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POLLINATION BIOLOGY OF <u>POLYGALA ALBA</u> NUTT.

(POLYGALACEAE)

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THE POLLINATION BIOLOGY OF POLYGALA ALBA NUTT. (POLYGALACEAE)

CHAPTER I

INTRODUCTION

The genus Polygala L. contains about 500 species of trees, shrubs, and herbs. With the exception of New Zealand, Polynesia, and the Arctic the group is cosmopolitan (Lawrence, 1951; Hutchinson, 1967; Willis, 1966; Miller, 1971). The most recent treatment of North American species includes 179 species (Blake, 1924) most of which are annuals or perennials of diverse habitats.

The characters used to distinguish species as well as sections and subgenera of Polygala (Chodat, 1893; Blake, 1916, 1924) include: presence or absence of crests and beaks on corolla; persistence of calyx; types of stigma lobes; and fruit and seed characters. Such characters are thought to be related to either breeding mechanisms or seed dispersal in most groups of plants (Grant, 1949; Faegri and van der Pijl, 1966).

The pollination and reproductive biology of the genus is poorly known. Hildebrand (1867), Müller (1883), Knuth (1898, 1904), Venkatesh (1956), Percival (1965), and Faegri and van der Pijl (1966) have described pollination mechanisms in certain European and Indian species. The approach of many early studies, as Fryxell (1957) pointed out, was that of floral mechanisms and structural adaptation rather than experimental. Cleistogamy, an obvious mechanism leading to autogamy, is well known in several species in the genus (Knuth, 1904; Shaw, 1904; James, 1957). Miller (1971) and Gillett (1968) have proposed other possible mechanisms for self-pollination in the absence of out-crossing but agree that careful field observations are needed.

The present study was designed to investigate the relationship of floral morphology, phenology, ecological conditions, and insect behavior to pollination and breeding systems in the genus *Polygala*. *Polygala alba*, a long-lived perennial of the central and southwestern United States and adjacent Canada and Mexico, was selected for study because of its local abundance, diverse ecology, extensive distribution, and morphological similarity to many other species.

CHAPTER II

MATERIALS AND METHODS

Observations on P. alba were made in the field and on greenhouse-grown plants. The field studies were conducted on eleven populations in Texas and Oklahoma (Table 1) representing a diversity of ecological conditions. Herbarium vouchers of P. alba, as well as associated species in each of the study areas, have been deposited in the Bebb Herbarium of the University of Oklahoma.

Fresh, dried, and preserved flowers were examined under dissecting and compound microscopes and the morphological structures related to pollination (Baker and Hurd, 1968; Faegri and van der Pijl, 1966; Percival, 1962; and Beattie, 1969a) were noted. Flowers were preserved in two solutions--modified Carnoys and a 9:1 mixture of 70% ethanol and glycerol (Beattie, 1969b). The latter was used to prevent loss of pollen from the stigma and style lobes.

Phenological and periodic floral phenomena of P. alba were studied. Data collected included: beginning of flowering, peak of flowering and fruiting, number of flowers opening per day, time of anthesis, time and cyclic fluctuations of floral structures and nectar production, fruit

Table 1. Population localities and transplant sources.

Population	Locality
1 Johnson's Pasture Roadside	3 mi W of intersect. of HW9 & IH35, McClain Co., Okla.; periodically disturbed roadside margins of tall grass prairie; Transplants 2016-1, 2016-2, JP99.
2 Johnson's Pasture Hilltop	3 mi W & 1 mi S of intersect. of HW9 & IH35, McClain Co., Okla.; sandstone outcrop in grazed tall grass prairie; Transplant G41.
3 Polygala Flat	1.8 mi E of Tabler, Grady Co., Okla.; open rocky areas of tall grass prairie; Transplant 1881-3.
4 Palo Duro	4.8 mi N of Palo Duro, Armstrong Co., Tex.; limestone outcrops, short grass.
5 Clarendon	Greenbelt Lake, Clarendon, Donley Co., Tex.; sand over limestone outcrops, sand sage grasslands.

Table 1 (Continued)

Population		Locality
6	Meridian Creek	2.5 mi N of Clifton on HW6, Bosque Co., Tex.; limestone outcrops, Juniper woodland; Transplant MC-A.
7	Panther Junction	Park Headquarters, Panther Junction, Big Bend National Park, Brewster Co., Tex.; creosote bush desert; Transplant 2339-2.
8	Sotol Vista	Sotol Vista turnout, Big Bend National Park, Brewster Co., Tex.; sotol grassland; Transplant SV-1.
9	Falfurrias	9 mi E of Falfurrias on HW285, Brooks Co., Tex.; Roadside in mesquite grassland; Transplant Fal-1.
10	Boca Chica	Boca Chica, 24 mi E of Brownsville on HW4, Cameron Co., Tex.; Transplants BC-1,2,3.
11	Hebbronville	3 mi SW of Hebbronville on HW16, Jim Hogg Co., Tex.; rocky disturbed roadside, margins of mesquite grassland.

Table 1 (Continued)

Population	Locality
12 Kiowa County	5 mi E of Snyder on HW62, Kiowa Co., Okla.; limestone outcrop, disturbed roadside; Transplant 2132.
13 Harmon County	8 mi N & 4 mi E of Hollis, Harmon Co., Okla.; gypsum hills, grazed prairie; Transplant 2169.
14 Mt. Ord	McLean Ranch, 13 mi S of Alpine, Brewster Co., Tex.; limestone slopes, Piñon-Oak woodland; Transplant 2230.
15 Nickel Creek	5 mi N of Nickel Creek on HW180, Culberson Co., Tex.; Transplant 2231-1, 2231-2.
16 New Mexico	10 mi S of White City on HW62-180, Eddy Co., New Mexico; Transplant 2234.
17 Karnes County	Karnes Co., Tex.; limestone outcrop, roadside margin of mesquite grassland; Transplant KC-1, collected by P. Whitson

Table 1 (Continued)

Population	Locality
18 Woods County	Woods Co., Okla.; outcrop in mixed grass prairie; Transplant PN-1, collected by P. Nighswonger.
19 Grady County	Grady Co., Okla; disturbed roadside; Transplant PN-2, collected by P. Nighswonger.

abscission and dehiscence, seed germination, and coincidence of flowering of *P. alba* and associated species.

The degree of selfing and outcrossing in three populations and transplants under greenhouse conditions was investigated. Large populations (more than 200 individuals) were divided by evenly spaced, variable-length transects, and the plants were randomly selected on each transect. In small populations (less than 200 individuals) all plants were counted and labeled and test plants randomly selected. Three types of exclosures were used: paper pollination bags (commercial type), screen wire cages, and screen bags covered with cheesecloth. The cages and screen bags (Fig. 4) gave best results, probably due to greater air flow and less shading. Another advantage of the screen-cheesecloth bags was that they allowed some inflorescences to be used as controls and others on the same plant to be enclosed. Thus a comparison of outcrossing and selfing on the same plant could be made. All open flowers were removed and discarded from both control and experimental inflorescences or plants. At the conclusion of each experiment inflorescences or plants were collected and the number of flowers and mature fruits recorded.

