CHEMICALLY INDUCED MALE STERILITY, A NEW TOOL IN PLANT BREEDING?

MET SAMENVATTING

CHEMISCHE INDUCTIE VAN MANNELIJKE STERILITEIT, EEN NIEUWE METHODE IN DE PLANTENVEREDELING?

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CHEMICALLY INDUCED MALE STERILITY, A NEW TOOL IN PLANT BREEDING?

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ABSTRACT

Plants of red clover, white clover, beets, turnips and perennial ryegrass were sprayed with solutions of Na 2.3-dichloroisobutyrate a short time prior to flowering.

Four to five weeks after treatment with definite dosages a moderate to very high degree of male sterility occurred in the first three crops, while no reduction of growth or flowering could be established.

Perennial ryegrass and turnips showed little or no response. Presumably for these crops the treatment was applied at too late a stage.

Observations on fruit set gave the impression that in beet even at a high degree of male sterility, there may be little or no influence on female fertility. In red clover the selectivity of the gametocide effect was much less manifest.

Some prospects were discussed concerning the application of chemical induction of male sterility for breeding purposes.

1. INTRODUCTION

In a fairly large number of bisexual or monoecious crops plants have been found which by their specific hereditary constitution produce little or no functional pollen whereas their ovular fertility is completely or almost completely unimpaired (VAN DER KLEY, 4).

Such male-sterile plants are effective females for a crossing programme; their employment makes the laborious procedure of emasculation superfluous. The application of male-sterility may permit the production of hybrid seeds and the commercial exploitation of heterosis in crops where emasculation on a large scale is not feasible. However, this procedure requires a time-consuming breeding programme when male-sterility must be combined with a large number of agronomically desirable characters.

In recent years several attempts have been made to induce male sterility by treating plants with maleic hydrazide, tri-iodobenzoic acid, α-naphthalene acetic acid or 2.4-dichlorophenoxiacetic acid (JAIN, 3). ROSSMAN (5) reported some experiments with gibberellins. In some cases encouraging results have been obtained but none of the treatments seems to have been successful in practice.

Quite recently, however, the compound FW-450 of Rohm and Haas Cy, Philadelphia, U.S.A. (α, β-dichloroisobutyrate) has been shown to partially prevent pollen shedding in cotton (EATON, 2). The highly selective gametocide effect seems to permit the commercial production of hybrid cotton (1).

An easy, cheap and reliable induction of male sterility would allow in several crops a more economical production of hybrid seeds. Moreover it would offer new possibilities for the breeding of crops in which effective hereditary male sterility has not yet been discovered.

Cross-fertilizing plants might offer the most favourable prospects. Therefore some tests were carried out in 1959 to determine whether effects similar to those in cotton could be obtained in other crops by treatment with FW-450.

2. MATERIAL AND METHODS

In May 1959 we received a small quantity of FW-450 from Dr. W. VAN DER ZWEEP, Head, Weed Control Department of the Institute for Biological and Chemical Research on Field Crops and Herbage (I.B.S.), Wageningen.[1])

Plants of beets, red and white clover, perennial ryegrass and turnips were treated with solutions in water in the months of May, June and July 1959, from some days to some weeks prior to flowering. The concentrations varied from 0.5 to 0.9%, the quantities from 0.3 to 2.0 cm^3 solution per dm^2 area. The solution was applied by means of a spray mounted on a calibrated cylinder. A wetting agent was added in a concentration of 0.5% (Tween 80). All plants were grown in the open.

After treatment observations were made periodically on the degree of damage of leaves and flowers, the dehiscence of anthers and the appearance of pollen. The latter was treated with acetocarmine and studied under the microscope. In the beets and red clover some data were collected on the fruit set and on the percent germination of the seed obtained.

3. RESULTS

Male fertility in beets

The experiments with beets were carried out with annual plants of *Beta patula*, *B. Webbiana* and the 0-type of a male-sterile line of *B. vulgaris*. The plants were sprayed about three weeks prior to flowering. Plants of the first two species were treated with 0.9, 1.8, 2.7, 5.4 and 8.1 mg FW–450 per dm^2, those of the latter species with 1.0, 2.0, 4.0, 8.0 and 16.0 mg. Each treatment was carried out in duplicate.

Within a week after treatment the plants that had received more than 4.0 mg per dm^2 showed slight scorch symptoms in old leaves and chlorotic discolorations in young leaves. However, no serious damage was observed.

