SPECIES HYBRIDIZATION IN VETCH

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Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1960

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1963

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ACKNOWLEDGEMENTS

The author wishes to express special appreciation to his major adviser, Mr. Charles E. Denman, for his valuable advice and helpful criticisms throughout this study. The author also wishes to thank the other members of his committee, Dr. Ralph S. Matlock, Mr. W. C. Elder, Dr. Douglas E. Bryan, Dr. J. Q. Lynd, and Dr. Robert D. Morrison.

Grateful acknowledgements are extended to the Agronomy Department and the U.S.D.A. Grass Research Laboratory of Oklahoma State University for the use of their facilities.

Appreciation is expressed to Mrs. Joan Wilbanks for typing the final manuscript.

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INTRODUCTION

Vetches, Vicia (Tourn.) L. species, are grown in Oklahoma primarily as winter pasture crops and to a lesser extent as green manure and cover crops. Hairy vetch, V. villosa Roth, which behaves as an annual (13) is the most commonly grown species in Oklahoma.

Characteristics desired in vetch are high forage and seed yield, winter hardiness, resistance to shattering, high percentage of hard seed coats, resistance to the pea aphid, Macrosiphum pisi Harr. and vetch bruchid, Bruchus brachialis Fahr. Winter hardiness is necessary where vetches are fall planted in areas where freezing temperatures occur. Age, rate of growth, plant vigor, soil moisture, and winter protection are all factors involved in winter killing. The vetch species commonly grown in Oklahoma are shatter-types which lose seed soon after the pods mature. Because of indeterminate growth habit, the vetch plant does not mature all of its seed pods simultaneously and shatter-types lose much of their seed before harvest. Slow deterioration of the vetch seed coat insures re-establishment of the crop in the subsequent season by preventing germination in the early summer months. Thus most of the hard seeds germinate in the fall and give rise to a new crop the following year. Pea aphids suck sap from the plants causing the leaves to turn yellow, and if the crop is heavily infested considerable loss of forage tonnage may result. The vetch bruchid is a weevil which lays its eggs on the green pods in the spring. The larvae gain access to

the seed by eating holes in the pods. Both the quality and quantity of the seed are reduced by larval damage. The lack of resistance to the vetch bruchid has resulted in a gradual decline in seed production in recent years. Hybridization among Vicia species having one or more agronomically desirable characteristics should lead to the development of improved varieties by combining some of these characteristics. Species hybrids are difficult to make in vetch. This study was undertaken to gain information on crossing techniques among several vetch species.

REVIEW OF LITERATURE

Studies (1,2,3,4,5,9,10,12) conducted on the vetches have dealt primarily with forage and seed yields as affected by degree of winter hardiness, percentage of hard seed, shattering, earliness, and resistance to the vetch bruchid and pea aphid.

Winter hardiness (3,9,12) is necessary for survival of vetches that are grown in colder regions and where fall seeding is desired. Degrees of winter hardiness vary among the species. Henson and Scoth (12) reported that V. villosa Roth is the most winter hardy of the commercial vetches grown. They also reported that V. pannonica Crantz and V. dasycarpa Ten. are slightly less winter hardy than V. villosa, but if adequate winter protection is provided they will withstand 0°F. temperatures. Willamette, a variety of V. sativa L. can withstand temperatures slightly below 0°F. (12). Investigators reported that V. ervilia (L.) Willid., V. atropurpurea Desf., V. articulata Hornem., and V. angustifolia L. are not winter hardy. Therefore, they are grown very little in the north (4,5,12).

Vetch species differ in percentage of hard seed (3,12). Some species are good seed producers, but the yield is low due to their inability to retain the seed in the pods after ripening (3,12). Vicia angustifolia has approximately 90 per cent hard seed (11). Vicia villosa and V. dasycarpa each have from 5 to 25 per cent hard seed, and V. sativa, V. pannonica, V. atropurpurea, and V. articulata have very little if any hard seed (12). Some wild vetches that have hard seed and shatter badly, will

volunteer and good stands will result without reseeding.

The influence of earliness on seed production was studied by Albrecht (2). He found that V. villosa, V. pannonica, and V. sativa are uncertain seed producers and are all late maturing. A shattering type, V. dasycarpa and a non-shattering type, V. monantha Retz. were good seed producers and matured early. Early maturing vetches were generally higher seed producers because they matured before insects and diseases became prevalent. They also escaped high temperatures and high humidity.

Pinckney and Stitt (15) tested vetches for resistance to the vetch bruchid by growing several species and varieties in infested fields. They found heavy infestations on V. villosa, V. dasycarpa, and V. atropurpurea. Eggs were deposited on V. sativa and V. pannonica. No infestations occurred on V. ervilia, V. angustifolia, V. hybrida, or V. lutea.

Albrecht (1) reported species and varietal differences in resistance to pea aphid injury among vetches. He found sources of resistance to the pea aphid in accessions of V. villosa, V. dasycarpa, V. angustifolia, V. hybrida, and three lines of V. sativa. Susceptibility was observed in V. pannonica, V. monantha, and six lines of V. sativa. The highly susceptible species were V. atropurpurea, V. ervilia, and six lines of V. sativa.

Attempts at hybridization among vetch species by several investigators have been only partially successful. Moriya (14) successfully crossed V. sativa (2n = 12), a non-tendrilled type. The absence of tendrils is inherited as a simple recessive. Selfing was thus indicated when the progeny lacked tendrils, whereas hybrids could be identified at an early stage by the presence of tendrils and intermediate expression of stem height and thickness, branching habit, and flowering as compared to the

parents. An increase in pollen fertility from 4.5 to 30.2 per cent in the F_1 generation to 92.4 to 96.2 per cent in the F_3 generation was attributed to the increased compactness of bivalent conjugation of chromosomes in the F_2 and F_3 generations.

