THE EFFECT OF FRUIT MATURITY ON SEED DEVELOPMENT IN CERTAIN XEROPHYTIC SPECIES OF CUCURBITA L.

by

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ABSTRACT

The development and maturity of seed and fruit of certain xerophytic species of Cucurbita was studied. Daily diameter measurement of fruit in C. palmata was determined for a period of 15 days after pollination and was found to increase with age up to the 10th day. Final fruit diameter at 18, 20, and 22 days did not show an increase on that reached at 10 days.

Fruit weight and seed number was determined for fruits of C. foetidissima and C. digitata-palmata hybrid at different ages and was found to be not correlated with age of fruit. Correlations of weight per 100 seeds with age of fruit, and seed number with fruit weight for different ages of the above mentioned two species were highly significant.

The effect of storage of fruit before seed extraction in both species was studied and was found to influence seed germination but not weight per 100 seeds. The best germination percentage obtained was 95% and 78% for seeds extracted from fruits harvested at 32 and 30 days and stored for 20 to 25 days.

A condition of seed dormancy was suggested. This condition prevailed even when the germination tests were extended to 28 days.

INTRODUCTION

Quite a number of workers in the field of seed production have studied problems dealing with the development of seed and its many interrelationships with the fruit. Their objectives behind this kind of research were many. Among them was the determination of the best harvesting practices and harvesting dates of many vegetable crops.

A very important item of basic research in this field is to gain knowledge of and answer questions about the different stages of seed development. The problem studied here specifically deals with seed and fruit development in the xerophytic species of Cucurbita.

The concensus among researchers is that, in general terms, the development of a fruit and its enclosed seed or seeds occurs concomitantly and in a reciprocally coordinated fashion. The process of pollination and fertilization exert marked influence on the development of most fruits and seeds. Normally, the process of double fertilization sets in motion the development of the embryo and the endosperm, respectively, and exerts a promotive influence on the development of the ovule into the seed and frequently, also, on the development of the fruit. The endosperm, in the majority of species, grows rapidly during the early stages of seed development but is later digested in many species and used as a source of food by the developing embryo.

LITERATURE REVIEW

Many research workers have done several investigations on the effect of the presence of seeds on the development of the fruit.

Denne (1963), working on apple fruits, reports that the mean fruit weight at harvest increased with increasing seed number and that there was some evidence that equatorial fruit diameter was increased proportionally more than length as the seed number increased. She also reports that fruitlets with more seeds have a higher rate of growth until about forty days after blossom. She also confirms the relationship between fruit size and seed number reported by Rodrigues and Menezes (1951) and by Schander (1955).

Murneek and Schowengerdt (1955), working on apples, reported a higher correlation between fruit size and seed number in small fruits than in larger fruits. This relationship, however, seems to be questionable in other reported studies. Luckwill (1959), working on pears, reports that although this relationship seems to be a rather general phenomenon, it may be masked in some fruits where there is an inherent tendency toward parthenocarpy. Schander (1955), working on apples and pears, adds that usually where the seed content is low, a stimulating effect of seeds on flesh development can be demonstrated, but high seed numbers may depress fruit growth through competition for available carbohydrates.

Sinnot (1921), Reed (1927), and Ashby (1932) report that fruit size is determined largely by the original size of the ovary. The

ultimate size at maturity is determined by cell size and cell number acting in a specific and orderly fashion together.

Murneek (1954), working on apples, Malus sylvestris, reports that with the exception of parthenocarpic fruits, most fruits require the presence of an embryo and an endosperm for their setting and growth. He adds that the polyembryonic apple fruit usually does not set unless fertilization has occurred in a considerable number of ovules. He mentions that when the crop is fairly heavy, fruits with less than three seeds usually abscise. He also reports that there is a relationship, not only between seed number and fruit size but also between leaf area and fruit volume. When the fruit set is relatively large, then foliage becomes a limiting factor; however, when it is light, seed number seems to determine the size of the fruit.

Reincke (1930), working with pears, reports that in seeded fruit the most extensive tissue development takes place in the immediate vicinity of the carpels, apparently at the expense of tissue development both at the stalk and the calyx ends, giving rise to a squat, smooth-necked pear with its greatest transverse diameter in the seed region of the fruit.

Dempsey and Boynton (1965), working on tomatoes, report that maturity or ripeness of fruits was significantly correlated with seed number. They observed that the largest fruits ripened much earlier than did the smaller ones.

Mann (1950) found that in Cucumis melo about 180 seeds were required to insure fruit set and 400 for normal fruit development, but

Dempsey (1965) suggests that a threshold for fruit set may not exist in tomatoes, especially under conditions of poor fruit set.

Nitsch et al. (1960) have reviewed the physiological role of seeds in fruit development. They show that greatly increased amounts of auxin are produced by the fertilized ovules at the time of endosperm and embryo formation. They went on to explain this by saying that this stimulates rapid ovary enlargement in the grape, swelling of the receptacle in the strawberry, and similar cell enlargements in developing fruits of other species. Marre and Murneed (1953) have shown that seeds acting as centers of auxin production have a controlling influence on carbohydrate metabolism of the fruit and adjoining plant parts. From these observations Dempsey et al. (1965) conclude that fruits with more seeds may have a greater metabolic activity, resulting in increased cell size and more rapid maturity.

When we consider the problem from another angle as the title of this thesis implies; that is, "The Effect of Fruit Maturity On The Development Of The Seed," we find that there also had been a considerable amount of work done in this direction.

Sakr and Mahmoud (1952), working on a group of vegetables, including watermelons and cantaloupes, report that maximum germination percentages of 40% could be obtained in Chilean black seeded watermelon at a fruit age of 35 days after anthesis and 68% in the variety Tom Watson at a fruit age of 23 days. However, germinable seeds could be obtained after 12 days from anthesis. In cantaloupes, they report that 92% germination could be obtained in Hales Best variety at an age of 27

days after full bloom. No increase in percent germination in this variety could be obtained after that period.

Dempsey and Boynton (1965), working on tomatoes, report a significant correlation between seed number and fruit maturity.

Scheer, Ellison, and Johnson (1959), working on asparagus, reaffirm earlier findings that a relation exists between seed germination and fruit maturity.

Harrington (1959), working on Cucumis melo, found that the stage of the maturity of the fruit has an extreme effect on germination of the seed, even after the light and small seeds are removed by milling and that this maturity effect was probably not a post-harvest dormancy for it persisted for nine months - end of the experiment.

