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Induction of Pollen Sterility in Grapes (Vitis vinifera)*)

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Introduction

Male sterile mutants are often isolated in plants that normally possess bisexual flowers and such individuals grow to maturity and set seed if artificial pollination is affected. This shows that the change from male fertile to male sterile conditions does not affect their physiology to such an extent as to make normal growth impossible. Taking advantage of this knowledge many attempts have been made in some crops by different authors to induce pollen abortion so as to eliminate the tedium of hand emasculation. Jain (1959) has reviewed in detail the different aspects of male sterility in flowering plants along with results obtained with the use of many chemical substances.

Attempts so far made have shown that pollen sterility could be induced in a wide variety of crops. But it is not known, however, whether these chemical methods could be of use in a field scale because of certain problems linked to their use. Firstly, the pollen sterility is accompanied by a certain degree of ovule sterility which, if occuring at a very high degree, renders this method useless. Secondly, the sensitive stage at which these chemicals are to be applied is very short, making the use of very homogeneous plants necessary, with the chemical application well timed. The above cited problems often arise while attempting the use of chemically induced pollen sterility in the field crops. In fruit trees another important aspect to be taken care of is the extent of phytotoxicity caused by these chemicals since the trees being perennial, the damage once induced may be carried through for many seasons.

Keeping the above mentioned problems in mind, an attempt was made in grapes to explore the possibility of using chemicals to substitute the conventional hand emasculation. So far no attempts seem to have been made in this crop to try this method although such a method, if standardised, would be greatly beneficial since the morphology of the flower in grapes renders hand emasculation tedious and time consuming. In this paper, the results of such an experiment will be discussed. A preliminary report on the present experiment has been published earlier (Iyer and Randhawa, 1965).

Material and methods

Three grape varieties Bhokri, Hussaini and Rosem T. Lahore, were used in the present investigation. Three chemicals, namely, maleic hydrazide (MH), tri-iodobenzoic acid (TIBA) and α-, β-dichloroisobutyrate (FW-450) were tried at various concentrations as given below.

Chemicals	Concentrations
Maleic hydrazide	250, 500, 750, 1000 ppm
Tri-iodobenzoic acid	200, 300, 400, 500 ppm
FW-450	0.50, 0.10, 0.20, 0.30 %

*) This study formed a part of the Ph. D. thesis submitted by the senior author to the Post Graduate School, I. A. R. I., New Delhi, India

All chemicals were applied as foliar sprays using a hand sprayer. Only the shoot which bore the inflorescence was treated and care was taken to see that the solution did not fall on the leaves of other shoots. This was ensured by covering the untreated shoots with an alkathene sheet. The spraying was done on each shoot on both sides of the leaves and also on the young leaves and growing points till the excess solution started dripping from the leaves. "Triton" was used as a wetting agent and was added to the solution at the rate of one drop in 1000 cc of the solution. To some shoots only one application was given while the rest had two, the second spray following three days after the first. For each treatment at least ten panicles were used.

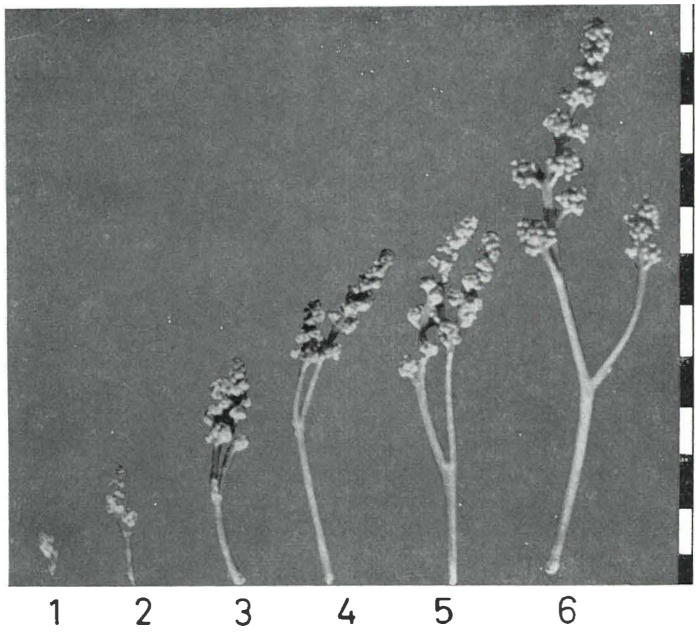

Fig. 1: Stages of development of panicles in grapes (var. Hussaini)

To find out precisely the stage of development of the inflorescence at which the treatments are most effective in inducing complete pollen sterility with the least ovule sterility, the development of the panicle was arbitrarily divided into six stages on the basis of distinct morphological differentation (Fig. 1). All the treatments were given on each stage in all the three varieties used. The distinct features of different stages are given below.

