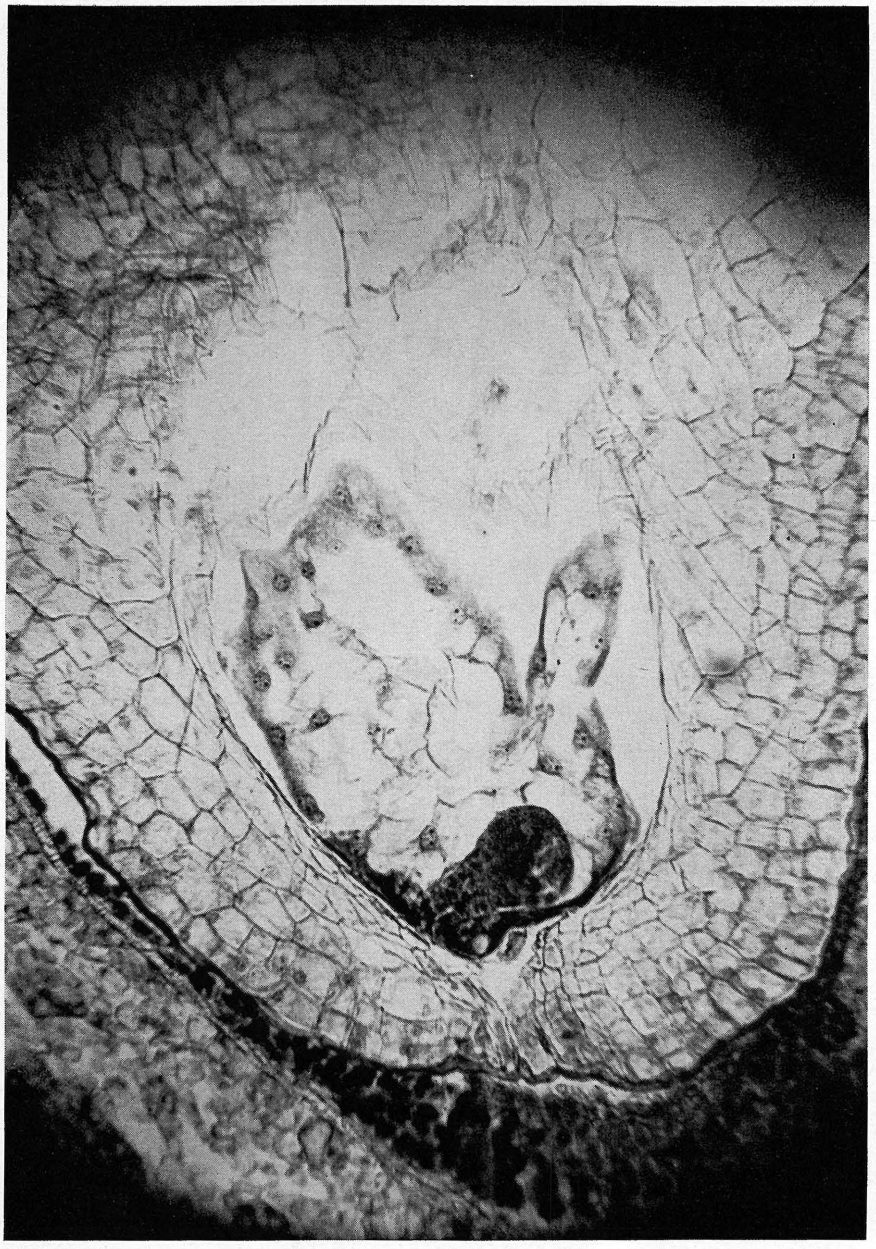


Fig. 30.—Seed from young fruit of control (unsprayed) collected 16 days after petal fall showing embryo and endosperm nuclei. (182X).