Young (1949) compared the seeds from mature Butternut squashes and immature ones picked when sufficiently marketable. Seeds from the first category germinated 91% and weighed 7.8 gms per 100 seeds. Seeds from immature fruit germinated only 19% and weighed 5.32 gms per 100 seeds.

Borthwick (1931), working with carrots, reports that lower germination of seeds was obtained from the third order umbels than from the first and second, and that average seed weight from second order umbels increased with maturity at harvest to 60 days.

Other investigators have indicated that cucurbit fruits should be allowed to reach full maturity if they are to be used for seed.

Harrington (1959) has shown that the stage of maturity of the fruit (muskmelon) has a drastic effect upon germination of seed.

Bisson and Jones (1932), working on peas, report an increase in fresh weight of the fruit (pods) up to about the 32nd day and a decrease due to loss of moisture after that. However, they also report that the average dry weight of fruit increased throughout the entire period. The increase in dry weight of the fruit up to the 22nd day was due to an increase in weight of both pods and peas, but after the 22nd day the increase in dry weight of the fruit was due entirely to the growth of the peas (seeds).

METHODS AND MATERIALS

The main objective behind this study is to find out how long a Cucurbita fruit should be allowed to develop and mature on the vine or in storage to produce a viable and well-developed seed. This objective is often an essential preliminary step in a breeding program. The Department of Horticulture at The University of Arizona is conducting an extensive breeding program on cucurbits, and it was anticipated that such a study on cucurbit seed and fruit development might prove to be useful, not only as basic research but also as a source of answering questions dealing with age, maturity, size, and weight of a cucurbit fruit when its seeds will produce the highest percentage of germina tion. The answers obtained in this respect might well be used for a time-saving procedure where multiple seasons of growing the species could be attained.

Originally, the experiment was planned to include growing, selfing, and harvesting fruits from two species of Cucurbita. The first species to be used was Cucurbita lundelliana Bailey, a mesophytic and perennial species producing a long hard tap root with coarse lateral fibers. The leaves of this species are broadly ovate to nearly uniform in outline. The fruits are green and striped and about 3 to 4 inches in diameter. The second species to be used was Cucurbita palmata Watson, a perennial xerophytic species found in Southern California, Mohave Desert, and southward in interior valleys and low elevations (Whitaker and Bemis, 1964). The root system has large,

fleshy storage roots. The fruit is a relatively hard-shelled, round pepo 2 to 3 inches in diameter and contains from 200 to 600 seeds. The leaf has five palmate lobes with sinuses developed to approximately one-third the length of the leaf veins.

The above mentioned two species were started from seeds in small pots and transplanted when they were 6 inches tall into beds in the greenhouse at Campbell Avenue, Tucson, Arizona. The vines were trained on strings tied to the ceiling of the greenhouse.

Female flowers were tagged before they opened and at anthesis they were pollinated using male flowers from the same species. Daily measurements of fruit growth were done on a four-fruit sample of Cucurbita palmata. This was accomplished by measuring the diameter of the fruit daily, using outside calipers for as long as there was an increase in the daily measurements. When this increase stopped, the fruit then was assumed to have reached maximum size. At this stage a schedule was prepared to harvest the fruits at the required stage of maturity. These different ages or maturity stages start from ten days after pollination to thirty-four days with a two-day period between one age and the one following it. When the fruit was harvested, the date of harvest was recorded on the other side of the tag. The fruits were then taken to the laboratory and weighed and their polar and equatorial diameters measured. Weight and measurements were recorded. There were four replicates of each treatment, i.e., age or stage of maturity except when difficulties developed where it was not possible to get the required number of fruits for the entire set of fruit ages. For those

species where the entire set of ages was complete, two fruits of each treatment were stored at room temperature (77°) for a minimum period of twenty days, after which seeds were extracted from them. Seeds were extracted from the remaining two replicates directly after harvest. This procedure was adopted to find out whether any development in the seed took place during storage and away from the vine.

Seed Extraction

Seed was extracted by cutting the fruit into two halves. The knife was pressed to cut through the rind and not deeper in order not to injure the seeds. The two halves were then twisted in different directions to get them apart. The contents, i.e., seed and pulp, were emptied by hand into a bucket of water. The seeds were separated from the pulp while in the bucket and then were strained, using a 2 mm. strainer. The seeds were then placed on a paper towel which was placed on a paper plate where they were dried for a week at room temperature. This drying period was found to be sufficient to obtain a room dry seed. Seed weights and seed numbers were recorded. An arbitrary classification of the seed, depending on its visual color and fullness, was done. This classification was by no means a real differentiation of mature and immature seed, but it gives an indication of the stage of maturity of the fruit.

Germination Tests

Germination tests were made in the laboratory. Random samples of seed were taken from those fruits that produced well developed seeds. Fruits that produced empty seeds containing only seed coats

were not tested. The sample of seed was weighed and placed between two paper towels, wetted with distilled water and both folded into a rag doll shape, tied at both ends and placed in a beaker containing distilled water at the bottom. Water was added at intervals and seeds were not allowed to be submerged. The beakers were placed in a germinator, the temperature of which was kept constant at 84°F. Together with the samples, a control sample was also included. This control sample of mature seeds of Cucurbita digitata Gray was tested earlier in the same germinator and was found to have an 80% germination in a period of one week. Percent germination of all samples was calculated and recorded after a ten-day period.

During the time the experiment was in progress, some difficulties developed that required some modification of the original plan. The production of female flowers was not attained in great enough numbers to allow selfing of the complete set of ages in the earlier mentioned two species. This was believed to be due to changes in temperature as can be seen from Fig. 1. The effect of temperature on female flower production was reported by Nitsch et al. (1952). This temperature change could not be regulated and because of the press of time, modifications in the original plan were the only alternative left. The plan was modified thus to include the species, Cucurbita foetidissima and an interspecific hybrid, Cucurbita digitata-palmata, where the production of female flowers was enough to allow for the selection of all ages of fruits.