To investigate the pollen cytology of *P. alba*, floral buds and flowers were preserved in modified Carnoy's fluid and stained according to Snow (1963). Anthers were dissected in 45% acetic acid and mounted in Hoyer's medium.

Pollen development was studied and the number of nuclei present in the microspores at anthesis determined. Meiotic preparations were made using the schedule of Estes (1969). Pollen fertility was determined for Populations 2 and 5 (Table 1) by removing anthers from three flowers per plant and mounting the anthers from individual flowers on a slide in a drop of .08% lacto-phenol blue. These slides were examined and the number of pollen grains in six transects per slide counted. Only darkly stained pollen was counted as fertile.

Nectar composition for two field populations (Pop. 1 and 3, Table 1) and transplants in the greenhouse was determined by paper chromatography. Three flowers from each of 20 plants were used. The nectar was collected by the insertion of Whatman #1 filter paper strips under the adaxial sepal and subsequently eluted with 50% ethanol for 24 hours. The eluates were spotted on Whatman No. 1 paper previously washed with methanol-*n*-butanol-acetic acid-water (507.6: 122.5:54:338.4 v/v/v). The chromatograms were developed in two dimensions with *n*-butanol-acetic acid-water (63:10:27 v/v/v), BAW, followed by isopropanol-butanol-water (70:10:30 v/v/v), IBW. Tests for the sugar reactions were determined with benzidine reagent (Smith, 1960). Known solutions of sucrose, fructose, and glucose were chromatographed in a similar manner. R_f values and colors of the benzidine reaction were determined for both knowns and unknowns.

The replenishment of nectar was investigated twice daily (10:30 am and 4:30 pm) for five consecutive days, and the presence or absence of a droplet under the adaxial sepal was recorded. Filter paper strips, used to extract the droplet, were treated with benzidine reagent to confirm the visual records.

Insect visitors on *P. alba* were observed with a Bausch and Lomb Balscope Zoom 60 telescope. These observations were made under various weather conditions and included: visitors and their behavior, duration of visits, number of flowers visited, and time of day. Insect visitors were collected and pollen loads analyzed. Pollen was scraped from the insects and mounted on a slide in a drop of .08% lactophenol blue. These slides were examined and the number of pollen grains in six transects per slide were counted. *Polygala alba* pollen, which is quite distinctive, was counted and its percentage of the total pollen load determined. The number of stainable and non-stainable pollen grains was also recorded. Insect specimens representing 209 collections were deposited in the United States National Museum where they are to be determined by Dr. Paul D. Hurd, Jr., Curator of the Division of Hemiptera and Hymenoptera.

CHAPTER III

OBSERVATIONS

Floral Morphology

The zygomorphic flowers of *P. alba* are borne in elongate, flexible racemes (Fig. 3). The pedicels (1 mm long), which vary from light green to white, blend with the petaloid calyx, giving the appearance of a white spike. Racemes are terminal on the branches, with secondary ones arising from middle and upper leaf axils. The stems branch from the basal caudex, and each branch is terminated by a long pedunculate inflorescence (0.5 cm-1 dm long). The number of branches varies from one on first-year seedlings to as many as 30 on old plants, but the numbers are greatly increased by mowing and grazing. The flowers, mostly 3 mm long, are subtended by three bracts--two laterals about 0.1 mm long and an abaxial one 0.5-1 mm long. These early caducous bracts vary in color from greenish to white.

The calyx is zygomorphic and consists of five sepals. The two abaxial ones (Fig. 5) are 1-1.2 mm long, lanceolate to ovate, and green with petaloid margins. The ovate-oblong adaxial sepal (Figs. 6, 7) is 1.6-1.8 mm long and petaloid. The two laterals (wings) are petaloid, elliptic-obovate, obtuse, short-cuneate at base, 3 mm long, and 1.5-2 mm wide (Figs. 5-7). The calyx persists on the fruit.

The corolla (Fig. 8) of three abaxially connate petals is papilionate and white. The keel, with a crest of 4-8 white to purplish clavate fimbriae, is 2-3 mm long. A hood (Fig. 12) or cupiform pouch (about 1 mm long) arising below the crest is yellow to greenish white and closes the corolla tube. The lateral petals overlap adaxially (Fig. 7), completing the corolla tube. The corolla is cauducous and falls intact as the fruit matures.

The eight stamens are basally united into a sheath and occur in two groups of four on either side of the gynoecium (Figs. 9, 10). The configuration of the corolla is such that the anthers within each group of four stand one above the other. Anthers open by a V-shaped slit on the inner face and extrude pollen with amazing accuracy into the spoon or saddle between the stigmas.

The gynoecium consists of a bicarpellate ovary, a two-lobed style, and two stigma lobes. The apical stigma is brush-like (Fig. 10) and stands above the receptive one, which is at first directed toward the base of the corolla. The spoon between the lobes has winged margins extending from the base of the brush to the receptive lobe. These stigmas stand directly above the receptacular nectary (Fig. 8), a distance of 2-2.5 mm.

Pollen is shed either in the bud before a flower opens or just as the wings expand. The pollen mass is at first golden yellow but becomes white with age. The color is apparently due to a volatile oil-like coating--tryphine (Erdtman, 1969)--on the grains. This tryphine makes the pollen adhere in a mass.

Phenology

In pollination studies the primary interests in the phenology of a species are time and duration of flowering. Linsley (1958), Percival (1965), and Macior (1968) cite examples of the phenological bonds existing between flowers and insects. These bonds refer not only to the time of flowering but also to daily activities of insects and the time flowers are open. The phenology of flowering can often be easily determined by study of herbarium specimens supplemented by field observations. Examination of herbarium vouchers of *P. alba*, however, gives a rather distorted picture of flowering of this species in undisturbed habitats. A common plant of outcrops and disturbed areas, this species is often abundant on roadsides and in grazed prairies. Plants of these disturbed habitats (Fig. 2) have been observed to flower most of the growing season, while adjacent undisturbed plants (Fig. 1) do not. Unfortunately, many herbarium collections contain specimens of these disturbed areas rather than from undisturbed ones, often without stating so on the specimen label.