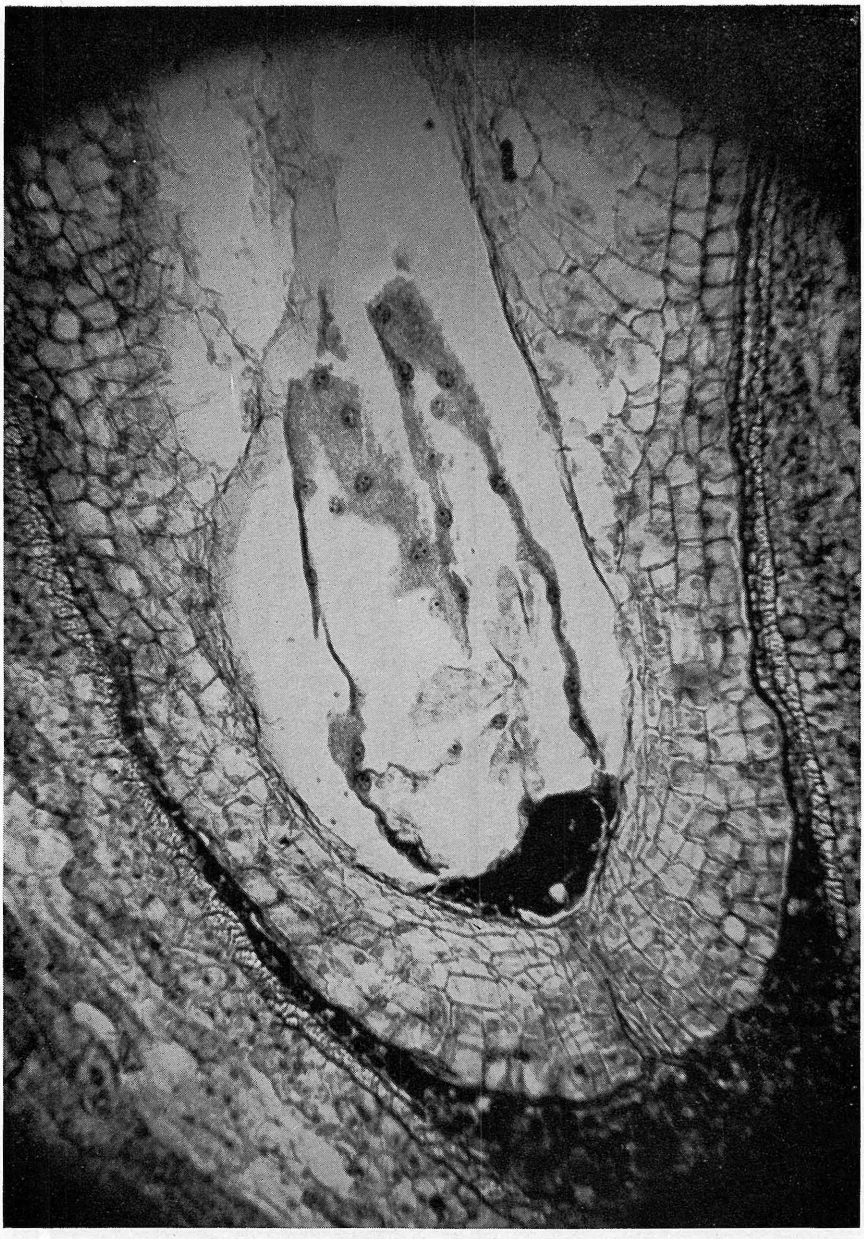


Fig. 31.—Seed from young fruit sprayed with 37 ppm NAA at 11 days after petal fall and collected 16 days after petal fall showing degenerated embryo. (182X).

