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EVALUATION OF MENDOK AND DALAPON AS MALE GAMETOCIDES AND THEIR EFFECTS ON GROWTH AND YIELD OF LINSEED

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Summary

Linseed plants were sprayed with aqueous solutions of Mendok (sodium 2,3-dichloroisobutyrate) and Dalapon (sodium 2,2-dichloropropionate) at 250, 500 and 1000 ppm with a view to inducing male sterility. Both compounds produced more or less similar responses except that Mendok was slightly more effective than Dalapon. Toxicity symptoms consisted of hyponasty, chlorosis and slight to severe burning of young leaves and flower buds. Treated plants showed profuse branching, enhanced vegetative growth and heavy flower production. However, flowers became progressively smaller and gradually closed. Production and germination of seeds were both considerably reduced in the treated plants. Gamete fertility was reduced by almost all treatments. Female sterility was caused by decrease in size and abnormal morphology of pistils, and non-separation of styles and stigmas. Male sterility was induced in two forms: developmental and functional. The former was characterized by fully or partially barren anthers and non-viable pollen. Rhythms of pollen non-viability interspersed with periods of restoration of viability were noted. Functional male sterility resulted from lack of anthesis, fusion and non-dehiscence of fertile anthers and agglutination of pollen.

The tapetal cells in the anthers of undersized flowers were highly vacuolate, radially stretched and they persisted up to the mature pollen grain stage. Another feature of morphological interest was the differentiation of microsporangia on petals.

It is concluded that neither Mendok nor Dalapon can be used as a specific male sterilizing agent in linseed.

Introduction

The use of male sterile plants for replacing laborious hand-emasculation in polycross studies or for production of hybrid seeds on a large scale is well known. Although the detection and maintenance of cytoplasmic male sterile lines have been accepted procedures for achieving these, chemical induction of male sterility has been frequently attempted. The results obtained with the gametocidal compounds, along with a discussion on their possible use, have been summarized by Mohan Ram and Rustagi (1966). Induction of effective male sterility by the use of selective gametocidal compounds would be of immense economic value.

This paper describes the results obtained with two herbicidal compounds with reported gametocidal properties, namely Mendok (sodium 2,3-dichloroisobutyrate; formerly called FW-450) and Dalapon (sodium 2,2-dichloropropionate), on linseed (*Linum usitatissimum* L. variety N.P. (R.R.)9). A preliminary report of this work and results obtained with wheat have been published elsewhere (Rustagi, 1967; Mohan Ram and Rustagi, 1968, 1969).

The experimental plant is highly self-fertile with a very small percentage of natural cross pollination (Richharia, 1962). However, controlled crossing is possible. The present investigation was undertaken with the following objectives: (a) to determine if the test

compounds selectively induce male sterility; (b) if they do, to find the optimum dosage required to produce the maximum effect; (c) to check if ovular fertility is also affected; (d) to investigate the morphological and histological changes resulting from the treatment; (e) to assay the treatment effects on growth and yield; and (f) to explore the feasibility of employing these compounds for raising male sterile plants for large scale breeding programmes.

MATERIALS AND METHODS

Preliminary experiments were carried out during 1964–65, and repeated in 1965–66 and in 1966–67 on a planned lay-out. Sowing was done each year in November in three rows in plots measuring 7.8 m long and 1.7 m wide, with cemented boundaries. No fertilizers were added to the soil. Seedlings were thinned when about 15–20 cm high, to retain about thirty plants in each row. Every row was then divided into three equal parts. Treatments were laid out in a factorial randomized block design (see Panse and Sukhatme, 1967) and were replicated four times. The plants were sprayed two, three or four times with aqueous solutions of 250, 500 and 1000 ppm Mendok or Dalapon to the point of run-off, using Triton X-114 (0.01%) as the wetting agent. The first application was made when the flower buds were initiating. The control plants were not sprayed since no appreciable differences were observed in preliminary experiments between plants sprayed with the wetting agent alone and those which were not sprayed. Effects of a single spray at all the three concentrations were also studied during 1966–67.

The treated plants were observed periodically for toxicity symptoms and morphological changes. Voucher specimens of these have been preserved in the Herbarium of the University of Delhi. Six plants from each subplot were harvested at maturity and data on their height, weight, number of branches, yield, etc. were recorded. Four lots of 100 seeds each from every treatment were germinated on moist filter paper and the percentage of germination was scored after 7 days.

Linseed being a predominantly self-pollinated crop, data on fruit set and seed production gave a fairly good indication of the effectiveness of the gametocides at inducing male sterility. Additional information was obtained by pollinating the emasculated control flowers with the pollen collected from the treated plants. Female fertility was determined following hand pollination of emasculated flowers of treated plants with control pollen. For each treatment twenty flowers were taken and pollinations were done every 10 days during the flowering period.

The duration of male sterility was calculated by counting the percentage of non-dehiscent anthers collected from twenty flowers from each treatment after every 5 days. Attempts were also made to germinate pollen grains in hanging drop cultures. Viability of the pollen was studied at 4-day intervals using tetrazolium (2,3,5-triphenyl tetrazolium chloride), acetocarmine and lactophenol–aniline blue stains. For this study thirty-two flowers from each treatment were collected and their anthers mixed. From this sample four slides were prepared separately in each of lactophenol–aniline blue, a mixture of acetocarmine and glycerine, and 4% tetrazolium in 60% sucrose solution. At concentrations below 50% sucrose, bursting of pollen grains was of common occurrence. After staining for 24 hours, counts of viable and non-viable pollen were made from four fields in each preparation and their frequency was scored on 100% basis. Large, plump and deeply stained pollen grains were considered viable; small, shrunken and empty or faintly stained pollen as non-viable. The number of abortive pollen was also noted.

