

STUDIES ON THE EFFECT OF
F, W-450 AND MH AS MALE GAMETOCIDES
IN CHILLIES (*Capsicum frutescens* L.)

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THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF
MASTER OF SCIENCE (AGRICULTURE) IN
(CYTOGENETICS AND PLANT BREEDING)
OF THE UNIVERSITY OF KERALA

DIVISION OF AGRICULTURAL BOTANY
AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE
VELLAYANI, TRIVANDRUM

1967

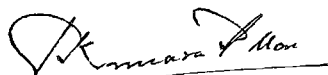
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C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Shri S. Sadasivan Pillai under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.



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A C K N O W L E D G M E N T

The author wishes to express his deep sense of gratitude and indebtedness to Prof. P. Kumara Pillai, M.Sc., M.S.(U.S.A) Vice-Principal and Head of the Division of Agricultural Botany and Shri K. Srinivasan, B.Sc., B.Sc.(Ag.), D.H., M.Sc.(Ag.), Junior Professor of Agricultural Botany for their inspiring guidance throughout the course of the study and in the preparation of this thesis.

The author is grateful to Dr. C.K.N. Nair, M.Sc., Ph.D. (Cornell), D.R.I.P. (Oak Ridge), Principal and Additional Director of Agriculture (Research), Agricultural College and Research Institute, Vellayani, for providing all the necessary help and facilities during the course of this study.

The author expresses his sincere thanks to Shri E.J. Thomas, M.Sc., M.S. (Iowa), Junior Professor of Agricultural Statistics, Agricultural College and Research Institute, Vellayani, for his valuable advice in the analysis and interpretation of the data.

The author also express his sincere thanks to Shri K. Karunakaran, M.Sc.(Ag.), Research Assistant, for his valuable help.

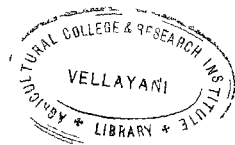
Finally, the author wishes to acknowledge with gratitude to all the staff members of the Division of Agricultural Botany and to all of his colleagues and friends for their kind co-operation and assistance.

S. SADASIVAN PILLAI.

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INTRODUCTION

INTRODUCTION

One of the most important practical applications of Genetics in crop improvement programme is the exploitation of Hybrid vigour or Heterosis. Male sterility is a device receiving increasing attention, as an aid to the production of large amount of hybrid seed, for raising hybrid varieties on a commercial scale. Isolation of male sterile lines and their large scale use for production of hybrid seeds in maize have inspired the waster minds of plant breeders to an extensive search to isolate spontaneously occurring male sterile lines in other crops.

The spontaneous occurrence of male sterile lines is a factor beyond human control. Hence a search for a means of artificial induction of male sterility was necessitated for a more extensive utilization of this phenomenon in breeding programmes. Investigation on the possibilities of using chemical gametocides, which aid in eliminating the manual labour of emasculation, as well as complete dependence on spontaneous male sterile lines is a revolutionary change in the 20th century chemical studies. A 'gametocide' is a substance which kills gametes and makes controlled pollination easier. If with the aid of such gametocides chemical emasculation can be successfully practise, it can augment the programme of hybrid seed production in crops lacking heritable male sterility. Only the chemicals which show selective male sterility can be used as a gametocide. The chemicals which had been tried as

gametocides are sodium, 2,3-dichloroisobutyrate (F,W-450),
1,2-dichloro 3,6-Pyridazine dione (Maleic hydrazide or MH) EIDA, NAA etc.

Chillies is an important vegetable and condiment crop, especially in southern regions of India. Its adaptability, the ease with which it can be cultivated, and its economic importance in every day human diet, make it an important vegetable crop in our state. If hybrid vigour can be exploited in this crop for yield and quality, and hybrid seed can be produced on a large scale, it will be a boon to the cultivators.

Hybrid vigour has been reported in chillies for height, number of branches and the number and weight of fruits per plant (Deshpande, 1953 and Pal, 1945). But the small and tender nature of the hermaphrodite flowers render the process of emasculation costly and laborious. Any kind of male sterility can eliminate this kind of cumbersome work, and can facilitate the technique of crossing, to make the maximum utilization of hybrid vigour or heterosis.

In chillies, heritable male sterility has not yet been reported. Hence an experiment for inducing male sterility by foliar application of chemical gametocides, F,W-450 (Sodium, 2,3-dichloroisobutyrate) and Maleic hydrazide (1,2-dichloro 3,6-Pyridazine dione) was undertaken. This investigation appears to be the first of its kind in this crop, and the practical application of it may aid cultivators for raising hybrid varieties on a commercial scale.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

I. Chemical induction of male sterility

Chemical induction of male sterility, a recent development in the application of chemicals in Agricultural practices, has become one of the branches of plant breeding programmes. This is a more recent development in India and only a few works have been reported. The first report was from Gujarat Agricultural Department in 1960, using the chemical gametocide, F,W-450 on cotton. The same experiment was also conducted by the Indian Cotton Committee, Bombay. These experiments showed that the chemical can be utilized for the induction of male sterility and for the production of hybrid seeds in cotton. The chemical emasculation is a valuable tool in the production of hybrid seeds on a mass scale at low cost. Some of the chemicals used for the induction of male sterility and their application in crop plants are reviewed.

(a) Sodium 2,3-dichloro isobutyrate (F, W-450)

F,W-450, is a selective chemical gametocide which inhibits the normal development of gametes. This chemical induces functional male sterility in crop plants without adversely affecting female fertility.

The chemical is water soluble and 100 per cent active. It can be applied as foliar sprays and is easily absorbed by plants. The translocation of the chemical has been reported to be greater towards apical meristems, especially to flowers and flower buds (Phom and Haas Company, 1960). Studies with C¹⁴ labelled F,W-450 by Hitton (1958) showed that this chemical accumulates to a greater extent in the anthers than ovules. The foliar characteristics, like pubescence, thickness of cuticle, growth condition and environmental factors, are considered to be the factors which govern the absorption and translocation of F,W-450.

The exact mechanism of action of the chemical for inducing male sterility has not yet been revealed convincingly. But the spray timing in most of the crops indicates that the action of the chemical is in some way concerned with the reduction division process during male gametogenesis. Hitton (1958) gives a biochemical explanation for the effect of this chemical. His experimental studies on cotton showed that F,W-450 is competent with pantoate, an enzyme which synthesises pantothenate, the absence of which causes sterility in plants. He states that the chemical accumulates to a greater extent in the anthers than in the ovules.

Dicotyledonous plants show greater response to F,W-450 than the monocotyledonous plants (Phom and Haas Company, 1960).

Cotton

Cotton is the first and best studied crop for inducing male sterility and for the production of hybrid seed with chemical emasculation. The first report on the chemical induction of male sterility using F,W-450 was made by Eaton Frank (1957) on cotton. According to him flowering was delayed in all the treatments. The plants failed to produce pollen grains when they were sprayed with 1.2 per cent solution of F,W-450; but when hand pollinated with pollen from control plants, normal bolls with viable seeds developed.

Meyer et al. (1958) reported that plants sprayed with F,W-450 produced a negatively significant increase of growth over the checks owing to the death of the apical meristems.

Bocanegra et al. (1958) state that, after treating the cotton, variety Tanguis, with 1.0 per cent solution, a reduction in size of the flowers, and capsules and in the number of viable seeds was observed. Four to six weeks after the treatment 90 - 100 per cent of the flowers contained a high proportions of sterile pollen. During the third and fourth weeks some degree of female sterility was also observed. Hitton (1958) and Roux and Chirinian (1959) studied the effect of the chemical on cotton and reported that the chemical inhibited the enzymatic synthesis of pantothenate and accumulated to a greater extent in the anthers than in the ovules. Investigations of Pate and Duncan (1960) on cotton revealed that all the concentrations used in their studies showed male and female sterility except 0.20 per cent which gave a selective sterility for male gametes.



Santhanam et al. (1961) conducted an experiment at PIRACOM, Coimbatore to study the effect of F₂W-450 on cotton. The initial spraying was given eight weeks after sowing and subsequent sprayings on weekly intervals following the initial spray. Disregarding some phytotoxic effects at high concentrations (0.25 and 0.50 per cent) they obtained cent per cent male sterility for a long time, especially in plants sprayed with higher concentrations. Female sterility was examined by crossing with pollen from sea island cotton, without emasculation. Seed sterility was usual as in the control.

Singh (1964) reported that this chemical is very toxic to cotton causing acute burning of leaves and shoot apices and reduction in ~~flower~~ size of flowers. He also reported successful induction of male sterility in cotton by the use of F₂W-450.

Tomato

To study the effect of F₂W-450 on tomato Moore (1959) conducted trials under Green house and field conditions. Green house studies on 146 varieties showed complete male sterility for 10 - 15 days without causing female sterility at concentrations of 0.075 and 0.15 per cent.

Field trials were conducted on 11 early varieties with four concentrations (0.075, 0.15, 0.3 and 0.6 per cent) having three intervals each. The lower concentration (0.075) did not show any kind of pollen sterility. There was an increase in the percentage

of pollen sterility as the concentrations of the chemical increased. The concentration, 0.15 per cent gave a high percentage of sterility for 15 days with a slight reduction in female sterility. The high concentrations 0.3 and 0.6 per cent almost prevented fruit set, when selfed. The third concentration (0.3 per cent) gave complete male sterility for about 12 days beginning from 12 days after treatment and female fertility was also satisfactory. The highest concentration, 0.6 per cent, gave complete male sterility for 19 days beginning from 12 days after treatment. Pollen production was normal after 37 days of application. Fruits did not set when normal pollen was applied between 15 and 22 days after treatment to the flowers of plants treated with 0.6 per cent concentration. This was taken to indicate female sterility. Female fertility was again normal after 37 days of treatment.

Balakrishnan (1963) studied the effect of F, W-450 on tomato. Three varieties with three concentrations (0.15, 0.3 and 0.6 per cent) were tried. Spraying was done when flowers of the first truss were undergoing anthesis. Cent per cent sterility was noted in all concentrations with certain phytotoxic effects. Ovule fertility was normal as in the control.

Watermelon

Henz and Mohr (1959) tested the effect of F, W-450 on watermelon. The initial spraying was done as the first pistillate blossoms appeared and spraying was repeated after six days. Here

the chemical prevented the opening of the mature staminate buds. The effect of F,W-450 in preventing the opening of staminate buds appeared to be localised in the tip of the bud. Pinching of the tip permitted the immediate uncurling of petals. Anthers dehisced with production of apparently normal pollen inside the mature unopened staminal buds. No deleterious effects upon female fertility were associated with the application of F,W-450.

Boswell (1960) conducted the same experiment on watermelon variety Charleslon gray, with concentrations of 0.12, 0.6, 2.0 and 2.5 per cent. All these concentrations caused phytotoxicity with male flowers being prevented from opening for a period of 7 - 10 days.

Beet root and Clovers

Veslag Landbouwkunding Onderzoek (1954) applied F,W-450 on beets. He reported a moderate to a high level of male sterility induced by this chemical without any adverse effect on the female fertility on beets.

Wit (1960) reported an effective treatment of F,W-450 in red clovers, white clovers and beet species with an aqueous solution shortly before blooming. He stated that a moderate to a high degree of male sterility was obtained without adversely affecting the vegetative growth or flowering. To detect the percentage of ovule fertility he made controlled crosses with pollen of untreated plants and concluded that the ovules of clover varieties were little affected than in beet species.

Rubenbauer and Schuttis (1960) applied F₄₅₀ on sugar beets with varying concentrations. The normal fruit setting in a control plant was 79.3 per cent while in treated plants a much reduced percentage of fruit setting was noted. In plants treated with 3 per cent solution the reduction in fertility was to 30.5 per cent and it reached up to 6 per cent when the same concentration was applied twice to the plant. The highest concentration caused a certain amount of damage to the plants.

Dudley (1960) conducted an experiment to study the effect of F₄₅₀ on sugar beets, under green house and field conditions and recorded that the timely application of the chemical delayed pollen shedding without adversely affecting seed yield or subsequent generation. The same experiment on the same crop was conducted by Butterfass in 1960. He sprayed twice with 0.33 per cent and got a high percentage of pollen sterility.

Alfalfa

Pederson (1959) studied the effect of F₄₅₀ on alfalfa with concentrations of 0.5 and 1.0 per cent. There was an adverse effect of this chemical at the above concentrations on the vegetative growth of the plant and the treated flowers, when self pollinated showed a reduction in pods per raceme, and seeds per pod. His experimental data indicate that damage had occurred to the female as well as to the male gametes. He also reported an increased flowering in F₄₅₀ treated plants. Moderate leaf burn occurred at 0.5 per cent

and severe damage at 1.0 per cent.

Miller and Hittle (1963) in their experiment on alfalfa with F,W-450 reported that the chemical caused burning of shoot apices and leaf margins, chlorosis of leaves and drying of young flower buds; but they have failed to get any significant induction of sterility.

Grapes

To evaluate the effect of F,W-450 on earliness, yield and fruit quality on grapes an experiment was conducted at I.A.R.I., New Delhi (1961). It revealed that 0.25 per cent F,W-450 induced a high degree of pollen sterility.

Iyer and Handhawa (1965) studied the effect of F,W-450 on four varieties of grapes. They reported that all the treatments caused various types of leaf abnormalities and the degree of damage was depended on the concentration of the chemical used. The extent of pollen sterility that could be induced was found to be dependent on (1) the stage of development of the panicle at which sprays were given (2) concentration of the chemical (3) number of application and (4) the variety used for the study. Two applications were found to be effective in the induction of complete pollen sterility. F,W-450 (0.3 per cent) at an early stage of the development of the panicle (about 15 days after emergence) induced complete pollen sterility. In all the treatments the germination of the seeds obtained was normal. Hence they reported that this method can be used with profit in large scale grape hybridization.

Soybeans

Starness and Hadley (1962) reported that in soybean F,W-450 induced male sterility by the non-dehiscence of anthers and caused pollen abortion. They sprayed aqueous solution of the chemical, three weeks after planting on four varieties of soybean. They stated that sodium, alpha, beta-dichloroisobutyrate appeared to have become more effective as a gametocide by virtue of its interference with anther dehiscence rather than as a result of causing pollen abortion. They failed to get complete pollen sterility.

Bocanegra et al. (1956) stated that the French bean, Canario sprayed during flowering with 1.5 per cent solution of F,W-450 resulted in 80 per cent sterility of the pollen grains a fortnight after treatment whereas the plants treated with 0.5 and 1.0 per cent solution had normal or nearly normal pollen. There was general reduction in vigour in all the three treatments and the pod set was lowest in plants receiving maximum concentration.

Bhindi

Nair (1964) conducted an experiment to study the effect of F,W-450 on bhindi using the concentrations of 0.1, 0.15, 0.20, 0.25 and 0.30 per cent. The chemical was applied at three stages of the plant growth viz., 30th, 37th and 44th day after planting. A high percentage of sterility was induced in all concentrations at all stages of application. Increase in sterility was obtained by an increase in concentration. The maximum sterility was obtained in plants treated with 0.25 and 0.30 per cent

at the first stage of application. The higher concentrations showed a reduction in fruit setting.

Brinjal

Leelamma (1965) carried out an experiment to induce male sterility in brinjal with F,W-450. The chemical was applied at three stages of plant growth and it was found to decrease the size and number of clusters of flowers. She reported the complete absence of dehiscent anthers in the treated plants. Such plants showed cent per cent sterility. This complete sterility lasted for a period of two weeks, beginning from two weeks after the treatment. There was a marked reduction in the percentage of fruit setting on selfing. A reduction in fruit setting was obtained with the increase in the concentration. The size and weight of the selfed fruits were also found to decrease as the concentration increased. The fruit setting on crossing was normal in almost all cases. The size and weight of the crossed fruits were found to be unaffected by the chemical. The seeds from selfed fruits showed a marked reduction in germination while the seeds from crossed fruits were found unaffected.