Gillett (1968) in his treatment of the Canadian polygalas gives the flowering time of P. alba as May to mid-August, and the range in Texas is given as March to October (Correll and Johnston, 1970). These periods represent essentially the northern and southern limits of P. alba. My observations of local populations and herbarium specimens extend the flowering time to include February and December. Whitson s.n., collected on 25 December 1969 in Karnes Co., Texas, and Goodman #8014, collected 26 March 1969 from the southern end of Padre Island, Texas, extend the known flowering range. Goodman #8014 had been in flower for some time, likely extending well into February. Therefore, considering P. alba throughout its distribution, flowering specimens could probably be found at any time of year. Of the local populations studied (Table 1), those in central Oklahoma began flowering about 28 April in 1969-1971 and reached their peak of flowering between 20 May and 1 June in 1969. The Meridian Creek population (Pop. 6) peaked about 10 May, the Panther Junction (Pop. 7) and Sotol Vista (Pop. 8) populations about 20 April. Local weather conditions can, of course, alter this time considerably. Table 2 indicates the approximate flowering peaks in the United States.

Transplants grown in the greenhouse exhibited no dormancy and are apparently day-neutral. A Karnes County, Texas, transplant has, with occasional removal of old inflorescences, flowered continuously for three years. Another aspect of flowering in this species is the presence of axillary inflorescences. These are typical but appear

Table 2. Range of peak of flowering in *P. alba* in the United States.

Locality	Peak of Flowering
Southern and western Texas	Mid-March to mid-April
Central and northern Texas	April to mid-May
Central Oklahoma	Mid-May to late May
Western Oklahoma, New Mexico, Colorado	May to June
Kansas	Late May to mid-June
Nebraska to North Dakota	Mid-June to July

to be greatly influenced by summer rains. In central Oklahoma most undisturbed populations have few flowers by 15 July. In dry years this reduction of flowering may occur in late June. At these times it is not uncommon to find populations with no open flowers and with fruits terminal on the inflorescences. This summer break in flowering may continue into September. After fall rains axillary inflorescences appear, and a flowering peak occurs in mid- to late September. In undisturbed sites flowering is sporadic, while mowed or grazed populations may resemble the spring and early summer peaks.

As mentioned above, open flowers are generally of greatest importance to pollination studies. The exception is cleistogamy, which is known to occur in the genus but is apparently lacking in *P. alba*. Opening of flowers of this species was observed to have a diurnal rhythm, with most flowers opening after 8:00 am, C.D.T. and closing by 8:00 pm, C.D.T.

The time of opening for ten transplants from different localities is given in Table 3. These data show the variation in opening on two inflorescences per plant. Flowers open throughout the day with the peak reached by 4:00 pm, C.D.T. In most cases flowers started closing between 5:30 and 6:30 pm, and most flowers were sufficiently closed by 8:00 pm to exclude visitors. Similar observations have been made under field conditions where time of opening appears to

Table 3. Variation in opening of flowers of transplants.

Pop. No.	Transplant No.	Inflor.	Opening Times of Flowers: 1st Flw.	2nd Flw.	3rd Flw.	4th Flw.
7	2339-2	(A)	10:00 am	1:30 pm		
		(B)	10:00 am	12:30 pm		
1	2016-2	(A)	10:00 am	12:00 noon	12:30 pm	12:30 pm
		(B)	10:30 am	11:30 am	11:30 am	11:30 am
13	2169	(A)	10:30 am			
		(B)	8:30 am	10:30 am	2:30 pm	
14	2230	(A)	9:30 am	10:00 am	12:00 noon	
		(B)	9:30 am	1:30 pm	3:00 pm	
1	2016-1	(A)	8:30 am	11:30 am	3:30 pm	
		(B)	1:00 pm	4:00 pm		
15	2231	(A)	11:30 am	3:30 pm	4:00 pm	
		(B)	8:30 am	8:30 am	8:30 am	9:00 am
19	PN-2	(A)	10:30 am	10:30 am	11:00 am	11:30 am
		(B)	12:00 noon	3:00 pm		

Table 3 (Continued)

Pop. No.	Transplant No.	Inflor.	1st Flw.	2nd Flw.	3rd Flw.	4th Flw.
18	PN-1	(A)	8:30 am	11:00 am	12:30 pm	
		(B)	8:00 am	11:30 am		
12		(A)	8:30 am	10:00 am		
		(B)	10:30 am	1:00 pm		
3	1881-3	(A)	9:00 am	9:30 am	9:30 am	3:00 pm
		(B)	10:30 am	11:30 am	5:30 pm	

depend on local weather conditions. Flowers open later on cool, cloudy days than on warm, clear days.

An additional aspect of suspected entomophilous species is the distribution of flowers through time; i.e., how many flowers are available to pollen vectors? Greenhouse observations on the number of flowers per day are given in Table 4. These values are averages of the number of flowers open per day on two inflorescences per plant. These data indicate that flowering is a gradual but continuous process, with new flowers opening daily and available to visitors.

A similar check was made under field conditions in Pop. 4, a population consisting of 146 individuals. All open flowers were removed from randomly selected test plants and these individuals allowed to continue flowering for seven days (15 May to 21 May). At the end of this period these individuals had 127 inflorescences and 1,211 open flowers. This is an average of 9.54 flowers per inflorescence with 3.35 inflorescences per plant. Expanding this, one could expect about 4,666 flowers in this population.

Maturation of fruits, like flowering, is a gradual process and ripe fruits abscise and dehisce as they mature, with the seeds falling with the fruit or just before. Germination does not appear to occur until the following spring. Seedlings have been observed to flower the first year.

The species associated with *P. alba*, as might be expected for any species with such ecological amplitude,

Table 4. Average number of open flowers per day on transplants.

Pop. No.	Transplant No.	7 May 1969	8 May 1969	5 March 1970	6 March 1970	7 March 1970	8 March 1970	9 March 1970	10 March 1970
3	1881-3	4.0	6.0						
12	2132	2.0	4.5						
18	PN-1	3.5	5.0						
19	PN-2	3.0	6.0						
7	2339-2	3.0	6.5						
1	2016-2	5.0	8.0						
1	2016-1	3.0	5.0						
13	2169	2.5	5.0						
14	2230	3.5	6.5						
15	2231-1	5.5	8.5						
1	JP99			2.5	3.5	5.0	8.5	9.5	10.0
2	G41			2.0	3.0	4.0	5.0	6.0	6.0
6	MC-A			5.0	8.0	12.0	17.0	20.0	20.0
15	2231-1			2.0	4.0	7.0	10.5	14.0	14.5
8	SV-1			3.0	5.0	7.0	9.0	10.0	9.0
17	KC-1			2.0	2.5	4.0	6.0	6.5	7.0

are numerous and represent many plant families. Some of those present at early and peak stages of flowering of *P. alba* are presented in the Appendix.