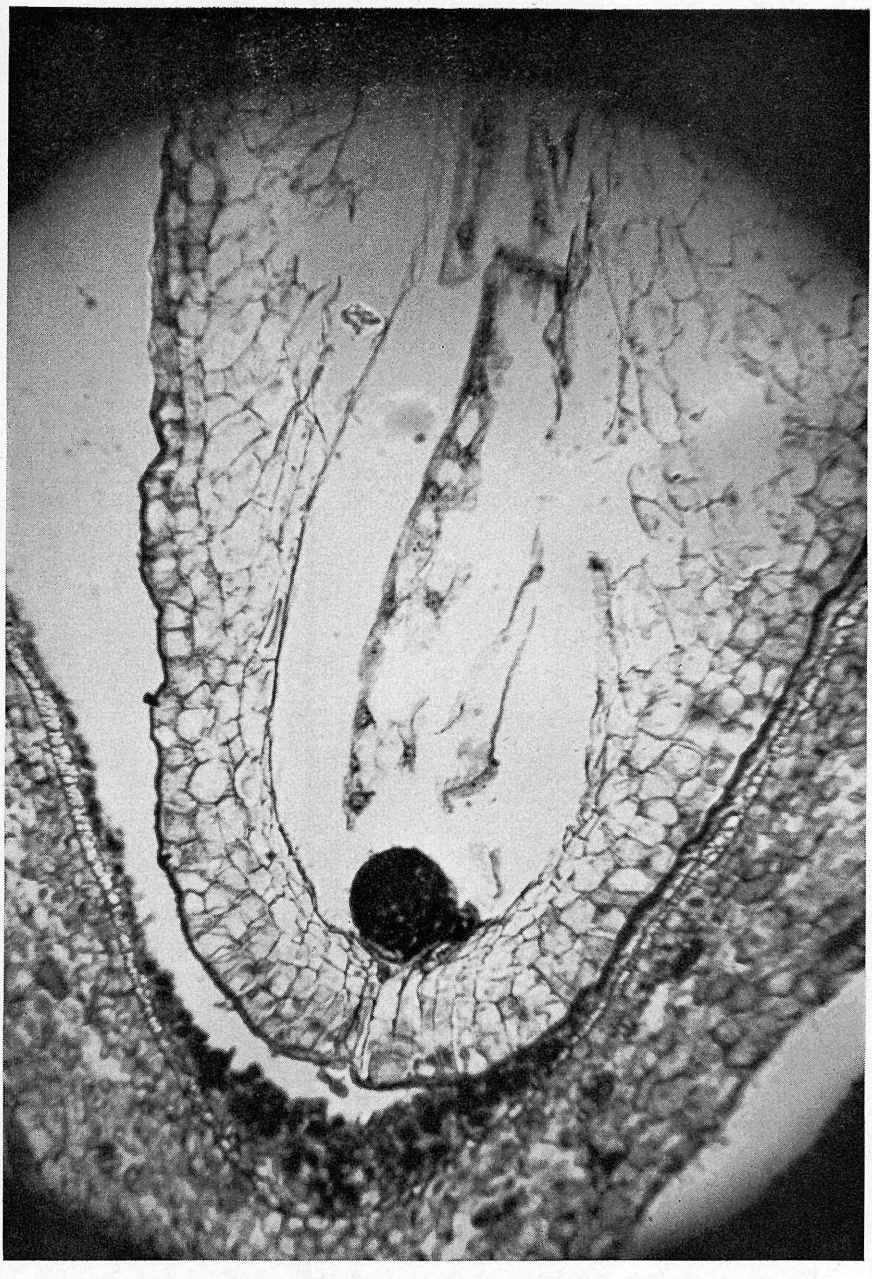


Fig. 32.—Seed from young fruit sprayed with 37 ppm NAA at 11 days after petal fall and collected 16 days after petal fall showing degenerating endosperm nuclei. (182X).