Flower buds, flowers and ovaries were fixed in formalin–acetic-alcohol for embryologi-

cal studies at a time when maximum toxicity was noted. The fixed materials were washed and stored in 70% ethanol. They were dehydrated in alcohol–xylene series, and embedded in paraffin. Microtome sections were cut at a thickness of 5–13 μ, and were

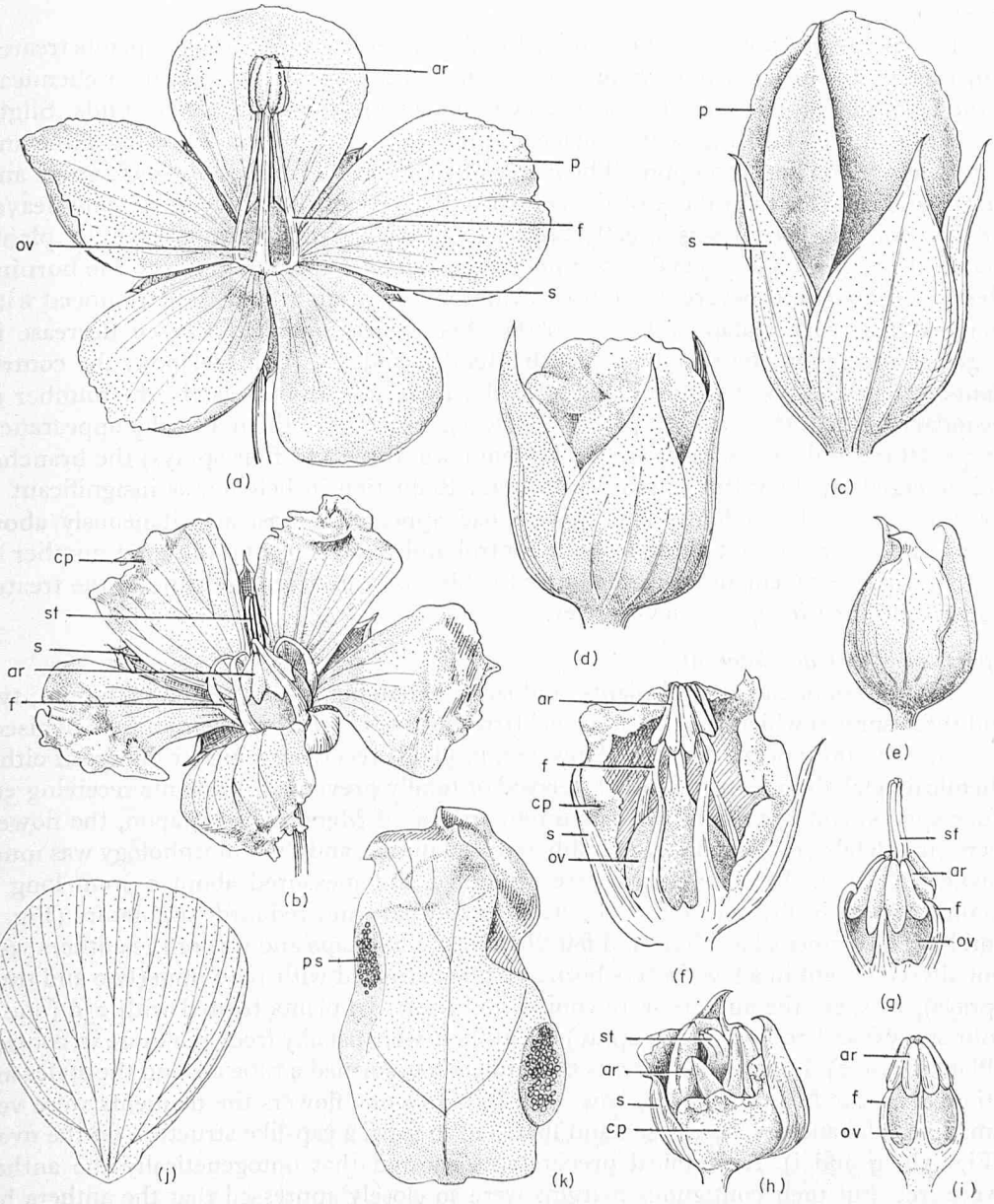

Fig. 1. (a) Open flower from control plant. × 1.75. (b)–(i) Flowers from treated plants showing gradual reduction in size, lack of anthesis and shortening of filaments and styles. Anthers are fused and form a cap-like structure over the ovary in (b), (g) and (i). In the same figures filaments are seen inflexed. × 2.5. (j) and (k) Normal and stamenoid petals from control and treated (closed and undersized) flowers respectively. In (k) the petal is broader at base, has reduced vasculature and bears two pollen sacs along the margins. × 3.5. The symbols used are as follows: ar, anther; cp, crumpled petal; f, filament; mt, microspore tetrad; ov, ovary; p, petal; pg, pollen grain; ps, pollen sac; s, sepal; sp, stamenoid petal; st, styles; t, tapetum.

stained in safranin and counterstained with fast green or orange G. Whole mounts of flower parts were also prepared for examination.

RESULTS

Effects on growth

The spray symptoms were first noticed 10 days after the initial spray in plants treated with 1000 ppm, and subsequently in those treated with lower dosages of either chemical, in the form of slight hyponasty and yellowing of young leaves and flower buds. Slight to severe marginal necrosis of the foliage and flower buds occurred following three and four sprays at 500 and 1000 ppm. The leaves of these plants had become dark green and thicker and broader than those of the controls. The size of the newly developing leaves and flowers (Fig. 1c–e) was greatly reduced. This was more pronounced in plants treated at 500 (four sprays) and 1000 ppm (three and four sprays), in which the burning effects were also quite severe. Phytotoxic symptoms were slightly more pronounced with Mendok than with Dalapon. Injury to the shoot apical meristem caused decrease in height at 1000 ppm (by about 15% with Mendok and 8% with Dalapon; the control plants were about 78 cm high). Consequently, there was an increase in the number of secondary branches* in treated plants (Table 1), which gave them a bushy appearance. At 500 (three and four sprays) and 1000 ppm (two, three and four sprays) the branches had emerged all along the length of the stem. Reduction in height was insignificant at lower dosages. Although the first flowers had appeared almost simultaneously about 10–15 days after the first spray in both control and treated plants, the total number of flowers was greatly enhanced in the latter (Table 1). Moreover, flowering in the treated plants continued for 30–50 days longer.