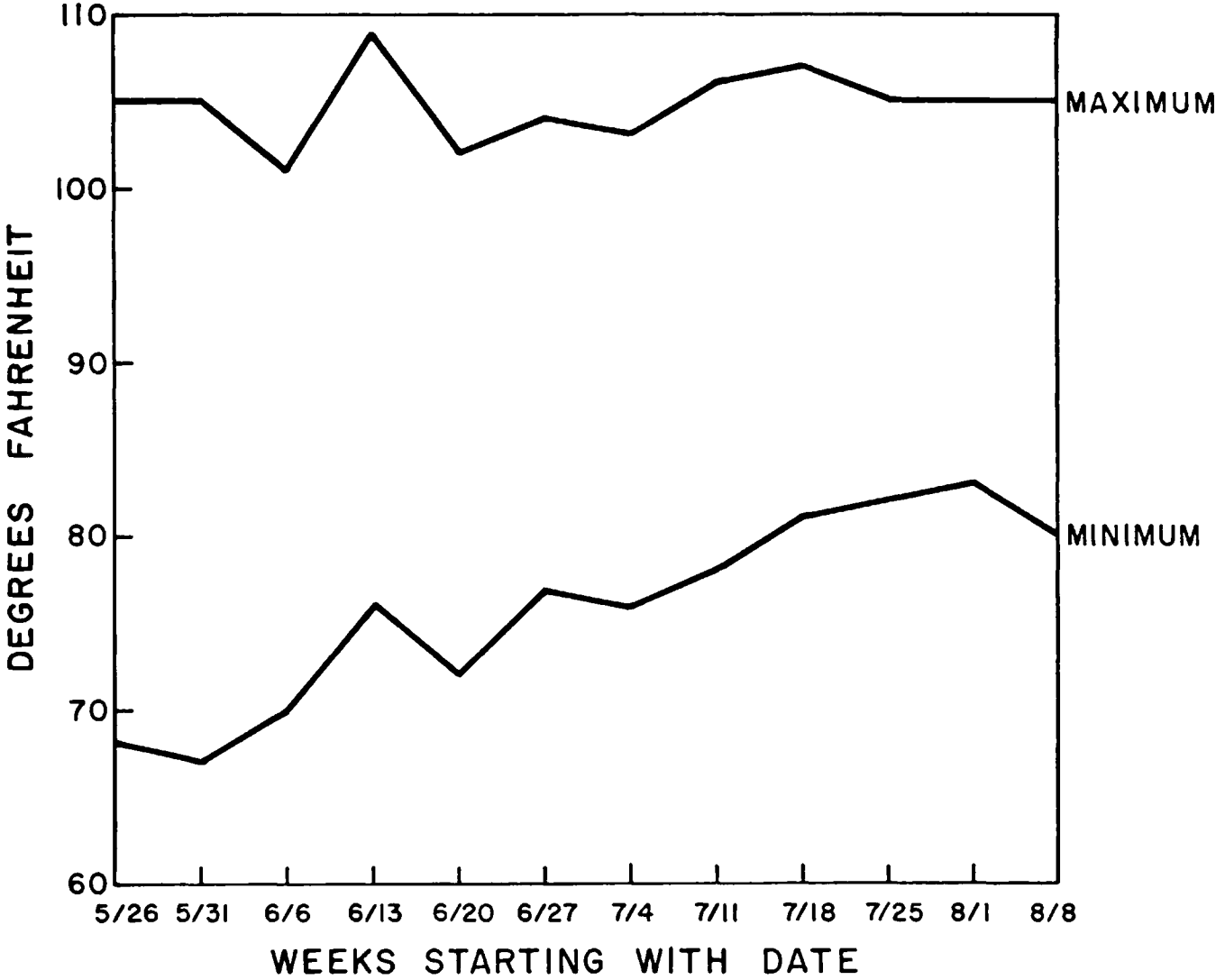

Fig. 1 Temperature in the greenhouse during the period the plants ceased producing female flowers

(1) Cucurbita foetidissima HBK. This is a xerophytic species that happened to be growing in the form of a colony of vines initiating from a common plant outside of the greenhouse. The leaves are large and triangular and with numerous hairs on both the upper and lower surfaces. Its fruits are striped and about 4 inches in diameter.

(2) Cucurbita digitata-palmata hybrid. This is an interspecific hybrid obtained by crossing the above two species. The hybrid is fertile and has seed and fruit that resembles the parents in morphology. The leaf shape, however, is strikingly different from both parents. The F_1 has a length-to-width leaf lobe ratio of 5. Parents have a leaf lobe ratio of 10 and 2, respectively, according to Bemis and Whitaker (1965). All other procedures outlined earlier were also used on the fruit and the seed of these two species, except the pollinating which was done by the bees. Most of the reported work in this thesis was done on the last mentioned species and species hybrid.

EXPERIMENTAL RESULTS

The following data was collected for all fruits harvested:

1. Equatorial and polar diameter of fruits
2. Fresh weight and weight after storage period
3. Seed weight per fruit
4. Seed weight per 100 seeds
5. Seed number per fruit
6. Germination percentages for each treatment - age of fruit

Fruit Size and Development

During the early stages of fruit development, a sample of four fruits from Cucurbita palmata plants was chosen and the diameter measured at daily intervals for 15 days starting from the day of pollination. These measurements are shown in Table 1. The equatorial diameter for the fruits in the above table was plotted against age of the fruit, Fig. 2. The curves obtained indicate a phase of rapid growth up to about the 10th day after pollination. The curves then tend to level off and stay as such through harvest, indicating that the growth or increase in equatorial diameter stays almost constant. Polar and equatorial diameters measured at harvest time for all fruits at different ages show little or no indication of fruit sizes being affected by age after the fruit passes the phase of rapid enlargement. Fruits of the same species showed low inherent variation in size. In Cucurbita foetidissima the mean diameter of fruit for all the 13 ages

Table 1. Equatorial diameter measurements in cms. of Cucurbita palmata fruits at daily intervals from pollination.

Days from pollination	Fruit and plant number			
	1-3	2-3	1-2	2-2
0	1.43	1.59	1.75	1.75
1	2.07	2.07	2.07	2.07
2	2.54	2.70	2.70	2.54
3	3.18	3.34	3.34	3.34
4	3.66	4.13	4.13	4.00
5	4.29	4.93	4.93	4.45
6	4.93	5.57	5.57	4.93
7	5.57	6.36	6.04	5.41
8	6.20	6.52	6.36	-
9	6.20	6.84	-	6.04
10	6.68	-	6.84	6.20
11	6.84	7.16	7.00	6.36
12	-	7.16	7.16	6.52
13	7.00	7.31	7.16	6.52
14	7.00	7.31	7.16	6.68
15	7.00	7.31	7.16	6.68
..				
..				
* 18				6.68
..				
* 20		7.31	7.16	
..				
* 22	7.00			

* Age at harvest.