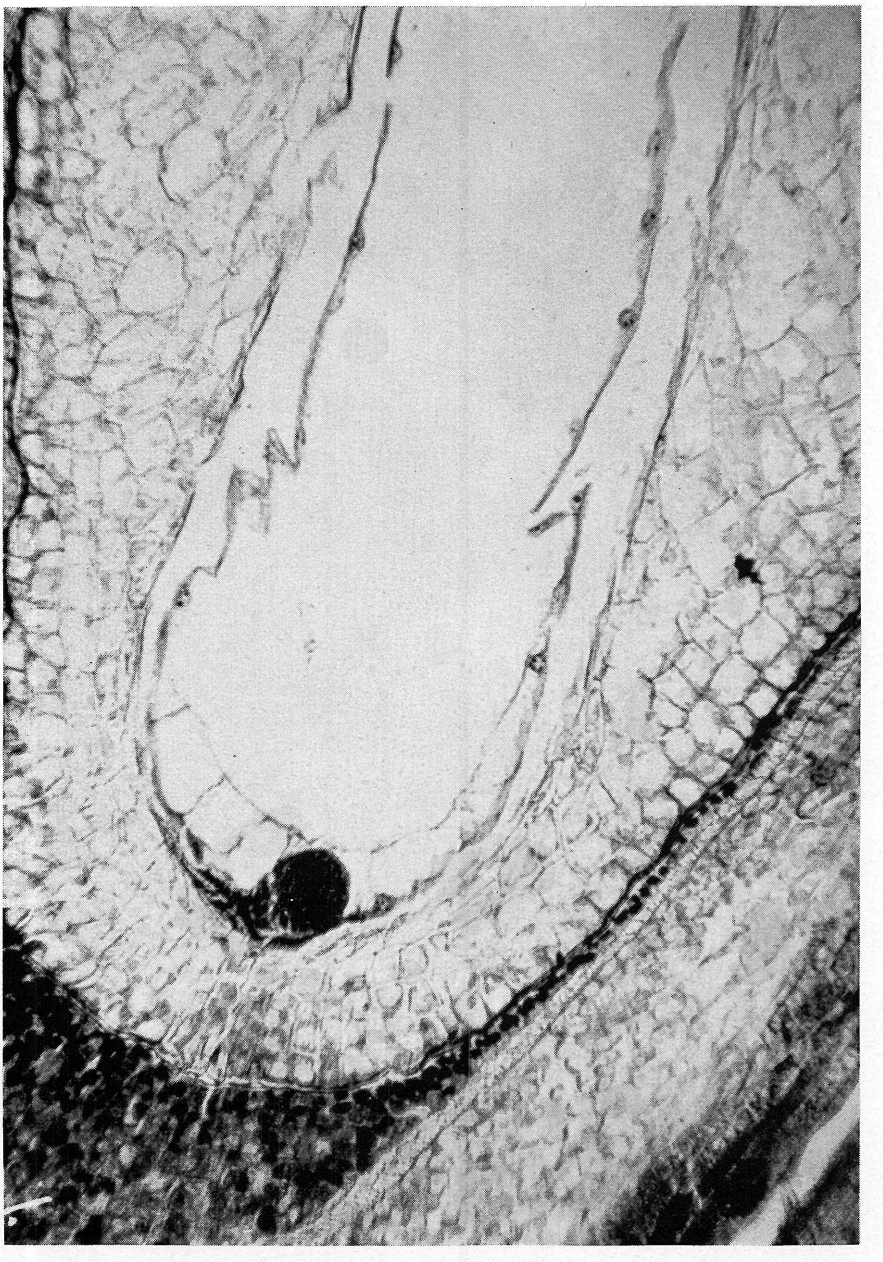


Fig. 33.—Seed from young fruit of control (unsprayed) "expected drop" collected 16 days after petal fall showing degenerating embryo and endosperm. (186X).

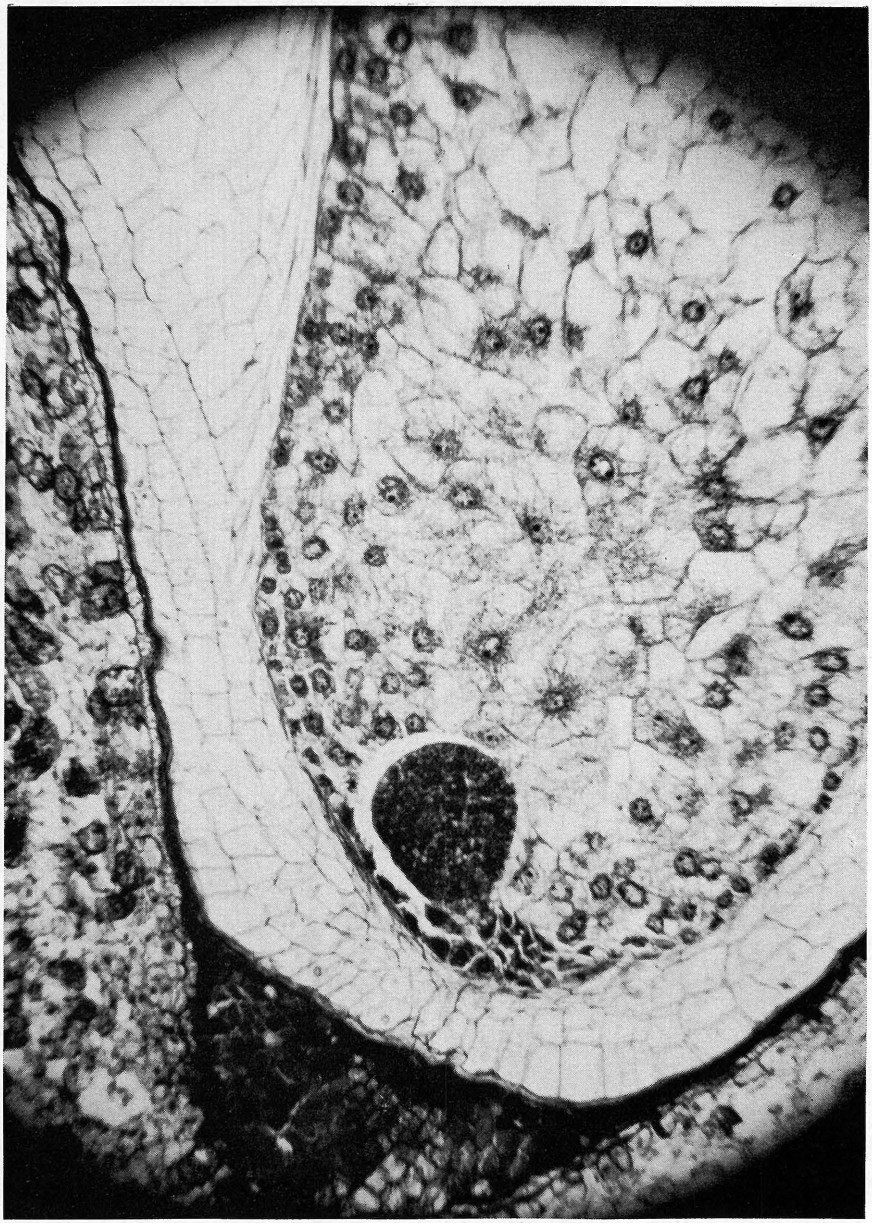


Fig. 34.—Seed from young fruit of control (unsprayed) collected 22 days after petal fall showing embryo and cellular endosperm. (182X).

Fig. 35.—Seed from young fruit sprayed with 37 ppm NAA at 11 days after petal fall and collected 22 days after petal fall showing developing embryo, but degenerating endosperm. (186X).