Effects on flower development

All flowers of the control plants, and some belonging to the treated (250 ppm, two and three sprays) which appeared normal (irrespective of whether their anthers dehisced or not), shed their petals by noon. However, in plants receiving a higher dosage of either chemical petal abscission was either delayed or totally prevented. In plants receiving 500 (four sprays) and 1000 ppm (three and four sprays) of Mendok or Dalapon, the flowers were completely closed and considerably reduced in size, and their morphology was much changed (Fig. 1a–k). The petals were crumpled and measured about 0.2 cm long as against 0.8 cm in the controls. They had a broad base and reduced vasculature (Fig. 1j and k). The stamens had short and flat filaments. The shape and size of the anthers were not altered except in a few flowers borne on plants treated with 1000 ppm (two and three sprays). In these the anthers were conical and short. In plants treated with 250 (one to four sprays) and 500 ppm (one spray) the anthers were usually free, like those in controls (Plate 1, No. 2). In other treatments the anthers had formed a tube around the styles and stigmas by confluence (Plate 1, Nos. 3 and 4). In some flowers the filaments were very small and the anthers were fused and flattened to form a cap-like structure on the ovary (Fig. 1b, g and i). Anatomical preparations showed that ontogenetically the anthers were free, but their contiguous margins were so closely appressed that the anthers had become connivent. Fusion of anthers with petals was also occasionally observed (Plate 2, Nos. 5 and 6).

In flowers which were fully or partially open, the length of the styles was comparable

* The branching pattern in linseed is characteristic. Near the surface of the soil the main stem breaks up into four to seven branches (referred to in the text as basal branches) and each of these produces acropetally axillary branches, in the form of a corymb towards the apex.

Table 1. *Effect of Mendok and Dalapon on growth and yield of linseed*

Treatment		Central basal branch: No. of secondary branches	Central basal branch: No. of flowers	Central basal branch: No. of fruits: Total	Central basal branch: No. of fruits: Seeded	Weight of seeds (g)	Weight of seeds/ plant (g)	Weight/ 100 seeds (g)	% germination of seeds
Control		7.1	60.0	37.3	37.1	2.665	12.617	1.013	96.7
Mendok:	250 ppm (2 sprays)	11.6	117.0	69.3	39.1	1.484	5.832	0.973	80.0
	(3 sprays)	18.7	173.4	84.6	27.5	0.707	1.935	0.872	33.7
	(4 sprays)	22.4	172.9	60.4	14.2	0.293	1.284	0.678	18.2
	500 ppm (2 sprays)	21.9	203.8	59.4	20.1	0.500	1.851	0.858	29.0
	(3 sprays)	29.4	203.3	30.1	10.6	0.181	0.544	0.581	20.0
	(4 sprays)	34.3	204.2	19.0	1.9	0.024	0.100	0.451	–
	1000 ppm (2 sprays)	27.8	199.5	31.5	6.0	0.103	0.479	0.663	16.5
	(3 sprays)	36.8	209.5	7.8	0.3	0.004	0.053	0.554	–
	(4 sprays)	40.7	217.2	8.2	0.6	0.002	0.033	0.098	–
Dalapon:	250 ppm (2 sprays)	7.4	76.4	52.9	49.4	2.090	9.796	1.012	43.5
	(3 sprays)	8.8	95.1	52.1	43.2	1.674	7.411	0.982	33.2
	(4 sprays)	13.4	126.8	59.0	38.5	1.217	5.565	0.956	23.0
	500 ppm (2 sprays)	18.4	169.6	58.4	36.5	1.172	4.828	0.900	38.2
	(3 sprays)	22.7	166.1	38.1	24.9	0.726	2.946	0.718	33.5
	(4 sprays)	25.2	189.9	36.9	25.1	0.661	2.357	0.718	18.7
	1000 ppm (2 sprays)	28.1	189.6	31.9	22.1	0.561	1.912	0.716	35.2
	(3 sprays)	25.8	169.0	26.5	16.7	0.456	1.465	0.759	29.2
	(4 sprays)	32.5	184.0	18.9	10.6	0.226	0.803	0.630	14.7
Source of variation						S.E. mean			
Treatments		2.1	17.1	6.1	4.2	0.180	0.812	0.070	2.3
Control *vs* treatments		1.5	12.4	4.4	3.1	0.414	0.587	0.014	–
Mendok *vs* Dalapon		0.7	5.7	2.0	1.4	0.060	0.264	0.042	–
Concentrations or sprays within Mendok or Dalapon		1.2	9.9	3.5	2.4	0.100	0.469	0.031	–
Concentrations × sprays within Mendok or Dalapon		2.1	17.1	6.1	4.2	0.180	0.812	0.070	–

to that in controls (0.5–0.6 cm; Fig. 1a). In a few flowers in which the filaments were short and the anthers had formed a tube around the ovary, the styles were seen projecting beyond the corolla (Fig. 1b and g)—a contrivance for avoiding self-pollination. However, in flowers which were completely closed and undersized, the length of the styles was reduced to a variable extent until a few showed only sessile stigmas (Fig. 1h and i).

Effects on yield

The total fruit set was enhanced in plants treated at low dosages (250 ppm, two to four sprays; 500 ppm, two sprays) of Mendok and Dalapon, but it was gradually decreased with higher dosages (Table 1). Plants treated three or four times with 1000 ppm Mendok or Dalapon had few or no fruits until 40 days after treatment. After this period the plants recovered and started bearing fruits, although most of the latter were either empty or contained only a few, shrivelled seeds. The actual number of seeded fruits in treated plants was thus considerably lower than that in control plants (Table 1). Seed yield as well as 100-seed weight were also significantly decreased. Mendok was more effective than Dalapon in inducing sterility effects, and these were largely due to increase in the concentration rather than the number of sprays. Germination tests could not be carried out for treatments of 500 (four sprays) and 1000 ppm (three and four sprays) Mendok, due to insufficient number of seeds. However, there was considerable reduction in the germination percentage with either chemical (Table 1). Further, at high dosages a few seedlings died within 10–15 days due to softening and rotting of the root and hypocotyl.

Effects on gamete fertility

For studying the effects of Mendok and Dalapon on gamete fertility the following points were considered: any change in the number of stamens; dehiscence or non-dehiscence of anthers; shedding, amount and viability of the pollen; loss in female fertility; and persistence of sterility effects.