A first reaction of the flowers was retarded dehiscence of anthers. Both in the treated and the untreated plants most flowers opened in the early morning hours. On the untreated plants nearly all stamens dehisced before noon. The stamens of the treated

[1] Dr. VAN DER ZWEEP gave information on the gametocide effect of FW-450.

plants of all three species often remained closed on the first day. This phenomenon was studied more closely in *B. vulgaris* in the second week of flowering – five weeks after the treatment. In the afternoon 50 to 100 newly opened and 50 to 100 one-to-two-day-old flowers of four to five large side stalks of all the treated and of some untreated plants were examined by means of a magnifying glass. In this way a classification could be made as to flowers with closed anthers, and those where all or a few anthers had opened. The results are summarized in table 1.

TABLE 1. INFLUENCE OF DOSAGE ON THE DEHISCENCE OF ANTHERS IN *Beta vulgaris*

Treatment		Percent flowers with closed anthers	
Conc.	mg/dm^2	Young flowers	Old flowers
0	0	4	0
0.2	1	35	19
0.4	2	43	15
0.2	4	93	70
0.8	4	87	70
0.4	8	90	93
0.8	16	98	72

From these data it appears that after treatment with at least 4 mg per dm^2 the anthers of some 90% of the flowers did not dehisce on the first day. In about 70 to 90% of the flowers they remained non-dehiscent on the next day. They then had turned from yellow to light or dark brown. Many anthers also showed a somewhat different colour on the first day, even in the bud.

The observations on flowers were supplemented with a microscopical examination of the contents of the anthers. In the non-treated plants these consisted for some 95% of fairly globular well-filled pollen kernels which could be stained light red by aceto-carmine. They had a diameter of about 20 μ (fig. 1). Such kernels are called normal in the following discussions. In the flowers with non-dehiscent anthers of treated plants of *B. patula* and *B. Webbiana* at most 15% of such kernels were found. The others were only partly filled or entirely empty, could not be stained by acetocarmine (or only very slightly) and had a diameter of about 12 μ. Mostly all kernels were abnormal (fig. 2).

In the beginning of flowering and in the then following week a number of flowers with dehiscent and with non-dehiscent anthers of *Beta vulgaris* were examined. The data are presented in table 2.

TABLE 2. PERCENTAGE NORMAL POLLEN IN DEHISCENT AND NON-DEHISCENT ANTHERS OF *Beta vulgaris*

Treatment	Age of flowers	Anthers	Percent good pollen					
			Beginning of flowering			Second week of flowering		
			min.	max.	average	min.	max.	average
Non-treated	young	dehiscent	90	96	94	85	96	94
Treated	young	dehiscent	42	96	77	3	81	51
		non-dehiscent	0	30	10	0	28	8
Treated	old	dehiscent	–	–	–	0	18	7
		non-dehiscent	–	–	–	0	2	1

The percentages stated in the table are not very accurate: per group only some tens of flowers were examined and per flower at most some hundreds of pollen kernels. However, the data show clearly that from the anthers that did not dehisce on the day of opening of the flowers few or no normal pollen kernels emerged on the following days. It may be a question whether such seemingly normal pollen kernels might have been able to cause fertilization.

From the combined examination of anthers and pollen it can be concluded that plants treated with a least 4 mg FW-450 per dm^2 were highly male-sterile during full bloom (five weeks after the treatment). Presumably this male sterility was less strongly developed at the beginning and at the end of flowering. In various cases on the side stalks of the second order higher percentages of flowers with dehiscent stamens were found. However, on a few plants which had been treated with a large dose even on the smallest side stalks almost exclusively flowers with non-dehiscent anthers were found. These plants were almost entirely male-sterile during a large part of the flowering period. The anthers mostly were pale and waxy and were very similar to those of the hereditary male-sterile counterpart of the 0-line. The pollen could be liberated only after vigorous pressing, it was mostly clustered together and consisted of very small, empty and markedly shrivelled kernels.

Female fertility

The stand was too dense to be able to determine the total seed production per plant at maturity. In order to gain an impression of the effect of the treatment on female fertility in *B. patula* and *B. Webbiana* 5 branches of the same position and size were collected of all the treated and of 5 adjacent non-treated plants. In *B. vulgaris* those branches of which flowering had been observed and noted were harvested. Unfortunately a comparison of corresponding branches of treated and untreated plants was not possible, since the latter through a misunderstanding were harvested in bulk.