Stage I: Panicle is very compact and the buds are least differentiated and covered with hairs and bract-like structures. The panicle is about 0.80 cm in length.

Stage II: The rachis of the panicle is elongated and the different buds start making their appearance. The hairs and the bract-like structures still persist at the bases of different groups of buds. Individual buds are not yet distinct and the panicle at this stage is about 1.1 cm in length.

Stage III : The different buds are quite distinct but all remain in a single group. The diameter of an individual bud is about 0.8 mm and the total length of the panicles about 2.0 cm.

Stage IV : The pedicle is elongated, each bud is separated from the other, measuring about 1 mm in diameter. The contours of the buds are distinct and the buds form secondary groups. The panicle is about 3.5 cm in length.

Stage V : The buds further increase in size, each measuring about 1.6 mm in length and 1 mm in diameter. The buds assume a bell-shape at this stage. The panicle length is about 5.5 cm in length.

Stage VI : The buds are oblong in shape and there is a loosening of the buds within a group, perhaps due to the elongation of the panicle. The length of the individual bud is about 2 mm with a diameter of about 1.2 mm. The length of the panicle is about 8.5 cm. The flowers start opening at the completion of this stage.

Determination of sterility

The pollen sterility was determined by acetocarmine staining test. In each treatment three panicles were chosen to study the extent of pollen sterility. All buds which opened at the first day of anthesis were removed and five flowers of this kind were used to study the sterility. The same panicles were used the succeeding days, removing all newly opened flowers until anthesis was complete. The percentage of sterility was calculated for each day of anthesis in all treatments.

The extent of total sterility (both pollen and ovule) was estimated by merely bagging the treated panicle and calculating the percentage of fruit-set on the basis of initial number of buds. The ovule fertility was determined by hand pollinating treated panicles with good pollen.

Observations

General effects of the chemicals

In case of maleic hydrazide treatments, the treated shoots were less vigorous than the check-plants. Particularly at high concentrations (750 and 1000 ppm) the young leaves were distorted. The old leaves were more or less normal in appearance. Some of the leaves faded to yellow about two weeks after the treatments and many started curling outwardly. The "apical dominance" was broken in the shoots sprayed with 500, 750 and 1000 ppm which was indicated by the active growth of axillary buds. Even the new growth that started from these buds showed deformities like reduction in leaf area, thickening of the leaves, wavy margins and prominent appearance of the veins. The appearance of the inflorescence developed from the treated shoots was normal at low concentrations (250 and 500 ppm), whereas at high concentrations (750 and 1000 ppm) they tended to be yellowish in colour. In contrast to the effects of MH, the effects of TIBA, even under low concentrations, were drastic and prominent three days after application. At the lowest concentration (200 ppm) there was a slight distortion of the leaves without change in colour. In the shoot sprayed with 300 ppm TIBA, the leaves were distorted and the old leaves tended to curl outwardly. At higher concentrations (400 and 500 ppm) the effects were more drastic. The colour of the inflorescences changed towards yellowish, intensity of colouration depending upon the concentration. The treated inflorescences were open in contrast to the compact nature in controls of similar age.

The damage to the foliage by the application of FW-450 was evident within two or three days after treatment. The leaves treated with different concentrations developed marginal burning, chlorosis and many burnt spots. The colour of the panicles also turned to yellow, but the prominent effect was the elongation of the whole panicle.

Effect of the chemicals on the pollen sterility

The observations on the pollen sterility after various treatments in 1962 and 1963 in the varieties Bhokri, Hussaini and Rosem-T-Lahore are given in Table 1, 2 and 3. The data presented in this table are only the values obtained when the treatments were given at stage III (as described under materials and methods) since in

Table 1

Effect of MH on the extent of pollen sterility (in per cent)

Variety	No. of applications	Control		250 ppm		500 ppm		750 ppm		1000 ppm	
		1962	1963	1962	1963	1962	1963	1962	1963	1962	1963
Bhokri	—	1.8	2.8								
	1	—	—	43.3	(36.3)	69.3	(61.2)	73.2	(61.3)	92.9	(84.8)
	2	—	—	57.7	(46.5)	100.0	(100)	100.0	(100)	—	
Hussaini	—	2.2	3.2								
	1	—	—	27.1	(24.4)	65.8	(51.4)	71.4	(61.5)	77.6	(78.3)
	2	—	—	38.8	(40.5)	100.0	(100)	100.0	(100)	—	
R. T. Lahore	—	2.3	4.5								
	1	—	—	8.5	(6.9)	22.2	(13.8)	75.3	(51.5)	76.7	(67.7)
	2	—	—	12.2	(9.5)	37.5	(22.0)	100.0	(100)	100.0	