Exclosure Experiments

Flower production and seed set of open-pollinated plants and those from which insects were excluded are summarized in Tables 5-9. The range of population means was 23.7 to 63.1% for controls and 0.0 to 1.03% for exclosures. During a period of sporadic flowering in the Polygala Flat population (24 June to 3 August 1969) the mean fruit set of control plants was 20.7% and that of plants in exclosures 0.12%.

Although populations were collected, data recorded, and calculations made in the same manner, it should be pointed out that indeterminate flowering and early abscission of fruits made collection of inflorescences with flowers and immature fruits necessary. Three points should be considered in interpreting these data. First, fruit set on control plants may be considerably greater than indicated. Secondly, the incidence of pollination is likely greater, since all immature fruits were scored as flowers. The third problem is the possibility that some flowers, not open at the time of bagging, had previously been open and pollinated. This could account for some of the fruit set in bagged plants.

Late-season conditions were studied by selecting and observing seven plants in Population 1 from 5 September to 10 October 1969. Flowering was sporadic and insect activity

Table 5. Comparison of per cent fruit set of control and test inflorescences in Johnson's Pasture Roadside population (Pop. 1, 13 May-13 June 1969).

Plant	Control			Exclosure		
	No. Fls.	No. Fruits	% Fruit Set	No. Fls.	No. Fruits	% Fruit Set
105	61	42	68.9	49	1	2.0
70	41	28	68.3	54	0	0
39	53	25	58.1	54	1	1.9
60	52	30	57.7	67	1	1.5
89	30	19	63.3	50	0	0
23	43	35	81.4	32	1	3.1
63	34	14	41.2	70	1	1.4
31	35	20	57.1	25	0	0
52	27	24	88.9	32	0	0
32	41	31	75.6	58	0	0
14B	15	5	33.3	33	0	0
Population mean			63.1			0.83

Table 6. Comparison of per cent fruit set of control and test inflorescences in Johnson's Pasture Roadside population (Pop. 1, 6 May-11 June 1970).

Plant	Control			Exclosure		
	No. Fls.	No. Fruits	% Fruit Set	No. Fls.	No. Fruits	% Fruit Set
7	30	16	53.3	59	2	3.4
14B	19	10	52.6	43	2	4.7
14C	20	9	45.0	44	0	0
18	46	20	43.5	55	0	0
18B	35	9	25.7	54	2	3.7
21	40	13	32.5	84	0	0
34	45	15	33.3	77	0	0
38	45	13	28.9	36	0	0
53	3	3	100.0	77	0	0
60	48	12	25.0	56	1	1.8
62	43	10	23.3	74	0	0
85B	51	22	43.1	55	1	1.8
95	34	7	20.6	69	0	0
201	41	20	48.8	69	0	0
201B	37	10	27.0	54	0	0
Population mean			40.17			1.03

Table 7. Comparison of per cent fruit set of control and test plants in Polygala Flat population (Pop. 3, 7 May-12 June 1970).

Control				Exclosure			
Plant No.	No. Fls.	No. Fruits	% Fruit Set	Plant No.	No. Fls.	No. Fruits	% Fruit Set
A5III	25	3	12.0	A1II	329	0	0
E3III	57	19	33.3	A1IVa	133	0	0
E4II	103	20	19.4	A1IVb	144	3	2.1
E4II	10	0	0	A3-1	54	0	0
E6II	8	5	62.5	A3III	112	1	0.9
F3II	34	7	20.6	A3IV	4	0	0
F8I	98	14	14.3	A41	0	0	0
F8II	29	8	27.6	A51	86	0	0
F10II	23	15	65.2	A5III	90	0	0
G4III	12	2	16.7	A5IV	209	7	3.4
I1I	55	9	16.4	E1I	247	0	0
F8IV	30	12	40.0	E1III	81	0	0
R-1A	50	23	46.0	E1IV	89	0	0
R-2A	32	18	56.3	E3II	47	0	0
R-3A	20	14	70.0	E4II	79	3	3.8
R-4A	88	38	43.2	F6I	8	0	0
				E6II	43	0	0
				F8I	78	0	0
				F8II	57	0	0

Table 7 (Continued)

Control				Exclosure			
Plant No.	No. Fls.	No. Fruits	% Fruit Set	Plant No.	No. Fls.	No. Fruits	% Fruit Set
				F10II	98	0	0
				F14II	264	0	0
				G4II	44	0	0
				I1I	91	0	0
				I1II	17	0	0
Population mean			33.9				0.43

Table 8. Comparison of per cent fruit set of control and test plants in Polygala Flat population (Pop. 3, April-10 June, 1971).

Control				Exclosure			
Plant No.	No. Fls.	No. Fruits	% Fruit Set	Plant No.	No. Fls.	No. Fruits	% Fruit Set
A5III	407	118	29.0	C1II	300	0	0
B5II	328	89	27.14	C2II	272	0	0
I1II	721	272	37.73	C5IV	156	0	0
E2II	116	24	20.69	F7IV	98	0	0
E3IV	932	304	32.62	F1II	63	0	0
E4I	269	65	24.16	E2I	306	5	1.6
E1III	873	99	11.34	E3I	232	1	0.4
E5I	394	117	29.70	E5II	108	9	8.3
E6II	128	30	23.44	E4IV	11	0	0
F1I	207	38	18.36	E6I	25	0	0
F8I	656	92	14.02	E9I	34	0	0
E9IV	65	37	56.92	E12II	334	0	0
E12IV	251	37	14.74	E15I	289	1	0.4
E15IV	116	19	16.38				
C1I	354	45	12.71				
C2IV	241	24	9.96				
Population mean			23.70				0.82

Table 9. Comparison of per cent fruit set of control and test inflorescences in Sotol Vista population (Pop. 8, 12-24 August 1969).

Plant	Control			Exclosure		
	No. Fls.	No. Fruits	% Fruit Set	No. Fls.	No. Fruits	% Fruit Set
SV-1	3	2	66.7	7	0	0
SV-2	5	4	80.0	7	0	0
SV-3	2	1	50.0	5	0	0
SV-4	12	3	25.0	4	0	0
Population mean			55.4			0.0

reduced. No data were collected from controls because of a wind storm, and from the 139 flowers in the exclosures only one mature fruit was collected.

Transplants from Populations 4, 7, and 9 were grown in the greenhouse for three years and, despite numerous attempts to artificially self-pollinate, only six fruits were collected. No differences in flowers, nectar, pollen production, or fertility were detected when compared to natural populations. The paucity of fruit set in cultivated plants, therefore, was likely due to lack of pollinators rather than the influence of the artificial environment. Other species of Polygala--P. longa, P. tweedyi, P. scopariodes, and P. macradenia--which are sympatric with P. alba all produced numerous viable seeds under the culture conditions of P. alba.