For each plant the seed clusters harvested were classified into two groups, those with a diameter greater than 3 mm and those with a smaller diameter. The latter mainly consisted of undeveloped or only partly developed clusters which had been formed on the extreme end of the branches. In practice such small clusters are removed with threshing and cleaning. They germinated for only 0 to 36% (on the average 6%). The percentage large clusters can therefore be used as a standard for fruit set; the number of large clusters per dm stalk length was taken as a standard for productivity.

The seed clusters were rinsed for one hour 3 to 4 weeks after the harvest and then placed on moist filter paper in a thermostate. The temperature in this apparatus was kept at 20 °C by day and at 29 °C at night. Germination proceeded very slowly in the clusters of both the treated and the control plants. Possibly the inhibitors occurred in abnormally high quantities because the clusters were harvested prematurely and were not allowed sufficient time to ripen out.

This explanation is supported by the fact that the germination was most regular and most rapid in *B. patula* of which the clusters at the harvest had most nearly approached maturity; it was slowest and most irregular in the seed of *B. vulgaris* which was harvested in a green condition.

The date obtained on female fertility of the species *B. Webbiana* and *B. patula* have been summarized in table 3, together with those of informative observations on the damage of the plants and on male sterility. The number of clusters formed per dm stalk showed no correlation with the dosages and therefore was not included in the table.

TABLE 3. INFLUENCE OF THE DOSAGE ON THE PLANT AND ON THE MALE AND FEMALE FERTILITY IN *B. Webbiana* AND *B. patula*

Treatment		*Beta Webbiana*				*Beta patula*			
Conc. %	Dosage mg/dm²	Plant damage	Pollen sterility	Fruit set %	Percent germination	Plant damage	Pollen sterility	Fruit set %	Percent germination
0.0	0	0	0	79	83	0	0	78	86
0.3	0.9	0	0	70	75	0	±	76	93
0.6	1.8	0	±	81	91	±	+	71 (76) [1]	71 (96)
0.3	2.7	±	±	71	92	±	++	80	95
0.9	2.7	±	+	76	96	±	++	*68*	*60*
0.6	5.4	+	+++	77	91	+	+++	*55*	*58*
0.9	8.1	++	+++	*59*	*71*	++	+++	*63*	*71*

± very slight + slight ++ moderate +++ strong

[1] The duplicates were widely different here because the largest clusters of one of the plants had already fallen at the harvest. The values of the completely harvested plant have been indicated between brackets.

From the table it is apparent that the treatment has exerted a greater influence on the male fertility than on the female. After definite dosages a fairly high degree of pollen-sterility was obtained while the growth of the plant, the fruit set and the percent germination of the clusters were affected little or not at all. Furthermore the data point to a clear difference in sensitivity between the two species, both as concerns the gametocide and the herbicide effect.

The most favourable effect was obtained in *B. Webbiana*, after a dosage of 5.4 mg/dm². The plants of *B. patula* gave the most satisfactory result after treatment with a solution of 0.3% and a dosage of 2.7 mg/per dm².

The results obtained in plants of *B. vulgaris* are presented in table 4. Because of the large differences between the duplicates the data of each separate plant have been mentioned.

Table 4 shows an irregular picture which is a little difficult to interpret. If we take as standards for fruit set of the untreated plants those of table 3, the following conclusion can be drawn. After a dosage of 4 mg/dm² with a solution of 0.2% a very high degree of male sterility was obtained, while the plant was not damaged and the fruit set was not reduced. After treatment with a larger dosage or with a higher concentration the fruit set was somewhat reduced, although it was normal again on one of the plants which had been treated with 16.0 mg/dm².

The value to be attached to the germination figures is uncertain. Even the samples of the untreated plants of *B. vulgaris* showed a rather slow irregular and poor germination. Of each pair of similarly treated plants appearing in table 4 the second plant was harvested a week later than the former. In general the percent germination of this more mature seed was considerably higher than that of the less mature seed. In a few cases it was even higher than that of the samples of the non-treated plants. It

TABLE 4. INFLUENCE OF THE TREATMENT ON PLANTS OF *B. vulgaris*

Treatment		Plant damage	Flowers with non-dehiscent anthers %	Fruit set %	Percent germination
Conc. %	Dosage mg/dm²				
Contr. 0-type	0				76
Contr. M.S.-type	0				73
0.2	1.0	0	65	75	45
0.2	1.0	0	2	82	85
0.4	2.0	0	33	76	47
0.4	2.0	0	52	70	34
0.2	4.0	0	98	74	50
0.2	4.0	0	83	79	62
0.8	4.0	±	80	61	31
0.8	4.0	±	92	67	82
0.4	8.0	+	79	67	66
0.4	8.0	0	100	63	77
0.8	16.0	++	99	78	11
0.8	16.0	++	98	71	24

seems justified therefore, to assume that the low germination figures are partly due to an insufficient maturity of the seed clusters. However, from the lower lines in tables 3 and 4 it must be concluded that the heavy treatments tend to be detrimental to the germination of the seeds.