The number of stamens remained unaltered by the treatments; no transformation of stamens into staminodes was observed. The anthers in control flowers were always free (Plate 1, No. 2), contained a profuse number of pollen grains (Plate 1, No. 1) and dehisced longitudinally. The two most common ways through which male sterility could be established as a result of treatment were fusion of anthers (Plate 1, No. 4) and lack of dehiscence (Table 2), thereby preventing the release of the pollen. Single sprays of Dalapon at 500 and 1000 ppm were successful in bringing about almost 100% male sterility (Table 2) through these responses, but this lasted only 2 or 3 days. This brief period of male sterility could be prolonged to about 3 weeks by repeating the applications. However, Mendok was less effective as compared to Dalapon at 500 ppm (one and two sprays). With Mendok only 50–90% anthers became non-dehiscent; the male sterility response was obtained about 20 days later than with Dalapon and it lasted about 1 week. At higher dosages, however, the effects of Mendok and Dalapon were more or less similar.

The amount of pollen grains produced in each anther, as examined from sections and whole mounts, in response to 250 (one to four sprays), 500 (one to three sprays) and 1000 ppm (one and two sprays) of either chemical was nearly the same as in control flowers. At 500 (four sprays) and 1000 ppm (three and four sprays) the quantity of pollen was reduced, particularly in those flowers which were small and closed (Plate 2, No. 7). Some anthers were totally empty, others showed degeneration of the sporogenous tissue or of the microspore tetrads. Moreover, a few flowers (500 ppm, four sprays; 1000

Table 2. *Effect of Mendok and Dalapon on non-dehiscent response of anthers*

Treatment*		% of non-dehiscent anthers							
		12 Feb.	17 Feb.	22 Feb.	27 Feb.	4 March	9 March	14 March	29 March
Control		0	0	0	0	0	×	×	×
Mendok:	250 ppm (1 spray)	0	0	10	10	0	×	×	×
	(2 sprays)	0	0	7	8	7	×	×	×
	(3 sprays)	–	–	–	15	12	3	0	0
	(4 sprays)	–	–	–	–	22	8	33	8
	500 ppm (1 spray)	0	12	51	45	13	4	0	0
	(2 sprays)	6	8	91	50	28	13	9	6
	(3 sprays)	–	–	–	100	90	25	22	13
	(4 sprays)	–	–	–	–	100	84	63	38
	1000 ppm (1 spray)	42	50	64	98	28	18	4	0
	(2 sprays)	47	98	100	100	78	69	14	9
	(3 sprays)	–	–	–	100	100	75	95	18
	(4 sprays)	–	–	–	–	100	100	100	39
		14 Feb.	19 Feb.	24 Feb.	1 March	6 March	11 March	16 March	31 March
Dalapon:	250 ppm (1 spray)	0	0	0	0	×	×	×	×
	(2 sprays)	0	48	30	0	×	×	×	×
	(3 sprays)	–	–	45	58	20	3	7	0
	(4 sprays)	–	–	–	–	55	39	9	0
	500 ppm (1 spray)	26	100	77	16	21	0	7	0
	(2 sprays)	60	100	100	80	24	6	12	0
	(3 sprays)	–	–	100	100	82	17	23	20
	(4 sprays)	–	–	–	–	89	63	33	45
	1000 ppm (1 spray)	48	100	100	36	18	0	2	20
	(2 sprays)	82	100	100	100	44	30	27	24
	(3 sprays)	–	–	100	100	100	86	52	39
	(4 sprays)	–	–	–	–	100	100	88	53

* Date of last treatment of Mendok: one spray, 30 January; two sprays, 9 February; three sprays, 19 February; four sprays, 1 March 1967.
Date of last treatment of Dalapon: one spray, 1 February; two sprays, 11 February; three sprays, 21 February; four sprays, 3 March 1967.
×, No flowers; –, data were not collected as the last treatment had not been given or had just been given.

ppm, three and four sprays) showed only one or two rows of microspore mother cells in each anther lobe as against four rows in the control flowers.

Data on pollen stainability were collected for normal-sized, fully or partially open flowers using acetocarmine, lactophenol–aniline blue and tetrazolium. For flowers which were undersized and closed, acetocarmine was used.

In open flowers neither compound was able to induce non-viability of pollen to any appreciable extent. In the closed and undersized flowers the pollen grains were agglutinated, shrunken and unstained. In plants given three sprays of 1000 ppm Mendok, the maximum non-viability obtained was 60% and it lasted only 4 days (Fig. 2a); with 250 and 500 ppm 10–20% non-viability was recorded for 4 days. Results with four sprays (Fig. 2b) followed a different pattern. About 18% pollen non-viability lasting 4 days was obtained at 250 ppm. However, this gradually declined and nearly 100% viability was restored 12 days after the last spray. At 500 ppm three peaks of pollen non-viability were noted on the fourth, twelfth and twentieth day after the last spray, the maximum being 40%. But at 1000 ppm the three peaks occurred on the fourth, sixteenth and thirty-second day registering 90, 90 and 52% non-viability respectively. The results with 500 and 1000 ppm (four sprays) thus indicated that there were rhythms of non-viability interspersed with periods of restoration of viability. The effects obtained with Dalapon were similar but less marked.

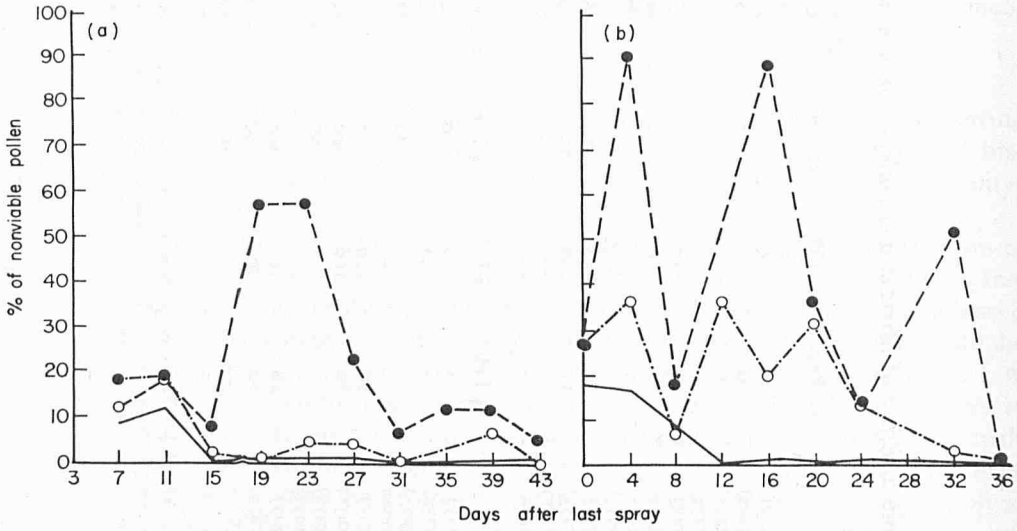

Fig. 2. Effect of (a) three and (b) four aqueous foliar sprays of Mendok on pollen viability in linseed. – – –, 1000 ppm; – · –, 500 ppm; ——, 250 ppm.