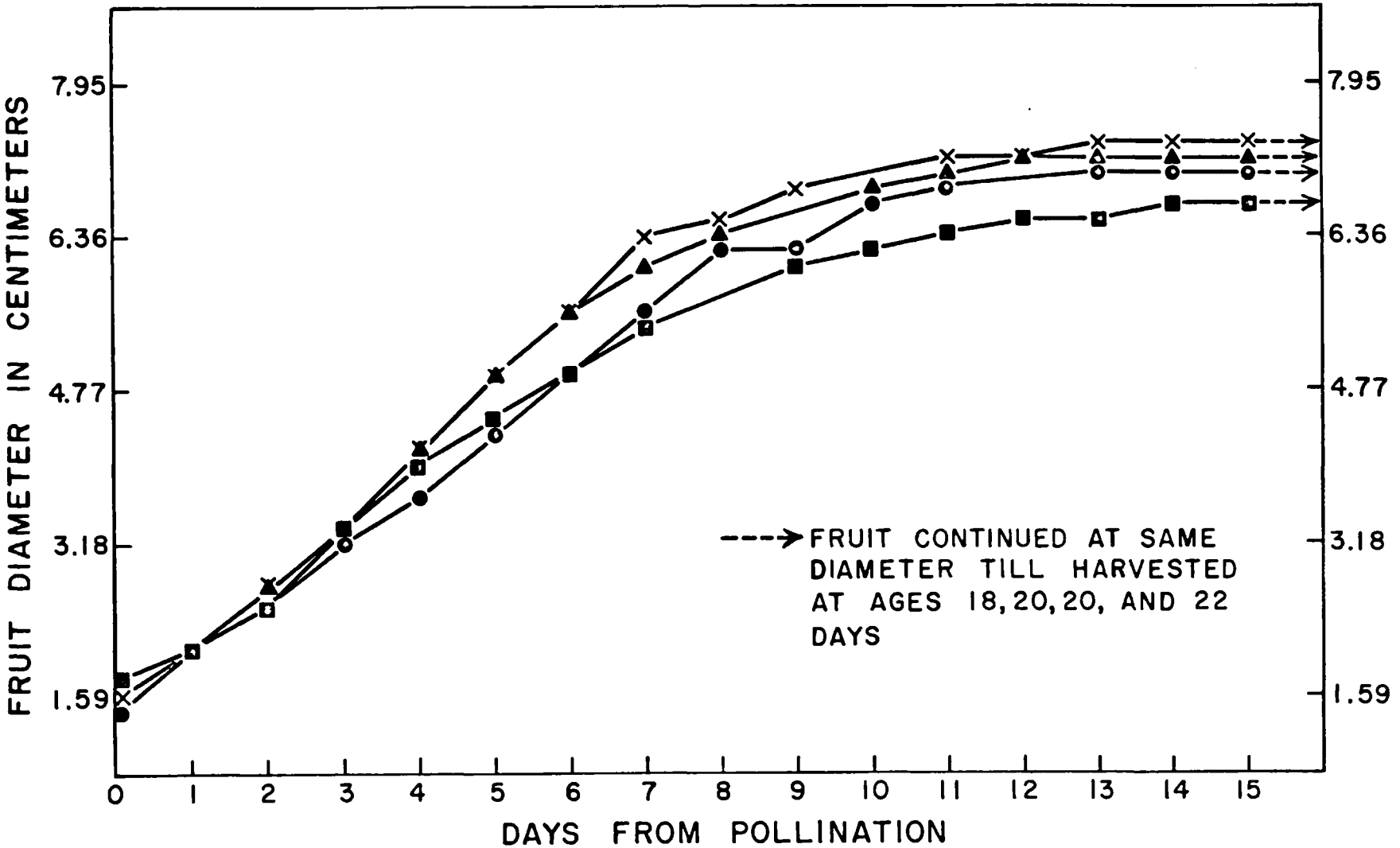

Fig. 2 Increase in fruit diameter during early fruit development of Cucurbita palmata

ranges from 5.88 cm. to 6.52 cm. This shows that the range is only 0.64 cm. among 52 fruits harvested in this species. Table 2 shows measurements of fruit diameter and fruit weight for Cucurbita foetidissima and C. digitata-palmata hybrid.

Fruit Weight

Table 3 shows the weights at harvest and weights after storage for fruits collected from Cucurbita foetidissima and Cucurbita digitata-palmata hybrid. The loss in weight after storage ranges from 11 to 28 grams in both species. The mean fruit weight for Cucurbita digitata-palmata hybrid was 152.31 grams $\pm$ 29.94 (32 fruits) and that for Cucurbita foetidissima was 119.60 $\pm$ 18.7 grams (52 fruits).

A summary of analysis of variance for fruit weight at different ages (Table 4) shows that fruit weight is not significantly different at different ages of the fruit.

Seed and Seed Number

During seed extraction seeds were observed to differ in color, plumpness, and buoyancy in water. Color could be recognized arbitrarily between seeds of different fruit ages and different species. However, these differences were very difficult to judge and evaluate quantitatively as an indication of seed germinability or maturity. The means of seed numbers shown in Table 5 do not indicate that all of these seeds are viable and well developed or that this is a measure of fruit maturity. The data listed, however, indicate the number of fertilized ovules in each fruit. Weight and germination percentages

Table 2. Mean fruit diameter and mean fruit weight of a four-fruit sample of C. foetidissima and C. digitata-palmata.

Fruit age days	C. foetidissima		C. digitata-palmata	
	Diameter cm.	Weight gms.	Diameter cm.	Weight gms.
10	6.12	114.50	-	-
12	6.44	126.75	6.92*	162.00*
14	6.12	113.5	6.52	139.50
16	6.20	125.5	6.60	149.50
18	6.28	124.5	6.28	118.00
20	6.36	123.5	6.60	156.75
22	6.20	117.25	7.08	186.00
24	6.20	114.50	6.84	177.75
26	5.88	104.50	6.44*	144.50*
28	6.52	124.25	6.04*	110.50*
30	6.12	109.25	6.76*	165.00*
32	6.44	130.50	-	-
34	6.34	126.00	-	-

r = 0.10 between age and size (non-significant)

* = means of two fruits only

Table 3. Fruit weight in gms. at harvest and after storage of Cucurbita digitata-palmata and Cucurbita foetidissima at different ages.