4. RESULTS OBTAINED IN RED CLOVER

The red clover material consisted of small pot-plants of early flowering Dutch local strains which were transplanted in May. Part of them were treated at the beginning of the first flowering stage. The rest were cut off and treated shortly before the second flowering stage.

In the first series, concentrations of 0.3%, 0.6% and 0.9% were used and dosages of 1.5 to 15 mg/dm². A week after the treatment almost all plants showed scorched spots on leaves and buds. Most flower buds were so injured that they did not develop further or they formed only small heads with withered flowers.

In the period between 6 and 8 weeks after treatment new flower heads appeared. The plants that had been treated with dosages of 1.5 and 3.0 mg per dm² produced a fairly large number of normal heads of which the male fertility was disturbed little or not at all. The dosage of 4.5 mg per dm² resulted in a slight reduction in the number of heads but in a great reduction of the pollen fertility. A large number of anthers did not dehisce and some 50% of the pollen was clearly abnormal. In plants that had received higher dosages the percentage of normal pollen kernels was reduced to 30% but these plants flowered only sparsely.

The treatment of the second cutting was accomplished partly with weaker concentrations and weaker dosages. During full bloom – a month after spraying – the number of heads per plant was counted; a given number of them were labelled. The stamens of some 5 flowers per head were examined; at maturity the number of seeds produced per head and the percent germination were determined. The results of these determinations are summarized in table 5.

The percentages mentioned in column 4 apply to flowers of which none of the anthers dehisced; of an approximately equally high percentage of flowers only part of the anthers dehisced.

TABLE 5. INFLUENCE OF THE TREATMENT ON RED CLOVER

Treatment		Average number of heads per plant	Percent flowers with non-dehiscent anthers	Percent normal pollen	Number of seeds per head	Percent germination
Conc. %	Dosage mg/dm²					
0	0	28.5	0	95	57	94
0.05	0.25	20.0	0	94	75	98
0.1	0.50	43.0	27	59	57	91
0.05	1.00	42.0	18	74	40	89
0.2	1.00	29.5	26	55	27	91
0.1	2.00	36.0	33	39	22	81
0.4	2.00	8.5	46	33	36	80
0.2	4.00	3.0	(75) [1]	–	–	–
0.8	4.00	11.0	(42) [1]	–	–	–
0.4	8.00	1.5	(67) [1]	–	–	–
0.8	16.00	1.5	–	–	–	–

[1] Less than 20 flowers examined.

The data mentioned in this table only have an informative value. Each treatment was carried out with only two plants; at most 5 or 6 heads per plant and only about 5 flowers per head were examined. Sometimes flowers occurred in one and the same head with 95% and with 10 or 0% normal pollen. In some flowers even both dehiscent and non-dehiscent anthers were found, with preponderantly good pollen and exclusively or preponderantly poor pollen respectively. Accurate determination of the percentage good pollen per plant or per head would therefore have been extremely time-consuming.

From table 5 it follows that concentrations of 0.05 to 0.2% and dosages of 0.5 to 2.0 mg per dm² induced a clear reduction of the male fertility, whereas no reduction in the number of heads could be established. After the most extreme of these treatments the reduction of male fertility was accompanied by a clearly reduced female fertility, indicated by the number of seeds per head.

If a practical application is possible this study shows that concentrations of approx. 0.05 and 0.1% and dosages of approx. 0.5 and 1.0 mg/dm² should be tried. Such treatments however are not likely to induce a high degree of male sterility, unless an important part of the outwardly normal pollen grains might be functionally abnormal. This will have to be investigated by pollination experiments.

5. RESULTS IN OTHER CROPS

The results of the experiments with white clover, turnips and perennial ryegrass will be recorded only summarily because they do not permit any clear conclusions. In white clover a treatment of 1.0 mg per dm² in a concentration of 0.2% gave a very high degree of male sterility, without damage to leaves or buds. During full bloom, in the fourth week after treatment, all anthers remained closed in 85% of the flowers. However, the influence on female fertility could not be established.