Sekizuka et al. (16) crossed the Japanese species, V. angustifolia var. segetalis (2n = 12), with V. sativa (2n = 12). Some satisfactory lines were isolated from the F_6 and F_7 generations. Certain lines showed improvement over the V. angustifolia parent in forage yield and seed production. Hybrids between V. sativa and V. angustifolia were obtained by Watanabe and Yamada (17) only when V. sativa was used as the female parent. The F_1 plants were phenotypically intermediate between the two parents except that the pods and seed resembled those of V. angustifolia. Heterosis in the hybrids was not observed until the full bloom stage. Pollen fertility in the F_1 plants was only 4.3 per cent. The V. sativa and V. angustifolia parents had 1.6 and 97.0 per cent of hard seed, respectively. Seeds from the F_1 plants were intermediate between the parents with approximately 50 per cent hard seeds.

Donnelly and Clark (11) also crossed V. sativa X V. angustifolia in 1958. However, they used flower color as a genetic marker for identifying hybrids in the seedling stage (8,11). The female parent, V. sativa, was from a white-flowered line, whereas the male parent V. angustifolia, was purple-flowered. Purple flower color was completely dominant to white flower color and also resulted in purple pigment in the plants. Therefore, purple pigment was observed in the stems of all hybrids while plants resulting from self-fertilization were identified by green stems. They also observed that more hybrid seeds were set when crosses were at-

tempted with emasculation than without emasculation. With emasculation, 54 out of 74 F_1 plants were hybrids. Without emasculation, only 20 out of 129 F_1 plants were hybrids. In comparison to either parent, the F_1 plants were more vigorous through maturity and had larger seed. Size of seeds from the F_2 plants ranged from smaller than those of the V. angustifolia parent to seeds larger than those of the V. sativa parent.

A cytological study conducted by Donnelly and Clark (11) showed regular chromosome pairing at meiosis in the hybrid resulting from a cross between V. sativa and V. angustifolia. They reported that the diploid chromosome number for V. angustifolia was 10. Chromosome pairing in the hybrid (2n = 11) ranged from one pair with nine univalents to five pairs with one univalent, the more frequent combinations being three pairs with five univalents and four pairs with three univalents. Pollen sterility in the F_1 was about 93 per cent.

Cooper (6) attempted to transfer disease and insect resistance to V. villosa, but was unable to obtain hybrids. He did obtain hybrids by crossing V. sativa and V. calcarata, but did not describe their characteristics.

A greenhouse study by Donnelly and Clark (11) in 1961 resulted in one pod of shrivelled seed when V. villosa was crossed onto 21 florets of V. dasycarpa. Because of the poor seed set they resorted to interplanting of a white-flowered selection of V. villosa in a field of purple-flowered V. dasycarpa. Of 132 seedlings produced from seed of one plant, 113 were apparent hybrids. The remaining seedlings resulted from either selfs or outcrosses.

Donnelly and Clark (11) made pollinations among 30 species pairs

involving 12 species of Vicia. Hybrids were obtained with two species pairs, but no hybrids were obtained for the other 28.

MATERIALS AND METHODS

This vetch species hybridization study was conducted at the Oklahoma State University Agronomy Farm from September 1960 to August 1962.

Table I lists the material used in this study for 1961 and 1962. In the fall of 1960, plants of 14 species of Vicia were established in the greenhouse. Sixteen interspecific crosses were attempted among the 14 species. In the fall of 1961, two plants each of 19 Vicia species and one vetch unclassified as to species, were established in the greenhouse. Twelve interspecific crosses were attempted among 9 of the 20 species established in 1961. Flowering of the various species extended over periods of approximately three weeks during March and early April. Flowers were emasculated by two methods. The first method entailed removing the antners using forceps without removing the petals. The second method involved removal of the entire corolla with the anthers intact. Care was taken to avoid injury to the stigma. Emasculations were made approximately 12 to 24 hours before the anthers would have matured. All flowers were bagged following emasculation to protect the stigma from foreign pollen.

Pollinations were usually made between 6:00 a.m. and 10:00 a.m. the day following emasculations. The anthers were taken from the flowers of species selected at the onset of anthesis. Time of flowering and development of the flowers were used as criteria for selection of the pollen parent. If they had not already dehisced, the anthers were

crushed and their pollen was transferred to the stigma of the emasculated flower with a tooth-pick or dissecting needle. Some flowers on all species that flowered were bagged to insure self-fertilization.