Pollen Cytology and Fertility

Pandy (1960), Brewbaker (1957, 1967), Brewbaker and Majumder (1961), and Solbrig (1970) have suggested a correlation between pollen cytology, incompatibility, and breeding mechanisms. Both binucleate and trinucleate pollen types are found in the Polygalaceae, but only trinucleate types are known in the genus Polygala, based on studies of four species (Brewbaker, 1967). The sequence of nuclear divisions from meiosis to time of anther dehiscence was observed in P. alba. Although few countable cells were found, the gametic number of 12 agrees with the reports of Lewis and Davis (1962) for Texas plants but not those of Mexico (Lewis and Davis, 1962; Lewis, 1962). My counts included five plants from Populations 1, 3, 4, 7, and 9.

Following meiosis the four nuclei were observed within the microspore mother cell wall. Cytokinesis occurs simultaneously and tetrads of tetrahedral configuration are formed (Fig. 13). The microspores separate and the nucleus of each remains near the center of the cell. At the onset of pollen wall formation, each microspore nucleus and most of the cytoplasm then moves to one end of the grain and the first post-meiotic division occurs. The wall, separating the small vegetative cell at the extreme periphery of the grain from the larger generative cell, then is formed. The generative nucleus remains near the center of the grain. The vegetative nucleus, although present in some cells almost to time of anther dehiscence, is generally absent shortly after the first division. It is not known whether it disintegrates totally or simply becomes too diffuse to observe. Prior to anthesis the generative nucleus divides to produce two sperm nuclei. These smaller nuclei are elongate and are difficult to observe; most preparations required squashing to release contents of the pollen grains. No observable differences were detected in the number of nuclei or the appearance of pollen removed from anthers prior to dehiscence and that removed from the spoon of the flower.

Pollen grains of _P. alba_ are 7-9 colporate, oblate to prolate spheroidal and average 29.33 x 24.46 microns (Larsen and Skvarla, 1961). Pollen fertility was calculated for both Johnson's Pasture Hilltop (Pop. 2) and the Clarendon (Pop. 5) populations. Population 2 had a mean fertility of 91.95% based on 39 individuals and included 26.7% of the total population, while Population 5 samples (16 individuals) had a mean of 91.36%. The range was 73-100%. No plants in any population sample were found to have more than 40% abortive pollen. Fertility of the pollen on insects ranged from 72.2-100% with a mean of 89.82%.

Nectar Composition and Production

Field and greenhouse studies were conducted to determine nectar composition. Samples from two field populations (1, 3) and transplants in the greenhouse were chromatographed separately. The R_f's in two solvent systems and the colors of the benzidine reaction of these samples, as well as those of known sugars, are given in Table 10. A comparison of these values indicates the presence of sucrose, glucose, and fructose, the three common nectar sugars (Percival, 1962).

It was noted in the field that flowers visited by insects during the morning contained nectar in the afternoon. Insects tended to visit these flowers as frequently as those which had opened shortly before a visitation. Ten test plants in the greenhouse were used to determine presence of nectar twice daily (10:30 am and 4:30 pm) for five consecutive days (6-10 March). Of the 39 flowers observed during

Table 10. Chromatography of *Polygala alba* nectar.

	R_f's on Whatnam No. 1[a]		Benzidine
Compound	BAW	IBW	Reagent
Glucose	.19	.46	Golden brown
Suspected Glucose	.19	.46	Golden brown
Fructose	.25	.49	Golden yellow
Suspected fructose	.24	.48	Golden yellow
Sucrose	.16	.40	Yellow
Suspected sucrose	.14	.41	Yellow

[a]See text for solvent systems.

this time interval, 63.3% produced nectar twice daily for 1.5 to 3 days. Approximately 30% of the total number had a supply for two days and 7.4% for three days.

Insect Visitors

Insect visitors collected on *P. alba* from 10 May 1969 to 23 May 1971 included members of five orders--Hemiptera, Coleoptera, Lepidoptera, Diptera, and Hymenoptera. Of these visitors 90% were Hymenoptera, including not only pollen and nectar feeders but also those parasitic on flower visitors. A total of 60 hours was spent observing insects in the field between 7:00 am and 6:00 pm, C.D.T. during the three-year period. The size of visitors and flower configuration presented problems in the determination of actual behavior within the flowers, but examination of insect collections narrowed considerably the number of potential pollinators. Some of these visitors carried no *Polygala* pollen, and it is doubtful that they could effect pollination. Members of the Coleoptera and Hemiptera, although often present on the inflorescences, did not visit flowers regularly but either chewed flowers or possibly captured thrips. In general, the duration of their visits ranged from several minutes to more than an hour.

An occasional visitor to the flowers of *P. alba* in the central Oklahoma populations was a gray lepidopteron which characteristically visited *Hedyotis nigricans* (Lam.) Fosb. and *Hymenoxys linearifolia* Hook. No *Polygala* pollen was found on this visitor. A similar case was found involving a dipterous visitor to Hedyotis.

Most of the P. alba visitors were medium to small, solitary or semisocial bees--cuckoos, andrenids, and halictids. The smaller ones use the crest or keel as a landing platform, and in so doing the hood is pulled back, thus opening the corolla tube. This exposes the brush and pollen in the spoon which are directly above the nectary and receptive stigma. After the first insect visit pollen was observed within the trichomes of the stigma brush. Those bees with probosces from 2 to 3 mm long could easily remove nectar. A proboscis extended to the nectary would pass the receptive stigma, and upon withdrawal, also contact the spoon and stigma brush. In addition to direct contact of the proboscis, the mandibles and anterior thorax are in contact with the stigma brush. A pollen forager while collecting would be raked on the ventral side of the head and thorax by the brush. Pollination can, therefore, be effected several ways. Larger bee visitors readily open the flowers as they clutch the crest and curve their abdomen back under the flower. Once the insect flies away the keel and hood spring back into place. Single flowers have been observed to be visited several times per hour by different bees, and it was not uncommon to find different species simultaneously visiting flowers on a single inflorescence. The rapidity and frequency of visits to P. alba by these bees suggest that they work the floral mechanism properly and obtain either nectar or pollen, or both. Visits ranged from one to 45 seconds, with most averaging about four seconds. Visitors were observed to visit only a few flowers on one inflorescence and generally only several inflorescences before moving to another plant. It was not uncommon for an insect to move from one plant to an adjacent plant and back to the first. Polygala alba visitors, particularly the green metallics, appeared to remain relatively constant, at least during the peak of flowering. In many central Oklahoma populations, Melilotus alba and M. officinalis began flowering at the peak of P. alba and were visited either by the same species of bees or very similar ones.