Turnips were treated shortly before or during the beginning of flowering. Not until the end of the flowering period, in the fourth week after treatment, were some non-dehiscent stamens with abnormal pollen grains found in some of the flowers. The highest percentage of flowers with aberrant stamens in this period was 35%. This occurred after the highest dosage and was accompanied with a reduced fruit set.

The plants of perennial ryegrass were treated shortly before or during the beginning of ear emergence. In the fourth week the plants came into flower, and in the fifth week observations were made. No non-dehiscent stamens or abnormal pollen kernels were found.

6. Discussion

From the results of the experiments described above it is apparent that 4 to 5 weeks after spraying with definite quantities of FW-450, plants of red clover, white clover and beet developed a moderate to very high degree of male sterility, while flowering was not clearly influenced unfavourably.

According to our provisional results a high degree of this male sterility in beets is not necessarily accompanied by a reduced female fertility or a reduction in the percent germination of the seeds. In principle this might offer the possibility of utilizing induced male sterility in breeding heterosis varieties, either as a temporary aid in the initial stages of the breeding programme, or as a permanent cultural measure for the production of hybrid seed. Even if the method might not be 100% effective, it could enable the breeder to produce in an economical way sufficient quantities of hybrid seed to test the value of large numbers of hybrid populations on a commercial scale. The most promising combinations could be stabilized later on by introducing a plasmatic factor for male sterility in one or more lines.

As a permanent measure the treatment could be used to support insufficiently stable forms of male sterility to prevent their break-down. However, the greatest prospects will be opened when a technique can be developed that guarantees an efficient male sterility without involving risks in the seed production. The production of hybrids and of triploids could be greatly improved in this way.

To ascertain how far the above-mentioned possibilities can be realized, more extensive investigations have to be conducted. The presence in beet clusters of several germs derived from flowers that bloomed at different days alone makes this necessary. Through observation of the flowers a reliable impression of the male and female fertility is obtained only when all flowers of a cluster are examined on these characters. Such investigations will be extremely difficult and time-consuming. Ultimately the main point is what percentage of seedlings from seed clusters of treated plants originated from crosses with untreated plants. This percentage can be established by planting rows of plants containing anthocyanin between rows of anthocyanin-free individuals and only treating the latter. After sowing the resulting seed clusters it can be ascertained whether the percentage of desired hybrids is sufficiently high for practical application.

In this way it can be established also whether a distribution of the dosages over more treatments is desirable for the maintenance of a sufficiently high degree of male sterility during a longer period. Finally the influence of the treatment on the seed production should be studied more closely.

The prospects for practical application are far less favourable in red clover, since the selectivity of the gametocide effect seems to be much less pronounced in this case. It is difficult to predict how far white clover offers better prospects.

The plants of perennial ryegrass and turnips were presumably sprayed too late, JAIN (3), in an attempt to induce male sterility in wheat by means of maleine hydrazide, obtained the most favourable effect in treating the plants in the early tillering stages. Perennial ryegrass was not treated before the beginning of ear emergence. The possibility is therefore not excluded that application of FW-450 in an earlier stage will be successfull in perennial ryegrass. The same holds for turnips.

Treatment with FW-450 apparently can lead to degeneration of pollen kernels in plants of widely different families. The dosage required and the quantity which can be tolerated without damage to female fertility seem to vary greatly from species to species.

Presumably also the developmental stage and the weather conditions will have some influence.

SAMENVATTING

Chemische inductie van mannelijke steriliteit, een nieuwe methode in de plantenveredeling?

Planten van rode klaver, witte klaver, bieten, stoppelknollen en Engels raaigras werden korte tijd voor de bloei bespoten met oplossingen van Na 2.3-dichlooriso-butyraat.

Vier à vijf weken na de behandeling met bepaalde doseringen trad bij de drie eerstgenoemde gewassen een matige tot een zeer sterke graad van mannelijke steriliteit op, zonder dat vermindering van de groei of van de bloei viel te constateren.

Engels raaigras en stoppelknollen vertoonden geen of slechts een zwakke reactie. Waarschijnlijk werd hier echter de behandeling in een te laat stadium uitgevoerd.

Oriënterende waarnemingen over de vruchtzetting gaven de indruk dat bij bieten zelfs bij een hoge graad van mannelijke steriliteit de vrouwelijke fertiliteit weinig of niet werd beïnvloed. Bij rode klaver kwam de selectiviteit van de gametocide werking veel minder tot uitdrukking.