TABLE I

VICIA SPECIES, ACCESSION NUMBER, PLANT INTRODUCTION NUMBER, DIPLOID CHROMOSOME NUMBER AND YEAR GROWN FOR THE PLANT MATERIAL USED IN THIS HYBRIDIZATION STUDY

	Species	Accession No. 1/	P.I. No. 2/	Diploid Chromosome Number 3/	Year Grown 4/
1	V. angustifolia L.	9272	234654	12	1962
2	V. angustifolia var. segetalis (Thuill) Koch	9275	249881	12	1962
3	V. articulata Hornem.	2076	170013	14	1961-62
4	V. atropurpurea Desf.	3968	------	14	1961-62
5	V. calcarata L.	4761	------	12 or 14	1961-62
6	V. dasycarpa Ten.	5548	------	14	1961-62
7	V. ervilia (L.) Willd.	1896	182312	14	1961-62
8	V. fulgens Battand.	3967	------	--	1961-62
9	V. galeata Boiss.	3887	200374	--	1961-62
10	V. graminea Sm.	9271	231420	--	1962
11	V. hirsuta (L.) S. F. Gray	9276	249883	14	1962
12	V. hybrida Crantz	2531	121151	12	1961-62
13	V. lutea L.	9274	249880	14	1962
14	V. macrocarpa Bertol.	9273	238379	10 or 12	1962
15	V. narbonensis L.	1757	------	14	1961-62
16	V. pannonica Crantz	2193	------	12	1961-62
17	V. sativa L.	2068	181831	12 or 14	1961-62
18	V. varia Host.	4760	------	14	1961-62
19	V. villosa Roth	Domestic	------	14	1961-62
20	Small-leaf (El Reno)	Domestic	------	--	1961-62

1/ Local accession numbers at Oklahoma State University
2/ Plant introduction numbers from U.S.D.A.
3/ Diploid chromosome number reported by Darlington and Wylie (7).
4/ Year or years they were used in this study.

RESULTS AND DISCUSSION

The results of the crosses attempted in the spring of 1961 are shown in Table II. In comparison to the number of florets pollinated relatively few seeds developed. Vicia atropurpurea was involved as one of the parents in all attempted crosses which resulted in seed set. This parent was suc-

TABLE II

NUMBER OF FLORETS POLLINATED AND THE NUMBER OF SEEDS SET FROM POLLINATIONS MADE AMONG THE VICIA SPECIES IN 1961

Pollinations	Number of Florets Pollinated	Number of Seeds Set
V. atropurpurea X V. calcarata	9	0
V. atropurpurea X V. dasycarpa	54	0
V. atropurpurea X V. ervilia	21	0
V. atropurpurea X V. fulgens	24	9
V. atropurpurea X V. galeata	37	10
V. atropurpurea X V. pannonica	23	0
V. atropurpurea X V. sativa	77	1
V. atropurpurea X V. villosa	16	0
V. dasycarpa X V. fulgens	32	0
V. dasycarpa X V. galeata	14	0
V. dasycarpa X V. sativa	12	0
V. dasycarpa X V. varia	41	0
V. fulgens X V. villosa	12	0
V. galeata X V. atropurpurea	5	0
V. galeata X V. dasycarpa	8	0
V. galeata X V. varia	35	0
V. galeata X V. villosa	20	0
V. pannonica X V. fulgens	1	0
V. sativa X V. atropurpurea	5	3
V. sativa X V. dasycarpa	16	0
	462	23

cessfully used more often, as the female parent, than any other species in the study because of its larger flowers and relatively greater petal strength, which allowed for removal of the entire corolla with little or no damage to the stigma. The wing petals enclose the keel loosely in V. atropurpurea permitting ready access to the anthers with emasculating equipment. In contrast the flowers of V. sativa exhibit an enclosed keel, with strong petals, which makes emasculation difficult. Successful emasculations were made by either removal of the entire corolla with the anthers intact or removal of the anthers individually with forceps. Some seed set resulted from florets emasculated by either method. However, not enough seeds were obtained to determine with which method a higher percentage of seed will result consistently. All seed set resulted when pollinations were made 12 to 24 hours after emasculation. However, a higher percentage of seed set could have resulted if this time interval was shorter or longer.

Seeds from three of the attempted crosses appeared to be normal. However, V. atropurpurea, which had received pollen from V. fulgens, set seeds which were shrivelled and appeared to be immature. Self-pollination studies were conducted in the spring of 1961 by bagging flowers of the various species before anthesis. Vicia atropurpurea, V. galeata, and V. sativa were the only species that set seeds under bags. Vicia hybrida, V. varia, and V. villosa failed to set seeds when cross-fertilization or self-fertilization was attempted.

The crossed seeds obtained in the spring of 1961 were germinated in vermiculite in a germinator at 20° to 30° C. in January 1962 and transplanted to separate pots in the greenhouse when the seedlings were about

one week old. Seeds from the following attempted crosses were germinated:

V. atropurpurea X V. fulgens - 3 seeds
V. atropurpurea X V. galeata - 3 seeds
V. atropurpurea X V. sativa - 1 seed
V. sativa X V. atropurpurea - 2 seeds

Nine plants were germinated from the nine seeds planted of the attempted crosses. In February 1962, ten seeds from each of the parent plants were germinated by the same method as used for the hybrid seeds. One plant of each parent was grown in the greenhouse for comparison. Since no genetic markers were used in this study it was necessary to attempt to identify any hybrids that might have resulted from cross pollinations. Methods used to detect hybrids were growth of the stems, percentage of normal seeds, number of seeds per pod, and total seed yield.