Attempts to germinate the pollen grains of linseed in hanging drop culture were discouraging because of their instantaneous bursting in water and in solutions up to 50% sucrose. At higher concentrations of sucrose, the pollen grains did not burst but they seldom germinated. The three-celled nature of the pollen could be responsible for this response (see Brewbaker and Majumdar, 1961).

Data from pollination experiments showed that both fruit and seed set were reduced markedly by either chemical at all levels of treatment, although more significantly by Mendok than Dalapon (Table 3). The reduction in seed yield was not only due to failure of anthesis or non-dehiscence of anthers, but was also brought about by loss of gamete

Table 3. *Effect of Mendok and Dalapon on fruit and seed set when flowers were selfed*

Treatment	Total no. of fruits	No. of seeded fruits	No. of seeds	Total no. of fruits	No. of seeded fruits	No. of seeds	Total no. of fruits	No. of seeded fruits	No. of seeds	Total no. of fruits	No. of seeded fruits	No. of seeds
	15 February			25 February			7 March			17 March		
Control	20	20	176	17	17	135	×	×	×	×	×	×
Dalapon: 250 ppm (1 spray)	18	28	124	13	13	72	×	×	×	×	×	×
(2 sprays)	20	20	113	8	6	32	×	×	×	×	×	×
(3 sprays)	–	–	–	–	–	–	19	14	36	20	14	45
(4 sprays)	–	–	–	–	–	–	19	7	11	19	11	52
500 ppm (1 spray)	11	8	12	4	3	11	19	15	53	14	12	58
(2 sprays)	10	0	0	1	1	1	17	11	43	16	11	49
(3 sprays)	–	–	–	–	–	–	12	7	21	15	11	58
(4 sprays)	–	–	–	–	–	–	12	1	2	13	3	8
1000 ppm (1 spray)	6	0	0	0	0	0	19	10	26	18	11	62
(2 sprays)	1	0	0	0	0	0	12	7	19	13	5	20
(3 sprays)	–	–	–	–	–	–	3	1	1	17	3	4
(4 sprays)	–	–	–	–	–	–	1	0	0	9	2	3
	23 February			5 March			15 March					
Mendok: 250 ppm (1 spray)	10	10	32	×	×	×	×	×	×			
(2 sprays)	5	2	8	14	5	14	×	×	×			
(3 sprays)	–	–	–	17	6	21	15	8	28			
(4 sprays)	–	–	–	8	4	27	16	3	7			
500 ppm (1 spray)	11	2	4	20	8	26	17	11	42			
(2 sprays)	2	0	0	6	1	10	15	10	44			
(3 sprays)	–	–	–	0	0	0	14	3	18			
(4 sprays)	–	–	–	0	0	0	4	0	0			
1000 ppm (1 spray)	12	0	0	15	5	21	14	5	16			
(2 sprays)	0	0	0	5	1	1	10	2	6			
(3 sprays)	–	–	–	0	0	0	7	0	0			
(4 sprays)	–	–	–	0	0	0	9	0	0			

×, No flowers; –, data were not collected as the last treatment had not been given or had just been given.

fertility. To understand whether this was due to male or female sterility, or both, two additional experiments were carried out. In one experiment the stigmas of the emasculated control flowers were pollinated with pollen from flowers of treated plants, while in the other the pollen of the control flowers were dusted on the stigmas of flowers of treated plants (Table 4). In both experiments a considerable reduction in yield with either chemical was observed, particularly at higher dosages (500 and 1000 ppm). Thus Mendok and Dalapon induce male as well as female sterility; the former is more effective than Dalapon.

The development of the ovules up to the formation of mature embryo sac was normal. Shrivelling of ovules was observed in some flowers which had been damaged by the spray treatments (500 ppm, four sprays; 1000 ppm, three and four sprays). No instance of parthenocarpy was observed, and whenever seeds were formed they invariably possessed an embryo.

Development of tapetum

In anthers of control and treated flowers which were normal in size, the tapetal cells were uniform in appearance and degenerated immediately after microsporogenesis (cf. Narayana, 1964). But in undersized and abnormal looking flowers, which had developed about 15 days after the last spray with Mendok or Dalapon at 500 (four sprays) and 1000 ppm (three and four sprays), the tapetal cells were markedly different (Plate 2, No. 5). They became radially elongated (although to a lesser extent towards the connective), occluded the locule, and remained intact and healthy even at the mature pollen grain stage.

Modification of normal petals into stamenoid petals

Another abnormal, yet interesting feature observed in most of abnormal-looking flowers was the development of anther sacs along the petal margins (Fig. 1k; Plate 2, Nos. 6 and 7). The wall of the adventitious anther sacs comprised the same four layers as in controls, i.e. epidermis, endothecium, middle layer and tapetum (Plate 2, No. 8). The tapetal cells were highly vacuolate, hypertrophied, radially elongated and persisted up to the mature pollen grain stage. The microspore mother cells underwent meiosis and produced spore tetrads, but the pollen were mostly shrunken and empty. Such microsporangia failed to dehisce and liberate the pollen.

DISCUSSION

To be of any commercial importance, a gametocidal compound should induce an appreciable degree of specific male sterility; it should not affect ovular fertility or have any other undesirable effects. It should preferably change the morphology of the flowers so as to increase the chances of natural cross pollination. Importantly, its residues on plants should be nontoxic to man and animals.