Muskmelon

Nripendra et al. (1965) recommended a multi direction foliar spray of aqueous F,W-450 for the induction of male sterility in this crop. The plants were severely damaged in the case of high concentrations and more number of sprayings. A concentration of 0.3 per cent as second spray showed the functional male sterility,

while 0.4 per cent concentration with two sprays showed the desired results by inducing pollen sterility. Concentrations of F,W-450 higher than 0.4 per cent produced deleterious effects.

Cajanus cajan

Kaul and Singh (1967) conducted an experiment to study the effect of F,W-450 on Cajanus cajan using the concentrations 0.5, 1.0 and 1.5 per cent. All the above concentrations proved rather injurious for the general growth of the plant. The treated plants flowered later than the control. All the treatments caused a slight reduction on size of flowers. Instances of failure of anther dehiscence were also noticed. All the treatments caused complete pollen sterility. Duration of complete pollen sterility ranged from 15 to 25 days depending upon the concentration and the number of sprays. After the period of complete male sterility, functional male sterility ranged from five to nine days. A significant decrease in the yield per plant in all the treatments was also noticed. They concluded that, out of the different effective treatments, 1.0 per cent solution applied before the bud initiation stage was the best treatment as far as the total yield of the plant and its components were concerned.

Maize

Dadax Nobles (1959), with a view to standardising suitable techniques for inducing male sterility, tried F,W-450 on maize. At 0.1 to 1.5 per cent concentrations white spots appeared on the leaves. At 2.5 to 3.0 per cent growth ceased, tassels and

ears showed abnormal development and pollen sterility was about 20 to 30 per cent and at 4 per cent and above all plants died.

Cameron and Eaton (1959) reported non-dehiscent anthers in treated plants. This non-dehiscence of anther was not a uniform feature of the anthers of all the treated plants. However they reported successful induction of male sterility in this crop.

Wheat

Chopra et al. (1960) tried the chemical F,W-450 on wheat at I.A.R.I. But they failed to get any positive result. Wheat plants were sprayed once or twice with 0.25, 0.5, 0.75 and 1.0 per cent aqueous solutions of F,W-450. Plants sprayed with lower concentrations were stunted and grass like in appearance. Those plants which survived showed a delay in flowering, small earheads with shorter awns and tougher glumes and with normal pollen grains.

Rye

Wit (1960) failed to get any response of F,W-450 for vegetative growth, flowering or even inducing male sterility in rye.

(b) 1,2-dihydropyridazine 3,6 dione (Maleic hydrazide or MH)

1,2-dihydropyridazine 3,6 dione (Maleic hydrazide or MH) is a hot water soluble salt and contains 40 per cent of active MH. It is an antiauxin and a growth inhibitor. MH is a weed controller and an anti-mitotic agent. MH was introduced in the year 1949 by the United States Rubber Company as a plant growth regulator (Narayana Swami 1960).

Several studies were conducted by various workers on the effect of this chemical on economically important crops. Varying kinds of growth inhibition, deformities in vegetative characters, reduction in fruit setting and in certain cases an inhibition of pollen production have been reported. This review is on the effect of MH on plants with special reference to the induction of pollen sterility.

MH is a synthetic compound which is frequently found to affect growth in a direction opposite to that of 2,4-D and to cause retardation and inhibition of cell division (Koser and Thompson, 1952). It proved more toxic to grasses than to broad leaved species (Currrier and Crafts, 1950). Few cases were reported where this substance was considered as a growth promoter (Pilet, 1956).

Maleic hydrazide is a unique growth regulant (Schoene and Hoffman, 1949) and a growth inhibitor (Naylor and Davis, 1950). Naylor (1950) studied the effect of MH on flowering of tobacco, maize and cocklebur. Certain cucurbits and corn showed male sterility at concentrations 0.025 - 0.50 per cent. In corn foliar sprays of the above concentrations, induced a high percentage of pollen sterility. Growth inhibition by MH was described by Currrier et al. (1950). Delay in flowering by treating the plants with MH was reported by David (1950) in Bristol black raspberries and Josephson (1951) in corn. Induction of partial male sterility in hybrid corn by the use of MH was also reported by Warren and Dismock (1954). However Naylor (1950) and Naylor and Davis (1950) reported complete male sterility in maize.

Poljakov (1966) reported that MH in certain cases induced 100 per cent male sterility in maize. The most effective treatments were 0.05 to 1.5 per cent solution applied at the time of meiosis in the archesporial cells of the stamen. Female sterility of 30 - 50 per cent was however, induced at the same time.

Rehm (1952) and Chopra et al. (1960) reported complete male sterility in tomato by the foliar application of MH. Rehm (1952) reported the period of complete pollen sterility to be of 10 to 14 days. Likewise Chopra et al. (1960) observed complete pollen sterility to last for only seven days. But Wit (1960) found complete male sterility for the full blooming period in red clovers.

In *Chrysanthemum* Beach and Leopold (1953) reported growth inhibition by the application of MH.

The use of MH as a chemical to reduce seed setting in weeds was reported by Miller et al. (1955). Fruit setting was considerably reduced at concentrations of 4000 and 8000 ppm. Williamson (1958) reported total deduction of fruit setting in wheat and rye. Robert (1959) studied the effect of MH on saint paulia and reported that the chemical inhibited pollen germination and showed a high degree of pollen sterility.

Wittwer (1959) generalised the effect of MH by comparing it with growth promoting substances and stated that MH produced effects opposite to these induced by growth promoting substances, such as IAA

and NAA. He reported that this chemical when sprayed during the late reproductive stage delayed the flowering in tobacco, corn, lettuce and cocklebur. According to him "The chemical offers promise as a means of 'chemically deflowering' the male inflorescences in the commercial production of hybrid seeds".

Ora Smith (1959) studied the effect of MH on vegetative growth and pollen production of various crops like corn, turkish tobacco, lettuce etc. He noticed all kinds of abnormalities in vegetative growth of the plant and reported that the concentration and time of application are important factors for its action. Turkish tobacco plants sprayed with MH during the initiation of flower bud at concentrations of 0.4 and 0.8 per cent stopped all elongation, killed the flower buds and prevented axillary bud development. The same result was also noticed in lettuce when applied to young head lettuce plants.

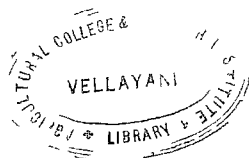
Maini and Sandhu (1959) conducted an experiment to study the percentage of seed set on Eruca sativa by treating it with some growth substances. All the chemicals were sprayed as aqueous solutions at flower bud stage. They showed that higher concentration of MH acted as an anti-auxin and interfered more with pollination than with pod set.

Roux and Chirinian (1959) studied chemical induction of male sterility with MH sprays in cotton. Their experimental results showed that male sterility could be induced in cotton by spraying with an aqueous solution of MH - 30 at the stage of micro-sporogenesis.

Increase in the number of secondary branches by the application of MH was observed in cotton by Currier et al. (1950) and Singh (1964). Reduction in flower size and induction of partial sterility were also reported by Singh (loc cit). Meyer et al. (1958) reported complete pollen sterility in cotton by the application of MH.

Singh et al. (1960) studied the varietal variation on the effect of MH and other chemicals on Loquat (Friobotrya japonica). The varieties studied were improved golden yellow and pale yellow. They got a significant variation on the effect of the chemicals on these two varieties. The treated plants showed a reduction in fruit size.

Jauhari and Ayodhya Prasad (1959) studied the effect of MH on growth and yield of brinjal. They sprayed the plants with 200, 400 and 800 ppm of MH. All these concentrations produced marked changes in the vegetative growth and yield of brinjal. The average number of branches under different treatments was materially affected. The highest concentration produced the minimum number of branches per plant, while the lowest concentration (200 ppm) gave the maximum and significantly highest number of branches per plant. The results indicate that the highest concentrations (400 and 800 ppm) showed deleterious effect on the growth of the plants. Higher concentrations were found responsible for delaying the flower bud formation, reducing the percentage of fruit set and causing delayed maturity of fruits. The number of fruits per plant was least under 800 ppm followed by 400 ppm.



Choudhury and George (1964) obtained pollen sterility of 90 - 100 per cent lasting for 5 - 12 days in two varieties of brinjal by spraying the whole plants with MH.

Chopra et al. (1960) studied the effect of various chemicals on wheat, and onion. MH at concentrations 50, 100, 250 and 500 ppm on wheat and 10, 50, 100 and 250 ppm on onion were studied. In wheat the initial spraying was done on 37 day - old plants and subsequent sprays on 15 days intervals before the emergence of flag leaf (3 sets) and a fourth set at the time of flag leaf emergence. Pollen sterility data showed a high percentage of sterility at 250 ppm and 500 ppm., abortion of anthers at 250 ppm at two or more times and 500 ppm once or twice at the time of flag leaf emergence.

Injection of 100 ppm on onion caused a synapsis and desynapsis and 250 ppm produced a very high degree of stickiness at microsporogenesis. Here pollen sterility was found to be cent per cent.

Choudhury and Ramphal (1960) studied the effect of MH on Cow pea (vigna sinensis). Plants were sprayed just before flower initiation with six concentrations (50, 100, 200, 400, 600, and 800 ppm) of MH, in order to interrupt or destroy the apical dominance and force up the number of lateral shoots. The apical dominance was interrupted with all concentrations. MH (800 ppm) completely destroyed the apical dominance, whereas with other concentrations the terminal growth was resumed after a temporary interruption. MH sprays (50, 100 and 200 ppm) significantly increased the yield of pod per plant. Higher concentrations

proved toxic and reduced the yield of pod. Maturity of the pods was delayed by about a week. There was no significant effect of the sprays on the size or viability of the seed.

Kumar (1963) reported an increase in the number of secondary branches in sesamum by the application of MH. He also reported a reduction in size of flower and a partial male sterility in sesamum.

Nair (1964) studied the effect of MH on bhindi with concentrations of 200, 400, 600, 800 and 1000 ppm. Marked suppression of plant growth along with deformities and malformation of leaves were observed in the highest concentration of MH (1000 ppm). Delayed flowering and reduction in the flower production were observed in higher concentrations. He failed to get cent per cent pollen sterility. Length of fruits and number of seeds per fruit were also reduced by all the treatments.

Iyer and Randhawa (1965) conducted an experiment to study the effect of MH on grapes. They chose three concentrations of MH (250, 500 and 750 ppm). All the treatments caused various types of leaf abnormalities resulting from the injury due to the spray of the chemicals. The degree of damage depended on the concentrations of the chemical used. Two applications of MH (500 ppm) at an early stage of the development of the panicle induced complete pollen sterility. MH 500 ppm., which induced complete pollen sterility gave the best reduction in fruit and seed set.

Kaul and Singh (1967) conducted an experiment on the effect of MH on Cajanus cajan using the concentration of 0.1, 0.5 and 1.0%. Burning of the leaf and shoot apices was noticed. All the treatments showed a significant decrease in plant height. A significant increase was observed in the number of branches and flowers. The size of the flowers was reduced in all the treatments. But they failed to get complete male sterility. All the treatments caused a significant decrease in the number of fruits per plant, number of seeds per fruit and total yield of grains per plant.

(c) Other chemicals

2,4-Dichlorophenoxy acetic acid (2,4-D), Trilode Benzoic acid (TIBA), Gibberellic acid (GA), M.C.P.B., Ethoxy caffeine, Uracil, Thymine, Nuclie acid, NAA, Coumarine etc. have also been tried for inducing male sterility.

Ehrenberg et al. (1956) studied the effect of Ethoxy caffeine for inducing mutation and sterility in barley. These studies revealed that the chemical induced sterilities which corresponded to the effect of x-rays at 6000 r. Srivastava (1958) while spraying M.C.P.B. at pre-flowering stage with 1000, 2500 and 5000 ppm on rice (variety 'Aman') noticed a high percentage of sterility and decrease in yield.

Nelson and Roseman (1958) tried the possibilities of chemical induction of male sterility in maize by means of GA at concentrations of 500 and 1000 ppm. Foliar spraying of the above

concentrations when the inflorescence was 1" in length showed sterile or partially sterile tassels. Yermanos and Knowles (1960) produced male sterile, self flower plants with a foliar spray of GA at 100 ppm in all the five varieties used.

Robert et al. (1961) reported 2,4-D as a pollenicide on grape. Dipping the flowering clusters in solutions of 2,4-D increased the number of seed-less berries. This was supposed to be due to the injurious effect of 2,4-D to pollen germination or in otherwards due to its action as a pollenicide.

Chopra et al. (1961) studied the effect of Uracil, Thymine, Yeast nucleic acid and TIBA on tomato and the above chemicals and sodium nucleate on onion. Aqueous foliar spraying was done about a week prior to the opening of the first flower. All the chemicals failed to produce any kind of male sterility in tomatoes. While in the case of onion cent per cent pollen sterility was noted in plants sprayed with TIBA at 10 to 100 ppm, Na-nucleate at 4 per cent and Thymine and Uracil at 250 and 500 ppm respectively.

Choudhury and George (1964) obtained pollen sterility of 90% - 100% lasting for 5 - 12 days in two varieties of brinjal by spraying the whole plant with NAA and 2,4-D. Spraying with 2,4-D at concentrations higher than 10 ppm. caused elongation, curling and cracking of tender parts of stem. NAA induced the conversion of stamens into miniature pistils, indicating some hormonal action in sex determination.

Iyer and Randhawa (1965) also studied the effect of TIBA on grape. Two sprays of TIBA (400 ppm) at an early stage of the development of the panicle (about 15 days after emergence) induced complete pollen sterility.

Kaul and Singh (1967) conducted an experiment to study the effect of Coumarine on Cajanus cajan as male gametocide. Application of the chemical at all concentrations proved rather injurious for the general growth of plants. Delayed flowering, reduction in size of flowers and induction of partial male sterility were also recorded by them.

II. Pollen studies

(1) Pollen morphology

Pollen grains serve best in distinguishing between and showing relationship among the higher groups of plants such as families, tribes, genera and sometimes species.

A great deal of work had been done on the morphology of pollen grains by several investigators. Pollen grains consist of an intine and an exine. The outer surface of the exine may be sculptured, reticulate or smooth and with or without spines. Wode House (1935), Lang (1937) and Erdtman (1954) studied the pollen morphology of solanaceae and reported that they are found and may be 2-3-4-5-6-colporate.

(ii) Pollen sterility

Cytological studies on the sterility of pollen grains were carried out by two methods.

- a) By staining methods, and
- b) Germination in artificial media.

a) Staining methods.

For the study of pollen sterility, acetocarmine staining technique was adopted even from the earlier days. Zirkle (1937) had described a method for testing the viability of pollen grains by acetocarmine staining method. Pollen grains were dusted on a slide containing one or two drops of acetocarmine. The slide was then examined under a microscope after 15 minutes when the grains were properly stained. The grains which stained well and looked plump and normal were taken as viable. Unstained and shrivelled ones were considered as non-viable or sterile.

Vietew (1952) used 2, 3, 5 - triphenyl tetra zonium chloride for testing the pollen viability of Zea mays. The best results were obtained, when the test was carried out at 50°C using a 2 per cent solution. Oberle and Watson (1953) while utilising the above method on peach, apple, pear and grape pollen remarked that this method was ineffective for the determination of pollen viability in these crops. Jacopini (1954) recommended the treatment of pollen grain with 2 per cent sodium biselenite for periods ranging from $\frac{1}{2}$ - 2 hours, depending on the species for a rapid reliable means of determining pollen viability in stone and pome fruit trees. Grains with full germinative power turned pale yellow while non-viable grains did not change. King (1959) recommended a peroxidase agar medium.