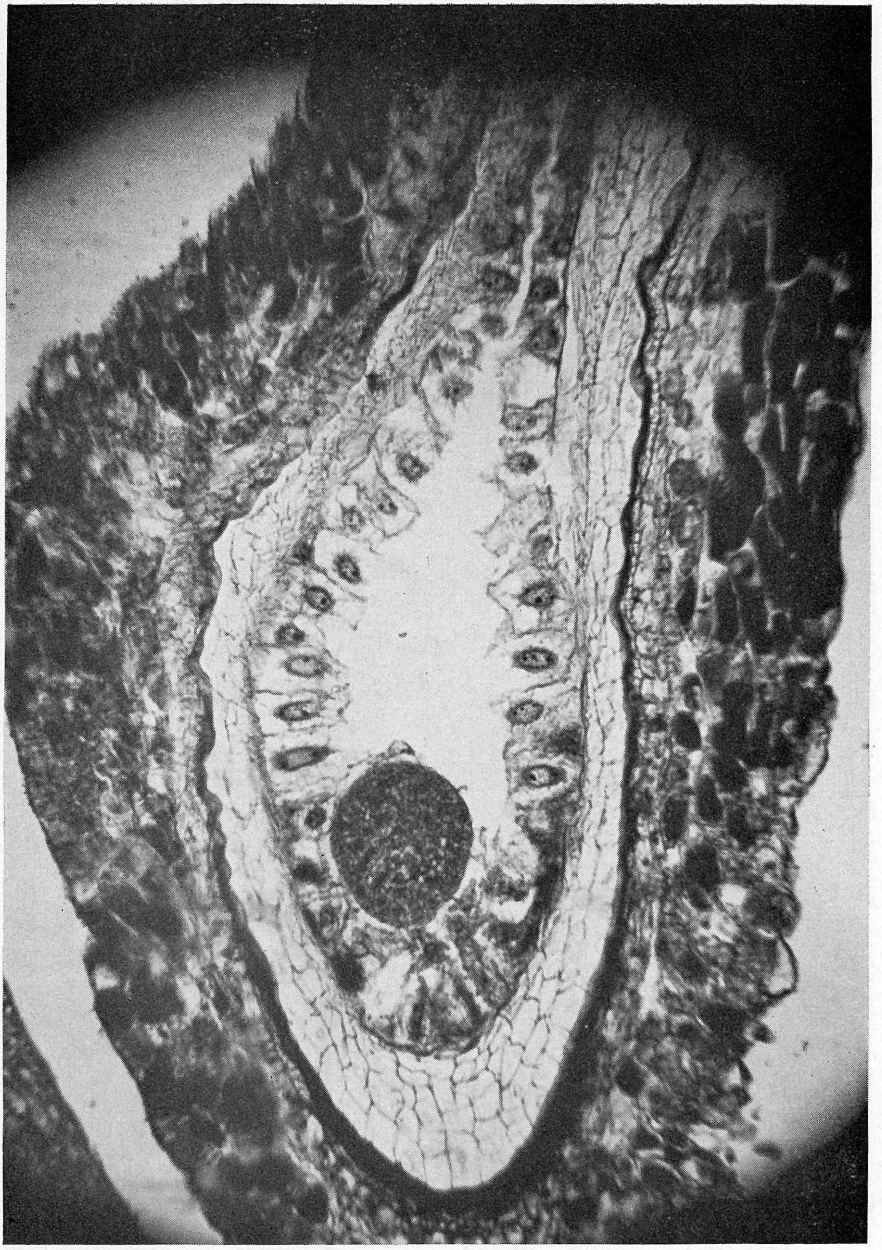


Fig. 36.—Collapsed seed from young fruit sprayed with 37 ppm NAA at 11 days after petal fall and collected 22 days after petal fall showing embryo and small amount of cellular endosperm. (182X).