High dosages of both Mendok and Dalapon proved toxic to linseed plants. The latter showed hyponasty, chlorosis, injury to apical bud, reduction in height, slight to severe burning of leaves and flower buds, gradual reduction in size of flowers and newly developing leaves, and low fruit and seed set. These symptoms were also reported for Mendok treated plants by other workers (e.g. Pedersen, 1959; Starnes and Hadley, 1962; Dulieu, 1963; Miller and Hittle, 1963; Sink and Gunesch, 1966; see also Rohm and Haas Co., 1959; Mohan Ram and Rustagi, 1966). Poor germinability of seeds collected from treated linseed plants (present work) suggests that probably these chemical

Table 4. *Effect of Mendok and Dalapon on gamete fertility as determined by pollination experiments*

Treatment*	Treated ♀ × control ♂						Control ♀ × treated ♂					
	Total no. of fruits	No. of seeded fruits	No. of seeds	Total no. of fruits	No. of seeded fruits	No. of seeds	Total no. of fruits	No. of seeded fruits	No. of seeds	Total no. of fruits	No. of seeded fruits	No. of seeds
	17 February			27 February			18 February			28 February		
Mendok: 250 ppm (1 spray)	18	18	132	12	5	19	19	19	161	20	18	150
(2 sprays)	16	16	94	14	3	6	11	11	75	19	18	138
(3 sprays)	–	–	–	13	3	4	–	–	–	15	12	109
(4 sprays)	–	–	–	–	–	–	–	–	–	16	12	73
500 ppm (1 spray)	11	8	58	12	5	21	14	14	113	17	13	97
(2 sprays)	2	0	0	6	0	0	5	5	14	15	10	51
(3 sprays)	–	–	–	0	0	0	–	–	–	8	2	2
(4 sprays)	–	–	–	–	–	–	–	–	–	6	0	0
1000 ppm (1 spray)	12	2	12	9	3	4	4	4	26	9	6	31
(2 sprays)	1	0	0	0	0	0	3	3	4	6	3	5
(3 sprays)	–	–	–	1	0	0	–	–	–	2	0	0
(4 sprays)	–	–	–	–	–	–	–	–	–	0	0	0
	19 February			1 March			20 February			2 March		
Dalapon: 250 ppm (1 spray)	0	0	0	14	14	97	20	20	178	19	18	148
(2 sprays)	0	0	0	14	13	70	18	18	151	17	17	145
(3 sprays)	–	–	–	11	2	3	–	–	–	17	16	121
(4 sprays)	–	–	–	–	–	–	–	–	–	15	13	109
500 ppm (1 spray)	11	2	2	15	12	38	18	18	154	17	17	131
(2 sprays)	0	0	0	16	3	5	13	13	110	18	14	93
(3 sprays)	–	–	–	8	2	3	–	–	–	15	12	74
(4 sprays)	–	–	–	–	–	–	–	–	–	11	5	35
1000 ppm (1 spray)	7	1	1	14	3	3	20	20	165	16	16	129
(2 sprays)	0	0	0	2	0	0	20	20	155	18	14	108
(3 sprays)	–	–	–	0	0	0	–	–	–	12	8	31
(4 sprays)	–	–	–	–	–	–	–	–	–	3	2	5

* For data of the control, refer to Table 3.

substances accumulate in the seeds and act on the young seedlings (see also Fürste, 1964). However, no carry-over effects were observed in wheat (Mohan Ram and Rustagi, 1969) and sugar beet (Funderburk and Davis, 1960) plants treated with Mendok and Dalapon respectively.

The treated plants showed two types of changes leading to male sterility: functional and developmental. Functional male sterility resulted from failure of pollen discharge through fusion and non-dehiscence of fertile anthers and agglutination of pollen. There was neither reduction in the number of stamens nor formation of staminodes. Developmental male sterility, on the other hand, manifested itself by the production of fully or partially barren anthers and formation of nonviable pollen. While functional male sterility was obtained without causing much phytotoxicity, developmental male sterility was induced at the cost of female fertility in those flowers which were closed, undersized and abnormal. In experiments in which the stigmas of the emasculated control flowers were pollinated with the pollen from treated plants, a good fruit and seed set was obtained only at dosages which failed to induce male sterility. Although there was evidence for regular occurrence of microsporogenesis in the treated plants, failure of reduction division could be suspected in certain microspore mother cells because of the abnormal size and shape of some pollen grains (see Brooks, Brooks and Chien, 1966). Detailed cytological investigations of the treated materials were not carried out by the present authors. It is worthwhile to know whether the applied compounds bring about changes in the chromosomes or in the cytoplasm.

One of the profitable responses was the combination of normal flower anthesis, non-dehiscence of anthers and stunting of filaments so as to place the anthers far below the stigmas. Since these flowers were totally male sterile, they were best suited for hand pollination without the need for emasculation. However, this ideal combination of features was observed only in a few flowers. The majority of flowers remained closed. The latter were most undesirable for breeding purposes because neither open nor artificial hand pollination was possible even if the flowers were fully male sterile, with no loss of ovular fertility. Opening such flowers for pollination would be as time consuming as emasculation.

Our experience with acetocarmine and lactophenol–aniline blue tests has made us skeptical about their reliability for checking pollen viability. For example steeping of anthers in concentrated hydrochloric acid did not decrease the intensity of staining of pollen. Positive staining was also obtained when the techniques were applied to the pollen collected from herbarium specimens. King (1960) and Hauser and Morrison (1964) have also expressed serious doubts about the reliability of staining reactions as criteria for viability. King believed that the above tests could at best indicate only degrees of pollen maturity. Thus comparable results obtained with iodine–potassium iodide, acetocarmine and hydrochloric acid tests (Dubey, 1963) or by lactophenol-aniline blue and Nitro-BT tests (Hauser and Morrison, 1964) can hardly justify the continued use of the classical stains.

Abnormal development of tapetum and its persistence till the mature pollen grain stage are in accordance with the observations made in other plants treated with Mendok—*Abelmoschus esculentus* (Dubey and Singh, 1968), *Beta vulgaris* (Ohta and Matsumura, 1962), *Spinacia oleracea* (Stainier, 1966) and *Trigonella foenum-graecum* (Kaul and Singh, 1967). In natural male sterile lines of flax also the tapetum presents similar features (Dubey and Singh, 1965) except that no cytoplasmic mass results from the disintegration of tapetal cells. Studies on naturally occurring male sterile lines (e.g. Artschwager, 1947;

Singh and Hadley, 1961; Zenkteler, 1962; Erichsen and Ross, 1963; Brooks *et al.*, 1966; Chauhan and Singh, 1966; Kaul and Singh, 1966) suggest that a correlation exists between the degeneration of the pollen grains and the abnormal behaviour of the tapetum (see also Singh, 1965).