Here, the sterile pollen grains showed a blue colouration while the viable pollen grains were colourless and swollen in appearance.

Ostapenko (1956) showed that the above mentioned staining methods would not give a good evidence for the sterility or viability testing of pollen grains. According to him these methods could only gave a relative value in determining the percentage of pollen viability.

b) Germination in artificial media.

There are experimental evidences to show that pollen grains could be grown in artificial media containing sucrose solutions. The concentrations of sucrose required by the pollen grains to germinate, varied between plants of different species and even within the same species.

The use of artificial media for pollen germination goes back to the later half of the 19th century. Adams (1916) studied the use of culture media for the germination of pollen grains of apple and other fruit trees. He found that a good percentage of pollen germination was obtained, in apple at 2.5 - 10 per cent, in pear at 4-8 per cent, in strawberry at 8 per cent, in longberry at 4 per cent and in raspberry and blackberry at 16 per cent sugar solution.

Kebel (1926) germinated pollen grains of certain fruit trees in sucrose solution and reported that an optimum concentration of sucrose solution of 5 per cent for quinces, 10 per cent for peaches

and apricot, 10 - 15 per cent for plums and pears, 5 - 15 per cent for apple and 15 per cent for cherries. Dikshit (1956) found that loquat pollen germinated successfully in 1 per cent sucrose solution. Singh (1956) studied the pollen grains of ten varieties of peach and reported that there was a varietal variation for requirements of sucrose solution for successful germination of pollen grains.

Various workers reported that Boron can induce pollen germination and pollen tube growth in various crops. Schumucker (1935) studied the pollen grains of various crops and discovered that Boron as borate was a stimulant to pollen germination and tube growth in many species. Thompson and Batjer (1950) studied the pollen of different crops and found that Boron in low concentrations 2.5 - 40 ppm stimulated the pollen germination and pollen tube growth, but inhibited pollen germination with higher concentrations. Raghavan and Sarash (1956) reported that arecanut pollen grains cultured in sucrose medium showed a considerable degree of pollen germination and pollen tube growth. Munzoer (1960) found that 0.001 to 0.01 per cent of Boric acid had a stimulating effect on pollen germination and pollen tube growth in more than sixty angiosperm species. Singh (1960) obtained an increase in pollen germination and tube elongation in mango with addition of 20 ppm Boric acid.

Hristov and Gencev (1964) studied the pollen germination of red pepper in connection with finding varieties suitable as components for hybridisation. Pollen tube growth of different red pepper varieties on a medium consisting of 10 per cent sucrose and 1 per cent agar was

stimulated when flower extracts of any one of the five varieties having good combining ability were added to the medium. Prasad et al. (1963) studied the pollen grains of eight varieties of tomato and discovered that 10 per cent sucrose solution gave the best results.

Dean (1964) reported that a medium containing 15 per cent sucrose and 30 ppm boron gave satisfactory results with fresh pollen of tobacco. Higher concentrations of both substances are however, required to stored pollen.

Addition of solidifying materials like agar and gelatin were used from the earlier days for the preparation of culture media for pollen grains. Iyengar (1939) used an agar-sugar medium for artificial germination of pollen grains of cotton. Johri and Vasil (1955) got an increase in the percentage of pollen germination on the above medium, when Boric acid was added to it. Agarwal, Khanna and Singh (1957) found that 1 per cent agar medium containing 6 per cent sucrose gave best results with pollen of Momordica charantia. Dvornic (1960) reported that the optimum condition of pollen germination of most of the vine varieties studied were 12 - 14 per cent sucrose-agar and 25 - 26°C. Girindra and Mohan Mishra (1962) reported that brinjal pollen was found to germinate well in media with 5.0 and 10.0 per cent of sucrose and pinch of agar-agar.

Balakrishnan (1962) stated that tomato pollen was found to germinate well in 15 and 20 per cent sucrose solution with addition of 100 ppm boric acid. Vilasini (1963) studied ten varieties of Hibiscus rosasinensis and recommended a suitable medium of 45 per cent

sucrose, 100 ppm boric acid and 1 g of agar. Hair (1964) with a view to standardise a suitable medium for bhindi pollen, obtained a medium of 20 per cent sucrose solution with 2 per cent agar which gave the best results and was selected the solid medium for the study of germination of pollen grains. Leelamma (1965) found that 1 gm of agar medium containing 15 per cent sucrose solution and 50 ppm boric acid gave best results with pollen of Solanum melongena.

MATERIALS AND METHODS

MATERIALS AND METHODS

The investigations reported here were carried out in the Division of Agricultural Botany, Agricultural College and Research Institute, Vellayani, Kerala, during the year 1966-1967. The purpose of the experiment was to study the effect of the chemicals, F, W-450 and MH on the induction of male sterility in chillies (Capsicum frutescens, L.)

A. MATERIALS

(1) Seed material.

Chillies (Capsicum frutescens, L.), variety, Dutta Series. I, was selected for the studies on the chemical induction of male sterility. The seeds were obtained from the College Farm, Agricultural College and Research Institute, Vellayani. Viability as well as the percentage of germination of the seeds were tested before being used for this investigation.

(2) Chemicals.

The chemicals used were sodium, 2,3-dichloroisobutyrate (F, W-450) and 1,2 - dichloro 3,6 - pyridazinedione (Maleic Hydrazide or MH).

Each chemical was tried in five concentrations with three stages of application. Control plants (no spray) and plants sprayed with distilled water thrice, at the same stages of growth as the chemical spray were also studied.

The chemicals were obtained from the Division of Agricultural Botany, Agricultural College and Research Institute, Vellayani, Kerala.

(3) Treatments.(i) Material used(a) F₀N-450 at five concentrations.

0.10%, 0.15%, 0.20%, 0.25% and 0.30%.

(b) MH at five concentrations.

25 ppm., 50 ppm., 100 ppm., 200 ppm., and 400 ppm.

(c) Distilled water(ii) Stages of application - Three stages.

(1) At the onset of flower production and anthesis.

(2) 15 days after the onset of flower production and anthesis.

(3) 30 days after the onset of flower production and anthesis.

The following symbols have been used for specifying the treatment combinations.

M	Maleic hydrazide (MH)
N	F ₀ N-450
T	Stages of application
L	Distilled water
C	Control
M ₁	MH at 25 ppm
M ₂	MH at 50 ppm
M ₃	MH at 100 ppm
M ₄	MH at 200 ppm



M_5	MH at 400 ppm
M_1	F, W-450 at 0.10%
M_2	F, W-450 at 0.15%
M_3	F, W-450 at 0.20%
M_4	F, W-450 at 0.25%
M_5	F, W-450 at 0.30%
T_1	First stage of application, on the onset of flower production and anthesis.
T_2	Second stage of application, 15 days after the onset of flower production and anthesis.
T_3	Third stage of application, 30 days after the onset of flower production and anthesis.

The different treatment combinations tried were as follows:-

1.	$L T_1$	12.	$M_4 T_2$	23.	$M_5 T_1$
2.	$L T_2$	13.	$M_5 T_2$	24.	$M_1 T_2$
3.	$L T_3$	14.	$M_1 T_3$	25.	$M_2 T_2$
4.	$M_1 T_1$	15.	$M_2 T_3$	26.	$M_3 T_2$
5.	$M_2 T_1$	16.	$M_3 T_3$	27.	$M_4 T_2$
6.	$M_3 T_1$	17.	$M_4 T_3$	28.	$M_5 T_2$
7.	$M_4 T_1$	18.	$M_5 T_3$	29.	$M_1 T_3$
8.	$M_5 T_1$	19.	$M_1 T_1$	30.	$M_2 T_3$
9.	$M_1 T_2$	20.	$M_2 T_1$	31.	$M_3 T_3$
10.	$M_2 T_2$	21.	$M_3 T_1$	32.	$M_4 T_3$
11.	$M_3 T_2$	22.	$M_4 T_1$	33.	$M_5 T_3$
				34.	C

B. METHODS

1. Lay out

A randomised block design with thirty four treatments and three replications was adopted. There were four plants under each treatment. Out of the four plants one was used for floral biology and pollen studies, one for breeding works, and two plants for the study of other morphological characters and yield. The spacing adopted was 80 cm x 80 cm.

2. Sowing

The seeds soaked for 20 hours in water were sown on 3rd October 1966.

3. Transplanting

One month old seedlings were selected for transplanting. Transplanting was done on 3-11-1966. Healthy and uniform seedlings were transplanted in the centre of each prepared pit. The seedlings were protected from the sun by giving a shade until they became well established.

4. Treatments

Application of the chemicals was carried out with the help of an atomiser and using distilled water as the medium.

The first spraying was given at the onset of flower production and anthesis and subsequent sprays were given at an

interval of 15 days. In all cases it was done with extreme care to obtain a thorough and uniform wetting of the plants especially the terminal buds.

5. Characters studied

I. Growth and morphological characteristics

1. Visual observations
2. Height of plants
3. Number of flowers
4. Pollen studies
 - a) Pollen morphology
 - (i) Pollen shape
 - (ii) Pollen size
 - b) Pollen sterility
 - (i) Acetocarmine staining method
 - (ii) Germinating pollen grains in artificial media.

II. Fruit set and fruit characters

1. Number of fruits per plant
2. Setting on selfing
3. Setting on crossing
4. Size of fruits
5. Number of seeds per fruit
6. Weight of fruits

III. Seed viability

I. Growth and morphological characteristics

1. Visual observations

Visual observations on the general growth of the plant under each treatment were made.

2. Height of plants

Height of plants was recorded by measuring the height of the main stem 15 days after the first application of the chemicals. Measurements were taken from the ground level to the base of the terminal bud. The data were analysed statistically.

3. Number of flowers

Opened flowers were counted daily in the morning from 5-12-1966 to 20-2-1967, at this time flower production ceased completely. The total number of flowers produced was estimated. The flowers produced by plants sprayed in the 1st stage of application of the chemicals were analysed statistically.

4. Pollen studies

(a) Pollen morphology

Pollen morphology was studied two weeks after the treatment. It was done by acetocarmine staining technique. Freshly opened flowers were collected and kept in a dessicator for one hour to obtain pollen grains for study. The shape and size of the pollen grains were studied.

(i) Pollen shape

Pollen grains were dusted on a slide containing a drop of acetocarmine - glycerine medium. The undehisced anthers were crushed with the help of the blunt end of the needle. It was then

covered with a cover glass. Ten pollen grain, at random from each treatment were taken from each replication and the shape of the pollen grains was studied.

(ii) Pollen size

In order to study the size of the pollen grains an ocular micrometer was used. The micrometer was standardised under the high power of a compound microscope. The size of hundred pollen grains taken at random from each treatment was measured and the mean diameter of pollen grains was calculated.

(b) Pollen sterility

Pollen sterility of the treated and control plants was estimated by two methods.

(i) Acetocarmine staining method

Collection of Pollen grains.

Flowers were collected at intervals of five days commencing from the fifth day after application. This was continued until the restoration of male fertility as in the control. Freshly opened flowers were collected and kept in a dessicator for one hour and then pollen grains were subjected to studies.

Staining and counting

Pollen grains were studied by mounting in an acetocarmine medium. All the three replications for each treatment were studied.

Pollen grains of each treatment were dusted on clean slide having one drop of acetocarmine and examined under the low power of a compound microscope after 30 minutes. Pollen sterility was estimated by counting the fertile and sterile pollen grains separately.

Staining

Well stained and plumpy pollen grains were taken as fertile and all other namely unstained and shrivelled ones were taken as sterile. Countings were made on 10 different microscopic fields with 3 replications. From these studies the mean percentage of pollen sterility of each treatment was calculated and the data were tabulated and statistically analysed using the analysis of variance method.

(ii) Germination of pollen grains in artificial media

With a view to study the germination percentage of pollen grains and pollen tube elongation, preliminary studies were done to standardise the culture media. The experiment was conducted in three stages as follows.

Stage I

On Sucrose medium

Sucrose solutions of varying concentrations of 5%, 10%, 15%, 20% and 25% were prepared and used to test the percentage of pollen germination and tube elongation. Small drops of sucrose solutions were placed on clean microscopic slides and pollen grains were dusted on it. After a few minutes the slides were inverted and placed on two glass rods in a petridish. A humid environment was provided by a wet

filter paper at the bottom of the petridish. Germinated and non-germinated pollen grains were counted after 24 hours from ten different microscopic fields.

Three concentrations, depending on the percentage of germination and tube length were selected.

Stage II

Sucrose Boric acid medium

The above three concentrations, viz., 10', 15' and 20' which gave the best result in the experiment, were used in combination with 50 ppm., 100 ppm., and 150 ppm. boric acid.

Stage III

Solidification media

The sucrose concentrations which gave the best result i.e., sucrose solution 15% + 50 ppm boric acid was tried in combination with 0.5%, 1.5% and 2% of agar.

The standard medium was taken in clean sterile petridishes. Pollen grains were sown uniformly in separate petridishes.

After 24 hours each petridish was examined under the low power of the microscope. The number of germinated and non-germinated pollen grains in microscopic field was counted. In a petridish ten such countings were made at random and the percentage of germination was determined.

The length of tubes of pollen grains selected at random from each petridish was measured using a standardised ocular micrometer and the mean tube length was found out.

The combination of 15 per cent sucrose solution with 50 ppm boric acid and one gram of agar gave the best results. Hence this was used for the studies on pollen germination and tube elongation on the experimental material.

Each treatment was taken in separate petridishes with a replication of three plates for each treatment. The germination test in the culture media was done when the maximum sterility was obtained in the three stages i.e., 15 days after the treatment in three stages.

The percentage of germination and the mean pollen tube growth were calculated as mentioned above.

II Fruit set and fruit characters

1. Number of fruits per plant

The number of fruits was counted at the time of each harvest and the data were recorded and analysed statistically.

In order to study the source of sterility the treated plants were selfed as well as crossed.

2. Setting on selfing

Selfing was effected by bagging method. The flower buds which would open next day were bagged in the previous evening because the normal anthesis in chillies is between 8 A.M. and 9 A.M. The bags were removed after five days.

3. Setting on crossing

The treatments which failed to set fruits on selfing were subjected to crossing. Hand pollination was done between 8 A.M. and 9 A.M. by collecting the pollen in a watch glass from control plants and pollinating with a camel's hair brush.

4. Size of fruits

The length of five selfed and five crossed fruits was recorded and the average length of selfed fruits as well as crossed fruits per treatment was calculated.

5. Number of seeds per fruit

The seeds extracted from selfed as well as crossed fruits were recorded separately and the average number of seeds per selfed fruits as well as crossed fruits per treatment were calculated.

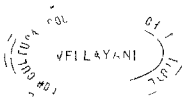
6. Weight of fruits

The fruits from each plant were weighed at the time of each harvest and the data were recorded and analysed statistically.

III Seed viability

Tested by germination

Hundred seeds were taken from each treatment and sown in moist filter paper in a petri dish. Germination counts were taken from 3 days up to 8 days and continued until the 8th day. The first test was to see if the seeds...



EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

The various effects of F,W-450 and MH on Chillies were studied and the data obtained are given below:-

I. Growth and morphological characteristics

1. Visual observations

Application of the chemicals, F,W-450 and MH at all the concentrations proved rather injurious for the general growth of plants. Comparing the two chemicals this effect was more pronounced in the case of MH. These effects were found to be directly proportional to the increase in the concentrations of the chemicals. Higher concentrations of both the chemicals showed a suppression of growth, lasting for one to two weeks. Leaves became yellow with burning tips. But normal leaves began to appear a few days after the treatments.