Fruit age days	C. digitata-palmata			C. foetidissima		
	Weight at harvest	Weight after storage	Loss	Weight at harvest	Weight after storage	Loss
10	-	-	-	95	79	16
	-	-	-	101	83	18
12	156	140	16	137	116	21
	-	-	-	136	116	20
14	137	122	15	115	101	14
	142	131	11	123	109	14
16	156	142	14	122	-	-
	144	129	15	107	87	20
18	133	115	18	169	143	26
	115	106	9	120	92	28
20	173	151	22	117	90	27
	171	148	23	148	120	28
22	184	162	22	100	77	23
	162	136	26	128	103	15
24	152	135	17	157	137	20
	222	195	27	96	74	22
26	165	147	18	105	86	19
	-	-	-	111	92	19
28	142	128	14	108	95	13
	-	-	-	134	112	22
30	174	155	19	136	119	17
	-	-	-	102	87	15
32	-	-	-	142	129	13
	-	-	-	131	115	16
34	-	-	-	131	116	15
	-	-	-	120	103	17
	$\bar{X} = 152.31 \pm 29.94$			$\bar{X} = 119.60 \pm 18.7$		

Table 4. Analysis of variance of the fruit weight of Cucurbita foetidissima as influenced by age of the fruit.

Source	Degrees of freedom	Sum of squares	Mean square	F value
Replications	3	773.68	257.89	
Treatments (age)	12	2915.77	242.98	0.62
Error	36	14119.07	392.21	
Total	51	17808.52		

Table 5. Seed number (means of a four-fruit sample) for different fruit ages in C. foetidissima and C. digitata-palmata hybrid.

Age of fruit	C. foetidissima	C. digitata-palamata hybrid
10	318	-
12	292	544*
14	258	459
16	332	444
18	257	378
20	264	394
22	306	457
24	253	498
26	294	326*
28	320	328*
30	310	376*
32	290	-
34	303	-
	$\bar{X}$ = 292 ± 26.45	$\bar{X}$ = 420 ± 17.67

* means of two fruits only.

which give indications of maturity will be considered later.

The number of seeds per fruit varied from 130 to 593 for Cucurbita digitata-palmata hybrid and from 192 to 405 for Cucurbita foetidissima.

Seed Number Versus Age of Fruit

A summary of analysis of variance for seed number versus age, Table 6, showed no significant differences in seed number among different ages of fruits of Cucurbita foetidissima.

Seed Number Versus Weight of Fruit

Irrespective of age, non-stored fruit weight of C. foetidissima was found to be highly correlated to number of seeds as shown in Table 7. The correlation coefficient calculated was $r = 0.829$ which was highly significant at 1% level.

Seed Weight

After the seeds had been extracted from the fruits and dried for about a week at room temperature they were weighed. The seed weight per fruit in Cucurbita foetidissima varied from 2.38 grams for a ten day old fruit to 10.42 grams for a 32 day old fruit. Seed weight per 100 seeds in the same species ranged from 0.82 grams for the ten day old fruit to 3.42 grams for the 34 day old fruit. In Cucurbita digitata-palmata hybrid the seed weight per fruit ranged from 2.80 grams for the 12 day old fruit to 8.30 grams for the 30 day old fruit. Seed weight per 100 seeds in C. digitata-palmata ranges from 0.51 grams for the ten day old fruits to 2.25 for 30 day old fruits. For both species the

Table 6. Summary of analysis of variance of seed number per fruit as influenced by age of the fruit in Cucurbita foetidissima.

Source	Degrees of freedom	Sum of squares	Mean square	F value
Replications	3	11140	3713.3	
Treatments (age)	12	33298	2774.8	1.23
Error	36	81315	2258.7	
Total	51	125753		

Table 7. Fruit weight versus seed number for non-stored fruits of Cucurbita foetidissima (means of two fruits).

Fruit weight	Seed number
131	354
117	298
108	230
136	380
104	249
114	265
120	298
102	218
101	267
122	292
99	287
124	294
126	304

$r = 0.829^{**}$

seed weight per fruit and seed weight per 100 seeds tends to increase with age of the fruit. Seed weight is one criterion from those studied that very clearly indicates the trend of seed development and may ultimately provide a clue to both seed and fruit maturity.

Seed Weight Versus Age of Fruit

Seed weight per 100 seeds, Table 8, was statistically analyzed and a summary of the analysis of variance appears in Table 9. Weight per 100 seeds was found to be highly significant in Cucurbita foetidissima. The correlation coefficient between weight per 100 seeds and age of the fruit appearing in Table 8 was found to be $r = 0.945$. This lends further support to the conclusion drawn from Table 9 that age influences weight per 100 seeds.

Figure 3 shows weight per 100 seeds, being the dependent variable, plotted against age of the fruit. The regression equation shows that there is an increase of 0.102 and 0.082 grams per 100 seeds for each increase of one day in the age of the fruit of Cucurbita foetidissima and Cucurbita digitata-palmata hybrid, respectively.

Storage Versus Non-storage of the Fruit

In order to study the effect of storage on seed development, the fruits were stored at room temperature (77°F) for a period of 20 to 25 days. The loss in fruit weight during storage is shown in Table 3. Seeds extracted from stored fruits were dried and weighed in the same manner as seeds from non-stored fruits. Mean weights per 100 seeds from stored and non-stored fruit were tested, using the T test and were

Table 8. Weight per 100 seeds for a four-fruit sample of C. foetidissima compared to age of the fruit.

Age of fruit days from pollination	Weight/100 seeds in gms.
10	.73 a
12	1.05 b
14	1.37 bc
16	1.24 bc
18	1.67 cd
20	1.61 cd
22	1.78 de
24	2.04 e
26	2.10 e
28	2.56 f
30	2.86 g
32	3.62 h
34	3.22 i

Means with the same letter (a, b, c, etc.) are not significantly different at the 5% level of significance.

Table 9. Summary of the analysis of variance for weight per 100 seeds versus age of fruit in C. foetidissima.