The modification of normal petals into stamenoid petals by Mendok and Dalapon treatments is perhaps reported for the first time in *Linum usitatissimum* (present work). In contrast to hundreds of genera with petaloid stamens, occurrence of stamenoid petals in nature is not a common feature (Meyer, 1966). It is somewhat surprising that the same substance which induced male sterility, also stimulated the differentiation of microsporangia along petal margins.

Whenever there was an appreciable degree of male sterility, it was invariably associated with reduction in ovular fertility. The latter was related to high phytotoxicity, decrease in size and abnormal morphology of the pistil, non-separation of stigmas and lack of fertilization, etc. Thus the absence of normal fruit set in treated flowers, which had been emasculated and pollinated with the control pollen, could also be attributed to nonreceptivity or injury of stigmas during hand pollination. Reduction in female fertility caused by Mendok and Dalapon treatment has also been reported for alfalfa (Pedersen, 1959; Miller and Hittle, 1963), *Antirrhinum* (Sink and Walker, 1963), cotton (Pate and Duncan, 1960; Richmond, 1962; Semenova, 1963) and tomato (Moore, 1959, 1964). Decrease in the size of pistil has been noted in Mendok-treated *Glycine* (Starnes and Hadley, 1962) and *Solanum nigrum* (Dulieu, 1963).

We have not carried out any studies on the uptake, translocation and site of accumulation of these compounds due to non-availability of labelled samples. However, observations like the presence of non-dehiscent anthers, cohesion of anthers and their adhesion with petals, and agglutination of pollen suggest that Mendok and Dalapon must be acting at the cell wall level. Failure of separation of microspores from tetrads and of stomium cells from one another may result from inhibition of enzymatic activities necessary for cell separation. However, biochemical and biophysical evidence will be needed to support this hypothesis.

It is concluded, therefore, that neither Mendok nor Dalapon can be used as a specific male gametocidal compound in linseed. Male sterility obtained at high dosages was invariably accompanied by toxicity. In general, higher concentrations had greater phytotoxic effects than increased number of sprays. This would suggest that the chemical substances should be applied repeatedly at comparatively low concentrations to have the least undesirable side-effects.

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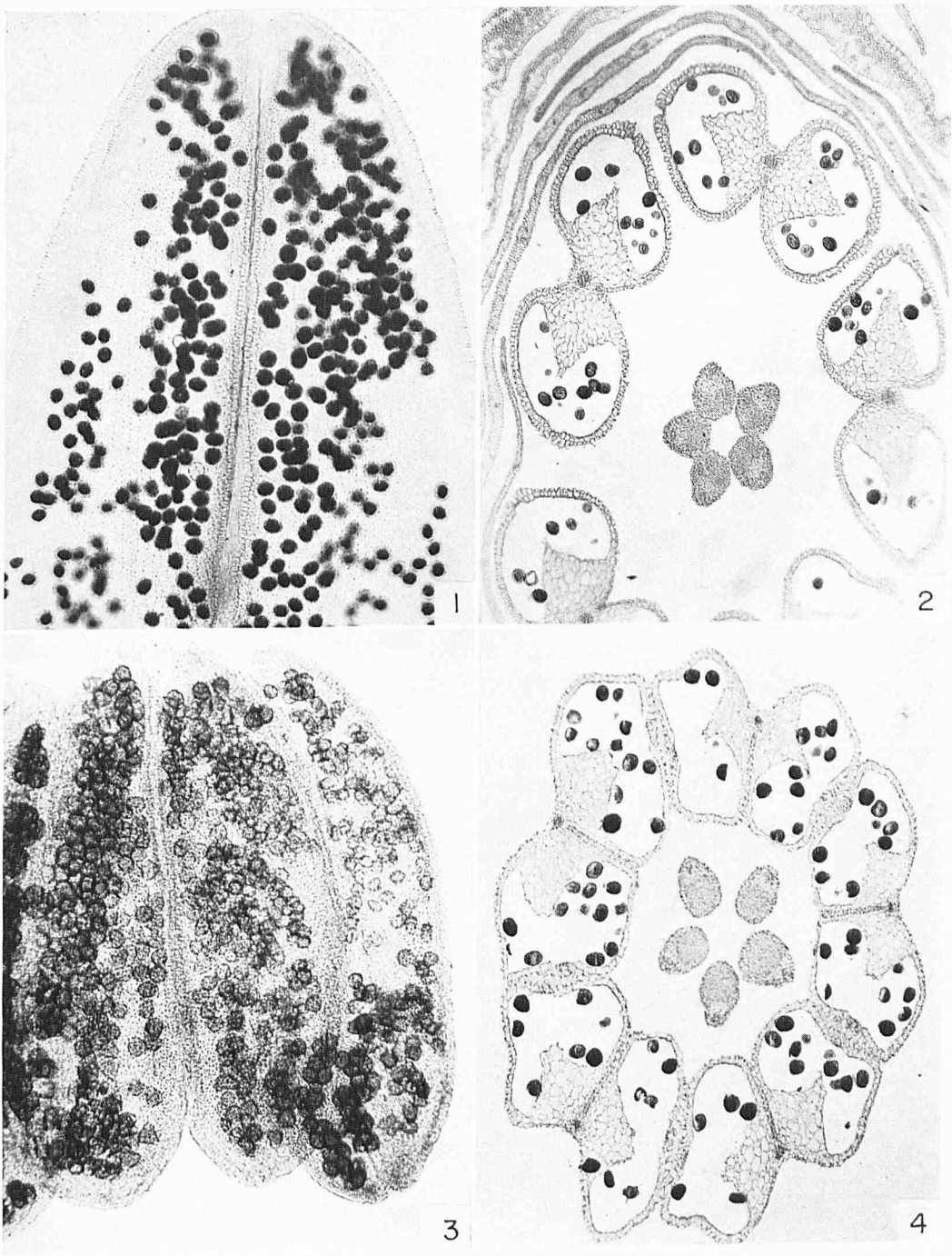
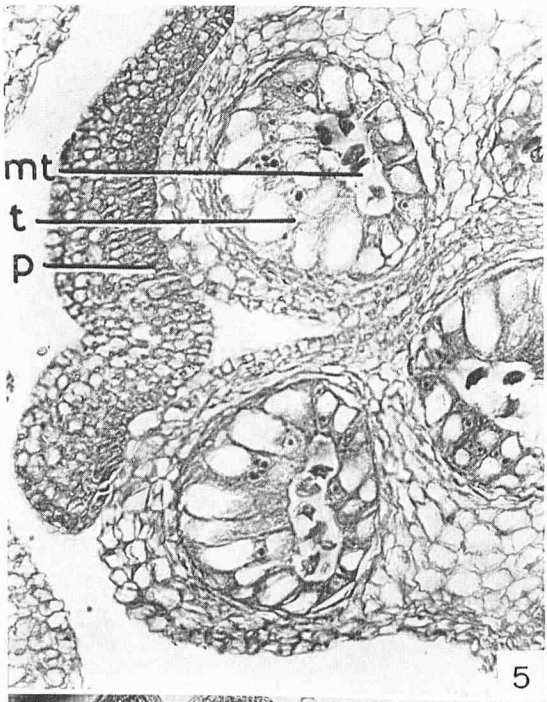