Plants sprayed with the 200 ppm and 400 ppm concentrations of MH showed the maximum suppression of growth. It was observed that this reduction of growth was brought about by the death of apical meristem. These plants showed delayed flowering. The leaves of these plants became thicker and larger in size and darker in colour. MH alone showed these marked differences. The effect of the chemical was more in the first stage of application. In general, plants sprayed with high concentrations of MH showed definite toxic effect.

2. Height of plants

Height of plants under different treatments 15 days after the first stage of application is presented in Table I and II.

These data indicate that the treatments with the chemicals in general reduced the height of plants. Of the two chemicals, MH showed significant reduction in height over the control, distilled water and F,W-450. The effect was found to be directly proportional to the increase in the concentrations of MH. Of the different concentrations of MH, 200 ppm and 400 ppm reduced the height significantly over other concentrations. In the case of F,W-450 also the higher concentrations 0.25% and 0.30% reduced the height considerably.

3. Number of flowers

Summary of results and the analysis of variance of the data are furnished in Tables III and IV respectively. The results showed that treatments had a striking effect on delaying the flowering of plants. The data also showed that there was a marked reduction in number of flowers within the period of 20 days after the first stage of application. All concentrations of MH showed a more marked effect in delaying flowering than F,W-450. The reduction and delay in flowering were found to be determined by the concentrations of the chemicals. The higher concentrations of MH (200 ppm and 400 ppm) and the highest concentration of F,W-450 (0.30%) showed significant reduction in the number of flowers and a delay in flower production.

TABLE I

Mean height of plants in cms. 15 days after the first
stage of application of the chemicals

Treatments	Rep. I	Rep. II	Rep. III	Mean
Control	20.12	25.50	22.12	22.58
D.W. sprayed	22.62	24.00	22.50	23.04
<u>E.1-450</u>				
0.10%	24.00	16.25	20.50	20.25
0.15%	17.50	22.00	22.87	20.79
0.20%	20.87	21.62	18.25	20.25
0.25%	20.25	17.00	21.25	19.50
0.30%	21.62	19.75	20.12	20.49
Mean	20.85	19.32	20.64	20.26
<u>MH</u>				
25 ppm	18.75	23.75	23.12	21.87
50 ppm	22.00	23.50	22.37	22.62
100 ppm	21.75	19.50	20.62	20.62
200 ppm	18.25	22.12	15.62	18.66
400 ppm	12.87	12.37	12.00	12.41
Mean	18.74	20.25	18.74	19.24
General mean	19.79	19.78	19.69	

Critical difference for comparison between concentrations of MH or
E, 7-450 = 3.91

“ “ “ “ between chemicals = 1.75

TABLE II

Analysis of variance for mean plant height, 15 days
after the first stage of application

Source	S.S.	df	Variance	F.ratio
Total	380.759	35		
Block	2.292	2	1.146	
Treatment	259.599	11	23.599	4.367**
Between chemicals	7.762	1	7.762	1.437
Between concentrations	103.931	4	25.931	4.799**
Between concentrations of F ₂ T-450	2.744	4	0.686	
Between concentrations of MH	201.692	4	50.423	9.332**
Error	118.868	22	5.403	

** Significant at 1% level.

A general comparison showed that the chemicals at all the concentrations tried, were significantly superior to the distilled water and the control causing reduction in the number of flowers and delay in flowering within the period of 20 days after the first stage of application.

The total number of flowers produced by the treated plants are given in Table V. Results showed that there was no marked reduction in number of flowers by the treatments. Regarding the stages, both chemicals reduced the number of flowers in the third stage of application over the first and the second stages of application of the chemicals. There was no such difference between the first and the second stages. Between the chemicals, F,W-450 produced lesser number of flowers than MH treated plants. Regarding concentration, the lowest concentrations of F,W-450 (0.10%) and MH (25 ppm) produced lesser number of flowers than other concentrations of the respective chemicals. The highest concentration of MH (400 ppm) in the first stage of application produced more number of flowers than in control, distilled water and F,W-450 sprayed plants.

All the treatments of F,W-450 at all the stages caused a slight reduction in size of flowers. This was not found in plants sprayed with MH. Another interesting observation in F,W-450 and MH treatments was that the flowers immediately after the application of the chemicals possessed smaller anthers than control. Such anthers

TABLE III

Mean number of flowers per plant, 20 days after the 1st stage of application of the chemicals

Treatments	..	No. of flowers
Control	..	39.66
D.W. sprayed	..	57.66
<u>F.W-450</u>		
0.10%	..	24.33
0.15%	..	24.33
0.20%	..	37.66
0.25%	..	22.66
0.30%	..	11.33
<u>Mean</u>	..	<u>24.06</u>
<u>MH</u>		
25 ppm	..	33.33
50 ppm	..	34.00
100 ppm	..	15.33
200 ppm	..	6.33
400 ppm	..	5.33
<u>Mean</u>	..	<u>18.66</u>
General mean	..	21.96

Critical difference for MH Vs F.W-450 = 7.47

.. .. concentrations of MH or F.W-450 = 3.93

TABLE IV

Analysis of variance for the total number of flowers per plant,
20 days after the first stage of application

Source	S.S.	df	Variance	F.ratio
Total	52229.817	101
Block	465.317	2	232.658	2.643
Treatment	10424.410	11	947.764	2.017*
Between chemicals	202.800	1	202.800	2.305
Between concentrations	2159.467	4	539.866	6.135**
Between concentrations of F, W-450	1047.600	4	261.900	2.976*
Between concentrations of BH	2373.064	4	593.266	6.128**
Error	41339.090	88	469.762	..

** Significant at 1% level

* Significant at 5% level

TABLE V
Mean number of flowers per plant

Treatments	Stages of application			Mean
	I	II	III	
Control	373.7	373.7	373.7	373.70
D.W.sprayed	472.3	366.7	305.7	408.23
<u>F₂W-450</u>				
0.10%	317.3	361.0	279.7	319.33
0.15%	351.0	437.0	330.7	372.90
0.20%	409.7	336.7	297.3	347.90
0.25%	380.3	420.0	320.7	373.66
0.30%	406.7	444.7	291.7	381.33
<u>Mean</u>	<u>373.0</u>	<u>399.88</u>	<u>304.02</u>	<u>359.02</u>
<u>MH</u>				
25 ppm	323.3	319.0	333.0	325.10
50 ppm	432.7	284.7	346.3	354.56
100 ppm	400.0	336.7	378.7	371.84
200 ppm	415.3	442.7	421.7	426.56
400 ppm	492.7	367.7	292.0	394.13
<u>Mean</u>	<u>412.80</u>	<u>350.16</u>	<u>356.34</u>	<u>372.43</u>
General mean	392.90	375.02	330.18	

did not dehiscence and they dried up. This was observed in all the concentrations of both the chemicals at all the three stages. This was more pronounced in the case of F,W-450 sprayed plants.

4. Pollen studies

a) Pollen morphology

(i) Pollen shape

The normal pollen shape, spherical and 2-6 corperate was found to be slightly affected by the chemical treatment. Some of the pollen grains of F,W-450 and MH treated plants were found to be irregular.

(ii) Pollen size

Table VI furnishes the summary of the results. The size of pollen grains of treated plants varied from 18.59 μ to 28.69 μ , when compared with 30.76 μ in control plants.

Between chemicals the difference was not significant. But in general the reduction of pollen size was more in treatments with F,W-450. The concentrations and stages of application of the chemicals also showed some effect on reduction of pollen size. In the case of F,W-450 the highest concentration (0.30%) produced maximum reduction in pollen size at the second and third stages of application, where the mean pollen diameter was only 23.65 and 24.24 μ respectively. The highest reduction in size vis., 18.59 μ obtained by MH was at a concentration of 50 ppm at the second stage of application. In the case

TABLE VI

Size of pollen grains in μ

Treatments	Stages of application			Mean
	I	II	III	
Control	30.76	30.76	30.76	30.76
D.W. sprayed	31.31	27.06	28.86	29.08
<u>F.V-450</u>				
0.10%	29.15	26.86	24.82	26.94
0.15%	28.82	25.10	24.53	26.15
0.20%	28.51	25.52	24.66	26.23
0.25%	26.79	24.82	24.79	25.46
0.30%	28.25	23.65	24.24	25.38
<u>Mean</u>	<u>28.31</u>	<u>25.19</u>	<u>24.61</u>	<u>26.03</u>
<u>MI</u>				
25 ppm	28.16	24.07	24.29	25.51
50 ppm	29.88	18.59	24.57	24.35
100 ppm	27.39	25.67	24.51	25.86
200 ppm	28.56	25.10	25.37	26.34
400 ppm	26.56	25.26	24.02	25.28
<u>Mean</u>	<u>28.11</u>	<u>23.74</u>	<u>24.55</u>	<u>25.47</u>
General mean	28.21	24.46	24.58	..

of MH the higher concentrations did not result in proportionately higher reduction in pollen size.

b) Pollen sterility

(i) Acetocarmine staining method

The percentage of pollen sterility was calculated at five days intervals starting from five days after the application of the chemicals till the recovery of complete fertility. The calculated pollen sterility and its analysis of variance are given in the Tables VII and VIII respectively.

Results of the Table VIII show that all the concentrations of both the chemicals at all stages of application were highly significant over control and distilled water sprayed plants for the induction of male sterility. Percentage of pollen sterility was found to vary for each five days period after treatment. The chemicals induced maximum sterility 15 days (third interval) after the application at all stages. After that percentage of sterility gradually decreased in the following intervals. The sterility obtained in the interval was statistically significant over other intervals. The manifestation of the effect of a particular concentration of the chemicals was quicker in the first stage of application and was found to slow down as the stages of application advanced. The duration and the percentage of sterility were found to be dependent upon the chemicals used, the concentrations of the chemicals and the stages of application.

Between intervals the sterility obtained was statistically significant in the case of both the chemicals, F,W-450 and MH, at all the concentrations and at all stages. Between treatment x intervals the sterility obtained was highly significant. Between stages and treatment x stages also the sterility obtained was highly significant.

Tables VII and VIII clearly show that between the two chemicals MH was more effective at all the stages of application than F,W-450, while F,W-450 showed its superior effect only in the first stage of application. The result showed that the higher concentrations (200 ppm and 400 ppm) of MH were more effective for the induction of male sterility than the other concentrations (25 ppm, 50 ppm and 100 ppm). The two concentrations, 200 ppm and 400 ppm of MH induced 100 per cent sterility 15 days after the first stage of application. In the case of F,W-450 the 0.25 per cent concentration was the most effective, producing 100 per cent sterility 15 days after the first stage of application.

The result showed that though the highest concentrations (200 ppm and 400 ppm) of MH induced maximum sterility, no such relation between increase in concentration and increase in pollen sterility was obtained in the case of F,W-450. The result also indicated that application of the chemicals at the onset of flower production and anthesis was more effective than later stages of application.

TABLE VII
Percentage of Pollen Sterility by acetocarmine staining
technique

Treatments	Five days intervals						
	1	2	3	4	5	6	7
First stage of application							
Control	14.44	10.24	15.26	13.58	11.87	12.11	13.67
D.W. sprayed	7.03	5.89	7.25	11.82	7.93	10.61	11.12
F.W-450							
0 10	10.91	14.00	100.00	70.71	50.10	17.92	10.00
0 15	34.70	52.25	2.64	61.00	15.94	12.15	7.93
0 20	10.81	14.80	28.00	13.92	13.14	14.56	10.93
0 5	31.42	35.80	100.00	51.42	35.64	17.31	8.72
0 30	15.0	4.4	62.50	23.42	16.83	14.94	10.31
mean	20.65	28.22	52.63	44.49	26.33	15.27	9.62
Second stage of application							
Control	10.11	7.82	8.83	9.64	8.43	9.52	8.67
D.W. sprayed	15.02	9.53	11.13	10.34	9.87	8.36	8.54
F.W-450							
0 10	12.34	22.86	2.74	17.10	21.43	19.21	10.70
0 15	15.26	37.00	60.19	16.26	13.14	12.70	8.84
0 20	10.76	12.47	4.76	21.72	14.26	13.24	9.87
0 5	21.22	44.82	54.47	14.06	13.96	13.37	10.21
0 30	12.50	8.82	51.89	6.62	12.74	1.13	8.49
mean	14.41	29.07	47.61	17.14	15.20	14.13	9.60
MEI							
25 ppm	10.28	13.26	42.94	11.56	8.64	9.52	8.43
50 ppm	7.92	13.20	23.67	17.64	13.53	13.72	9.39
100 ppm	7.48	17.23	61.82	57.86	17.00	11.93	9.32
200 ppm	9.26	4.22	56.15	53.12	34.83	33.71	10.93
400 ppm	14.52	45.92	52.63	29.17	27.64	13.89	9.91
Mean	9.89	2.97	47.44	34.27	20.23	16.55	9.59
Third stage of application							
Control	8.43	12.57	10.00	8.82	8.49	8.83	
D.W. sprayed	8.35	10.46	7.21	12.17	8.18	11.00	
F.W-450							
0 10%	14.55	28.36	33.34	21.56	15.26	9.52	
0 15%	19.83	43.58	52.22	21.96	16.13	9.67	
0 20%	17.82	23.37	35.38	16.50	14.72	9.28	
0 25%	13.36	14.70	27.00	14.56	14.62	9.73	
0 30%	20.63	28.23	35.00	31.18	12.94	9.33	
Mean	17.24	27.65	36.79	21.15	14.73	9.22	
MEI							
25 ppm	13.6	21.57	33.83	14.76	13.79	9.63	
50 ppm	22.89	24.73	46.00	21.82	19.4	9.78	
100 ppm	22.10	72	41.7	1.42	14.83	9.76	
200 ppm	22.49	28.97	63.60	46.13	20.34	12.63	
400 ppm	1	0	5	1.13	10.27	9.63	
Mean	18.67	28.74	52.09	2.25	16.83	10.89	

Critical difference for intervals with MEI or F.W-450 = 6.57
 " " " " MEI vs F.W-450 = 2.67
 " " " " between intervals = 4.64

TABLE VIII

Analysis of variance for the percentage of pollen sterility
by acetocarmine staining method

Source	S.S.	df	Variance	F.ratio
Total	52791.3538	215
Treatment	13134.2839	11	1194.0258	14.4268**
Between intervals	12090.3932	5	2418.0786	29.2204**
Treatments x intervals	5697.7395	55	103.5952	1.2518*
Between stages	5159.1861	2	2579.5930	31.1722**
Treatments x stages	6221.9543	22	282.8161	3.4175**
Intervals x stages	1384.9741	10	138.4974	1.6736
Error	9102.8227	110	82.7529	..

** Significant at 1% level

* Significant at 5% level

TABLE IX

Pollen germination and tube elongation in sucrose
media

Concentration of the sucrose solution	Mean percentage of germination	Mean pollen tube length in μ (after 24 hours)
5%	37.52	46.73
10%	61.36	95.28
15%	85.28	124.43
20%	72.50	109.71
25%	54.89	85.50

(ii) Germination of pollen grains in artificial media

(a) Standardisation of media

The results obtained during the three stages are presented as follows.

(1) Sucrose solution alone

Table IX shows the germination percentage and the length of pollen tube in a medium containing sucrose solution alone. All the concentration of sucrose solution induced the pollen germination and the tube elongation after 24 hours revealed that 10 to 20 per cent, sucrose solution gave a high germination percentage with maximum pollen tube length. The highest concentration viz., 25 per cent showed a retardation in the germination percentage and elongation of pollen tubes.