Source	Degrees of freedom	Sum of squares	Mean square	F value
Replications	3	00.78	0.26	
Treatments (age)	12	35.97	3.00	27.27**
Error	36	3.86	0.11	
Total	51	40.61		

** Indicates significance at 1% level.

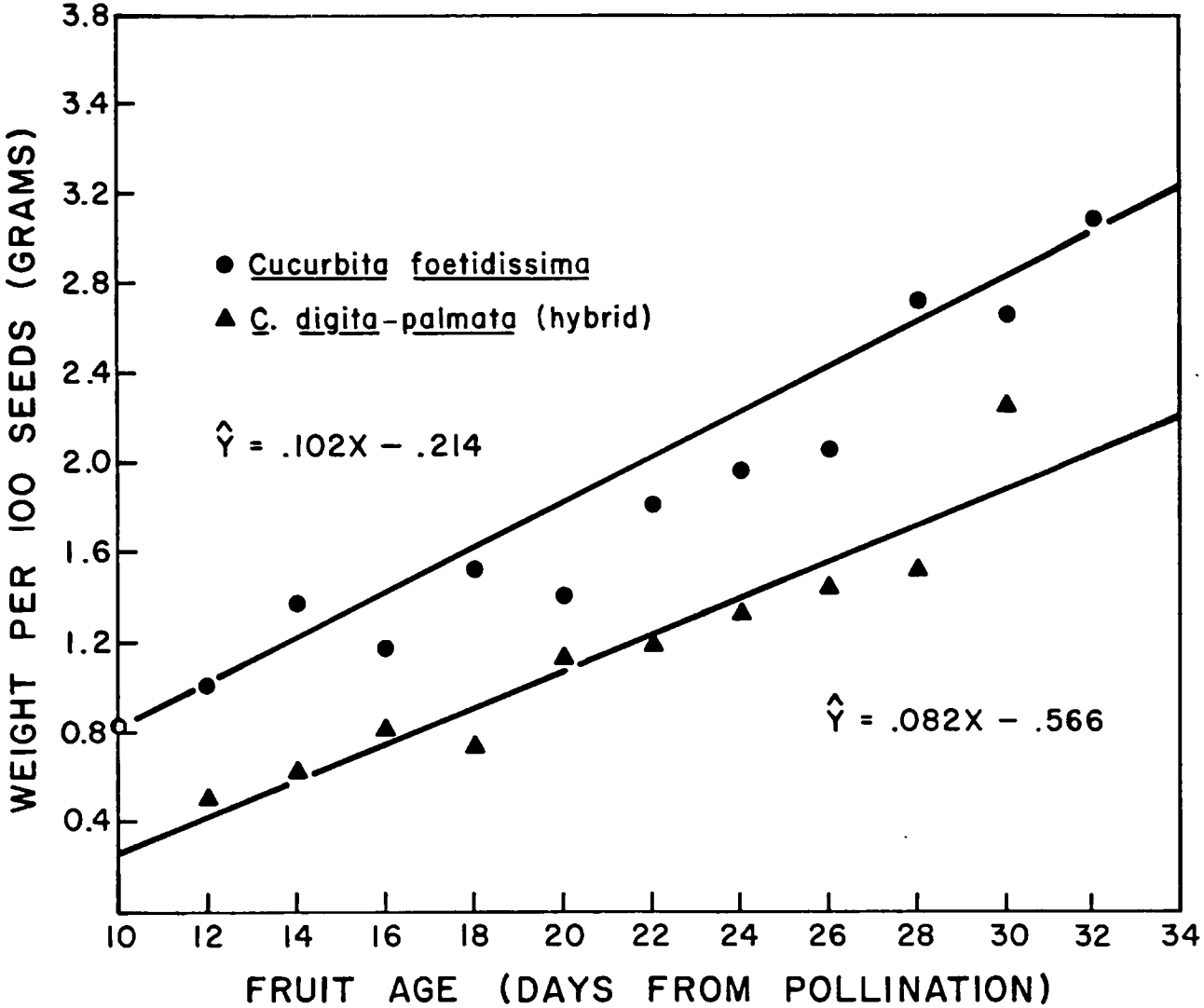

Fig. 3 Weight per 100 seeds vs. age of fruit of C. foetidissima and C. digitata-palmata hybrid

found to have no significant difference at the 5% level. Weights per 100 seeds are shown in Table 10 and represent means of two fruits from each treatment.

A complete analysis of variance for the data collected on storage and age, Table 11, for fruits of Cucurbita foetidissima showed no significant difference among the weights per 100 seeds from stored and non-stored fruits, for storage, or storage x age interaction, but a highly significant difference was obtained for age. This confirms the earlier result obtained when mean weights per 100 seeds extracted from stored and non-stored fruits were tested, as shown in Table 10.

Germinability of Seed

This criterion was studied from the standpoint of testing seed viability and maturity. Both characteristics were thought to give an indication or a measure of the fruit maturity. Samples of seeds from fruits with different ages were treated the same, as described earlier. Only fruits from which sufficient number of partly developed and plump seeds could be chosen to make up two samples were tested. The germination percentage counts were determined after a ten day period.

Germination Percentages

Mean germination percentages and mean weight of seed for stored and non-stored fruits are shown in Table 12. The percentages are means of eight samples of 25 seeds, making up a total of 200 seeds in each fruit age. These are split into stored and non-stored. For the stored fruits of Cucurbita foetidissima the percent of germination

Table 10. Means of weights per 100 seeds (two fruits in each treatment) for stored and non-stored fruits of C. foetidissima.

Age of fruit in days	Stored gms.	Non-stored gms.
10	0.64	0.82
12	1.09	1.01
14	1.35	1.38
16	1.29	1.18
18	1.68	1.53
20	1.83	1.40
22	1.74	1.81
24	2.11	1.97
26	2.14	2.06
28	2.37	2.73
30	3.06	2.66
32	4.14	3.10
34	3.02	3.42

t value calculated = 1.748 (non-significant).

Table 11. Summary of analysis of variance for weight per 100 seeds compared to different ages of fruit and storage.

Source	Degrees of freedom	Sum of squares	Mean square	F value
Main plots	25	37.340		
Treatments (age)	12	35.873	2.989	28.740**
Replications	1	00.222	0.222	
Error A	12	1.245	0.104	
Sub-plots	26	37.745		
Storage	1	00.175	0.175	1.750
Age x storage (interaction)	12	1.697	0.141	1.410
Error B	13	1.306	0.100	
Total	51	40.518		

**Indicates significance at .01 level.

Table 12. Percent germination of selected seed samples from stored and non-stored fruits of two species of Cucurbita.