P. N. RUSTAGI AND H. Y. MOHAN RAM—*MENDOK AND DALAPON AS MALE GAMETOCIDES* (*facing page* 132)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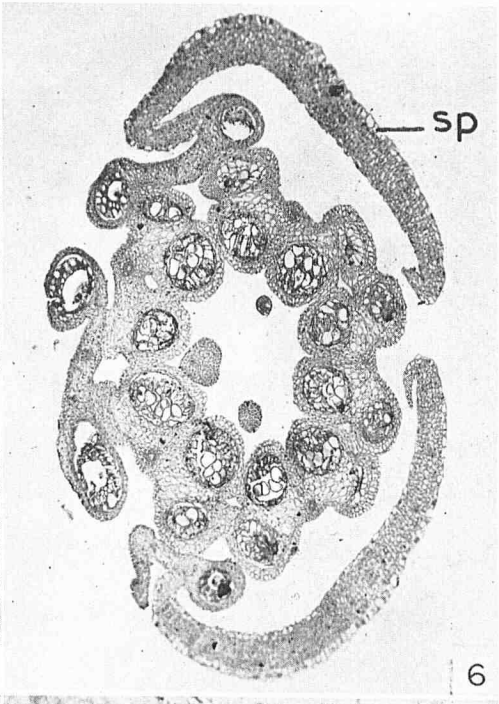
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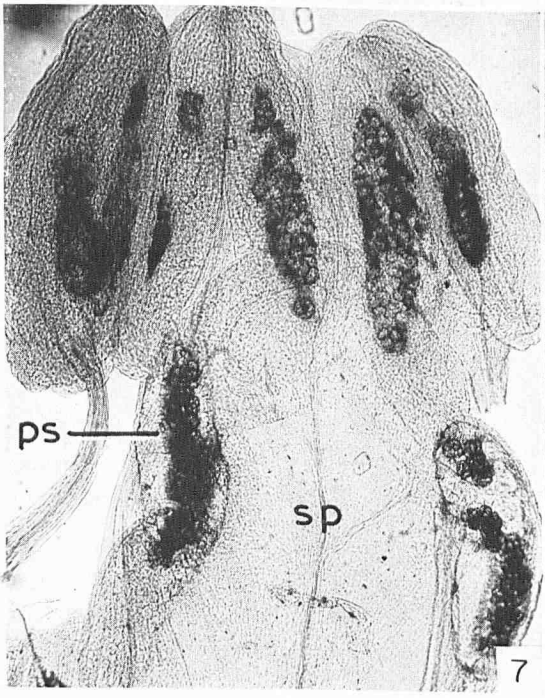
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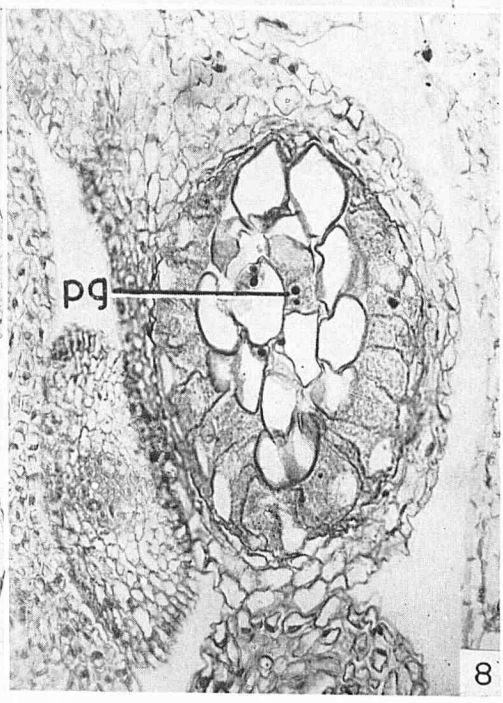
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EXPLANATION OF PLATES

PLATE 1

Nos 1–4. Structure of anthers of control flowers (Nos. 1 and 2) and of those treated four times with Mendok and Dalapon at 1000 ppm (Nos. 3 and 4).

No. 1. Cleared whole mount of a part of anther at pollen grain stage from a control flower. × 41.

No. 2. Part of a transverse section of a control flower showing mature, free anthers before dehiscence. × 185.

No. 3. Whole mount of marginally fused anthers from a treated flower. × 52.

No. 4. Part of a transverse section of a treated flower showing fused and non-dehiscent anthers. × 52.

PLATE 2

For explanation of symbols, see legend to Fig. 1.

No. 5. Portions of two adjoining anthers (in transverse section) from a treated flower enlarged to show their cohesion with each other and adhesion with a petal. The tapetal cells are highly vacuolate and hypertrophied; those lying towards the connective are less stretched than the others. Microspore tetrads are in the process of degeneration. × 275.

Nos. 6–8. Structure of staminoid petals.

No. 6. Transverse section of a flower bud showing supernumerary microsporangia. Petals have differentiated pollen sacs. Note the fusion of anthers and their adhesion with petals. × 50.

No. 7. Whole mount of a stamenoid petal with two pollen sacs along the margins; the regular anthers are fused and partially empty. × 50.

No. 8. Pollen sac from a stamenoid petal enlarged to show wall layers, hypertrophied tapetal cells and pollen grains. × 227.