(2) Sucrose solution with Boric acid

The germination percentage and the length of pollen tube in the sucrose boric acid solution are presented in Table X. Boric acid was found to increase the percentage of germination and tube growth. 15 per cent sucrose solution with the lowest concentration of boric acid (50 ppm) gave the best results. There was a retardation in the germination percentage and length of pollen tubes as the concentration of boric acid increased.

(3) Sucrose solution in combination with boric acid and agar

The summary of the results is presented in Table XI. Among the four concentrations of agar used, 1 gram of agar with 15%

TABLE X

Pollen germination and tube elongation in sucrose
with Boric acid media

Concentration of sucrose solution with Boric acid	Mean percentage of pollen germination	Mean pollen tube length in μ (after 24 hours)
10% + 50 ppm	74.18	118.21
10% + 100 ppm	71.49	102.67
10% + 150 ppm	69.35	98.43
15% + 50 ppm	91.20	134.56
15% + 100 ppm	88.32	121.27
15% + 150 ppm	80.94	110.50
20% + 50 ppm	85.00	127.34
20% + 100 ppm	79.35	110.21
20% + 150 ppm	72.43	97.67

TABLE XI

Pollen germination and tube elongation in Sucrose and Boric acid in
combination with agar agar media

Concentration of Sucrose solution with Boric acid and agar agar	Mean percentage of pollen germination	Mean pollen tube length in μ (after 24 hours)
15% + 50 ppm + 0.5 gm	78.38	119.00
15% + 50 ppm + 1.0 gm	92.41	136.47
15% + 50 ppm + 1.5 gm	80.37	123.61
15% + 50 ppm + 2.0 gm	74.42	116.93

sucrose solution and 50 ppm boric acid was found to be the best combination.

Percentage of sterility in culture medium

Germination percentage of pollen grains in artificial medium was found to be adversely affected by chemical treatments. Summary of results is presented in Table XII.

The percentage of sterility of the treated plants varied from 100 to 29.13, when compared with control having 14.05%. Cent per cent sterility was obtained at the higher concentrations (200 ppm and 400 ppm) of MH and 0.25 per cent of F₂W-450 for the first stage of application and the lowest sterility of 29.13 per cent in the second stage of application of MH at 50 ppm.

Between the chemicals MH was found to be superior for inducing a high percentage of sterility than F₂W-450. In the case of concentrations of the chemicals the difference was significantly different. The stage of application was found to have a major role in determining the percentage of sterility in the case of both the chemicals. First stage of application of the chemicals was significantly superior over the second and third stages of application.

(iii) Length of pollen tube

Summary of results in Table XIII revealed the effect of treatments on pollen tube elongation. The length of pollen tube was much affected by all the treatments. The reduction in length of

TABLE XII

Percentage of pollen sterility in culture medium

Treatments	Stages of application			Mean
	I	II	III	
Control	14.05	14.05	14.05	14.05
D.W. sprayed	15.34	14.05	15.67	15.02
<u>F.W-450</u>				
0.10%	57.23	31.12	35.24	41.19
0.15%	52.30	50.89	45.83	50.01
0.20%	31.95	42.01	36.51	36.82
0.25%	100.00	47.50	31.31	59.63
0.30%	52.24	50.77	36.27	46.42
<u>Mean</u>	<u>58.74</u>	<u>44.47</u>	<u>37.23</u>	<u>46.81</u>
<u>MH</u>				
25 ppm	33.77	40.92	35.55	36.75
50 ppm	51.41	29.13	42.71	41.08
100 ppm	52.84	51.82	40.23	48.29
200 ppm	100.00	48.51	52.89	67.13
400 ppm	100.00	46.49	60.20	68.89
<u>Mean</u>	<u>67.60</u>	<u>43.37</u>	<u>46.31</u>	<u>52.43</u>
General mean	63.17	43.92	41.77	..

TABLE XIII

Length of pollen tube in μ (after 24 hours)

Treatments	Stages of application			Mean
	I	II	III	
Control	137.21	137.21	137.21	137.21
D.W. sprayed	136.67	137.56	135.27	136.50
<u>7.7-450</u>				
0.10%	112.53	115.34	109.33	112.40
0.15%	114.48	105.21	98.21	106.90
0.20%	96.33	93.67	89.34	93.12
0.25%	4.47	38.27	66.43	36.39
0.30%	37.21	43.21	42.25	40.89
<u>Mean</u>	<u>73.00</u>	<u>79.34</u>	<u>81.11</u>	<u>77.94</u>
<u>III</u>				
25 ppm	113.21	117.55	121.23	117.33
50 ppm	102.33	99.67	93.32	100.44
100 ppm	98.30	93.17	94.23	95.23
200 ppm	3.43	67.54	82.55	61.11
400 ppm	4.67	39.61	44.27	29.51
<u>Mean</u>	<u>64.39</u>	<u>83.54</u>	<u>88.32</u>	<u>80.72</u>
General mean	68.69	81.44	84.71	..

pollen tube was found to be dependent upon the chemicals used, the concentration of the chemical and the stages of application.

The tube elongation of treated plants varied from 3.43 μ to 121.23 μ , when compared with control having 137.21 μ . Between the chemicals MH was found to be more effective than F,W-450 in reducing tube elongation. The highest reduction in tube elongation (3.43 μ) was obtained in MH at 200 ppm at the first stage of application. In the case of F,W-450 the highest reduction (4.47 μ) was obtained at 0.25 per cent in the first stage of application. There was a correlation between the stages of application and each concentration, that is as the stages of application advanced the same concentration, showed a reduction in its effect. Results showed that the effect of various concentrations of both the chemicals was significantly different.

II. Fruit set and fruit characters

1. Number of fruits per plant

Summary of results and analysis of variance are furnished in Tables XIV and XV respectively.

Analysis of variance in Table XV shows that the overall effect of the treatment was significant at 1 per cent level. The two chemicals did not differ in their effects significantly, eventhough there was a tendency of reduction in fruit set in the case of MH. The effect of concentrations in general was significant. In the case of individual chemicals the concentrations of MH alone were

TABLE XIV
Number of fruits per plant

Treatments	Stages of application			Mean
	I	II	III	
Control	175.16	175.16	175.16	175.16
D.W. sprayed	179.83	167.16	165.50	170.83
<u>F.W-450</u>				
0.10%	159.00	137.83	143.33	147.38
0.15%	166.83	192.00	156.50	171.77
0.20%	140.50	158.66	132.00	143.72
0.25%	143.50	144.33	135.00	140.94
0.30%	162.33	156.66	141.33	153.44
<u>Mean</u>	<u>154.43</u>	<u>153.89</u>	<u>142.03</u>	<u>151.45</u>
<u>MH</u>				
25 ppm	151.66	163.33	162.66	159.88
50 ppm	179.16	150.50	143.16	157.61
100 ppm	143.16	145.50	143.16	143.94
200 ppm	102.83	140.33	167.33	136.83
400 ppm	98.66	106.83	136.83	114.11
<u>Mean</u>	<u>135.09</u>	<u>141.69</u>	<u>150.63</u>	<u>142.47</u>
General mean	144.76	147.79	146.33	..

C.D. for comparison between stages for the same concentration = 43.91

.. = 19.64

.. Concentration of MH or F.W-450 = 22.65

.. chemicals = 11.33

TABLE XV

Analysis of variance for total number of fruits per plant

Source	S.S.	df	Variance	F, ratio
Total	391741.136	101
Replication	14271.342	2	7135.671	2.451
Treatments	186099.000	33	5639.364	1.945**
Between chemicals	7254.014	1	7254.044	2.502
Between concentrations	43762.155	4	10940.539	3.773**
Concentrations x chemicals	27324.467	4	6831.117	2.355
Between stages	1592.266	2	796.133	..
Chemicals x stages	14051.29	2	7025.645	2.423
Concentrations x chemicals	20102.845	8	2512.856	..
Chem. x conc. x stages	27810.333	8	3476.292	1.198
Between concentrations of F, W-450	21735.867	4	5433.967	1.874
Between concentrations of MH	49350.000	4	12337.5	4.255**
Control Vs. F, W-450	6324.938	1	6324.938	2.181
Control Vs. MH.	12020.889	1	12020.889	4.149*
Distilled water Vs. F, W-450	11264.948	1	11264.948	3.775
Distilled water Vs. MH	24121.481	1	24121.481	7.319**
Error	191370.794	66	2899.557	..

** Significant at 1% level

* Significant at 5% level

significantly different. Here the concentration of 50 ppm sprayed at the first stage gave the highest number of fruits per plant and this differed significantly from the higher concentrations of 200 ppm and 400 ppm sprayed at the first stage. The maximum reduction in number of fruit set (98.66) was obtained in plants sprayed with 400 ppm of MH, when compared with control having 175.16. Reduction in fruit set was found to be dependent on the concentration of the chemical and stages of application in the case of both chemicals. The effect of stages, chemicals x stages, concentration x stages and chemical x concentration x stages were not significant in the case of F,W-450.

Between stages in general, in the case of MH highest reduction in fruit set was found in the first stage of application. As the stages advanced fruit set gradually increased. But in the case of F,W-450 the maximum reduction in fruit set was noticed in the third stage of application.

2. Fruit setting on selfing

Summary of results is given in Table XVI. Results showed that the treatments were significant. Both the chemicals, F,W-450 and MH were found to be equally effective in inducing a reduction in fruit setting on selfing.

The number of fruits set on selfing of treated plants varied from 2.33 to 7.33 when compared with control having 21.63. This high reduction in fruit setting depended upon the concentration of the

TABLE XVI

Percentage of fruit setting on selfing

Treatments	Stage of application			Mean
	I	II	III	
Control	21.63	21.63	21.63	21.63
D.W. sprayed	21.66	22.00	21.67	21.77
<u>I.V-250</u>				
0.10%	3.33	5.63	7.00	5.32
0.15%	2.67	2.33	3.89	2.96
0.20%	3.66	3.33	4.67	3.88
0.25%	2.33	3.00	2.33	2.55
0.30%	2.33	2.67	3.33	2.77
<u>Mean</u>	<u>2.87</u>	<u>3.39</u>	<u>4.27</u>	<u>3.49</u>
<u>MH</u>				
25 ppm	4.67	5.00	7.33	5.66
50 ppm	3.66	2.33	5.37	3.78
100 ppm	3.00	3.67	2.66	3.11
200 ppm	2.33	3.33	2.33	2.66
400 ppm	2.37	2.33	2.66	2.45
<u>Mean</u>	<u>3.21</u>	<u>3.33</u>	<u>4.07</u>	<u>3.53</u>
General mean	3.04	3.36	4.15	..

chemical and stages of application. In the case of F,W-450 maximum reduction in fruit set was obtained in plants treated with the higher concentrations of 0.25 per cent and 0.30 per cent. Similarly in the case of MH also, maximum reduction in fruit set was obtained in plants treated with the higher concentrations of 200 ppm and 400 ppm. Between the chemicals, F,W-450 was more effective in the first stage of application while in the second and third stages MH was more effective than F,W-450. Between the chemicals the effect was not significantly different from one another in all the three stages.

3. Fruit setting on crossing

Summary of results is furnished in Table XVII. The results showed that the percentage of fruit set on crossing was not much affected by the treatments in the case of both the chemicals. However, in the case of F,W-450 the highest concentration of 0.30% at the second stage of application showed a reduction in fruit set i.e., 92 per cent, when compared with control having 100 per cent. The reduction in fruit setting percentage was found to depend upon the concentrations and stages of application of the chemicals.

Stages of application were found to have a major role in determining the percentage of fruit set on crossing. The maximum reduction was found to be in the second stage in the case of F,W-450 and in the first stage in the case of MH.

TABLE XVII

Percentage of fruit setting on crossing

Treatments	Stage of application			Mean
	I	II	III	
Control	100.00	100.00	100.00	100.00
D.W. sprayed	99.00	99.67	99.33	99.33
<u>E.W-450</u>				
0.10%	100.00	98.33	99.00	99.11
0.15%	95.67	96.66	96.00	96.11
0.20%	97.00	93.40	94.67	95.02
0.25%	94.00	93.00	93.33	95.44
0.30%	94.33	92.00	94.67	93.66
<u>Mean</u>	<u>96.20</u>	<u>94.67</u>	<u>95.33</u>	<u>95.47</u>
<u>MH</u>				
25 ppm	99.00	100.00	99.33	99.44
50 ppm	97.33	100.00	98.67	98.66
100 ppm	93.00	96.34	98.67	96.66
200 ppm	95.37	96.67	98.00	96.68
400 ppm	94.66	96.67	97.33	96.22
<u>Mean</u>	<u>95.87</u>	<u>98.33</u>	<u>98.40</u>	<u>97.53</u>
General mean	96.03	96.50	96.96	..

In general the results showed that both the chemicals did not adversely affect the female phase of the treated plants.

4. Size of fruits

(a) Length of selfed fruits

Table XVIII shows the mean length of selfed fruits in cm. Both the chemicals reduced the fruit length significantly. The length of fruits in the treated plants varied from 3.7 to 5.9 cm while it was 6.1 cm in control plants.

By comparing the effect of both the chemicals the data showed that MH markedly reduced the fruit length in all the stages than F,W-450. Maximum reduction of fruit length (3.7 cm) was obtained in plants treated with 25 ppm of MH and in the case of F,W-450 maximum reduction (4.0 cm) was obtained in plants treated with 0.25 per cent at the second stage. By comparing stages the maximum reduction was obtained in the first stage of application of both the chemicals. As the stage advanced this reduction gradually decreased. The results showed that plants treated with 0.25 per cent of F,W-450 recorded maximum reduction in fruit length compared to the other concentrations of the same chemical. In the case of MH the lowest concentration 25 ppm reduced the fruit length compared to the other concentrations of the same chemical. So in general the higher concentrations of F,W-450 and lower concentrations of MH reduced the fruit length in all the three stages.

TABLE XVIII

Length of selfed fruit in Centimeters

Treatments	Stage of application			Mean
	I	II	III	
Control	6.1	6.1	6.1	6.10
D.W. sprayed	5.8	5.5	5.4	5.54
<u>E. #450</u>				
0.10%	5.5	5.5	5.9	5.63
0.15%	5.0	5.5	5.0	5.16
0.20%	4.5	4.5	4.6	4.53
0.25%	4.5	4.0	5.0	4.50
0.30%	4.2	5.0	5.0	4.73
<u>Mean</u>	<u>4.75</u>	<u>4.90</u>	<u>5.5</u>	<u>4.91</u>
<u>MH</u>				
25 ppm	4.8	3.7	4.0	4.16
50 ppm	4.5	5.2	5.0	4.90
100 ppm	4.6	4.5	5.5	4.86
200 ppm	4.7	5.2	4.5	4.80
400 ppm	5.0	5.2	5.0	5.06
<u>Mean</u>	<u>4.72</u>	<u>4.76</u>	<u>4.80</u>	<u>4.65</u>
General mean	4.73	4.83	5.15	..

(b) Length of crossed fruits

Data relating to the length of crossed fruit are presented in Table XIX. The results showed that treatments had a significant effect on the reduction of fruit length. Here also the variation in length of crossed fruit was related to the stages of application, the concentrations of the chemical and the chemicals used.

Between the chemicals MH was found to be more effective in the reduction of fruit length than F,W-450. Maximum reduction in fruit length was obtained in plants treated with 25 ppm of MH in the first stage (4.0 cm) and in the case of plants treated with 0.25 per cent of F,W-450 in the third stage (4.3 cm). The length of fruit in treated plants varied from 4.0 to 5.8 cm, when compared with control having 7.0 cm. By comparing concentrations 0.25 per cent of F,W-450 and 25 ppm of MH produced maximum reduction in fruit length. In the case of stages maximum reduction was obtained in the first stage. As the stages advanced there was a tendency for increase in fruit length. This was true in the case of both the chemicals. In general the higher concentrations 0.20 per cent and 0.25 per cent of F,W-450 and the lower concentrations 25 ppm and 50 ppm of MH reduced the fruit length in all the three stages.

