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Crossing and Flowering Behavior In Spanish Clover, *Desmodium sandwicense* E. Mey., And Other *Desmodium* Species

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CROSSING AND FLOWERING BEHAVIOR IN SPANISH CLOVER, *DESMODIUM SANDWICENSE* E. MEY., AND OTHER *DESMODIUM* SPECIES [1]

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Desmodium species have been recognized for many years as being useful leguminous forage crops (1, 2, 3, 5, 9, 11, 13). However, there is very little information on their breeding behavior (6, 7, 8).

Hutton (6) indicated that *D. uncinatum* (Jacq.) D. C., *D. sandwicense* E. Mey., and *D. intortum* (Mill.) Urb. are cross-pollinated but that they self-pollinate readily. He found that *D. uncinatum* and *D. sandwicense* have similar breeding behavior and flowers of both species will trip spontaneously or as a result of insect visitation (7). He found considerable intrastrain in progenies raised from seed of *D. sandwicense* grown in adjacent plots. Under his experimental conditions, pollination was found to be complete by about 11 A. M. He found that tripping was necessary to obtain satisfactory pollination of *D. uncinatum* and suggested that cross-pollination was more important than self-pollination in the species.

There is little information about photoperiod response of *Desmodium* species (12). Under Hawaiian conditions, some species behave as short-day plants, others are long-day plants, and still others are indeterminate.

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[1] Portions of the material presented here were included in theses presented by Soon Jai Park and Amara Bromdep to the Graduate School of the University of Hawaii in partial fulfillment of the requirement for the Master of Science degree.

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D. canum (Gmel.) Schintz and Thellung, commonly known as kaimi clover, is a useful leguminous pasture plant for infertile soils and moderate rainfall conditions (5, 10). It has the ability to compete successfully with pangola grass and kikuyu grass and can upgrade quality of forage. Hosaka (5) first described its use as a pasture plant in Hawaii and studied its flowering behavior. He found that kaimi clover was highly self-fertile and that the length of time from flowering to maturation of seed pods was influenced by the length of day: the longer the day, the shorter the maturation period.

There is considerable confusion in the nomenclature of *D. sandwicense* and *D. uncinatum. D. sandwicense* has been mistakenly identified as *D. uncinatum* in Hawaii. Consequently, most of the literature referring to Hawaiian flora give *D. sandwicense* as a synonym for *D. uncinatum.* This is in error. The two species are entirely different in growth habit, time of flowering, seed size and shape, pod size, length of inflorescence, etc. Leaflet markings are similar. *D. sandwicense* is indeterminate in flowering behavior, has a prostrate to upright habit of growth, and is a smaller plant than *D. uncinatum. D. uncinatum* is a prostrate to semi-prostrate plant, and it flowers only during the short-day season. *D. sandwicense* is the proper name for our Spanish clover but Dr. Bernice Schubert, Curator, Arnold Herbarium, Harvard University, thinks it may be a form of *D. limense* Hook., which it resembles.[3] *D. sandwicense* (Spanish clover), *D. intortum* (greenleaf desmodium, kuru vine, intortum, or giant Spanish clover), *D. uncinatum* (silverleaf desmodium), and a fourth species, as yet unidentified, make up a species complex, and hybrids are readily obtained among them.

The studies reported here are preliminary accounts of work underway to investigate the breeding behavior and flowering responses of several *Desmodium* species.

METHODS AND MATERIALS

Crossing and flowering behavior. Two methods of crossing were used in working with Spanish clover: a tripping method suggested by Dr. K. S. McWhirter[4] and conventional emasculation. The tripping method was as follows: From 6 A. M. to 8 A. M. each morning, before the flowers were open, the standard and keel petals were removed with forceps; and the anthers and pollen were blown away leaving the stigma exposed. Pollen was then applied to the stigma with a toothpick.

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[3]Personal communication.

[4]Personal communication. Dr. McWhirter is Senior Lecturer in Genetics, School of Agriculture, University of Sydney.