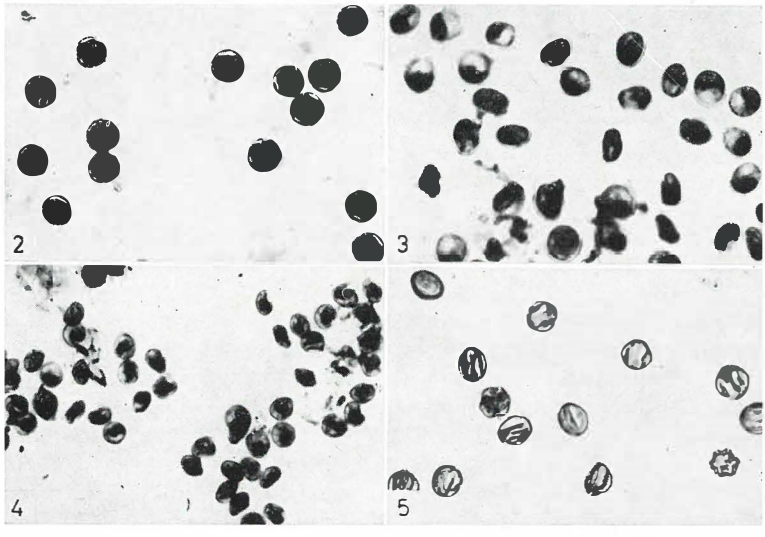

Fig. 2: Well stained pollen grains of the control (var. Bhokri)
Fig. 3: Completely sterile pollen grains, induced by MH 500 ppm (var. Bhokri)
Fig. 4: Completely sterile pollen grains, induced by TIBA 500 ppm (var. Bhokri)
Fig. 5: Empty pollen grains, induced by FW-450 (0.30%) (var. Bhokri)

terms of pollen sterility there were not many differences among stages I, II and III. None of the treatments during later stages gave complete sterility and hence are not included in the table.

The effect of various concentrations of MH on the extent of pollen sterility is presented in Table 1. It is evident from these data that the degree of sterility was proportional to the concentration of the chemical and the number of applications. In the varieties Bhokri and Hussaini two applications of 500 and 750 ppm of MH induced complete pollen sterility (Fig. 2 and 3). Two applications of 250 ppm failed to induce complete sterility although in this case the sterility was much higher as compared to the controls. A single application of 1000 ppm induced sterility as high as 92.9 and 77.6 per cent in Bhokri and Hussaini respectively. Two applications of 1000 ppm MH were not tried since minor concentrations had induced complete sterility in a preliminary trial in 1961. The effective minimum concentration of maleic hydrazide applied on the variety Rosem-T-Lahore was found to be higher. Two applications of 750 and 1000 ppm were necessary to induce complete sterility, whereas two applications of only 500 ppm which, on the contrary, had given good results with the varieties Bhokri and Hussaini, in this case failed. This could be mainly due to the presence of heavy tomentum on the leaves of this variety which prevents the sticking of the solution on the leaf surface.

Table 2

The effect of TIBA on the extent of pollen sterility (in per cent)

Variety	No. of applications	Control		200 ppm		300 ppm		400 ppm		500 ppm	
		1962	1963	1962	1963	1962	1963	1962	1963	1962	1963
Bhokri	—	1.8	2.8								
	1	—	—	5.8	3.9	10.0	6.9	57.4	53.88	82.0	69.1
	2	—	—	9.1	7.3	17.1	14.6	100.0	100.0	100.0	100.0
Hussaini	—	2.2	3.2								
	1	—	—	5.0	5.1	9.5	11.7	60.5	56.4	86.0	79.7
	2	—	—	5.7	7.0	15.1	22.5	100.0	100.0	100.0	100.0
R. T. Lahore	—	2.3	4.5								
	1	—	—	2.5	4.6	7.1	13.8	58.7	50.1	80.4	60.1
	2	—	—	5.1	6.8	10.5	21.7	82.7	65.2	100.0	100.0

The results obtained with the use of TIBA are presented in table 2. In this case also a direct relationship was obtained between the concentration of the chemical and pollen sterility induced.

Single applications could not induce complete pollen sterility in any case. In the varieties Bhokri and Hussaini two sprays of 400 ppm TIBA, applied during stage III or earlier, induced complete pollen sterility (Fig. 4), whereas in Rosem-T-Lahore 500 ppm were needed.