5. Number of seeds per fruit

(a) Number of seeds per selfed fruit

Summary of results is furnished in Table XX. The effect of chemicals was markedly significant. This was found to be dependent

TABLE XIX
Length of crossed fruit in cm

Treatments	Stages of application			Mean
	I	II	III	
Control	7.0	7.0	7.0	7.00
D.W.sprayed	5.8	5.8	6.3	5.96
<u>F.W-450</u>				
0.10%	5.6	5.5	5.5	5.53
0.15%	5.7	5.8	5.5	5.40
0.20%	5.2	5.5	4.6	5.10
0.25%	5.6	5.0	4.3	4.96
0.30%	5.5	5.5	5.3	5.43
<u>Mean</u>	<u>5.52</u>	<u>5.46</u>	<u>5.40</u>	<u>5.48</u>
<u>III</u>				
25 ppm	4.0	4.7	4.8	4.50
50 ppm	5.5	4.5	4.5	4.83
100 ppm	4.2	5.0	5.0	4.73
200 ppm	5.5	5.5	5.5	5.50
400 ppm	5.5	5.1	5.0	5.20
<u>Mean</u>	<u>4.95</u>	<u>4.96</u>	<u>4.96</u>	<u>4.95</u>
General mean	5.23	5.21	5.18	..

upon the chemicals used, the concentrations of the chemicals and the stages of application. The number of seeds per fruit in treated plants varied from 15 to 60.5, when compared with control having 70.

Between chemicals the adverse effect of MH was found to be more than that of F,W-450. Maximum reduction in number of seeds i.e., 15 was obtained in plants treated with 400 ppm of MH in the second stage. In the case of F,W-450 the concentration of 0.30 per cent in the third stage resulted in the maximum reduction of seeds (31). In the case of concentration of the chemicals the highest concentration (0.31%) of F,W-450 and 100 ppm of MH produced the minimum number of seeds per fruit 36.66 and 29.33 respectively. Between stages the minimum reduction was obtained in the third stage in the case of both the chemicals. Maximum seeds were obtained in the first stage of application of the chemicals.

The data clearly showed that 0.15%, 0.25% and 0.50% of F,W-450 and 100 ppm, 200 ppm and 400 ppm of MH markedly reduced the number of seeds per fruit, when compared with control and distilled water sprayed plants.

(b) Number of seeds per crossed fruit

Table XII shows the summary of results. The treatments were markedly significant. In some cases certain concentrations of both the chemicals produced more number of seeds per fruit than the control and distilled water sprayed plants.

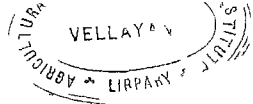


TABLE XI

Number of seeds per selfed fruit

Treatments	Stages of application			Mean
	I	II	III	
Control	70.0	70.0	70.0	70.0
D.W. sprayed	59.0	60.5	60.0	59.83
<u>P.W-450</u>				
0.10%	59.5	55.0	49.0	54.16
0.15%	52.0	48.0	43.0	47.66
0.20%	60.0	60.5	58.0	59.50
0.25%	45.0	41.0	39.0	41.00
0.30%	43.0	36.0	31.0	36.66
<u>Mean</u>	<u>51.3</u>	<u>48.1</u>	<u>44.0</u>	<u>47.77</u>
<u>MH</u>				
25 ppm	45.0	49.0	19.0	37.66
50 ppm	54.0	46.5	50.0	50.16
100 ppm	24.0	36.0	28.0	29.33
200 ppm	32.0	48.5	31.5	37.33
400 ppm	32.0	15.0	54.0	33.66
<u>Mean</u>	<u>37.4</u>	<u>39.0</u>	<u>36.5</u>	<u>37.63</u>
General mean	44.35	43.55	40.25	..

The number of seeds per fruit in treated plants varied from 50.0 to 87.5, when compared with 78.5 in control plants. In the case of F,W-450, 0.10 per cent in the first stage, 0.15 per cent in the first and second stages and 0.30 per cent in the first stage produced more number of seeds than control and distilled water sprayed plants. In the case of MH, 25 ppm in the first and second stages and 100 ppm and 200 ppm in the first stage produced more number of seeds than control and distilled water sprayed plants. 0.10 per cent of F,W-450 in the third stage produced the maximum number of seeds, 87.5 than all other treatments and control. The highest reduction of seeds 50.0 was noticed in 400 ppm of MH sprayed in the second stage. Between the concentrations of the chemicals 0.20 per cent of F,W-450 produced the maximum reduction of seeds i.e., 66.33 where as in MH the highest concentration, 400 ppm showed the maximum reduction i.e., 59.16. Between stages, the second stage of F,W-450 and third stage of application of MH showed the maximum reduction.

Both the chemicals were not markedly different in their effect. All the treatments of both the chemicals except the highest concentration (400 ppm) of MH, produced more number of seeds than distilled water sprayed plants.

6. Weight of fruits

Summary of results and the analysis of variance are furnished in Tables XXII and XXIII respectively. The over all effect

TABLE XXI

Number of seeds per crossed fruit

Treatments	Stages of application			Mean
	I	II	III	
Control	78.5	78.5	78.5	78.50
T.W. sprayed	65.0	71.0	60.0	65.33
<u>F.W-450</u>				
0.10%	72.0	61.0	87.5	73.50
0.15%	85.0	55.0	85.0	75.00
0.20%	68.0	62.0	69.0	66.33
0.25%	57.0	74.0	71.0	67.33
0.30%	82.0	63.0	60.0	68.33
<u>Mean</u>	<u>72.8</u>	<u>63.0</u>	<u>74.5</u>	<u>70.09</u>
<u>MH</u>				
25 ppm	80.0	95.0	74.0	83.00
50 ppm	65.0	72.5	70.0	67.53
100 ppm	81.0	63.0	62.5	69.83
200 ppm	82.0	75.0	60.0	72.33
400 ppm	57.5	50.0	70.0	59.16
<u>Mean</u>	<u>73.1</u>	<u>71.1</u>	<u>67.4</u>	<u>70.13</u>
General mean	72.95	67.05	70.95	..

of the treatment was significant at 5 per cent level. The two chemicals did not differ in their effects, even though there was a tendency of reduction in yield in the case of MH. The effect of concentration in general was significant. In the case of F,W-450 plants treated with 0.15 per cent gave the maximum yield whereas in MH, 25 ppm gave the maximum yield.

In the case of individual chemicals the concentration of MH alone was significantly different. Here the concentration, 25 ppm with the highest yield differed significantly from 400 ppm with the lowest yield. Other comparisons were not significant. The effect of stages, chemicals x stages, concentration x stages and chemical x concentration x stages were not significant in the case of both the chemicals. However the maximum yield was obtained with the chemical, F,W-450 for the concentration of 0.15 per cent at the second stage. This was higher than that of control. For MH the maximum yield was obtained for the concentration of 200 ppm sprayed at the third stage. But this was lower than that obtained for F,W-450. The maximum reduction in yield was obtained in plants treated with 200 ppm and 400 ppm of MH at the first stage.

Between the stages the maximum yield was obtained in the second stage of application of F,W-450 and third stage of application of MH. MH at the first stage gave the maximum reduction in yield.

TABLE XXII

Weight of fruits per plant in gram

Treatment	Stages of application			Mean
	I	II	III	
C Control	189.33	188.33	188.33	188.33
D.W.sprayed	192.55	168.33	164.16	175.01
<u>F.W-450</u>				
0.10%	176.83	139.33	164.66	160.27
0.15%	174.33	192.66	166.16	184.38
0.20%	136.44	170.50	141.66	149.53
0.25%	152.33	158.00	145.33	151.66
0.30%	146.83	174.33	150.00	157.05
<u>Mean</u>	<u>157.35</u>	<u>166.97</u>	<u>157.56</u>	<u>150.57</u>
<u>MH</u>				
25 ppm	160.00	178.50	161.83	166.77
50 ppm	170.60	152.16	143.33	157.69
100 ppm	163.83	144.16	170.16	159.38
200 ppm	98.33	146.33	187.50	144.38
400 ppm	99.16	110.00	147.50	118.88
<u>Mean</u>	<u>139.78</u>	<u>146.63</u>	<u>162.06</u>	<u>149.42</u>
General mean	148.56	156.79	159.81	..

C.D. for comparison between stages for the same concentration = 52.87

..	stages	= 23.70
..	concentration of MH or F.W-450	= 30.82
..	chemicals	= 14.43

TABLE XXIII

Analysis of variance for weight of fruits per plant

Source	S.S.	df	Variance	F.ratio
Total	576543.294	101
Replication	68944.647	2	34472.323	7.959**
Treatments	221753.627	33	6719.807	1.552**
Between chemicals	11178.311	1	11178.311	2.581
Between concentration	47916.178	4	11979.045	2.766*
Chemicals x concentrations	31247.078	4	7811.769	1.803
Between stages	8086.222	2	4043.111	..
Chemicals x stages	11125.256	2	5562.628	1.284
Concentration x stages	24953.223	8	3119.153	..
Chemicals x concentrations x stages	59008.188	8	7376.024	1.703
Between concentrations of F,W-450	27907.467	4	6976.867	1.611
Between concentrations of MH	51256.222	4	12814.055	2.958*
Control Vs. F,W-450	8625.066	1	8625.066	1.898
Control Vs. MH	16965.313	1	16965.313	3.917*
Distilled water Vs. F,W-450	6182.437	1	6182.437	1.427
Distilled water Vs. MH	19507.500	1	19507.500	4.504*
Error	285845.020	66	4330.985	..

** Significant at 1% level

* Significant at 5% level.

In the case of comparison with control and distilled water sprayed plants the effect of MH alone was significant. It tended to decrease the yield in comparison with control, distilled water and F.W-450 treated plants. So the data clearly showed that the higher concentrations (200 ppm and 400 ppm) of MH adversely affected the yield.

III. Seed viability

1. Percentage of seed germination in selfed fruits

The percentage of seed viability was calculated from an entirely random sample. The calculated percentage of seed sterility is given in Table XXIV.

Results showed that the treatments were significant. From the data it was seen that the percentage of seed sterility was highly correlated with the chemicals used, the concentration of the chemicals and the stages of application. The percentage of seed germination of treated plants varied from 40.67 to 80.21, when compared with control having 85.67 per cent.

In all the treatments germination was completed within eight days after sowing. In the controls complete germination was observed by the sixth day after sowing. Between chemicals MH was found to have more adverse effects on seed germination than F.W-450. Between concentrations, 100 ppm, 200 ppm and 400 ppm of MH and 0.25 per cent and 0.30 per cent of F.W-450 were found to give lesser germination

TABLE XXIV

Percentage of seed germination in selfed fruits

Treatments	Stages of application			Mean
	I	II	III	
Control	85.67	85.67	85.67	85.67
D.W.sprayed	83.33	85.67	81.33	83.44
<u>F.W-450</u>				
0.10%	78.67	80.21	76.49	78.46
0.15%	72.42	79.67	69.67	75.92
0.20%	79.24	69.34	69.24	72.61
0.25%	58.33	60.14	56.53	58.33
0.30%	51.49	48.41	52.49	50.79
Mean	68.03	67.55	64.88	66.82
<u>MH</u>				
25 ppm	72.64	73.67	69.41	71.91
50 ppm	69.24	64.66	65.00	66.30
100 ppm	57.33	51.43	54.67	54.47
200 ppm	52.73	53.13	49.49	51.88
400 ppm	43.33	40.67	45.55	43.11
Mean	59.05	56.67	56.82	57.53
General mean	63.54	62.11	60.85	..

percentage. Highest concentration of F, W-450 (0.30%) and MH (400 ppm) showed the maximum reduction in seed germination. The highest reduction in seed germination (40.67%) was found in plants treated with 400 ppm of MH in the second stage, whereas the maximum percentage of seed germination was obtained in plants treated with 0.10% of F, W-450 in the second stage. This was lower than that of control and distilled water sprayed plants. Comparing stages the maximum reduction was obtained in the third stage in the case of both the chemicals. As the stages advanced the percentage of seed germination also decreased.

2. Percentage of seed germination in crossed fruits

The germination percentage of seeds from crossed fruits is given in Table XXV. The data indicated that the germination was only slightly affected by the treatments. The percentage of seed germination of treated plants varied from 63.66 to 91.13 when compared with control having 92.27.

Between chemicals the adverse effect was more in the case of MH. MH at 400 ppm in the third stage of application produced the maximum reduction in seed germination. Other concentrations of both the chemicals were not significantly different. Between stages the third stage of application showed the maximum reduction of seed germination.

TABLE XXV

Percentage of seed germination in crossed fruits

Treatments	Stages of application			Mean
	I	II	III	
Control	92.27	92.27	92.27	92.27
D. N. sprayed	93.69	90.62	89.33	91.28
<u>F. V. 450</u>				
0.10%	89.67	90.33	91.13	90.37
0.15%	89.33	84.27	83.27	85.62
0.20%	82.27	83.13	80.17	81.86
0.25%	78.66	75.43	74.00	76.03
0.30%	74.33	69.23	71.43	71.66
<u>Mean</u>	<u>82.85</u>	<u>80.48</u>	<u>80.00</u>	<u>81.11</u>
<u>ppm</u>				
25 ppm	83.33	88.43	85.67	85.81
50 ppm	80.67	81.65	79.24	80.52
100 ppm	71.00	79.67	72.17	74.28
200 ppm	76.38	73.43	74.13	71.65
400 ppm	69.17	67.27	63.66	66.70
<u>Mean</u>	<u>76.11</u>	<u>78.09</u>	<u>74.97</u>	<u>76.39</u>
General mean	79.48	79.28	77.48	..

DISCUSSION

DISCUSSION

The practical utilization of chemical gametocides, though it is in its infancy, has attracted the masterminds of plant breeders all over the world. Chemically induced male sterility has been successfully utilized in the exploitation of hybrid vigour, in sexually propagated crop plants like cotton, tomato etc.

The yield of chillies can be increased by exploiting hybrid vigour as already demonstrated by Dashpande (1933) and Pal (1945). The difficulty in the production of large scale hybrid seeds can be overcome by the use of male sterile lines. So far no male sterile lines have been discovered in chillies. As such, chemical methods of inducing male sterility appears to be the only way out of this difficulty. The present study was thus undertaken to evaluate the selectiveness of maleic hydrazide and P, V-450 on chillies as male gametocides and to study their side effects on the general growth and yield of the crop.

I. Growth and morphological characteristics

1. Visual observation

In the present investigation it was observed that in general all the concentrations of both the chemicals produced side effects like suppression of growth, yellowing of leaves with burning tips and burning of younger buds. The application of the higher concentrations at every stages of growth were comparatively more

injurious. This is in agreement with the results in other crops reported by Turkey (1959), Hammer and Turkey (1959), Pate and Duncan (1960), Krishnan Nair (1964) and Kaul and Singh (1967).

The injurious effects were more pronounced in the case of treatments with MH as compared to F,W-450. Comparatively higher toxic effects with MH than with F,W-450 has been reported by Nair (1964) in bhindi and Kaul and Singh (1967) in Cajanus cajan. The suppression of growth as well as other injurious effects persisted only for a few days and plants recovered within one week. This is in agreement with report of Nair (1964) in bhindi.