Insect activity was greatest on clear, bright, warm days and was almost nonexistent in cool, wet weather. A week of such cool weather in May, 1971, was reflected by the decreased fruit set of P. alba in the Polygala Flat population (Pop. 3). In addition to the rapid and frequent visits, P. alba visitors were observed only on those flowers with yellow to yellow-green hoods exposed and held perpendicular to the axis of the racemes. Pedicels of old flowers, fruits, and floral buds were found to point toward the apex of the inflorescence and the withered fimbriae to cover the colored hood.

Pollen loads were examined on 94 insect visitors. The results are presented in Table 11.

Table 11. Per cent composition of Polygala alba pollen of insect pollen load.

Population	Insect Coll. No.	Pollen Load (No. of Grains)	P. alba Pollen (%)
1	29	53	0.0
	32	32	37.5
	33	255	82.4
	144	430	0.0
	145	192	91.6
	147	47	89.4
	149	103	97.1
3	122	149	98.7
	123	51	80.4
	124	90	96.7
	128	44	95.5
	130	53	83.0
	132	126	96.0
	133A	82	93.7
	133B	327	99.4
	134	188	98.4
	135	378	97.6
	137	18	88.9
	139	226	98.7
	140	237	100.0
	141	240	0.0

Table 11 (Continued)

Population	Insect Coll. No.	Pollen Load (No. of Grains)	*P. alba* Pollen (%)
	142	471	90.8
4	38	23	60.9
	43A	59	86.4
	43B	62	98.4
	45	34	94.1
5	152	200	0.0
	156	31	38.7
	162	151	98.6
	159	258	0.0
	155	382	90.6
	153	164	92.7
	163	155	95.5
	161	62	91.9
	160	46	89.1
	157	16	0.0
6	4	288	1.7
	9A	80	0.0
	9B	70	87.3
	11	53	0.0
	12	221	1.4
	13	162	4.9
	14A	124	1.6
	14B	36	11.1

Table 11 (Continued)

Population	Insect Coll. No.	Pollen Load (No. of Grains)	*P. alba* Pollen %
	15	61	8.2
	17	395	0.5
	19	200	54.5
	20	187	100.0
	22	80	87.5
	23	513	97.1
	25A	321	99.4
	25B	240	98.3
7	55	9	0.0
8	59	89	98.8
	58	57	98.2
	64	79	93.7
	82A	279	94.3
	88A	145	94.5
	88B	148	99.3
	84	140	98.6
	75	81	96.3
	82B	156	94.9
	88C	201	98.0
	66	283	98.9
9	169	550	0.0
	167	160	0.0
	173	150	0.7

Table 11 (Continued)

Population	Insect Coll. No.	Pollen Load (No. of Grains)	*P. alba* Pollen %
	174	10	0.0
	181	365	13.9
11	184	107	17.8
	185	296	0.7
	183	220	0.0
	182	201	1.0
	191	106	0.0
	193	108	7.3
	203	31	0.0
	187	67	98.5
	189	171	96.5
	190	218	98.6
	195A	49	95.9
	195B	177	98.8
	196	71	25.4
	201	31	93.5
	202	7	14.3
	204	35	97.1
	205	18	72.2
	206	59	79.7
	193	91	2.2

CHAPTER IV

DISCUSSION AND SUMMARY

Polygala alba Nuttall is a sexually reproducing species of extensive ecological amplitude. No evidence of vegetative reproduction has been found in the three years of field and greenhouse studies. Plants of sandy areas of southern and coastal Texas were found to develop secondary caudices where stems were buried but no adventitious roots developed. Exclosure experiments support the hypothesis that this species is predominantly insect pollinated. Whether geitonogamy or xenogamy occurs still remains to be tested. The fruit set of less than 5% by plants in exclosures indicates that self-pollination is rare. Therefore, the mechanism for autogamy--collapse of the apical stigma (brush) against the receptive stigma--suggested by Müller (1883), Gillett (1968), and Miller (1971) rarely functions in this species, if at all. At the time the brush collapses, the receptive stigma no longer appears viscid and has changed from greenish white to yellow brown. Although anthers dehisce in the bud and extrude pollen, little, if any, is present on the stigma at the time the flowers open.

The phenology of flowering, diurnal fluctuations of flowers, floral morphology, nectar replenishment, frequency of insect visitors, and fruit set of control plants suggest that *P. alba* is adapted to pollination by insects which are capable of depressing the keel, collecting pollen, or extending their probosces to the nectary. No insects were observed to remove nectar by piercing the corolla or inserting a proboscis between the corolla and the adaxial sepal.

Corbicular and mandibular pollen loads were found on insect visitors, but most were of the latter type. The pollen present on the mandible and anterior thorax (included here as mandibular load) may be the result of a visitor's nectar collecting rather than pollen foraging. A proboscis extended to the nectary would upon retraction pass the spoon and brush. It was noted that even after most of the pollen was removed from the spoon some remained on the stigma brush, which comes in contact with the mandibles and anterior thorax as a visitor enters a blossom. Thus pollination could result from either direct contact of a proboscis or by the brush which has been forced down by the visitor.

Faegri and van der Pijl (1966) referred to flowers of the type of *P. alba* and many members of the Fabaceae as flag blossoms and the presence of pollen outside the anther as a secondary pollen presentation mechanism. Flowers of this type are often melittophilous (Faegri and van der Pijl, 1966; Baker and Hurd, 1968). *Polygala alba* is, therefore, not only morphologically adapted to bee pollination but may well represent another case of flower constancy. Grant (1950) and Linsley (1958) have presented evidence for oligolecty for the same genera of bees which regularly visit this plant species. A consideration of this evidence with the frequency of visits and high pollen load percentage suggests the same may well be true for P. alba.

Although pollen is of the trinucleate type and cytokinesis simultaneous, the presence of fruits in exclosures indicates that P. alba is either self-compatible or apomictic. Whether self-incompatibility is present in those individuals which set no fruit remains to be tested.

The extensive distribution, extensive ecological amplitude, and colonizing habit of this species are consistent with the pollination mechanism and breeding behavior described. The capacity for autogamy, as Baker (1960) has pointed out, is important for colonizing species. Following dispersal a plant which is perennial and capable of selfing stands a good chance of becoming established and building up a population even in the absence of insect visitors. The same should also be true of facultative apomicts. It seems probable that the capacity for allogamy, and thus genetic recombination, would have played an important role in the establishment of this species in diverse habitats.