In the second method, flower buds were emasculated in the late afternoon by opening the ends of the keel petals with forceps and carefully removing the anthers from the closed buds. The racemes, bearing the emasculated buds, were covered with fiber pollinating bags. Wet cotton was inserted at the base of the raceme inside the bags to increase the relative humidity around the emasculated flowers. Pollinations were completed the next morning between 8 A. M. and 10 A. M.

Notes were made on the flowering behavior, number of flowers opened per raceme per day, and total number of flowers opened per raceme in kaimi clover and Spanish clover. Observations were made on the time of anther dehiscense in Spanish clover. Ovule number per ovary and average number of seeds per pod were obtained for a number of *Desmodium* species.

Photoperiod studies. Flowering response to photoperiod was studied in five species of *Desmodium.* The experiments were repeated three times with the same experimental conditions but with the controls growing under decreasing or increasing daylength. The first experiment was started in August 1964, and the last experiment was started in January 1965. The longest day in Hawaii has a light period of 13 hours 20 minutes without twilight effect (14 hours 10 minutes including twilight), and the shortest day in Hawaii has a light period of 10 hours 50 minutes without twilight effect (11 hours 40 minutes with twilight).

Plants of five species of *Desmodium* were used for photoperiod studies. The accession numbers given are those assigned by the Department of Agronomy and Soil Science to the accession when it was received.

Species	*H. A. E. S. Accession No.*
D. canum	5334
D. sandwicense	5053
D. motorium	5208
D. motorium	5135
D. gyroides	5140

Plants were grown from seed in plastic pots containing vermiculite. A complete nutrient solution was applied twice a week, and water was supplied as needed. All plants were kept outside for eight hours a day and then in the growth chambers according to the treatment. The controls were allowed to grow continuously under the normal daylight conditions outside. The two daylength conditions (long-day treatment 16-hour light and 8-hour dark, and short-day treatment 8-hour light and 16-hour dark) were obtained by combined outside and growth chamber time. The temperature in both chambers was kept at 75 degrees F. during the daytime and 65 degrees F. at night.

Nine pots of each species were used in each experiment. The plants were kept for 30 days under long-day treatment and short-day treatment. Then two-thirds of of the short-day plants were transferred to long day, and two-thirds of the long-day were transferred to short day. The plants were kept under these conditions for 21 days. After 21 days, one-half of the short-day plants in the long-day treatment were brought back to short day; and one-half of the long-day plants in short-day treatment were brought back to long-day treatment. The plants were now kept in this new treatment for 90 days. During the treatment the date of flowering was recorded.

From the above procedures the following treatments were obtained: long-day, short-day, long-short day, short-long day, short-long-short day and long-short-long day.

RESULTS AND DISCUSSION

Flowering behavior in D. sandwicense. The flowers are borne on racemes, derived from axillary and terminal buds. The racemes are indeterminate in flowering, and the basal flowers open about two weeks before the terminal flowers. The flowers are ephemeral, lasting but three to four hours or more depending upon environmental conditions. On warm days they open shortly after sunrise and are wilted by about 11 A. M. On cool moist days they may last all day. Once the flower is tripped it quickly wilts and the standard petal is folded over the stigma within an hour's time. Along with this the usual bright color fades.

Only a few flowers open per raceme per day. Several days before the flowers open, the part of the stem containing that set of flowers elongates rapidly such that the flowers are separated from each other on the axis. Two flowers occur at each node, and the pairs are arranged in a spiral on the axis. The day before the flowers open, the closed petals expand and project beyond the sepals. Flower color can be determined at this time.

In the late afternoon of the day before the flowers open, the anthers are white (viewed with a 20 X lens); by about 8 P. M., the anthers start to turn yellow, indicating pollen maturation. The anthers dehisce about midnight and the pollen will fall out of the anthers if disturbed. The stigma is extended beyond the anthers at this time and little if any pollen touches the stigma. By dawn the petals have completely expanded but the flower is still closed. Shortly after daylight, the standard petal becomes erect and the flower is receptive to pollinating insects. If the flower is tripped at this time, pollen is discharged in a cloud around the stigma. The stigma does not strike the standard petal as in alfalfa, but stops abruptly about midway between the standard petal and keel. Observations on pollen maturation were made during the summer months, and possibly the time of pollen maturation will vary according to the season of the year.