Enkele perspectieven die de toepassing van chemische inductie van mannelijke steriliteit voor de veredeling zou kunnen bieden, werden besproken.

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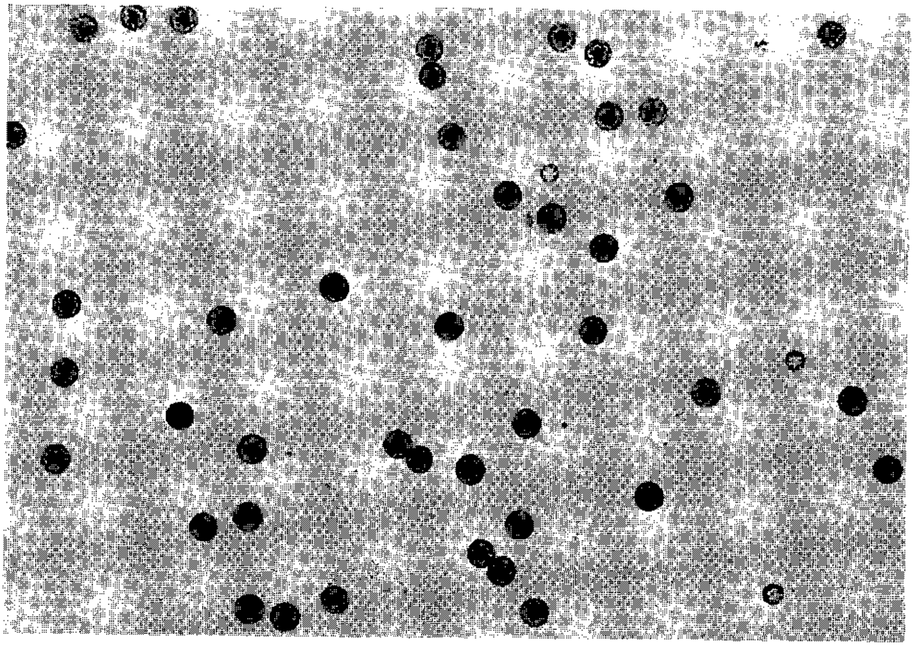

FIG. 1. POLLEN GRAINS OF NON-TREATED PLANTS OF *Beta vulgaris;* DIAMETER 20 μ

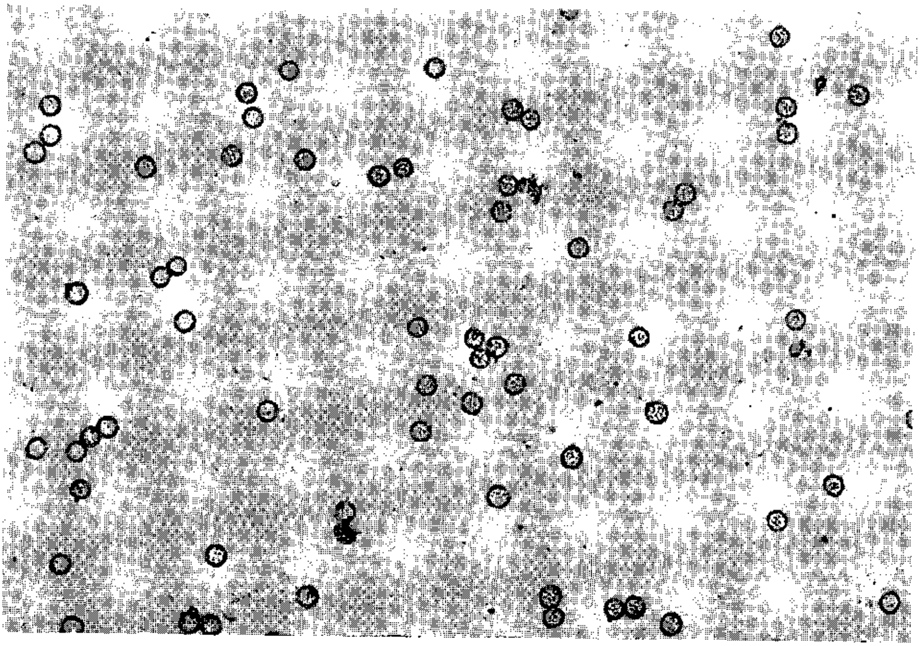

FIG. 2. POLLEN GRAINS OF TREATED PLANTS OF *Beta vulgaris;* DIAMETER 12 μ

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