FIGURES

Habitats and living plant of *Polygala alba* and types of exclosures used

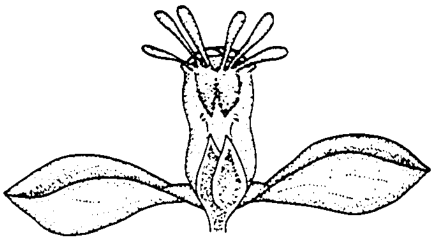
1. Undisturbed habitat--tall grass prairie, Polygala Flat (Pop. 3).
2. Disturbed roadside habitat, Johnson's Pasture Roadside, (Pop. 1).
3. Living specimen of *P. alba* Nutt.
4. Cage and screen bag exclosures.


2
3

FIGURES

Floral morphology of *Polygala alba*

5. Abaxial view of flower showing abaxial sepals, wings, and fimbriae.
6. Adaxial view of flower--adaxial sepal (nectary-holding sepal).
7. Adaxial view of flower--nectary exposed.
8. Section through flower showing configuration of corolla, androecium, and gynoecium.



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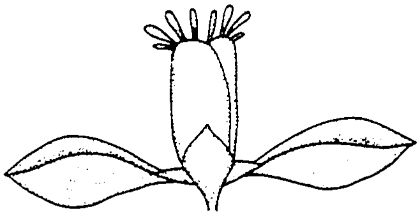

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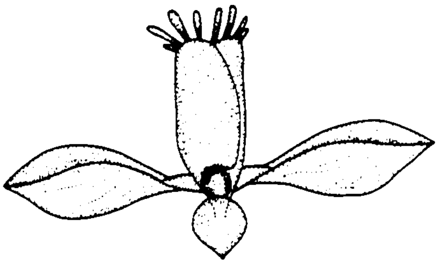

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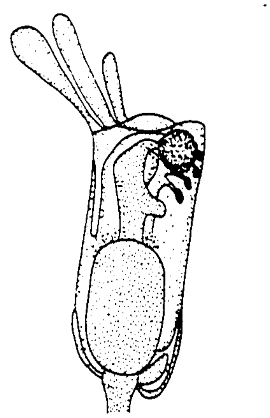

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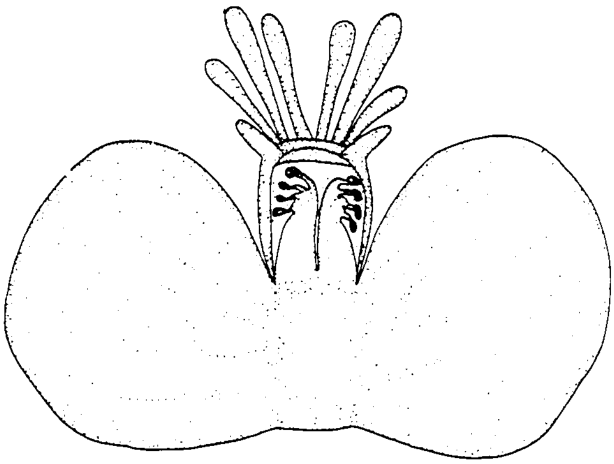
FIGURES

Floral morphology of _Polygala alba_

9. Corolla and androecium--showing stamen arrangement.
10. Gynoecium--brush, spoon, receptive stigma.
11. Corolla, androecium, gynoecium, nectary, and adaxial sepal.
12. Hood on the keel.



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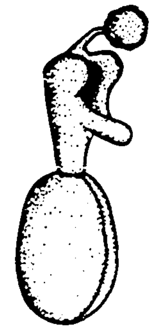

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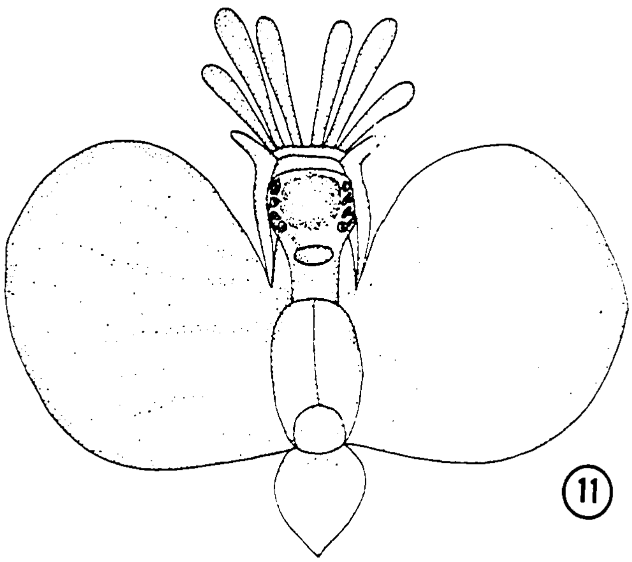

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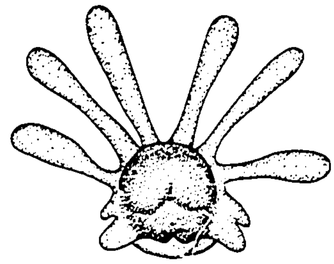

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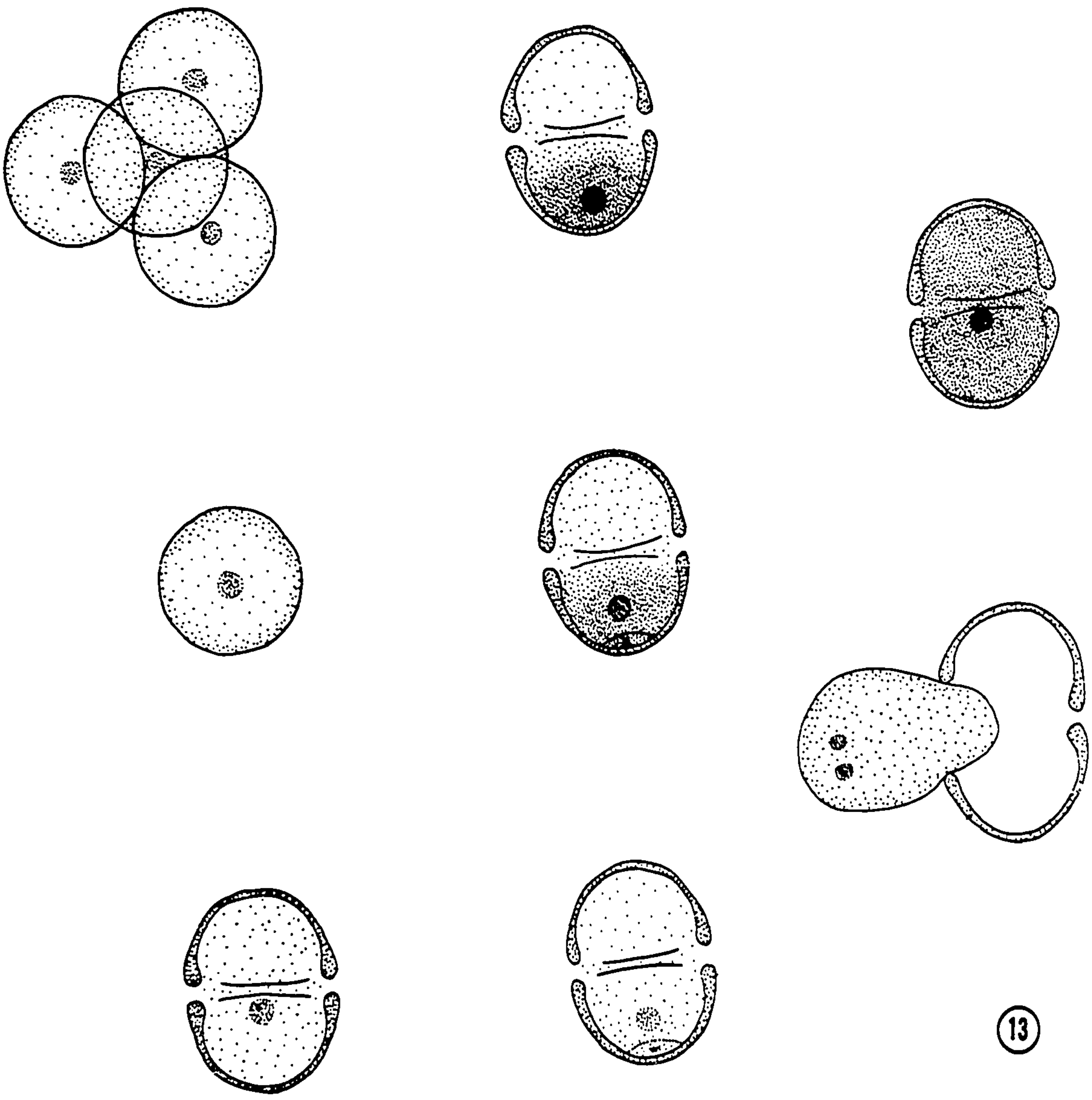
FIGURES