One hundred and eight racemes of five clones of Spanish clover were observed for flowering behavior. Depending on the clone, the average length of time for flowering per raceme varied from 7.5 to 9.7 days. From 4.9 to 5.8 flowers were opened per raceme per day. Total numbers of flowers per raceme varied from 28.6 to 36.7 with an average of 31.6 flowers per raceme. Flowering was nearly complete by 11 A. M. Untripped flowers set seed just as readily as those which were tripped. The average number of flowers open per day per raceme is presented in Table 1.

Crosses made by the tripping method on greenhouse materials were unsuccessful during the fall of 1964. The flowers were very sensitive to heat and low humidity. Over 300 crosses were attempted and only seven pods were formed. Crosses made outside were more successful than those in the greenhouse. Results in Table 2 were obtained from 47 combinations among 10 plants. Percentage of pods set from individual crosses varied from 3.0 to 37.0 per cent. Very poor results were obtained by emasculation unless wet cotton was placed at the base of the raceme in the pollinating bag. Although tripping involved less work and a larger number of seeds per pod were obtained than by emasculation and wet cotton, the number of true F_1 hybrid as identified by flower color segregation in the F_2 progenies was much lower than the emasculation and wet cotton method (39.8 per cent as compared to 59.3 per cent). Generally, the average number of seeds per pod from the three methods of crossing (3.0) was much lower than the number of seeds per pod (8.5) from open pollinations. Best results were obtained during cool moist weather. Similar observations were made by Hutton (6) who found that pollen germination was poor when relative humidity was low.

Results from studies on amount of outcrossing in Spanish clover are presented in Table 3. Plants from open-pollinated seed obtained from white flowered plants growing adjacent to purple flowered plants were checked for flower color (purple flower color is dominant over white flower color). An average of 18.8 per cent outcrossing was observed. This is a low estimate since there was no way to identify the hybrids from crosses among the white flowered plants growing in the same block.

Flowering in D. intortum and D. uncinatum. *Desmodium intortum and D. uncinatum* flower in the same manner as Spanish clover. They flower during the short-day period with *D. uncinatum* starting to flower by the end of September and ending by mid March or early April. Several lines of *D. intortum* flower in early September and continue to mid June or later. The greenleaf desmodium types (H. A. E. S. 4331) begin to flower in mid November and finish about mid March. Influence of flowering on vegetative growth varies considerably within *D. intortum.* Some lines will continue to initiate vegetative buds during the flowering season, and considerable vegetative growth is produced. Others produce only flower buds, and very little vegetative growth occurs. Preliminary observations indicate that not only daylength but also temperature plays an important role in controlling flowering in *D. intortum* and *D. uncinatum.*

Table 1. Average number of flowers open per day per raceme in kaimi clover and Spanish clover

Species	Average number of flowers per raceme at day of observation Days																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Spanish Clover*	7.4	6.9	6.3	5.6	3.8	3.4	2.4	1.9	2.3	1.4	1.4	.7	1.3	.9	.3	.7	.3	.4
Kaimi Clover**	2.6	3.3	3.4	3.6	3.3	2.5	1.7	1.3	.7	.3								

* 108 racemes from 5 clones were observed.
** 54 racemes from 27 clones were observed.

Table 2. Average percentage of pods set and number of seeds per pod obtained by three different methods of crossing in Spanish clover*

Method of crossing	No. of flowers manipulated	No. of pods set	Percentage of pods set	Av. no. of seeds per pod	Total no. of seeds obtained	Percentage of true hybrids from crosses
Tripping	1629	205	12.6	3.2	645	39.8
Emasculation only	354	5	1.4	1.6	8	—
Emasculation and use of wet cotton in pollinating bag	1175	113	9.6	2.7	309	59.3

* Results presented are from 10 plants in 47 combinations. Crosses were made outdoors on the Manoa Campus of the University of Hawaii.