Growth measurements were obtained as the seedlings developed by determination of the length of the branches arising from the crown node of each plant. Figures 1 and 2 show the height of the hybrids at eight days after germination. Growth measurements were started at eight days after emergence and continued at ten day intervals for 68 days. These measurements were not started at the same time for the parent plants, but were taken for the same length of time. Total growth of the plants was obtained by adding together the length of all branches from one plant. In Figure 3 the rate of growth of the hybrid of V. atropurpurea pollinated with V. fulgens was less than either parent. Since it showed even less vigor than either parent this is an indication that it may have resulted from self-fertilization instead of cross-fertilization. In Figure 4 all three plants showed about the same rate of growth and continued for about 30 days at which time the hybrid began increasing

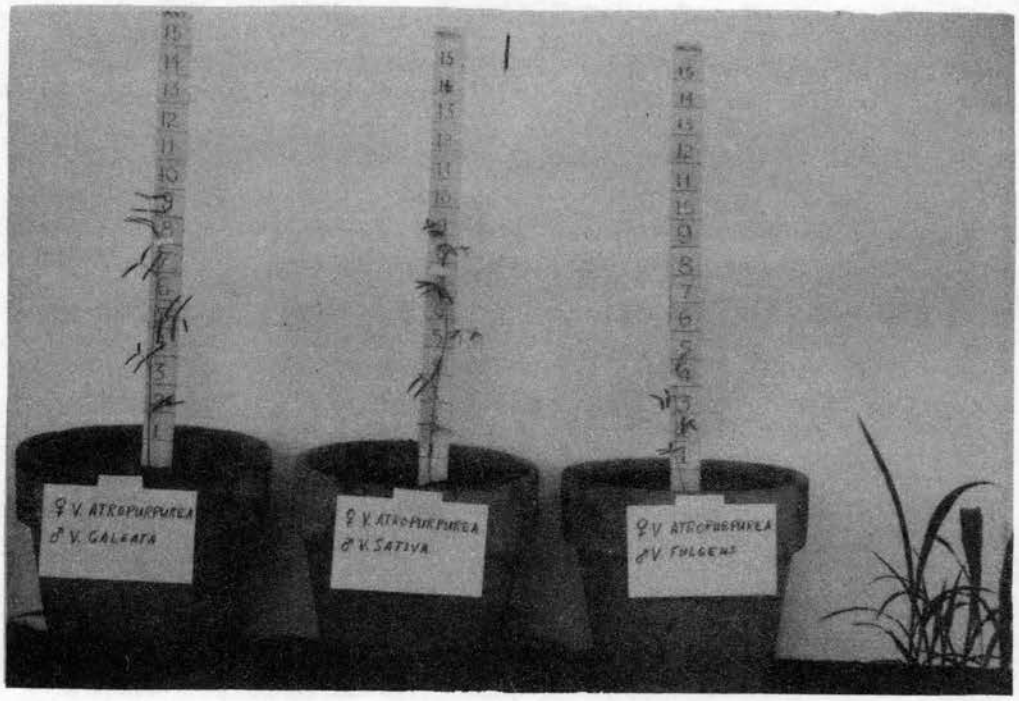

Figure 1. Relative height of the hybrids at eight days after emergence where V. atropurpurea was used as the female parent in all three crosses.

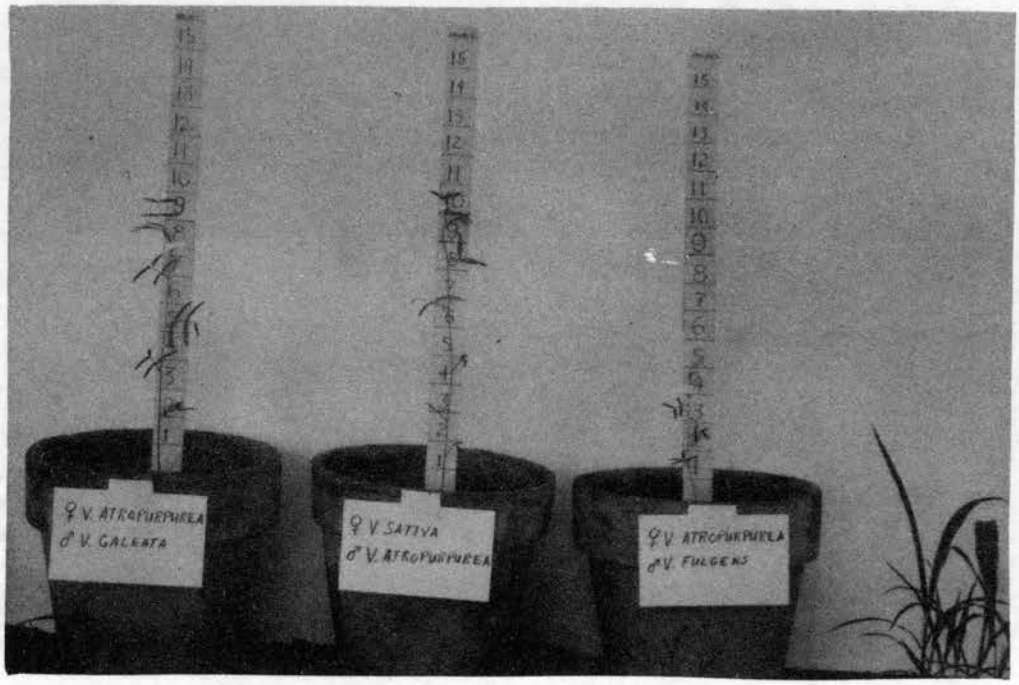

Figure 2. Relative height of the hybrids at eight days after emergence using V. sativa as the female parent in one cross.