The effects of FW-450 have been presented in table 3. Complete male sterility was induced in the varieties Bhokri and Hussaini with 0.30% of FW-450 taking into account both pollen sterility and the presence of non-dehiscing anthers. In Rosem-T-Lahore, too, the same concentration gave a similar result in contrast to the other two chemicals used in the present experiment. In this case also the time of spray was

Table 3
The effect of FW-450 on the extent of pollen sterility (in per cent)

Variety	No. applications	Control		0.05%		0.10%		0.20%		0.30%	
		1962	1963	1962	1963	1962	1963	1962	1963	1962	1963
Bhokri	—	1.8	2.8								
	1	—	—	3.0	3.6	4.9	4.6	24.0	28.1	62.2	61.7
	2	—	—	6.2	6.9	9.5	7.8	40.1	48.2	100.0	100.0
Hussaini	—	2.2	2.3								
	1	—	—	3.6	5.9	5.0	12.9	32.2	37.3	69.4	74.6
	2	—	—	4.8	9.1	8.0	18.4	63.0	57.0	100.0	100.0
R. T. Lahore	—	2.3	4.5								
	1	—	—	2.4	4.7	4.1	6.7	13.7	26.1	47.1	60.8
	2	—	—	2.9	6.7	5.6	11.5	21.4	50.0	100.0	100.0

very important, since only early stages (I, II and III) were sensitive to the chemical. Two applications of the chemical were necessary to induce complete male sterility.

The effects of FW-450 on the grape flower buds were different from those of MH and TIBA. The treatments resulted in nondehiscence of anthers and prevention of anthesis in addition to the formation of sterile pollen grains (Fig. 5). In all concentrations of FW-450 except the lowest concentration (0.05%), the anthers of the treated buds dried up and failed to dehisce. The treated buds failed to open and there was absolutely no anthesis. The calyptra cap which overturns from the bud during anthesis in the controls, was observed to have detached from the base but failed to

Table 4
Percentage fruit set on selfing in different treated panicles (two sprays)

Variety	Stage at which sprayed	Control	MH				TIBA				FW−450			
			250	500	750	1000	200	300	400	500	0.05	0.10	0.20	0.30
			ppm				ppm				%			
Bhokri	—	41.8	—	—	—	—	—	—	—	—	—	—	—	—
	I	—	13.7	0.0	0.0	—	23.9	10.6	0.0	0.0	10.2	4.3	2.1	0.0
	II	—	15.2	0.0	0.0	—	24.2	11.9	0.0	0.0	13.1	8.1	2.5	0.0
	III	—	16.2	0.0	0.0	—	25.1	12.7	0.0	0.0	14.7	8.9	3.7	0.0
	IV	—	18.1	10.6	8.7	—	27.6	14.1	9.6	7.0	16.8	13.1	3.9	3.6
Hussaini	—	36.4	—	—	—	—	—	—	—	—	—	—	—	—
	I	—	18.1	0.0	0.0	—	24.3	12.1	0.0	0.0	12.6	4.1	1.7	0.0
	II	—	21.3	0.0	0.0	—	25.2	13.7	0.0	0.0	14.9	5.9	2.1	0.0
	III	—	24.1	0.0	0.0	—	25.6	13.9	0.0	0.0	18.7	5.2	3.6	0.0
	IV	—	26.7	16.0	11.5	—	28.9	17.2	15.4	11.8	19.2	9.6	4.2	3.1
Rosem-	—	31.6	—	—	—	—	—	—	—	—	—	—	—	—
T-	I	—	21.6	18.6	0.0	0.0	17.5	8.3	1.7	0.0	7.6	5.1	2.1	0.0
Lahore	II	—	22.5	15.0	0.0	0.0	18.4	11.9	2.1	0.0	9.1	6.2	2.6	0.0
	III	—	23.3	19.6	0.0	0.0	19.5	12.7	2.3	0.0	10.8	8.3	2.4	0.0
	IV	—	27.1	24.0	14.0	11.5	20.2	16.2	3.6	0.0	13.8	10.1	3.2	0.0

overturn, thereby covering the stigma throughout. Hence, in such treatments the changes of these buds getting crosspollinated are very poor. The ovaries of buds treated with higher concentrations of FW-450 were distorted and many were found to be elongated.

Fruit set by selfing the treated panicles

To confirm the extent of male sterility, calculated by the staining method and visual observations of the buds, data were collected on the extent of fruit set, when the treated panicles were merely bagged. The observations for the year 1962 are given in Table 4 and are represented diagramatically in Fig. 6 for the varieties Bhokri and Rosem-T-Lahore. The observations recorded during the year 1963 were not included in Table 4 since analogous results were obtained in both seasons.