Table 3. Percentage of outcrossing in Spanish clover under open pollinated conditions from white-flowered plants grown adjacent to purple-flowered plants*

White flowered plant no.	No. of open-pollinated seeds obtained	No. of white-flowered plants from o. p. seed	No. of purple flowered plants from o. p. seed	Percentage of outcrossing
1	36	27	9	25.0
2	43	36	7	16.3
3	39	33	6	15.4
4	24	17	7	29.2
5	43	36	7	16.3
6	39	33	6	15.4
Average	37.3	30.3	7.0	18.8

* Purple flower color is dominant to white flower color.

Seed set in *D. intortum* is good, and under natural conditions both tripped and untripped flowers appear to set seed equally well. There are some differences in preference by pollinating insects between *D. intortum* and *D. uncinatum* flowers. When plants of both species are grown side by side, bees will approach *D. uncinatum* flowers and may leave without touching the flower. They go readily to the *D. intortum* flowers and trip them by going in directly over the keel petals and allowing the stigma to strike them on the ventral side of the thorax. A tripped flower is rarely visited by another bee. *D. sandwicense* flowers are readily visited by pollinating insects. Flowers of all three species have been observed to remain untripped all day and then wilt. Many of these fall within 24 hours; the remaining evidently have been selfed and have set seed. It appears that cross pollination is still more important than self-pollination in these species.

Flowering in D. canum. Kaimi clover has a somewhat different flowering behavior than *D. intortum, D. sandwicense,* or *D. uncinatum.* Three to five flowers occur per node with two developing together and then about a week later the remaining flowers open. This behavior was most pronounced in certain accessions (H. A. E. S. 5146 and H. A. E. S. 5148). Kaimi clover flowers are much smaller than those of the other three species. They open in the early morning shortly after sunrise and are usually wilted by noon. They are, for the most part, self-pollinated and the flowers are seldom tripped by insects. However, one accession, H. A. E. S. 5194, was actively sought by pollinating insects, and all flowers were tripped as soon as they were opened. Two accessions (H. A. E. S. 5146 and H. A. E. S. 5148) had flowers twice the size of the other accessions, and these were occasionally visited by pollinating insects. Although there was considerable variation among the different accessions of kaimi clover, no variation was observed within any one accession, except for accessions 5194 and 5146.

Table 4. Number of days from date of planting to first visible flowers for 27 accessions of kaimi clover planted July 8, 1966

	Days to flowering from planting date 7/8/66											
	53	54	57	58	59	60	61	62	63	65	68	81
No. of accessions observed to flower/day*	1	2	2	4	5	3	1	2	2	1	3	1

* Two plants were observed per accession.

During the winter of 1965-1966 over 300 crosses were attempted between *D. canum* and *D. intortum,* with *D. canum* used as the male parent. No seeds were obtained. The pollinated flowers usually fell within 24 hours. Occasionally there would be some elongation of the ovary indicating that fertilization had taken place but these would abort within 72 hours.

Flowering behavior was observed on 54 racemes from 27 lines of kaimi clover. An average of 22.6 flowers per raceme were produced with a range of 8 to 50 flowers per raceme. From one to eight flowers were open on any one day. Peak flowering occurred on the fourth day and then tapered off until all were finished at the end of the tenth day. The average number of flowers open per day per raceme is presented in Table 1.

Length of time from planting to the first visual appearance of flowers in kaimi clover is summarized in Table 4. These observations were made with the planting date in July. Flowers appeared from 53 to 81 days after planting. The length of time to flower may be influenced by the time of year. Kaimi clover usually flowers all year with peaks in May-June and in September-October.