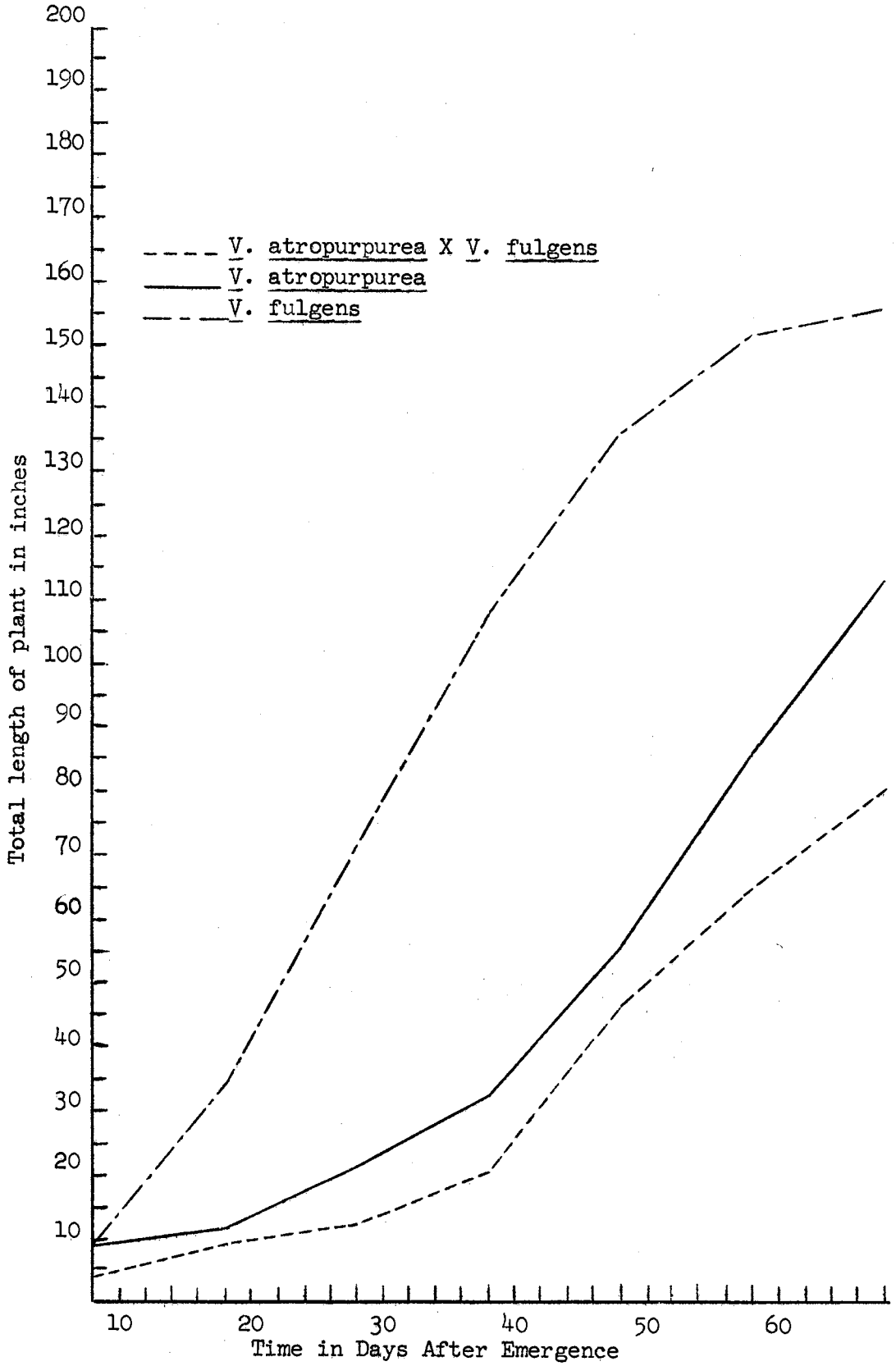

Figure 3. Combined length of stems of V. atropurpurea X V. fulgens and the parent plants on successive days after emergence.