2. Height of plants.

Plants sprayed with higher concentrations (200 and 400 ppm) of MH significantly reduced the height, whereas there was no marked reduction in plant height in the treatment with F,W-450 (Plate, II, III, IV and V). Reduction in plant height with MH has been reported by Currier et al. (1950) and Singh (1964) in cotton, Chowdhury and Kamphal (1960) in cowpea and Yaul and Singh (1967) in Cajanus.

3. Number of flowers.

Results presented in the Tables III and IV clearly show that both the chemicals significantly delayed flowering. In this case the effect of MH was found to be superior to that of F,W-450. Higher concentrations (200 and 400 ppm) of MH delayed flowering significantly. Delay in flowering by treating the plants with MH was reported by David (1950), Josephson (1951), Wittwer (1959) and Maini and Sandhu (1959) in several crop plants. In the case of F,W-450, Eaton (1957)

reported the same results in cotton. Kaul and Singh (1967) reported that both the chemicals caused delayed flowering in Cajanus. Davis (1950) suggested that delay in flowering was due to the toxic effects of the chemical, which retarded the growth of the plants. Consequently the treated plants flowered later than the control.

All the plants sprayed with F, W-450 showed a slight reduction in size of flowers. (Plate VI and VII). The reduction in flower size was also reported by Kumar (1963) in sesame and Singh (1964) in cotton. Another interesting feature observed in F, W-450 and MH treatments was that the flowers produced immediately following the application of the chemicals possessed smaller anthers which get dried up without dehiscence. Such results were also reported by Kaul and Singh (1967) in Cajanus.

Summary of results in Tables V shows that F, W-450 and MH did not show any marked reduction in the total number of flowers produced. (Fig. I). A slight reduction was observed in plants sprayed at the third stage of application. In the case of F, W-450 this line of results was reported by Laelamma (1965) in brinjal. In the case of MH the highest concentration in the first stage of application resulted in significant increase in the total number of flowers produced. Such results reported by Currier et al. (1950), Kumar (1963) and Singh (1964). Kaul and Singh (1967) suggested that this increase was brought about by the increase in the number of branches due to the death of apical meristems.

4. Pollen studies

(a) Pollen shape.

The shape of the normal pollen grains of chillies is spherical and 2-6 colporate. For short periods following each treatment with the chemicals the percentage of pollen grains with normal shape was small and there were large number of pollen grains which were deformed in shape, small and shrivelled. This observation is in conformity with the findings of Saton (1957), Narayana Swami (1960) and Chopra et al. (1960)

(b) Pollen size.

Results presented in Table VI indicate that the pollen size was much affected by the chemical treatment. (Fig. II). The reduction in pollen size was comparatively more in the MH treated plants. Among the concentrations of F,W-450 the highest concentration resulted in maximum reduction in size of pollen grains. Nair (1964) and Leelamma (1965) also recorded reduction in pollen size in plants treated with F,W-450.

5. Sterility.

(i) Non-dehiscence of anthers.

Both F,W-450 and MH treated plants showed the presence of non-dehiscent anthers. This is an indication of pollen sterility. Plants sprayed with F,W-450 showed more number of non-dehiscent anthers than plants sprayed with MH. This effect was more pronounced with higher concentration of the chemical. Instances of failure of anther dehiscence

because of the application of F₁W-450 were reported by Hensz and Mohr (1959), Cameron and Eaton (1959), Pate and Duncan (1960), Santhanam (1961), Nair (1964) and Kaul and Singh (1967). Cameron and Eaton (1959) reported that the non-dehiscent character was not a uniform feature of the anthers of treated corn plants. But Kaul and Singh (1967) reported that the effect was quite uniform in Cajanus, where the plants were treated with F₁W-450 and ME. In the present experiment however, the effect was not uniform.

(ii) Pollen sterility.

(a) Acetocarmine staining method.

Pollen sterility determination by acetocarmine staining method revealed marked variation in the effect of chemicals on inducing sterility. (Fig. III A & B, IV A & B and V A & B).

Though there were differences between the treatments for the maximum percentage of sterility induced and for the period of its retention, all the treatments of both the chemicals could induce male sterility.

In the case of F₁W-450 complete pollen sterility was obtained in plants treated with 0.10% and 0.25% on the 15th day of the first stage of application only. Other concentrations induced only partial male sterility in all the three stages. Successful induction of male sterility with F₁W-450 has been reported by Eaton (1957) and Singh (1964) in cotton, Moore (1959) in tomato,

Cameron and Eaton (1959) in corn, Kumar (1963) in sesame, Nair (1964) in bhindi, Leelamma (1965) in brinjal, Vit (1960) in red clovers and Kaul and Singh (1967) in Cajanus. But Pedersen (1959), and Miller and Hittle (1963) working on alfalfa and Starness and Hadley (1962) with Soybeans could obtain only partial pollen sterility.

MH could bring about 100% pollen sterility only in the plants sprayed with 200 and 400 ppm at the first stage of application. Induction of complete male sterility by the use of MH was reported by Naylor (1950), Naylor and Davis (1950) and Poljakov (1966) in maize; Meyer (1956) in cotton, Rehm (1952) and Chopra et al (1960) in tomato and Iyer and Randhawa (1965) in grapes. However, there were other reports like Warren and Dimmock (1954) in hybrid corn, Robert (1959) on Saint paulia, Kumar (1963) in sesame, Singh (1964) in cotton, Nair (1964) in bhindi and Kaul and Singh (1967) in Cajanus, where maleic hydrazide was found to induce only partial male sterility.

As is seen from data in Table VII, this induced male sterility was of temporary nature, because after some time pollen sterility was gradually reduced and the plants regained their original fertility. Table VII also shows that in all the treatments with the chemicals the sterility percentage increased for a period and after reaching the maximum of each concentration and the stages of application got gradually decreased and after a few weeks pollen viability was restored. This is in line with the reports of various investigators like Moore (1959), Santhanam (1961) and Leelamma (1965) with F₁W-450

and Nair (1964) with F, W-450 and MH. In the present experiment maximum pollen sterility was obtained on the 15th day after the application of the chemical in the early stages of application. Kaul and Singh (1967) also reported the same effect of F, W-450 and MH on Cajanus.

Complete pollen sterility in the present experiment lasted for 12-17 days, depending upon the concentration and stages of application of the chemicals. In tomato Rehm (1952) reported the period of complete pollen sterility to be of 10-14 days. Chopra et al (1960) observed complete pollen sterility to last for only 7 days. Kaul and Singh (1967) reported the period of complete pollen sterility to be of 15 to 25 days in Cajanus. But in red clovers Wit (1960) found male sterility for the full blooming period. Choudhury and George (1964) obtained pollen sterility of 90-100% lasting for 5-12 days in two varieties of brinjal by spraying the whole plants with MH.

(b) Germination of pollen grains in artificial media.

The results of the present investigations on this aspect presented in Tables IX, X and XI indicated that a medium containing 15% sucrose, 1% agar and 50 ppm of boric acid gave the maximum percentage of pollen germination and pollen tube elongation. Stimulation of pollen germination and tube elongation by the addition of boric acid to the culture media was reported by Schurucker (1955), Thompson and Batjer (1950), Munzeer (1960), Sing (1960) and Dean (1964). Hristov and Gancev (1964) found that 10 per cent sucrose and 1 per cent agar

stimulated pollen germination and tube elongation in red pepper when flower extracts were added to the medium. Higher concentrations of sucrose retarded the pollen germination and tube elongation in the present study. This is in line with the reports of Prasad and Muzaffapur (1962) in the species of Malvaceae.

(c) Percentage of sterility.

In nutrient medium also the pollen grains of different treatments showed a high percentage of sterility (Table XII and Fig. VI). P, W-450 at 0.25% in the first stage of application showed 100% sterility. Other concentrations were not capable of inducing 100% sterility. The effect was more in the first stage than in the second and third stages of application. This is in agreement with the results reported by Nair (1964) in bhindi and Leelamma (1965) in brinjal.

MH had also reduced the germination percentage in the culture medium. MH at concentrations of 200 and 400 ppm in the first stage of application showed 100% sterility. It was found that the higher concentrations resulted in higher percentages of sterility and that maximum sterility was obtained in the first stage of application. Such results were also reported by Nair (1964) in bhindi.

A comparison of the two methods discussed above revealed that the sterility percentages were always higher in the culture medium than those obtained under acetocarmine staining method. This is in agreement with the findings of Ostapenko (1956), Nair (1964) and

Leelamma (1965). This variation may be due to the fact that in agar medium only the pollen grains which are completely fertile will germinate, while in staining technique, pollen grains having less viability may also take stain and will be counted as fertile ones.

(d) Pollen tube elongation.

All the treatments had an effect of reducing pollen tube elongation compared to control. (Table XIII and Fig. VII). Maximum reduction was obtained in plants sprayed with 0.25% of F,W-450 and 200 ppm and 400 ppm of MH at first stage of application. Such reduction in pollen tube elongation was observed by Nair (1964) in bhindi with F,W-450 and MH and Leelamma (1965) in brinjal with F,W-450.

II. Fruit set and fruit characters.

The effects of the chemicals on the number and weight of fruits per plant are given in Tables XIV and XXII respectively. The results clearly showed that both the chemicals significantly reduced the number and weight of fruits per plant. (Fig. VIII). The two chemicals did not differ in their effects significantly eventhough there was a tendency of reduction in fruit set and weight of fruits in the case of MH.

In the case of F,W-450 the number and weight of fruits per plant in general showed a negatively significant increase over the control. Such reduction in fruit setting and yield were reported by

Bocanegra et al. (1958) in French bean, Rubenhauer and Schuttis (1960) in sugarbeets, Pate and Duncan (1960) in cotton, Nair (1964) in bhindi, Leelamma (1965) in brinjal and Kaul and Singh (1967) in Cajanus. However, Dudley (1960) reported no reduction in yield in sugarbeets sprayed with F,W-450.

Plants treated with MH showed maximum reduction in yield and fruit setting per plant. The higher concentrations 200 ppm and 400 ppm produced the maximum reduction in number and weight of fruits per plant. Other concentrations also showed a negatively significant increase over the control. The result also showed that early stages of application had a profound effect in decreasing the yield when compared to other stages of application. Such results were also reported by Williamson (1958) in wheat and rye, Jaubhari and Ayedhya Prasad (1959) in brinjal, Nair (1964) in bhindi, Iyer and Rhandhawa (1965) in grapes and Kaul and Singh (1967) in Cajanus. Choudhury and Ramphal (1960) reported that in cowpea MH sprays (50, 100 and 200 ppm) significantly increased the yield of pods per plant. Higher concentrations (400, 600 and 800 ppm) reduced the yield of pod.

2. Fruit setting on selfing.

Results presented in Table XVI show a high reduction in fruit setting on selfing consequent on F,W-450 and MH sprays.

The maximum reduction in fruit setting of F,W-450 treated plants was observed in higher concentrations (0.25 and .30%) at the

first and third stages of application. Reduction in fruit setting on selfing was also reported by several workers like Moore (1959) in tomato, Pederson (1959) in alfalfa, Nair (1964) in bhindi and Leelamma (1965) in brinjal.

MH also reduced fruit setting on selfing. The highest concentrations (200 and 400 ppm) showed maximum reduction at all the three stages of application. The fruit setting on selfing was found to be dependent upon the concentration of the chemical and the stages of application. These findings are in agreement with the results obtained by Nehm (1952), Narayana Swami (1960), Chogra *et al.* (1960) and Nair (1964).

3. Fruit setting on crossing.

Results presented in Tables XVII did not show any significant difference between the treatments and control for the percentages of fruit set on crossing.

In the case of F,W-450, this reduction was more with higher concentrations and it was likely that fertility of the female phase was also slightly affected. These findings are in line with the observations of several workers like Eaton (1957), Moore (1959), Nair (1964) and Leelamma (1965).

In the case of MH also more reduction in fruit setting on crossing was obtained in the higher concentrations. This is in agreement with the findings of Nair (1964) in bhindi.

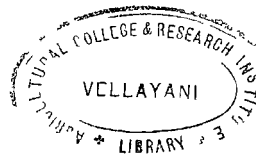
4. Length of fruits.

Results presented in Tables XVIII and XIX show that both the chemicals reduced the length of fruits to a small extent both in selfing as well as in crossing. (Plate VIII & IX). Plants sprayed with MH showed more reduction in fruit length than F,W-450. The same trend of results were observed in F,W-450 by Moore (1959), Nair (1964) and Leelamma (1965) and in MH by Narayana Swami (1960), Singh et al. (1960) and Kaul and Singh (1967).

5. Number of seeds per fruit.

With regard to the effect of chemicals on the seed setting of selfed as well as crossed fruits, a marked difference was observed in the case of both the chemicals. These results are presented in the Tables XX and XXI. The number of seeds per fruit was found to be markedly reduced in selfed fruits (Fig.IX), whereas the reduction was not so marked in crossed fruits. In the case of F,W-450 the maximum reduction of number of seeds per fruit on selfing was obtained under the higher concentrations of 0.25% and 0.30 per cent at the first stage of application. This is in agreement with the results obtained by Pederson (1959), Santhanam (1961) and Nair (1964).

Maximum reduction in seed setting with MH was obtained in selfed fruits of plants treated with 100, 200 and 400 ppm at the first stage of application. Narayana Swami (1960) reported the effect of MH in the reduction of seed setting in various crops. Reduction of



seed setting in plants treated with MH was also reported by Miller et al (1955), Nair (1964), Iyer and Randhawa (1965) and Kaul and Singh (1967).

III. Seed viability.

The percentage of germination of seeds in selfed fruits as well as crossed fruits revealed that the chemicals had brought about marked differences in germination. Seeds in selfed fruits of both the chemicals showed significant reduction in germination (Fig.X) when compared to the seeds in crossed fruits. In the case of F, V-450, comparable results were obtained by Bocanegra et al. (1960) in cotton and Leelamma (1965) in brinjal. Higher concentrations of both the chemicals showed marked reduction in germination of seeds in selfed fruits. While the data presented in Table XXV show that there is no marked reduction in germination of seeds in crossed fruits. Iyer and x Randhawa (1965) in grapes reported that there was no reduction in germination of seeds obtained from plants treated with F, V-450. In the case of MH, Choudhury and Ramphal (1960) also reported similar results in cow pea.

Reduced fruit set on selfing may be due to pollen sterility, non-dehiscence of anthers or female sterility or due to a combination of two or more of these factors. In this study there was marked reduction of fruit set on selfing in the treatments with the chemicals. The fruit set on crossing was however, not reduced to any appreciable extent in any of the treatments. This suggests that the female side is not much affected.

To further clarify this point the number of seeds per fruit on selfing as well as crossing was compared. The number of seeds per fruit on selfing was found to be markedly reduced in the treated plants whereas number of seeds per crossed fruit was not much affected. This further confirms that the functioning of the ovary was not much affected. The other observation that germination of seeds was more affected in selfed fruits than in crossed fruits again confirms that female fertility generally remained unaffected by the treatments.

SUMMARY

S U M M A R Y

The present investigation was undertaken in the Division of Agricultural Botany, Agricultural College and Research Institute, Vellayani during 1966-1967, with a view to evaluate the selectiveness of F.W-450 and MH on chillies as male gametocides and to study their side effects on the general growth and yield of the crop.

F.W-450 at 0.10, 0.15, 0.20, 0.25 and 0.30% and MH at 25, 50, 100, 200 and 400 ppm were applied as foliar spray at three stages of plant growth. The first spraying was given when the first flower was undergoing anthesis. Subsequent sprayings were given at an interval of 15 days in between two sprayings. Control plants (no spray) and plants sprayed with distilled water thrice, at the same stages of plant growth as the chemical spray, were also studied. Plants were raised in a randomised block design.