Studies on Ovule Number. Observations on the number of ovules and number of seeds per pod for several *Desmodium* species are presented in Table 5. *D. intortum* had a range of 8 to 14 ovules per ovary depending upon the particular plant type. *D. uncinatum* and *D. sandwicense* had from 8 to 10 ovules per ovary. Ovule number was much lower in *D. canum* which had a range of five to eight ovules per ovary. There appeared to be distinct differences among the accessions as to the number of ovules per ovary. Preliminary studies on pollen abortion in *D. canum, D. intortum* and *D. sandwicense* indicated the presence of very little abnormality, with over 95 per cent stainable and filled pollen.

Photoperiod Studies. The average results from the three experiments are presented in Table 6. All of the accessions had been previously observed for one year under field conditions. It was quite obvious that three of the accessions (*D.*

sandwicense, H. A. E. S. 5053; *D. canum*, H. A. E. S. 5334; and *D. motorium*, H. A. E. S. 5135) were indeterminate in their flowering behavior. The other two accessions (*D. motorium*, H. A. E. S. 5208 and *D. gyroides*, H. A. E. S. 5140) behaved as short-day plants. Under long-short day and short-long-short day treatments, the effect of the long-day treatment prevented the short-day treatment from becoming effective during the time alloted for the experiments. The three experiments may be briefly summarized as follows:

D. sandwicense flowered continuously in Hawaii under natural conditions. In each of the three experiments, it flowered regardless of the treatments imposed. Evidently the environmental conditions in the growth chambers were not adequate for *D. sandwicense* since the controls flowered from 23 to 31 days ahead of the treatments.

Table 5. Ovule number per ovary and number of seeds per pod in several *Desmodium* species

Plant species		No. of ovules per ovary*		No. of seeds per pod	
		Range	Average	Range	Average
D. canum	H. A. E. S. 5321	7-8	7.6	—	—
	H. A. E. S. 5146	5-7	6.3	—	—
	H. A. E. S. 5155	5-6	5.4	3-6	4.5
D. distortum	H. A. E. S. 4525	6-7	6.2	3-7	5.3
D. intortum	H. A. E. S. 4247	10-14	12.3	3-7	—
	H. A. E. S. 4248	8-10	9.1	—	—
	H. A. E. S. 4331	8-11	9.9	—	5.0
	H. A. E. S. 5149	10-12	11.1	3-7	—
D. sandwicense	H. A. E. S. 5053	8-10	9.2	3-10	7.9
D. scorpiurus	H. A. E. S. 5328	5-6	5.7	2-5	3.9
D. tortuosum	H. A. E. S. 4383	5-7	6.0	3-6	4.3
	H. A. E. S. 5178	5-6	5.7	1-5	3.7
	H. A. E. S. 5179	—	6.0	3-6	4.2
	H. A. E. S. 5180	5-7	6.0	2-6	4.5
D. velutinum	H. A. E. S. 4348	6-7	6.1	1-6	3.9
D. uncinatum	H. A. E. S. 5020	8-10	8.7	—	—

* **Data presented on ovule number are averages from 10 flowers of the same plant.**

Table 6. Average number of days required to induce flowering in four *Desmodium* species when subjected to varying conditions of day length

Plant		Number of days to flowering						
		Control	L. D.*1*	S. D. *2*	L. S.*3*	S. L. *4*	L. S. L.*5*	S. L. S.*6*
D. sandwicense	(H. A. E. S. 5053)	65	96	88	89	91	94	89
D. canum	(H. A. E. S. 5334)	86	90	88	90	92	87	89
D. motorium	(H. A. E. S. 5208)	89	—	86	—	—	—	—
D. motorium	(H. A. E. S. 5135)	88	90	85	92	92	93	90
D. gyroides	(H. A. E. S. 5140)	122	—	105	—	—	—	—

1 Long-day treatment.
2 Short-day treatment.
3 Long-short day treatment.
4 Short-long day treatment.
5 Long-short-long day treatment.
6 Short-long-short day treatment.

D. canum flowered continuously under natural conditions in Hawaii, with peaks in May-June and September-October. *D. canum* flowered under all treatments used. There were only six days difference among the controls and the treatments.