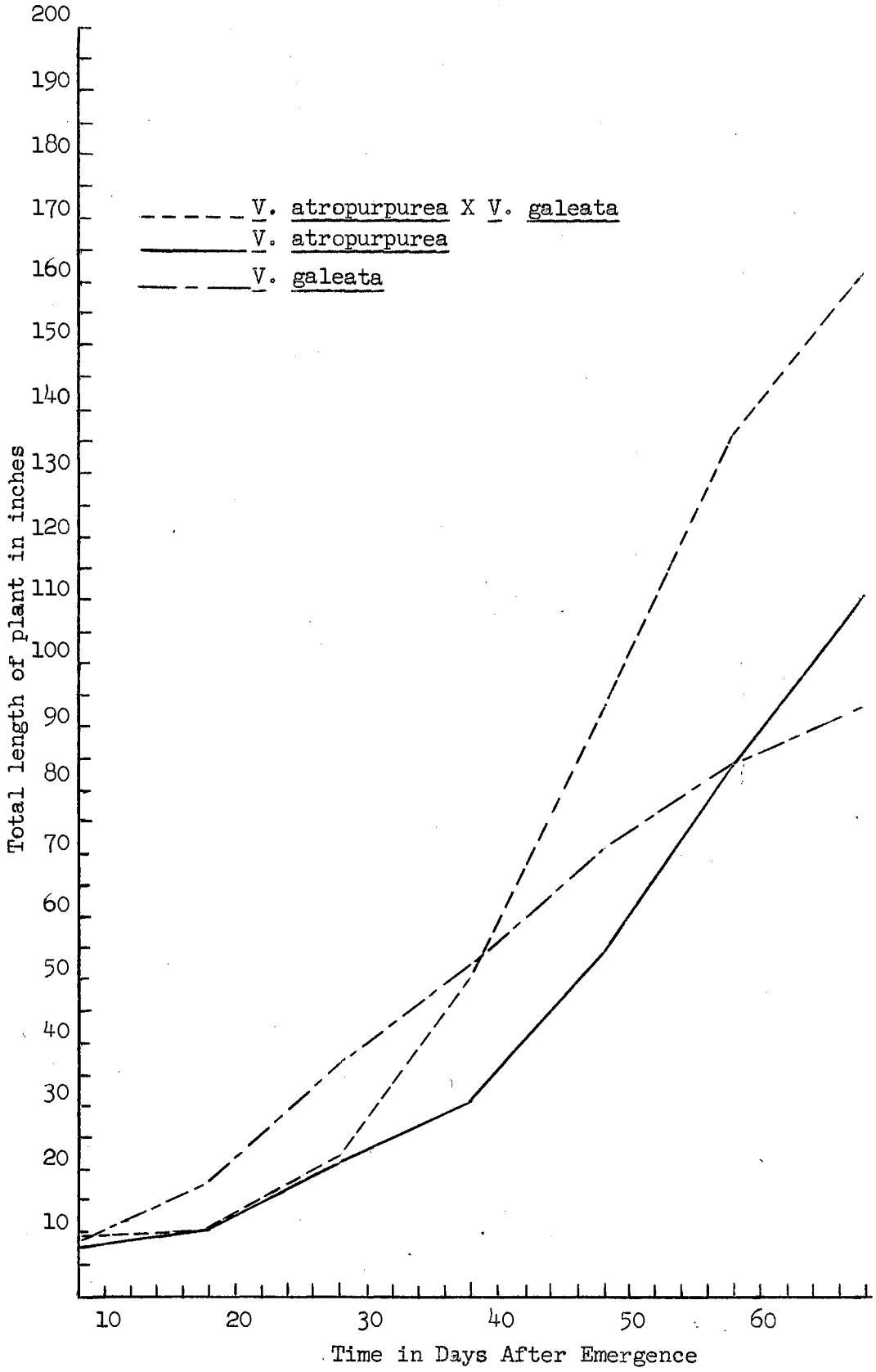

Figure 4. Combined length of stems of V. atropurpurea X V. galeata and the parent plants on successive days after emergence.

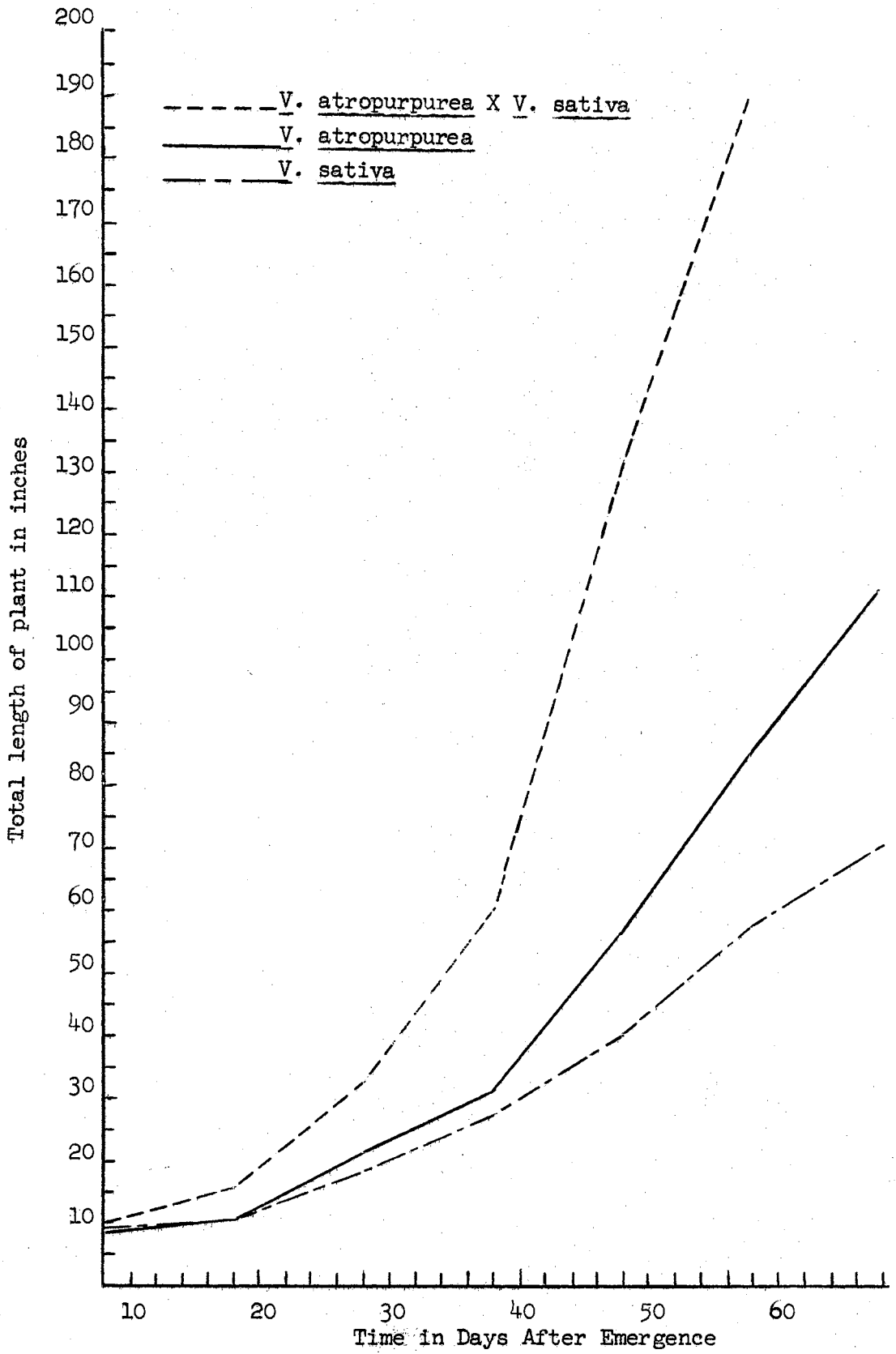

Figure 5. Combined length of stems of V. atropurpurea X V. sativa and the parent plants on successive days after emergence.