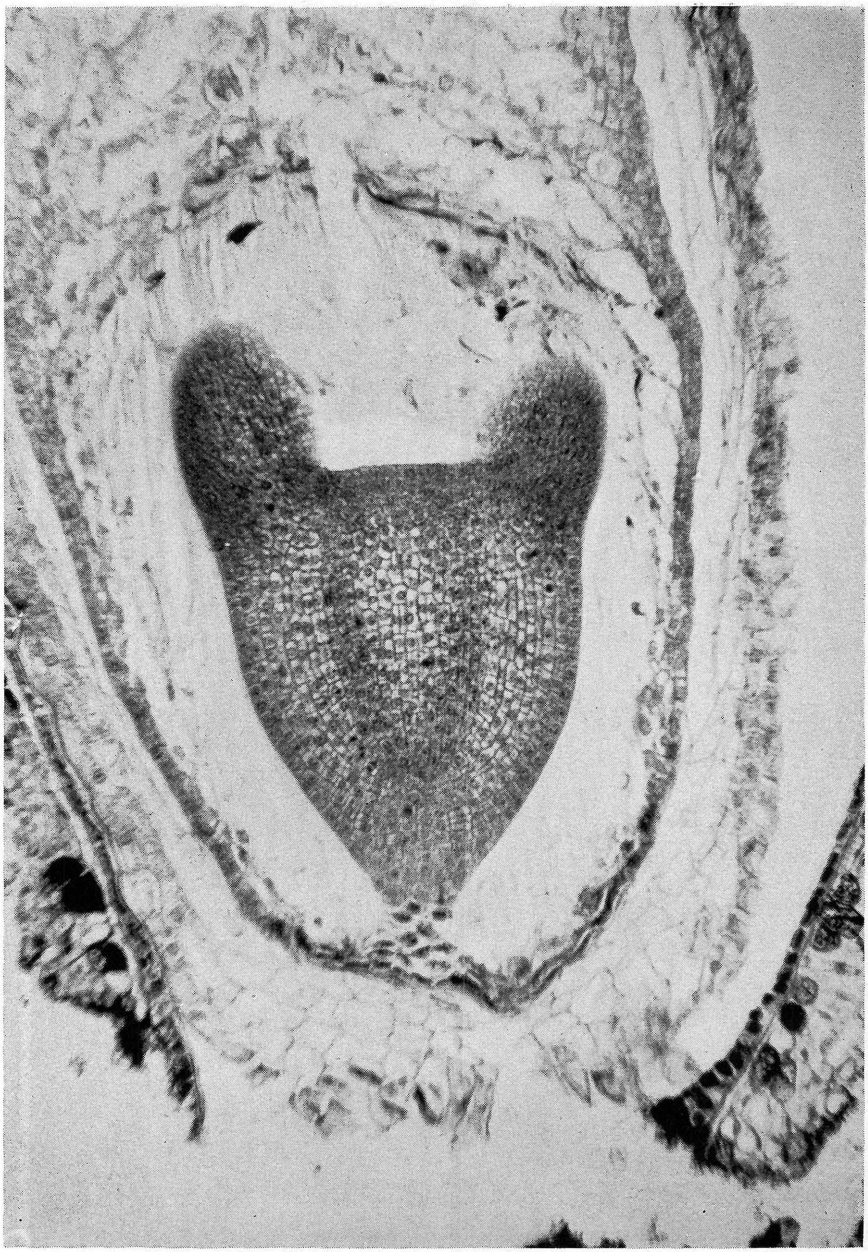


Fig. 37.—Collapsed seed from young fruit sprayed with 37 ppm NAA at 11 days after petal fall and collected 28 days after petal fall showing well developed embryo. (186X).

some extent in fruits of the first drop at the time the spray was applied which was three days after petal fall.

Effects on Fruit Size

In some cases where fruit set was reduced, fruit size showed an increase. However, in other cases, such as the 20 and 50 ppm sprays (Figure 2), there was very little evidence of increase in fruit size even though fruit set was reduced appreciably. This substantiates reports in the literature (5, 11) that NAA may have a dwarfing effect on fruit growth.

Why the 30 ppm treatment resulted in larger fruit size than the 20 ppm when the final fruit set was so close may be the result of a larger proportion of small fruits removed by the former. As the number of seeds is less in fruits under 2 inches in diameter (Figure 4) of the 30 ppm treatment than in the 20 ppm, one might assume that fruits of the former would be more prone to abscise.

Evidently fruit size is a combination of the result of the thinning action of NAA plus some direct effect on reduction of fruit enlargement. The result obtained depends upon the concentration of NAA and the time of application.

Effects on Seed Number and Weight

It appears from the data presented on seed number and weight of mature fruits of Golden Delicious that seed number is affected more pronouncedly than is individual seed weight when NAA is applied at or a few days after petal fall. However, delaying the NAA spray for a week or two subsequent to petal fall apparently had a somewhat greater influence in reducing individual seed weight and at the same time less effect in reducing seed number as compared to those sprays applied at petal fall. Several investigators (11, 12, 17) have found little or no difference in seed number and/or weight of seeds in fruits examined at harvest. This disagreement with results presented above might be due to differences in time of application or concentration of the spray, and to the system used to classify the seeds as to number and weight. Seeds in a given fruit and treatment were found to vary greatly in size and weight. If all aborted seeds below a given size were eliminated, the results might be quite different. In this work only those structures larger than 2 mm were counted as seeds. Most of what appeared to be aborted seeds were between 2 to 5 mm in length.