Age of fruit in days	Stored fruits		Non-stored fruits	
	Wt/100 seeds gms.	Germination %	Wt/100 seeds gms.	Germination %
		Cucurbita foetidissima		
18	2.20	14	1.53	-
20	2.45	24	1.83	-
22	2.07	32	2.05	-
24	2.55	53	2.80	-
26	2.45	85	2.45	-
28	2.90	72	3.10	-
30	3.25	91	3.10	1
32	4.60	95	3.30	1
34	3.90	95	3.70	18
		Cucurbita digitata-palmata hybrid		
18	2.95	10	1.20	-
20	3.45	27	1.85	-
22	2.25	22	2.15	-
24	3.25	42	2.25	3
26	3.20	44	3.10	-
28	3.70	54	2.60	-
30	3.80	78	4.00	4

ranges from 14% for the 18 day old fruits to 95% for the 34 day old fruits. In contrast to this, the non-stored fruits did not have any seeds germinating for ages 18 to 28 days. Germination in this group starts as a minimum of 1% in the 30 day old fruits and rises to only 18% in the 34 day old fruit. Similar tendencies are observed in Cucurbita digitata-palmata hybrid. The percent germinations shown in Table 12 indicate very clearly the difference in the germination percentage between stored and non-stored fruits and also among different ages of the same group. Inspection of the seeds which did not germinate reveals that most of them, especially those that are extracted from older fruits (28 days and older), are normal in appearance, and imbibed, but the embryo failed to break the seed coat and emerge.

DISCUSSION

The studies herein reported have considered a number of factors and characteristics related to fruit and seed development. The primary objective as mentioned earlier was to determine how long the Cucurbita fruit should be allowed to develop on the vine and in storage to give viable and well-developed seeds. To answer such a question, several considerations had to be made. The first among these was to determine at what age the fruit stops development on the vine and whether there is any need for fruit storage after it had been harvested. It was then thought appropriate to follow seed development within the fruit while it is on the vine and later on while it is in storage.

The developing fruit of C. palmata was studied during its early periods of growth and was found to increase in diameter in a striking manner for a period of 10 days after pollination. During this period the fruit diameter increased by an average of 0.55 cm. daily. During the next five days very slight increases in fruit diameter occurred. Final diameter measurement at harvest showed no increase. Data of diameter measurements at harvest for 52 Cucurbita foetidissima fruits of different ages showed no significant differences.

The analysis of variance in Table 6 shows no significant difference in seed number per fruit for the different ages; however, a high correlation was found between seed number and fruit weight (Table 7). The age of the fruit probably should not be a factor in

influencing the seed number of the fruit since the seed number is determined by the number of ovules that are fertilized following pollination. Since there was a high correlation between seed number and fruit weight, it can be suggested that either the increased seed number affects the ultimate weight of the fruit or the larger fruits have a greater potential number of ovules which may be fertilized. Increases in seed weight in both species give a very clear picture of the trend of seed development in the fruit and over the entire period of 10 to 34 days after pollination. Weight per 100 seeds, Table 8 and Figure 3, shows a steady increase up to 34 days. The summary of analysis of variance appearing on Table 9 shows a highly significant difference between different ages of the fruit and weight per 100 seeds. Figure 3 shows this result graphically with the regression line for both species. The estimated value of weight per 100 seeds was calculated from the graph as $0.102x - 0.214$ and $0.082x - 0.566$ for Cucurbita foetidissima and Cucurbita digitata-palmata hybrid, respectively. The correlation coefficient for age and weight per 100 seeds in C. foetidissima is highly significant, $r = 0.945$, which is very indicative of the relation that exists between age of the fruit and the weight of the seeds it contains.

The answer to the other part of the problem under study, i.e., effect of storage of the fruit on the quality of seed, can be discussed from the data obtained for stored and non-stored fruits.

Stored fruits were shown to have a considerable loss in weight after storage, Table 3, which could be attributed to moisture loss.

Also, when the mean weight per 100 seeds for the stored and non-stored fruits in Cucurbita foetidissima were tested, Table 10, the means were found to be not significantly different at the 5% level, indicating that storage did not affect the weight per 100 seeds. The summary of analysis of variance for the whole experiment appearing in Table 11 confirms this. The only significant difference detected was due to age. No significant difference was found for the interaction, age x storage. From this we can safely conclude that (1) storage of the fruit does not increase the weight of the seeds it contains, (2) age from 10 to 34 days, while the fruit is on the vine, increases seed weight, and (3) no effect could be detected for an interaction of age and storage.

Table 12 contains the results of the germination test and it shows that only 34 day old non-stored fruits contained seeds that germinated. In the stored fruits, germination of seeds could be obtained from all ages starting from 18 days to 34 days, with the maximum of 95% for the 32 day old fruits and higher. The germination percentages are quite conclusive in showing the difference between stored and non-stored fruit.

Since seeds extracted from 18 day old stored fruit showed a 14% germination while seed extracted from 28 day old non-stored fruit did not show any germination, it follows that to have any germinating seeds at all a fruit must be allowed to develop on the vine for at least 18 days and then stored for a minimum of 20 days. The other alternative would be to allow the fruit to develop on the vine for 30 days and

extract the seeds directly without storing the fruit. In the last mentioned condition, the percent germination is liable to be very low. The best germination percentage obtained, however, was 95% and 78% in Cucurbita foetidissima and Cucurbita digitata-palmata hybrid for fruits harvested at 32 and 30 days from pollination and stored for 25 days.

Inspection of seeds that have been extracted from fruits 20 days and older but not stored showed that non-germinating seeds are swollen and with an adequate amount of water being absorbed. The seed coats are not mechanically resistant to moisture. Jasim (1964) reports that there are no hard or mechanically resistant seed coats in Cucurbita and that Cucurbita species studied, including Cucurbita foetidissima, Cucurbita palmata and Cucurbita digitata, are permeable to water and do not have a hard seed coat. Whitaker et al. (1962) reports that much better seed can be obtained from fruits of all species of Cucurbita if they are placed in a cool, dry storage area for one month or six weeks before the seeds are extracted. He also reports that fruits of Cucumis melo, which are not more than 2 to 2½ inches long, severed from the vine by accident, insects or planned pruning, will develop a few good seeds if left intact until they commence to decay; whereas, if the seeds were removed immediately, only light ones, probably nonviable, would be obtained.

From the seed weight studies and other data presented, we can then establish that seeds reach their full maturity after the 30th day from pollination. However, low germination percentages can be obtained

from younger seeds if they are not extracted immediately from the fruit, but left in the fruit for a period of 25 days.

The question now arises as to why nearly mature seeds (30 days from pollination) fail to germinate.

An explanation that is suggested here for this case involves three possibilities. First, an inhibitor is being produced or synthesized by the fruit at the time the seed reaches maturity, or secondly, an after-ripening period commences at this juncture of fruit development that prevents seeds extracted at this stage from germinating. The third possibility might be seed dormancy, a condition in which germination is temporarily delayed because of some internal control mechanism. Whatever this condition is, it seems that fruit storage for 25 days seems to nullify its effect on germination. An extension of the germination test to 28 days showed no change in percent germination of the seeds extracted from non-stored fruit, indicating that this condition of dormancy still prevailed up to 28 days from the time the germination tests were started.