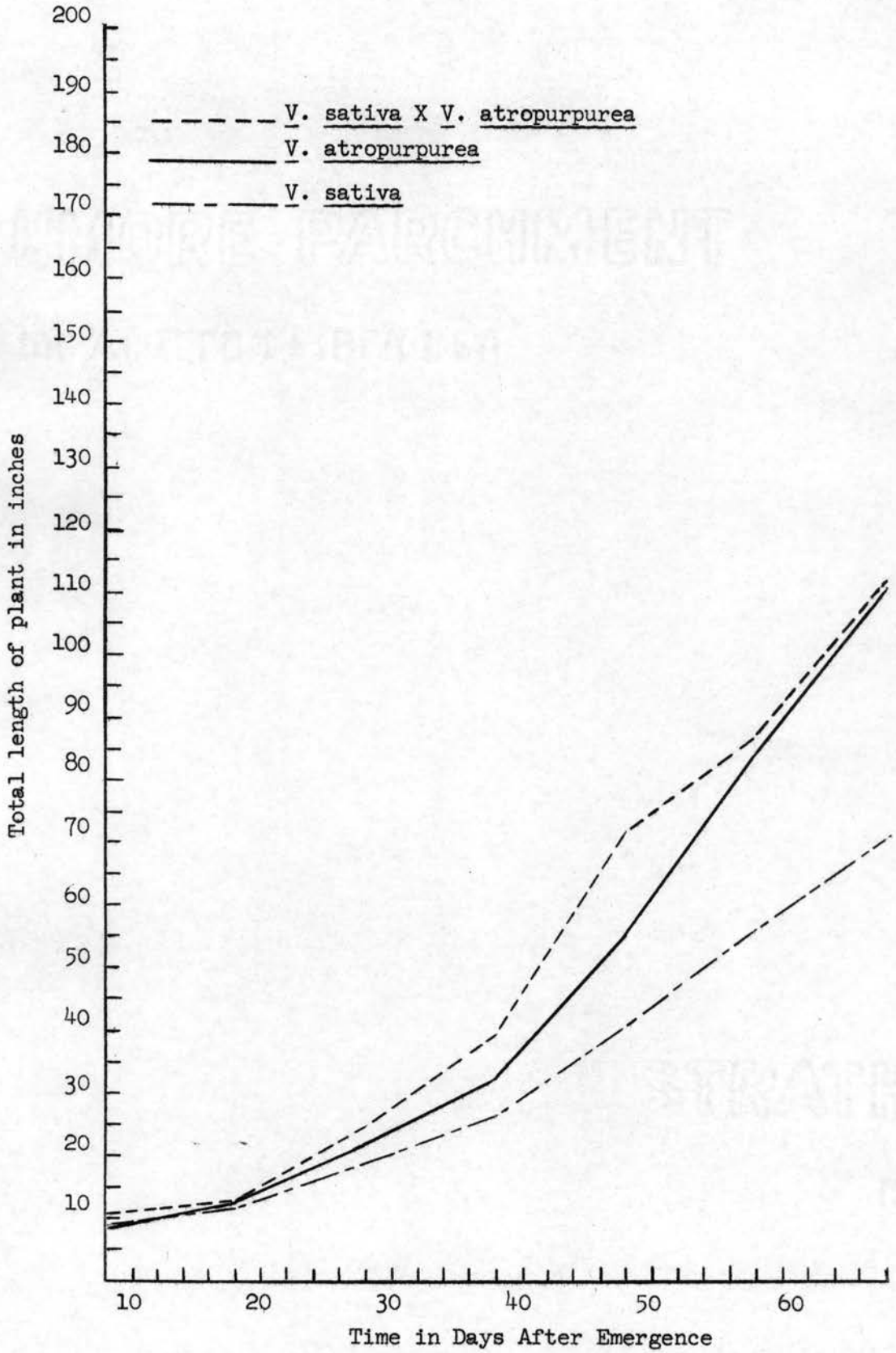

Figure 6. Combined length of stems of V. sativa X V. atropurpurea and the parent plants on successive days after emergence.

rapidly. At 68 days the V. atropurpurea X V. galeata cross had 165 inches of growth, whereas the parent plants had 97 and 118 inches of growth. From the growth curves it is highly possible that heterosis was observed as a result of a cross between the parents V. atropurpurea X V. galeata. The cross in Figure 5, V. atropurpurea X V. sativa, is probably the best indication of hybrid vigor since it more than doubled the growth of the larger parent after 68 days. Its growth was slightly above either parent when measurements were first taken and at 38 days growth increased sharply and was still growing vigorously at 68 days. In Figure 6 all plants were similar in size when measurements were first taken. Very little, if any, hybrid vigor was exhibited in the cross plant. This was probably an indication of the female parent being self-fertilized.