From the average seed weight and the number of seeds per fruit, the average weight of seeds per fruit was determined which showed a reduction by all of the spray applications from petal fall to two weeks after petal fall.

However, when the application was delayed until five weeks after petal fall, a concentration of 80 ppm failed to reduce the weight of seeds per fruit with the exception of those in small fruits below two inches in diameter. The seed weight per fruit measurements appeared to correlate quite well with those of fruit set.

The data also revealed that fruit size increased as seed number, individual seed weight, and average weight of seeds per fruit increased. This would indicate that some substance(s) produced by the seed might be a factor in fruit development.

Seed Analysis

A. Hormone Assay

One of several problems encountered in the hormone assay work was the difficulty of removing the ovules and young seeds from apple fruits at an early date. Consequently, few extractions were attempted prior to 3 weeks after petal fall.

Examination of the data of the hormone assays indicates that maximum production occurred from 37 to 52 days after petal fall depending upon variety, year, location and other factors. This variability is consistent with that reported by Luckwill (10) in working with several English apple varieties. The data also revealed that the peak hormone production apparently occurred about the end of the "June drop" or shortly thereafter. This also substantiates the work of the above worker which indicated the greatest hormonal stimulus at the end of the "June drop."

A comparison between hormone production of seeds from fruits from unsprayed trees vs. those from NAA sprayed trees revealed that in 1955 a somewhat greater response, although not significantly different, was obtained from the unsprayed trees. This would be expected if the effect of NAA on seed development and fruit set, as proposed by Luckwill (10, 11), is considered. The application of NAA affects the developing seed which results in a reduction in the production of the natural auxin thereby resulting in fruit abscission.

However, in 1956 the hormone assay showed little difference in hormone production from seeds of fruits sprayed with NAA vs. those that were unsprayed. This might be attributed to the time or method of sampling. The assay this year was made on June 27, 10 to 17 days later than those made in 1955. However, based on number of days after petal fall, there was little difference. Before drawing conclusions regarding the differences in hormone production of seeds from sprayed and unsprayed trees, additional experiments need to be carried out.

One would expect some differences to exist relative to time and amount of hormone production from seeds of the different apple varieties. Results presented herein revealed that Rome Beauty reached its peak production a week or two prior to that of the other varieties. This might be related to the fact that Rome Beauty usually sets a greater percentage of fruits than the other varieties included here with the exception of Yellow Transparent. With Yellow Transparent, there was an indication that a previous, but somewhat lesser peak in hormone production took place. This was evident in the 1956 assays which were begun earlier in the season. More work on the different varieties should reveal what relationship exists between hormone production and the fruit setting behavior of the variety.

When a second application of the apple seed hormone was applied to the tomato ovularies, the data indicated that increased growth occurred. It would appear from this that the hormone is exhausted or loses its effectiveness on ovulary growth after a number of days. When considered in regard to apple fruit set, one might propose that unless a constant supply of auxin is available, the fruit would abscise.

B. Cytological Examinations

It appeared from the cytological studies that the embryos were not all affected to the same degree by a given concentration of spray. Some degenerated rapidly while others continued to develop even though the seeds were collapsing. This difference might be attributed to the vigor of the seeds, or more specifically, to the embryos and/or to the amount of NAA translocated into the seeds.

In general, it has been observed by various workers that with a given date of application after petal fall, increasing the concentration of the NAA spray resulted in increased thinning. Also, using a given concentration, a reduction in degree of thinning occurred as the time of application after petal fall was delayed. Although no comparisons can be made in this regard from results obtained in any given year, if the same concentration or date of application of the 1954 and 1955 results are compared, the above observations tend to be substantiated. These comparisons might be permissible on the basis that the check treatments for these years were quite similar. Therefore, comparing the results of the 20 ppm sprays and likewise the 30 ppm sprays for 1954 and 1955, it is seen that as the date of application was delayed, embryo degeneration was less where the lower concentration was used.

It appeared that when embryo degeneration was rapid, it took place along with or even prior to the degeneration of the endosperm.

However, as noted in the 1956 material, where the spray application was delayed until 11 days after petal fall, the embryos were observed to continue development while the endosperm had largely degenerated.

From results obtained by other workers (19) and from these experiments it appeared that at a given date of application, increasing the concentration of the NAA spray resulted in increased thinning of young fruits and increased the retardation of growth and degeneration of the embryo.

This research indicated that application of NAA affected the developing seed as evidenced by effect on seed number and weight, degeneration of endosperm and embryo, and collapsed seeds. This resulted in a reduction in natural hormone production and fruit abscission. Earlier applications of NAA, within a week after petal fall, appeared to cause embryo abortion and seed collapse more pronouncedly than sprays applied 10 days or later after petal fall.