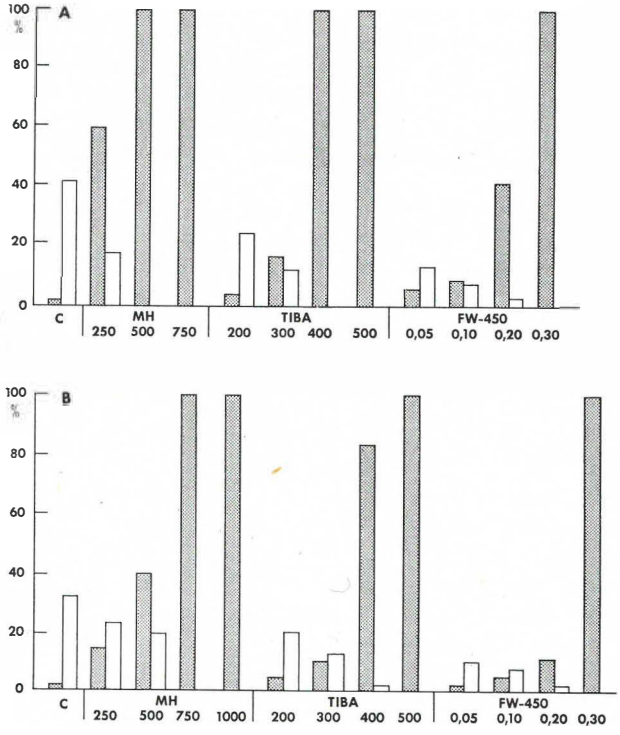

Fig. 6: Relation between pollen sterility and fruit set in treated grape panicles after self-pollination.
A: Bhokri, B: Rosem-T-Lahore; white columns: per cent fruit set; black columns: per cent pollen sterility, C: control.

As to be seen in table 4, the panicles of the variety Bhokri, that were treated twice with 250 ppm MH, set fruit after selfing indicating that complete sterility was not induced. However, a reduction in fruit set could be observed when the percentage was only 16.2 (stage III) as compared to the control (41.8). Treatments with MH 500 and 750 ppm inducing complete pollen sterility did not show any fruit set on

selfing showing complete absence of viable pollen. But when the same treatments were given during stage IV, fruit setting did take place since the pollen sterility was not complete. Treatments with TIBA at 400 and 500 ppm and FW-450 at 0.30% also gave no fruit set on selfing confirming the early observation that these treatments had induced complete pollen sterility. It was also observed that fruit set in treatments which had induced only partial pollen sterility went on increasing as the stage of application advanced. To quote one example, the fruit set in Bhokri was observed to be 13.7, 15.2, 16.2 and 18.1 per cent when 250 ppm MH was applied during stages I, II, III and IV, respectively. These differences in fruit set could be expected because of the differences in pollen sterility observed earlier in these treatments. The relationship between pollen sterility and the fruit set observed on selfing is shown in Fig. 6. As the amount of sterile pollen increases, fruit set drops until the value for fruit set reaches zero in treatments where complete sterility was obtained. The fruit set in FW-450 treatments was much lower even at concentrations which had caused very little pollen fertility. This could also be attributed to the adverse effect of FW-450 on the ovule fertility.

Table 5

Percentage of fruit set by hand pollination in treatments giving complete pollen sterility

Variety	Stage at which treated	Control		MH						TIBA				FW—450	
				500 ppm		750 ppm		1000 ppm		400 ppm		500 ppm		0.30%	
		1962	1963	1962	1963	1962	1963	1962	1963	1962	1963	1962	1963	1962	1936
Bhokri	—	53.1	51.9	—	—	—	—	—	—	—	—	—	—	—	—
	I	—	—	30.5	32.5	17.5	18.9	—	—	12.3	10.5	9.3	9.9	11.4	10.9
	II	—	—	32.1	35.3	20.1	19.2	—	—	13.5	14.6	12.8	11.7	14.5	16.7
	III	—	—	36.9	37.3	23.6	26.5	—	—	15.6	17.1	13.6	15.5	16.9	15.3
Hussaini	—	42.7	40.5	—	—	—	—	—	—	—	—	—	—	—	—
	I	—	—	26.5	24.4	13.6	12.7	—	—	11.6	9.2	8.5	7.1	10.1	8.6
	II	—	—	31.1	30.0	15.1	13.9	—	—	12.5	10.3	9.2	9.1	11.9	9.3
	III	—	—	33.6	31.7	16.8	15.7	—	—	15.6	13.7	11.2	8.5	12.2	11.6
Rosem-	—	37.1	36.3	—	—	—	—	—	—	—	—	—	—	—	—
T-	I	—	—	—	—	21.9	20.6	14.7	15.3	—	—	13.7	12.6	11.1	10.9
Lahore	II	—	—	—	—	23.6	22.3	16.3	17.2	—	—	14.5	12.9	13.5	11.4
	III	—	—	—	—	25.6	24.8	18.0	17.1	—	—	15.1	15.8	14.1	14.6