The number of mature seed per pod was determined for each attempted cross and parent plants as shown in Table III. In most instances a lower percentage of mature seed was observed in F_1 plants in comparison to the mature seeds of parent plants. In the nine attempted crosses that set seed, six had a lower percentage of mature seed than the average of the parents. One cross was intermediate and the percentage of two was slightly more than the average of the parents. The seed data of the parents were taken from original parent plants and not the parent plants grown for growth measurements. The parent plants grown for growth measurements died before setting seed because of the onset of hot weather. The number of seeds per pod of the crosses and parent plants was determinated to check for intermediate number of seeds between the parents as shown in Table III. The number of seeds per pod of the cross V. atro-

TABLE III

AVERAGE NUMBER OF SEEDS PER POD, THE AVERAGE NUMBER OF MATURE SEED PER POD, AND THE PER CENT OF MATURE SEED PER PLANT FOR THE PARENT PLANTS AND THE HYBRIDS OF THE VICIA SPECIES

	Average No. of seed per pod	Average No. of mature seed per pod	Per cent of mature seed per pod
Parent Plants			
V. atropurpurea	4.9	4.3	88.0
V. fulgens	1.3	1.0	77.0
V. galeata	3.9	3.7	95.0
V. sativa	7.2	6.5	90.0
Hybrids			
V. atropurpurea X V. fulgens #1	4.6	3.8	83.0
V. atropurpurea X V. fulgens #2	4.3	3.0	70.0
V. atropurpurea X V. fulgens #3	3.7	2.8	76.0
V. atropurpurea X V. galeata #1	4.5	2.8	62.0
V. atropurpurea X V. galeata #2	4.1	3.8	93.0
V. atropurpurea X V. galeata #3	4.2	3.8	90.0
V. atropurpurea X V. sativa #1	3.0	2.4	80.0
V. sativa X V. atropurpurea #1	5.3	4.5	85.0
V. sativa X V. atropurpurea #2	6.5	5.8	89.0

purpurea with V. fulgens was intermediate between the parents for the three plants. The cross V. atropurpurea with V. galeata also produced an intermediate number of seeds per pod between the parents. In the cross V. atropurpurea with V. sativa the expected average seeds number per pod was not observed for a hybrid or self. Some abnormality could have occurred. In the reciprocal of the above cross, an intermediate number of seed per pod was observed as would be expected if this were a hybrid plant.

Table IV gives the total seed production from the attempted crosses. These could not be compared to parent seed yields, because the parent plants did not produce seed. The average of the cross V. atropurpurea with V. galeata was greater than with either of the other crosses. In the cross V. atropurpurea with V. sativa the low seed yield was partially due to a loss of a large portion of the plant.

TABLE IV

TOTAL SEED YIELDS FOR EACH HYBRID PLANT AND THE MEAN OF EACH DIFFERENT CROSS AMONG THE VICIA SPECIES

	Total seed from each plant in grams	Mean weight of seed yield from each cross in grams
V. atropurpurea X V. fulgens #1	5.50	
V. atropurpurea X V. fulgens #2	3.85	3.45
V. atropurpurea X V. fulgens #3	.99	
V. atropurpurea X V. galeata #1	7.17	
V. atropurpurea X V. galeata #2	4.99	6.08
V. atropurpurea X V. galeata #3	6.08	
V. atropurpurea X V. sativa #1	1.93	1.93
V. sativa X V. atropurpurea #1	2.74	
V. sativa X V. atropurpurea #2	4.40	3.57

TABLE V

NUMBER OF FLORETS POLLINATED AND THE NUMBER OF SEEDS SET FROM POLLINATIONS MADE AMONG THE VICIA SPECIES IN 1962

	Number of florets pollinated	Number of seeds set
V. atropurpurea X V. dasycarpa	2	0
V. atropurpurea X V. ervilia	6	0
V. atropurpurea X V. fulgens	12	0
V. atropurpurea X V. galeata	6	0
V. atropurpurea X V. narbonensis	9	0
V. dasycarpa X V. fulgens	49	0
V. fulgens X V. atropurpurea	37	0
V. fulgens X V. galeata	59	0
V. galeata X V. atropurpurea	11	0
V. galeata X V. ervilia	7	0
V. graminea X V. atropurpurea	4	0
V. lutea X V. dasycarpa	2	0
V. narbonensis X V. galeata	1	0
V. pannonica X V. fulgens	9	0
	214	0

All cross pollinations made in 1962 produced no seed as shown in Table V. The results were apparently influenced by environmental conditions causing seed set on all species to be a failure.

SUMMARY

This study was conducted to gain information on hybridization techniques and to make interspecific crosses among the 20 Vicia species.

It was found that successful emasculations could be made by either removal of the entire corolla with the anthers intact or removal of the anthers individually with forceps. Seed set resulted from both methods. However, not all species were emasculated successfully by removing the entire corolla. Seed set resulted when pollinations were made 12 to 24 hours after emasculation.

Seed set was obtained when V. atropurpurea was pollinated with pollen from V. fulgens, V. galeata, and V. sativa. Also, seed was obtained when V. sativa was pollinated with pollen from V. atropurpurea. Plants were established from all crosses which set seed. Those plants resulting from seed of V. atropurpurea X V. galeata and V. sativa indicated hybrids because of vigorous growth, number of seed per pod, seed maturity, and total seed yields. In contrast, those plants resulting from seed of V. atropurpurea X V. fulgens and V. sativa X V. atropurpurea evidently resulted from self-pollinations since they lacked vigorous growth, had a low per cent of mature seed and low seed yields.

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