The endosperm began to become cellular about a week after the termination of the first drop, and the production of hormone reached a peak from 40 to 50 days after petal fall, depending on variety and year, which was a week or two after the June drop. It is believed that the NAA spray resulted in a reduction of hormone production in the developing seeds which eventually resulted in fruit abscission.

Relationship of Seed Development, Hormone Production and Fruit Abscission

In relating the findings of the experiments conducted to date, it appeared that at a given date of application, increasing the concentration of the naphthaleneacetic acid spray resulted in greater embryo degeneration and thereby increased the amount of abscission of young fruits. This substantiates and augments the results obtained by other investigators (19) which have shown that increased thinning resulted when the concentration of the spray was increased.

Investigations have shown that seed development is essential in the case of the apple for satisfactory fruit setting to occur. This work, as well as that of others, showed that the application of NAA sprays affected the developing seed as evidenced by the reduction in seed number and weight in mature fruits, and the presence of collapsed seeds and degenerated embryo and endosperm in young fruits.

It has been postulated that a factor which plays a role in apple fruit setting is the production of a hormone, or hormones, by the developing seeds. It is reasoned that if fertilization of ovules within a fruit is inadequate, insufficient hormone material is produced and abscis-

sion results. Luckwill's work (10, 11) showed that a hormone was produced in the seed of the apple and the amount present was correlated with fruit abscission periods.

The authors found that earlier application of NAA, within a week after petal fall, appeared to result in embryo abortion and collapse of seeds more pronouncedly than sprays applied ten days or more subsequent to petal fall. An understanding of this difference might be had from the results obtained from the hormone assays. These findings, as did Luckwill's showed an increase in hormone response as the seed developed. The hormone content appeared to reach a peak between 40 to 50 days after petal fall which was within a week or two after the June drop terminated.

It may be postulated further from the results obtained that the NAA spray by suppressing seed development, thereby reduced the production of the natural auxin which resulted in fruit abscission.

In addition to contributing toward an understanding of the mechanism of the thinning action of NAA, this work should serve to aid growers and research workers in understanding the greater effectiveness of this material when the application is made within a week to ten days after petal fall. Also, it helps to further explain the effectiveness of higher concentrations of NAA in increasing the amount of thinning. In addition, the comparison of the natural auxin production in the seeds of several apple varieties has contributed to a limited extent to an understanding of their fruit setting behavior.

CONCLUSIONS

Fruit Set and Development

1. At a given date of application subsquent to petal fall, increasing the concentration of the NAA spray resulted in increased thinning of young fruits.

2. Delaying the date of application of the NAA spray required an increase in concentration to result in comparable thinning. When delayed to 35 days after petal fall, no thinning resulted.

3. There was a temporary delay, of a week or two, of drops from trees sprayed with NAA when compared to those from unsprayed trees.

4. Fruits from trees sprayed with NAA contained fewer seeds when compared to those from unsprayed trees with the exception of those sprayed 35 days after petal fall.

5. Individual seed weight appeared little affected except where higher concentrations of NAA were used within 14 days after petal fall. In these instances, seed weight was less.

6. The weight of seeds per fruit was less for fruits from sprayed trees with the exception of those sprayed 35 days after petal fall. The reduction in weight of seeds per fruit increased as the concentration of the spray increased.

Hormone Assay

1. The maximum response in growth of tomato ovularies to apple seed extracts was variable depending upon variety, date of sampling and year.

2. Rome Beauty apple seed extracts gave a somewhat earlier peak response than did the extracts of other varieties.

3. The response in growth of tomato ovularies to apple seed extracts increased from petal fall to a peak between 36 and 53 days subsequent to petal fall and then decreased.

4. Less response was obtained from hormone extracts of seed of fruit from trees sprayed with NAA as compared to those from unsprayed trees.

5. A second application of the apple seed extracts applied two weeks after the first increased the amount of ovulary growth as compared to those receiving a single application.

Cytological Examinations

1. Fruits from trees sprayed with NAA contained a greater percentage of seeds with degenerating embryo and endosperm than did those from unsprayed trees.

2. The contents of seeds within the same fruit often were affected to different degrees. Some showed degeneration of embryo while in others the embryo appeared to be little, if any, arrested in development.

3. Higher concentrations of NAA resulted in somewhat more degeneration of embryos and showed a more pronounced effect earlier subsequent to spraying.

4. Retardation of growth and degeneration of the embryo appeared to be hastened with the earlier applications of NAA,—within a week or ten days after petal fall. With subsequent sprays the condition of the embryos was quite variable. Some degenerated while others appeared comparable to those from unsprayed trees although the seeds were collapsing.

5. When embryo degeneration was rapid, it occurred along with, or even prior to, the degeneration of the endosperm. However, when spraying was delayed the embryos were observed to continue development while the endosperm had largely degenerated.

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