Fruit set by controlled pollination

The percentage of fruit set obtained by hand pollination is given in Table 5 and shown in Fig. 7. Data for hand pollination are given only for treatments producing complete pollen sterility. It is evident from table 5 that in all cases fruit set was less than that of the control. In Bhokri the control gave a fruit set of 53.1 per cent whereas the highest fruit set obtained among treated panicles (MH 500 ppm) was 37.3 per cent in the two years, respectively. The lowest fruit set (13.6%), observed in Bhokri, resulted from treatments with TIBA (500 ppm) in 1962. Highest fruit set in Hussaini (33.6%) gave a treatment with 500 ppm MH and the lowest (11.2%) with 500 ppm TIBA as compared to 42.7 per cent in the control.

As to Rosem-T-Lahore MH 750 ppm set highest percentage of fruits by hand pollination (25.6%) followed by MH 1000 ppm (18.0%), TIBA 500 ppm (15.1%) and

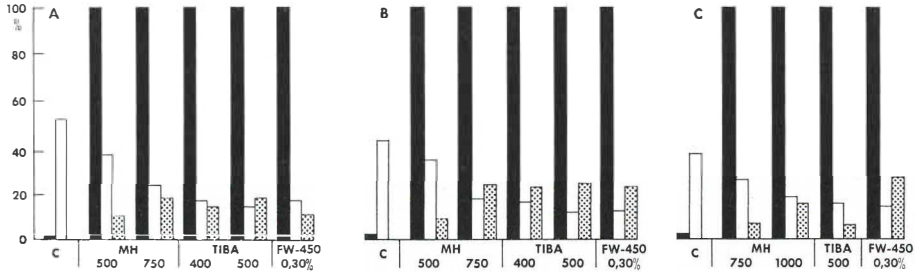

Fig. 7: Relationship between pollen sterility, fruit set (by hand pollination) and seed set in treatments giving complete sterility.

A: Bhokri, B: Hussaini, C: Rosem-T-Lahore; black columns: per cent pollen sterility; white columns: per cent fruit set; dotted columns: per cent reduction in seed set; C: control.

FW-450, 0.30 per cent (14.1%) during the year 1962 as compared to a set of 37.1 per cent in the control. In the succeeding year identical results were observed.

Furthermore, the table shows that the highest percentage of fruit set was obtained in all varieties from panicles treated at stage III. Within a treatment with a particular concentration stage I gave the least fruit set. This indicates that the chemicals, when applied at a very early stage, adversely affect the ovules while inducing pollen sterility. In order to get good fruit set, with the use of good pollen in a complety pollen sterile panicle, chemicals have to be sprayed at stage III.

Seed set in treated panicles

Seed set in the treated panicles giving complete male sterility was studied to find out whether there was any significant reduction in seed number per fruit. The observations are summarized in table 6. A significant reduction in seed set during all treatments compared to the controls was found in the varieties Bhokri and Hussaini.

In Bhokri, though all treatments differed significantly from the control, there were no significant differences in seed set between treatments MH (500 ppm) and FW-450 (0.30%). All other treatments differed significantly from each other. In the variety Hussaini all treatments differed significantly from the control, but no significant difference in seed set was noticed between treatments TIBA (400 ppm) and

Table 6

Effect of different chemicals on seed set*)

Variety	Control	MH 500 ppm	MH 750 ppm	MH 1000 ppm	TIBA 400 ppm	TIBA 500 ppm	FW—450 0.30%
Bhokri	1.38	1.24 (10.14)	1.12 (18.84)	—	1.18 (14.46)	1.14 (17.38)	1.24 (10.14)
Hussaini	1.64	1.50 (8.53)	1.26 (23.17)	—	1.28 (21.95)	1.26 (23.17)	1.28 (21.95)
Rosem-T-Lahore	1.5	—	1.40 (6.66)	1.26 (16.00)	—	1.40 (6.66)	1.10 (26.66)

*) The values given in parenthesis represent the percentage of reduction in seed set with regard to the control for each treatment.