13. Development of pollen from tetrad to mature grain with sperm nuclei.



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APPENDIX

ASSOCIATED SPECIES FLOWERING WITH *POLYGALA ALBA* NUTTALL

POPULATION 1--JOHNSON'S PASTURE ROADSIDE

Species	4 May 1970	21 May 1970	2 June 1970	11 June 1970	9 June 1971
Nothoscordum bivalve L.			x		
Sisyrinchium angustifolium Miller	x		x		x
Paronychia jamesii T.&G.					x
Lesquerella ovalifolia Rydb.	x		x		
Schrankia uncinata Willd.		x		x	x
Baptisia leucophaea Nutt.					
Medicago sativa L.				x	x
Melilotus officinalis L.		x		x	x
Melilotus alba Desv.		x		x	x
Psoralea tenuiflora Pursh		x		x	x
Petalostemum purpureum (Vent.) Rydb.				x	x
Petalostemum candidum Willd.				x	x
Amorpha canescens Pursh				x	x
Linum rigidum Pursh	x	x			x
Oxalis violacea L.			x		
Stillingia sylvatica L.					x
Euphorbia corollata L.					x
Callirhoe involucrata (Torr.) Gray		x			x
Calylophus serrulatus (Nutt.) Raven		x		x	x
Gaura sinuata Nutt.					x

POPULATION 1--Continued

Species	4 May 1970	21 May 1970	2 June 1970	11 June 1970	9 June 1971
Cornus drummondii C. A. Mey.		x			
Asclepias tuberosa L.		x		x	x
Evolvulus nuttallianus R.&S.		x			x
Verbena bracteata Lag. & Rodr.					x
Hedyotis nigricans (Lam.) Fosb.		x		x	x
Aphanostephus skirrhobasis (DC.) Trel.				x	
Erigeron strigosus Muhl. ex. Willd.		x			x
Rudbeckia hirta L.					x
Dracopsis amplexicaulis (Vahl.) Cass.				x	x
Ratibida columnaris (Sims) D. Don.					x
Hymenopappus tenuifolius Pursh		x		x	x
Hymenoxys linearifolia Hook.	x	x	x	x	x
Gaillardia lanceolata Michx. var. *fastigiata* (Greene) Waterfall				x	x
Achillea lanulosa Nutt.					x
Pyrrhopappus grandiflorus (Nutt.) Nutt.	x		x		x
Thelesperma ambiguum Gray		x		x	x

POPULATION 3--POLYGALA FLAT

Species	2 May 1970	6 May 1970	20 May 1970	11 June 1971
Sisyrinchium angustifolium Miller	x	x		
Lesquerella ovalifolia Rydb.	x	x		
Baptisia leucophaea Nutt.	x	x		
Melilotus officinalis L.		x	x	x
Melilotus alba Desv.		x	x	x
Psoralea tenuiflora Pursh				x
Krameria lanceolata Torr.				x
Linum rigidum Pursh				x
Stillingia sylvatica L.				x
Callirhoe involucrata (Torr.) Gray				x
Calylophus serrulatus (Nutt.) Raven			x	x
Penstemon cobaea Nutt.			x	
Hedyotis nigricans (Lam.) Fosb.			x	x
Erigeron strigosus Muhl. ex. Willd.			x	x
Hymenopappus tenuifolius Pursh				x
Hymenoxys linearifolia Hook.	x	x	x	x
Achillea lanulosa Nutt.				x
Pyrrhopappus grandiflorus (Nutt.) Nutt.	x	x		x

POPULATION 3--Continued

Species	2 May 1970	6 May 1970	20 May 1970	11 June 1971
Thelesperma ambiguum Gray			x	x
Berlandiera texana DC.				x

POPULATION 5--CLARENDON, 13 JUNE 1970

Paronychia jamesii T.&G.

Linum pratense (Nort.) Small

Stillingia sylvatica L.

Psoralea linearifolia T.&G.

Krameria lanceolata Torr.

Calylophus serrulatus (Nutt.) Raven

Calylophus hartwegii (Benth.) Raven

Cryptantha jamesii (Torr.) Pays.

Ratibida columnaris (Sims) D. Don.

Xanthisma texanum DC. var. drummondii (T.&G.) Gray

Melampodium leucanthum T.&G.

Lygodesmia texana (T.&G.) Greene

Psilostrophe villosa Rydb.

Thelesperma megapotamicum (Spreng.) O. Ktze.

POPULATION 9--FALFURRIAS, 21 MAY 1971

Tephrosia lindheimeri Gray

Verbena halei Small

Monarda punctata L.

Aphanostephus kidderi Blake

Dyssodia tenuiloba (DC.) Robins.

Verbesina encleioides (Cav.) Gray

Xanthisma texanum DC. var. *texanum*

Simsia calva (Engelm. & Gray) Gray