D. motorium (H. A. E. S. 5208) flowered only during the short-day period in Hawaii. This accession flowered only during the short-day treatment. There was no flowering as a result of the long-short and short-long-short treatments.

D. motorium (H. A. E. S. 5135) flowered continuously under natural conditions in Hawaii. It flowered under all experimental conditions but the short-day treatment appeared to be more favorable for flowering than the control. This accession of *D. motorium* behaved differently than H. A. E. S. 5208 which behaved as a short-day plant. This difference may be related to the difference in chromosome number between the two. H. A. E. S. 5208 has 22 chromosomes and H. A. E. S. 5135 has only 20 chromosomes (10).

D. gyroides flowered only during the short-day season in Hawaii. This accession flowered only as a result of short-day treatments. The controls flowered 17 days later than the short-day treatments.

SUMMARY

Observations on the flowering and breeding behavior of several *Desmodium* species are presented. *D. intortum* and *D. uncinatum* are short-day plants. *D. canum* and *D. sandwicense* are indeterminate in their flowering behavior. A comment is made on the confusion of *D. sandwicense* with *D. uncinatum.*

LITERATURE CITED

1. Bogdan, A. V. 1965. Cultivated varieties of tropical and sub-tropical herbage plants in Kenya. East African Agric. Jour. 30:330-338.
2. Boultwood, J. N. 1964. Two valuable perennial legumes–horse marmalade (*Desmodium discolor*) and kuru vine (*D. intortum*). Rhod. Agric. Jour. 61:70-72.
3. Burkart, A. 1943. Las leguminosas Argentinas silvestres y cultivades. Acme Agency. Buenos Aires. 590 pp.
4. Bromdep, Amara. 1966. Studies of nodulation of selected species of legumes in Hawaiian soils and photoperiod effects of flowering in *Desmodium* species. Univ. of Hawaii M. Sc. Thesis. Dept. of Agronomy and Soil Science.
5. Hosaka, E. K. 1945. Kaimi Spanish clover for humid lowland pastures of Hawaii. Hawaii Agric. Expt. Sta. Circ. 22. 10 pp.
6. Hutton, E. M. 1960. Flowering and pollination in *Indigofera spicata, Phaseolus lathyroides, Desmodium uncinatum* and some other tropical pasture legumes. Emp. Journ. Exp. Agric. 28:235-43.
7. Hutton, E. M. 1964. Plant breeding and genetics 79-92. *In* C. S. I. R. O. Some concepts and methods in tropical pasture research. Commonwealth Agricultural Bureaux Bul. 47. 242 pp.
8. Park, Soon Jai. 1967. Inheritance of flower color in *Desmodium sandwicense* E. Mey. Univ. of Hawaii M. Sc. Thesis. Dept. of Agronomy and Soil Science.
9. Rotar, Peter P., and Ukio Urata. 1966. Some agronomic observations in *Desmodium* species; seed weight. Hawaii Agric. Expt. Sta. Tech. Prog. Rpt. No. 147. 14 pp.
10. Rotar, Peter P., and Ukio Urata. 1967. Cytological studies in the genus of *Desmodium.* A report on some chromosome counts in the genus. Amer. Journ. Bot. 54:1-4.
11. Younge, O. R., D. L. Plucknett, and Peter P. Rotar. 1964. Culture and yield performance of *Desmodium intortum* and *D. canum* in Hawaii. Hawaii Agric. Exp. Sta. Tech. Bul. 59. 28 pp.
12. Wang, Chi-Chu. 1961. Growth, flowering and forage production of some grasses and legumes in response to different photo-periods. Journ. of the Agricultural Association of China. W. S. 36:27-52.
13. Whyte, R. O., G. N. Nilsson-Leissner, and H. C. Trumble. 1953. Legumes in agriculture. FAO Agricultural Studies No. 21. Food and Agricultural Organization of the United Nations. Rome. 367 pp.

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