FW-450 (0.30%) and between MH (750 ppm) and TIBA (500 ppm). As to Rosem-T-Lahore, only the treatment with FW-450 (0.30%) gave a significant reduction in seed set when compared with the control.

Seed germination

Seeds from the fruits of treated panicles were tested for their germinating capacity. This was studied only with the variety Bhokri, and seeds were used only from those treatments, which induced complete male sterility. 240 seeds were sown from each treatment using four replications. The comparison of the effects of different concentrations of different chemicals on the viability of seeds was made by adopting a randomised block design and the mean values of each treatment were compared for significance of difference with the corresponding S. E. (d) and C. D. The mean number of seeds germinated (out of 60 seeds) were 40.50 in MH (500 ppm), 37.75 in TIBA (500 ppm) and 32.25 in FW-450 (0.30%) as compared to 39.75 in the control.

The statistical analyses gave no significant differences between any treatments or with the control showing that none of the chemicals had any adverse influence on the viability of seeds.

Discussion

The extent of male sterility that could be induced was found to depend upon the stage of the development of the inflorescence at which the chemical is applied, the concentration of the chemical, number of applications and the variety used for the study.

A very important factor seemed to be the stage at which sprays are given and it was found that only the early stages of bud development responded well for the induction of pollen sterility.

When treatments were given at a later stage, a complete or even a high amount of sterility could not be obtained in any case. Although it is known that the application of the chemicals should be prior to the initiation of meiosis, the precise stage and the exact mechanism, by which pollen abortion occurs, has not been demonstrated so far. One possibility, however, has been shown to be the competition of the chemicals with pantoate for a site on the enzyme of pantothenate synthesis (Hilton 1958). During the present investigation it was noted that the stages of bud development, at which the chemicals were most effective, were at the pre-meiotic or at very early stages of meiosis. Ohta and Matsuma (1962) based upon their experiments with sugar beets using FW-450, concluded that the induced pollen sterility was due to the inability of the pollen grains to absorb nutrients from the tapetal cells, which developed abnormally and showed hypertrophy. Tapetal secretion of waxy material is important for the formation of exine.

The stage of development of the panicle was also found to influence the ovule fertility. It was observed that although complete pollen sterility could be obtained with certain treatments during the stages I, II and III, the ovule fertility in these three sets differed greatly among each other, the latter stages giving higher fertility. This suggests that the sensitivity of the ovules is higher at an early stage, and as they develop, the capacity to overcome the inhibitory effects become higher.

All chemicals used, viz. MH, TIBA and FW-450, induced pollen sterility in all the grape varieties tested, the extent of sterility depending on concentration of the chemical. The mode of action by which these chemicals bring about male sterility ap-

pears to be slightly different from each other since the appearance of the degenerated pollen looked different when different chemicals are used.

With MH and TIBA the pollen assumed a plasmolysed appearance, while shape and size of the sterile pollen obtained after a treatment with FW-450 were identical with those of the check plants. Besides inducing pollen sterility, FW-450 prevented the dehiscence of anthers which, as a rule, could be considered as male sterile. This was not observed with the other two chemicals.

Another interesting observation was the fact that the chemicals had to be sprayed twice in order to induce complete sterility thus showing that the sensitive phase is fairly long and the absorption of the chemical and its breakdown is slow.

Furthermore, intra-varietal differences in the responses to the chemicals could be demonstrated, since in the varieties Bhokri and Hussaini the minimum effective concentration of MH was 500 ppm and in Rosem-T-Lahore 750 ppm. This may partly be due to the presence of thick tomentum on the leaves of the latter variety which does not permit the solutions to penetrate the leaf. Inter-specific and inter-varietal variation in response to the chemicals have been demonstrated in many crops (see McIlrath 1953, Jain 1959 and Dudley 1960).

Effects on fruit set and ovule fertility

While attempting to standardise a technique to induce male sterility which will be of practical use, the extent of fruit set and seed set is to be taken into consideration. Many authors have observed a combined reduction of seed set and pollen sterility in similar experiments (Rehm 1952, Chopra *et al* 1960, Lippert and Hall 1961, Kho and de Bruyn 1962). Therefore, in the present investigation a comparison was made between the treatments (only of those which induced complete male sterility) to determine which one had the least adverse effect on the fruit and seed set. Undoubtedly, none of these panicles gave any fruits after self-pollination, thus indicating that pollen sterility was complete. However, the absence of fruit set when selfed could also be due to the cumulative effect of both pollen and ovule sterility. Therefore, panicles were also pollinated with good pollen to estimate the extent of ovule fertility. The results with hand pollination indicated that in Bhokri and Hussaini the highest fruit set obtained was with MH 500 ppm, within different treatments, although this was less than those of the controls. In Rosem-T-Lahore MH 750 ppm had the least drastic effect on ovule fertility. This made clear that among the three chemicals used, maleic hydrazide is the most promising one to be used as a selective gametocide, though it caused a reduction in fruit set in comparison to the control. But this reduction may not be a big handicap in a large scale hybridisation since using MH treatments it is possible to increase the number of cross-pollinated flowers within a limited time compared with conventional hand emasculation.