Jasim (1964) reports in a comparison of percent germination for seven species of Cucurbita in a 30 day test that Cucurbita palmata has only 7.1% germination. Small and large seeded Cucurbita digitata have 24.6% and 15.4% germination, respectively. Cucurbita foetidissima has only 18.1% germination in the same test. There was no mention of how and where the test was carried out, but the assumption is that it is a standard germination test with no special treatment of either fruit or seed.

It appears from previous work done on Cucurbita and results obtained in this study that a storage period of fruits after harvest is necessary for high seed germination percentages.

Indications of fruit maturity that have been reported by certain workers in other genera of Cucurbitaceae could not be used to advantage in the case of the xerophytic species used. Such indications as degree of stem slip, which is a measure of the degree of abcission between the stem and the fruit, degree of netting, ground color beneath the netting, and flesh color do not give reliable indications of fruit maturity here. Seed weights and germination percentages were the only two relatively reliable indications that had been used in this study.

SUMMARY

This work presents the results of a study conducted on fruits and seeds of three species of Cucurbita to determine the interrelationship between fruit and seed development on the vine and in storage in order to determine the length of time that a fruit should remain on the vine or in storage to produce well-developed and viable seeds.

Early fruit development involved a rapid increase in fruit diameter during the first ten days after pollination. After ten days from pollination there was no noticeable increase in fruit diameter. Weight per 100 seeds in fruit age continued to increase through the longest period considered, which was 30 days in C. digitata-palmata and 34 days in C. foetidissima. No significant difference in seed number and fruit weight was found for different ages of fruits, but a highly significant correlation was found between seed number and weight of the fruit in Cucurbita foetidissima. Significant differences were obtained among different ages in weight per 100 seeds, but weight per 100 seeds for stored and non-stored fruits of all ages proved to have non-significant differences.

Storage of 32 and 30 day old fruits for a period of 25 days gave the highest percent germination in Cucurbita foetidissima and Cucurbita digitata-palmata, respectively. Non-stored fruits 30 days old gave seeds with very low germination percentages. An after-ripening period was suggested. This after-ripening period was thought

to involve some physiological process which did not result in increase of seed weight. It is also concluded that the effect of this process on seed germination is nullified by a 25 day storage of fruits before seed extraction.

LITERATURE CITED

Ashby, E. 1932. Studies in inheritance of physiological characters. Ann. Bot. 44:457-467.

Bemis, W. P. and T. W. Whitaker. 1965. Natural hybridization between Cucurbita digitata and Cucurbita palmata. Madrono 18:39-47.

Bisson, C. S. and H. A. Jones. 1932. Changes accompanying fruit development in the garden pea. Plant Physiol. 7:91-105.

Borthwick, H. A. 1931. Carrot seed germination. Proc. Amer. Soc. Hort. Sci. 28:310-314.

Dempsey, W. H. and J. E. Boynton. 1965. Effect of seed number on tomato fruit size and maturity. Proc. Amer. Soc. Hort. Sci. 86:575-581.

Denne, M. P. 1963. Fruit development and some tree factors affecting it. New Zealand Jour. of Bot. 1:265-293.

Harrington, J. F. 1959. Effect of fruit maturity and harvesting methods on germination of muskmelon seed. Proc. Amer. Soc. Hort. Sci. 73:422-430.

Jasim, A. J. 1964. Factors affecting the germination of certain xerophytic species of Cucurbita. Univ. of Arizona M.S. Thesis.

Luckwill, L. C. 1959. Factors controlling the growth and form of fruits. Jour. Linn. Soc. Bot. 56:294.

Mann, L. K. 1953. Honeybee activity in relation to pollination and fruit set in the cantaloupe Cucumis melo. Amer. Jour. Bot. 40:545-553.

Marre, E. and A. E. Murneek. 1953. Carbohydrate metabolism in the tomato fruits as affected by pollination, fertilization, and application of growth regulators. Plant Physiol. 28:255-266.

Murneek, A. E. 1954. The embryo and endosperm in relation to fruit development with special reference to the apple, Malus sylvestris. Proc. Amer. Soc. Hort. Sci. 64:573-582.

_____ and G. C. Schowengerdt. 1935. A study of size of apples to number of seeds and weight of spur leaves. Proc. Amer. Soc. Hort. Sci. 33:4-6.

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

_____, C. Pratt, C. Nitsch, and N. J. Shauls. 1960. Natural growth substances in the Concord and Concord Seedless grapes in relation to berry development. Amer. Jour. Bot. 47:566-576.

Reed, H. S. 1927. Growth and differentiation in plants. Quart. Rev. Biol. 2:79-101.

Reinecke, O. S. H. 1930. The relation of seed formation to fruit development of the pear. South African Jour. Sci. 27:303-309.

Rodrigues, A. and A. Menezes. 1951. Sobre as Relacoes entre o Numero de Semetes, a Forma e as Dimensoes dos Frutos, a Perecra Rocha. Agron. Lusit 13:227-251.

Sakr, Sayed M. and Essam El-Din Mahmoud. 1952. Viability of seeds harvested from fruits at different stages of maturity. Proc. Amer. Soc. Hort. Sci. 36:652-654.

Schander, H. 1955. Uberden Einfluss Verschiedenen pollens aufdie Fruchtsgestaltung bei der birnsorte. Conference. Mitt Obsth Vers Anst Jork 11:259-265.

Scheer, D. F., J. H. Ellison, and M. W. Johnson. 1959. Effect of fruit maturity on asparagus seed germination. Proc. Amer. Soc. Hort. Sci. 75:407-410.

Sinnot, E. W. 1921. The relation between body size and organ size in plants. Amer. Nat. 55:385-403.

_____. 1939. Developmental analysis of the relation between cell size and fruit size in Cucurbits. Amer. Jour. Bot. 26: 179-189.

Whitaker, T. W. and W. P. Bemis. 1964. Evolution in the genus Cucurbita. Evolution 18:553-559.

_____ and G. N. Davis. 1962. Cucurbits. Leonard Hill (Books) Limited, London, and Inter-Science Publishers, Inc., New York.

Young, R. E. 1949. The effect of maturity and storage on germination of Butternut squash seed. Proc. Amer. Soc. Hort. Sci. 53:345-346.