A standardised method for the induction of pollen sterility must ensure that seed set and germination should not be seriously affected. Nevertheless, contradictory views regarding the effects on seed set in different plants have been reported. While Walkof (1958), Walker (1959), Putt (1959) and Ramakrishna (1964) observed that all treatments giving complete male sterility caused serious injuries to ovules, thus questioning the practical usefulness of these chemicals. Roble and Honma (1959), Chopra *et al* (1960) and Wit (1960) foresaw a good future to the use of these chemicals. In the present study, however, all treatments inducing complete pollen sterility also reduced seed set, the reduction in seed set was not high in MH treatments, thereby indicating that among the tested chemicals MH alone offers a possibility to be used

as a selective gametocide. With regard to seed germination, none of the treatments adversely affected the germination capacity of the seeds.

Effectof the chemicals on the foliage

All chemicals gave rise to abnormalities normally resulting frcm the application of plant growth regulators on plants. The extent of damage to the shoots depended on the concentration of the chemical. The immediate response to MH and TIBA applied to the leaves of grape varieties was epinasty or a downward curling and bending of the leaves. But in the TIBA treated shoots, some leaves tended to curl upwards also (hyponasty). The epinastic curling and distortion of leaves has been observed by many authors while experimenting with different growth regulators in a variety of crops (ZIMMERMAN and WILCOXON 1935; ZIMMERMAN 1951; MCILRATH 1953; LOUSTALOT and MUZIK 1953; CHOPRA *et al* 1960 and CHOUDHARY and GEORGE 1962). The epinastic effects might be due to the inhibition of cell enlargement and cell division in the young leaves. It appears that the effect of two of the chemicals (MH and TIBA) persists for a long time since even newly formed leaves showed many deformities during a certain period. The present study also revealed that the severity of damage differs from variety to variety. The varieties Bhokri and Hussaini showed symptoms of relatively higher toxicity than Rosem-T-Lahore. This may be due to the thick leaves with a dense tomentum of Rosem-T-Lahore. Another interesting observation made during the present investigation was, that the application of MH in two separate treatments gave response equivalent to that resulting from an equal dosage applied in one treatment. This leads to the conclusion that the breakdown of absorbed MH is slow and the effect of the chemical is cumulative.

Conclusions

The present investigation indicates that chemical induction of pollen sterility is possible in grapes although with a slight accompaniment of ovule sterility. All the three chemicals have been shown to have gametocidal activity in grapes but only MH holds promise since the adverse effect of this chemical on fruit and seed set was the lowest. The different responses of the varieties tested would be perhaps a hindrance to the immediate ccmmercial use of this method. Nevertheless, the present study has paved way to future line of work in elucidating different problems connected with chemical emasculation in grapes.

Summary

The morphology of the grape flower renders hand emasculation very tedious and hence hinders large scale hybridisation work. Chemical induction of male sterility which has been successfully achieved in some crops may likewise aid in grape breeding, if the method proves practicable. This paper deals with an experiment designed to achieve this object.

Three varieties of grapes, Bhokri, Hussaini and Rosem-T-Lahore (all belonging to *Vitis vinifera)* were tested with three different chemicals, viz. maleic hydrazide, tri-iodobenzoic acid and FW-450, using various concentrations. In the first two varieties the dosages of maleic hydrazide 500 ppm, tri-iodobenzoic acid 400 ppm and FW-450 0.30% was found to induce complete pollen sterility. However, the third variety needed higher concentrations. In all cases two applications of the chemicals were necessary to induce complete pollen sterility.

In addition to inducing pollen sterility, FW-450 prevented anthesis and anther dehiscence. Considering the fruit set and seed set obtained in the male sterile flowers (induced by various chemicals) by using good pollen, maleic hydrazide appears to be most promising. The chemical treatments did not influence seed germination. The most important factors with regard to the use of this method were (1) the variety used, (2) the chemical applied, (3) the number of applications of the chemical and (4) the stage at which the chemical treatments are made. The implications of these results have been discussed.

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