Studies were made on the effect of chemicals on the general growth and height of plants, number of flowers, pollen size and shape, pollen sterility by staining and culture methods, pollen tube elongation, number and weight of fruits per plant, and fruit setting, length of fruits, number of seeds, and seed viability in selfed as well as crossed fruits. Salient results obtained from the present investigation are summarised below:-

1. Side effects like suppression of growth, yellowing of leaves with burnt tips and burnt of younger buds followed the chemical

application, their intensity being more with higher concentrations at earlier stages. These effects were more pronounced with MH than with F,W-450.

2. Higher concentrations (200 and 400 ppm) of MH significantly reduced the plant height in the first stage of application. No such marked reduction in plant height was observed in plants sprayed with F,W-450.

3. Higher concentrations of both the chemicals at the first stage of application resulted in a general delay in flower production, the delay being significant with MH at 200 and 400 ppm.

4. Total flower production showed a slight reduction in plants sprayed at third stage of application. Highest concentration of MH in the first stage of application resulted in significant increase in the total number of flowers produced. Other treatments had no marked effect on this character.

5. All the plants sprayed with F,W-450 showed a slight reduction in size of flowers, and in the case of both the chemicals the flowers produced immediately following the application possessed smaller and non-dehiscent anthers.

6. Pollen analysis showed the presence of deformed and shrivelled pollen grains in the treated plants with a general reduction in size, the size reduction being more in the MH treated plants.

7. Complete pollen sterility was obtained in plants treated with 0.10 and 0.25% of F₂-450 and 200 and 400 ppm of MH at the first stage of application. This induced sterility was of temporary nature and got gradually decreased, and after a few weeks the pollen viability was restored.

8. Germination studies of pollen in 1% agar medium showed a higher percentage of sterility in culture medium compared with the staining technique.

9. The rate of pollen tube elongation was also much effected by the chemical treatment. The maximum reduction was noticed in the higher concentrations of both the chemicals at the earliest stages of application.

10. The higher concentrations 200 and 400 ppm of MH produced maximum reduction in number and weight of fruits per plant. In general the early stages of application had a profound effect in decreasing the yield when compared to other stages of application.

11. The fact that the fruit set was reduced on selfing and that it was almost normal on crossing in the treated plants suggested that in general the effect of chemicals at the concentrations used in this study was selective and affecting only the male side.

12. Both the chemicals inhibited the length of fruits to a small extent both in selfing as well as crossing. Plants sprayed with MH showed more reduction in fruit length than F₂-450.

13. The higher concentrations of both P,W-450 and LH induced maximum reduction of seed setting in selfed fruits. As in the case of fruit setting, the seed setting also was not markedly reduced in crossed fruits of treated plants again indicating that the ovule fertility was not much hampered with by the treatments.

14. Seeds of selfed fruits of treated plants showed reduced germination compared to seeds of crossed fruits suggesting that even some of the fertile pollen grains which could effect fertilisation of the ovules were not fully normal. This is also supported by the fact that the rate of pollen tube elongation was found affected in the treated plants.

In general, the results of the present investigation indicated the possible use of the two fumigicides, P,W-450 and LH for successful induction of male sterility in chillies without such harmful side effects. The higher concentrations of LH (200 and 400 ppm) and 0.25% of P,W-450 are found to be the most suitable concentrations of these chemicals which give the best results in inducing male sterility in chillies.

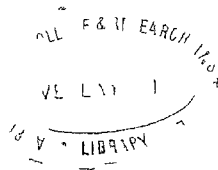
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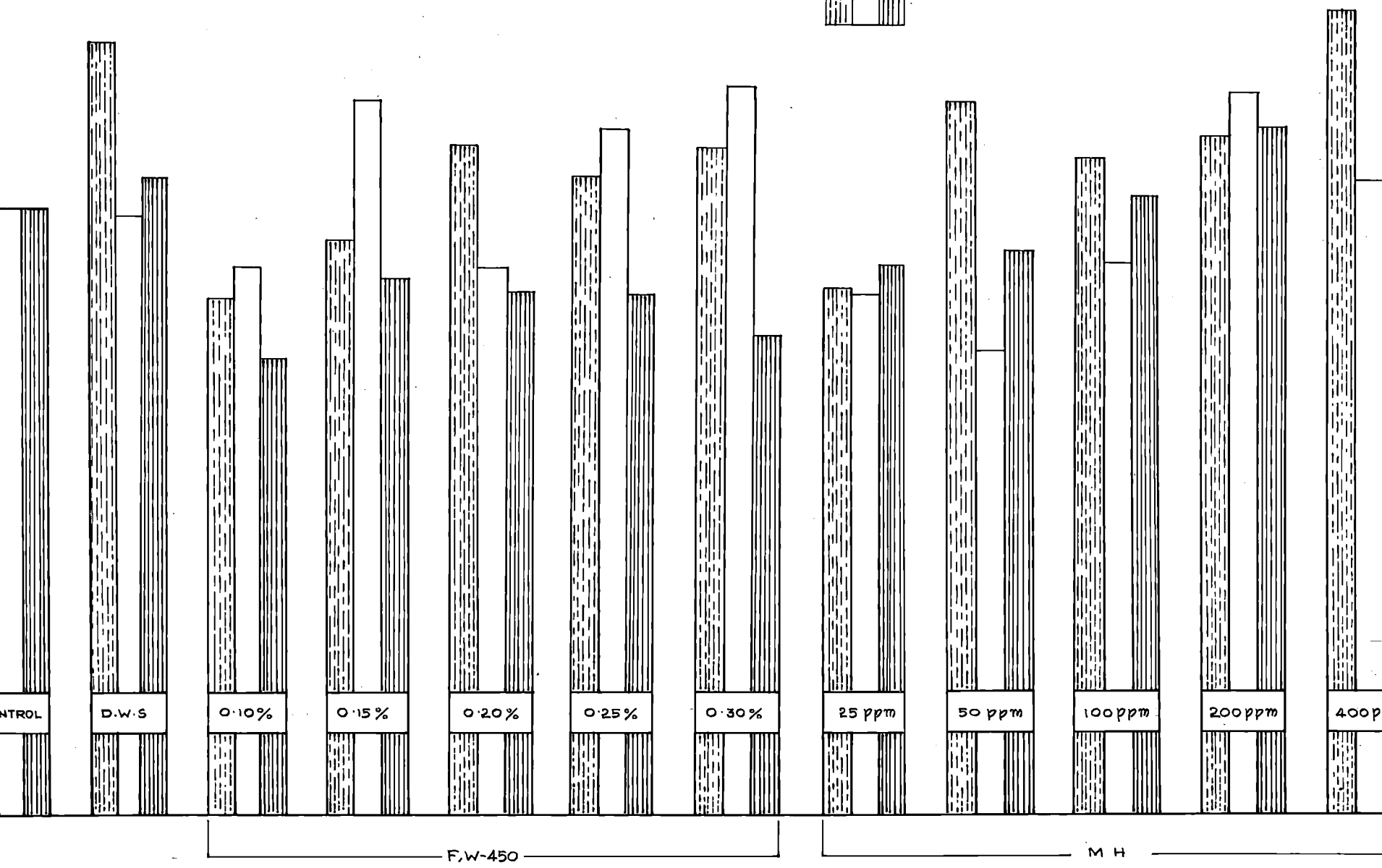
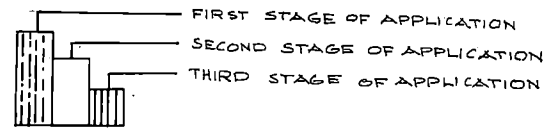
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FIGURE I

Bar diagram showing the total number of flowers per plant.



TREATMENTS

FIGURE II

Bar diagram showing the size of pollen grains in u.

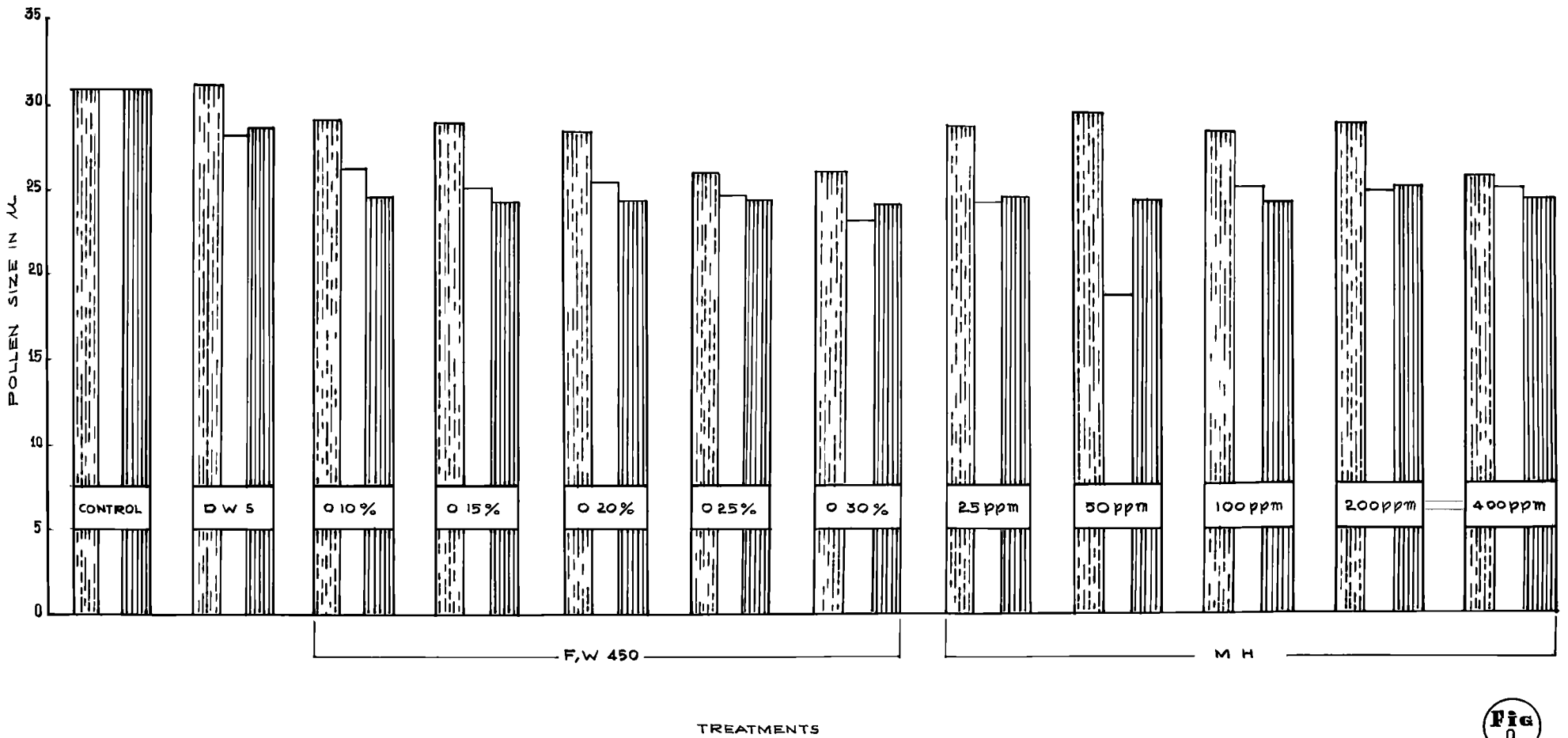
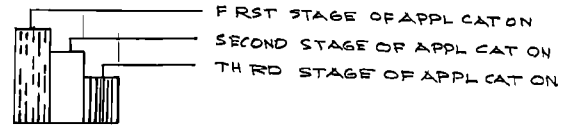


Fig 2

F I G U R E III

**Graph showing the percentage of pollen sterility by acetocarmine staining
technique in the first stage**

A. F, W-450

B. MH

**Note:- Observations were made 5 days after spraying in 5 days intervals,
until the restoration of fertility as in control.**

1 - 6 represents the intervals

C - Control.

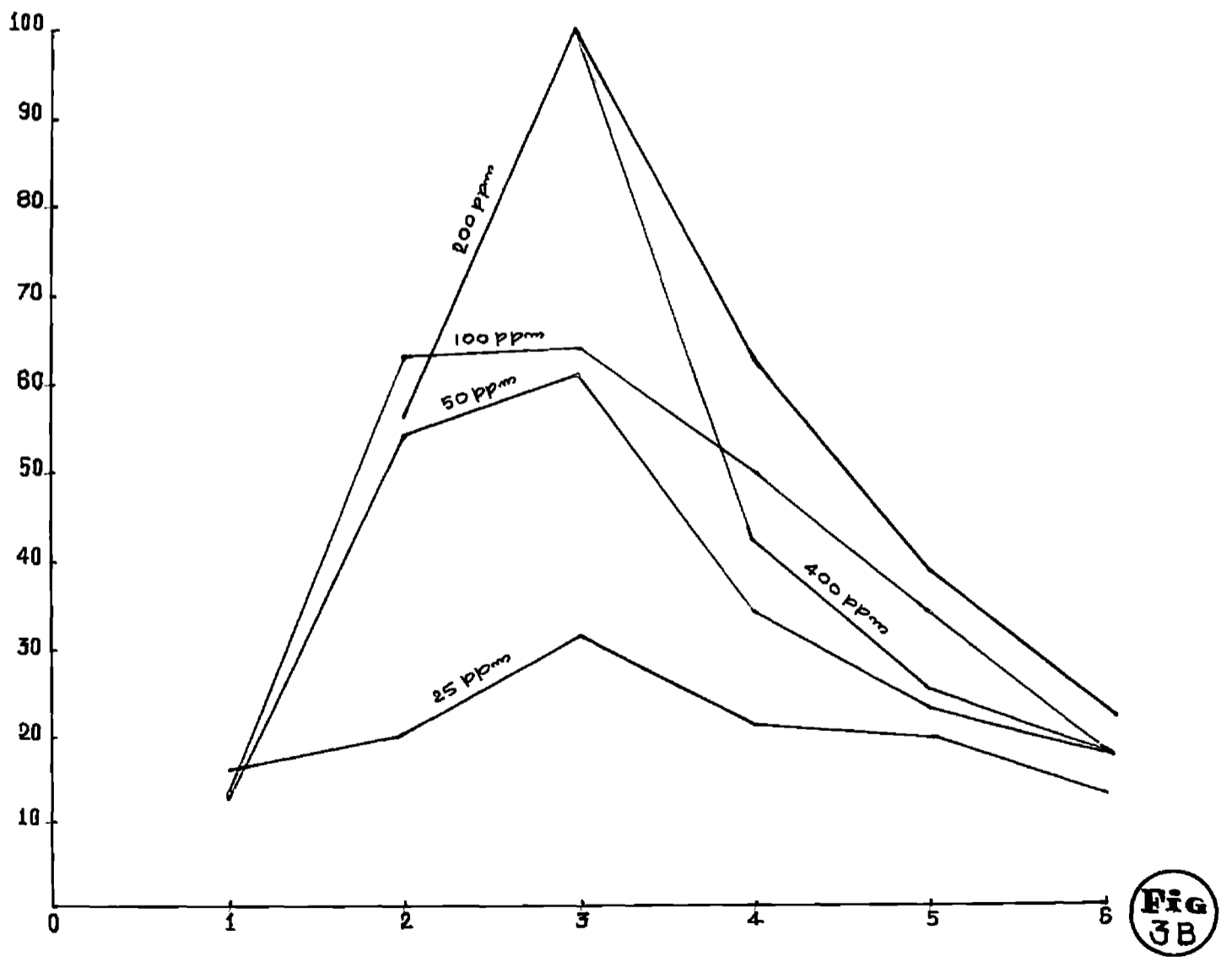
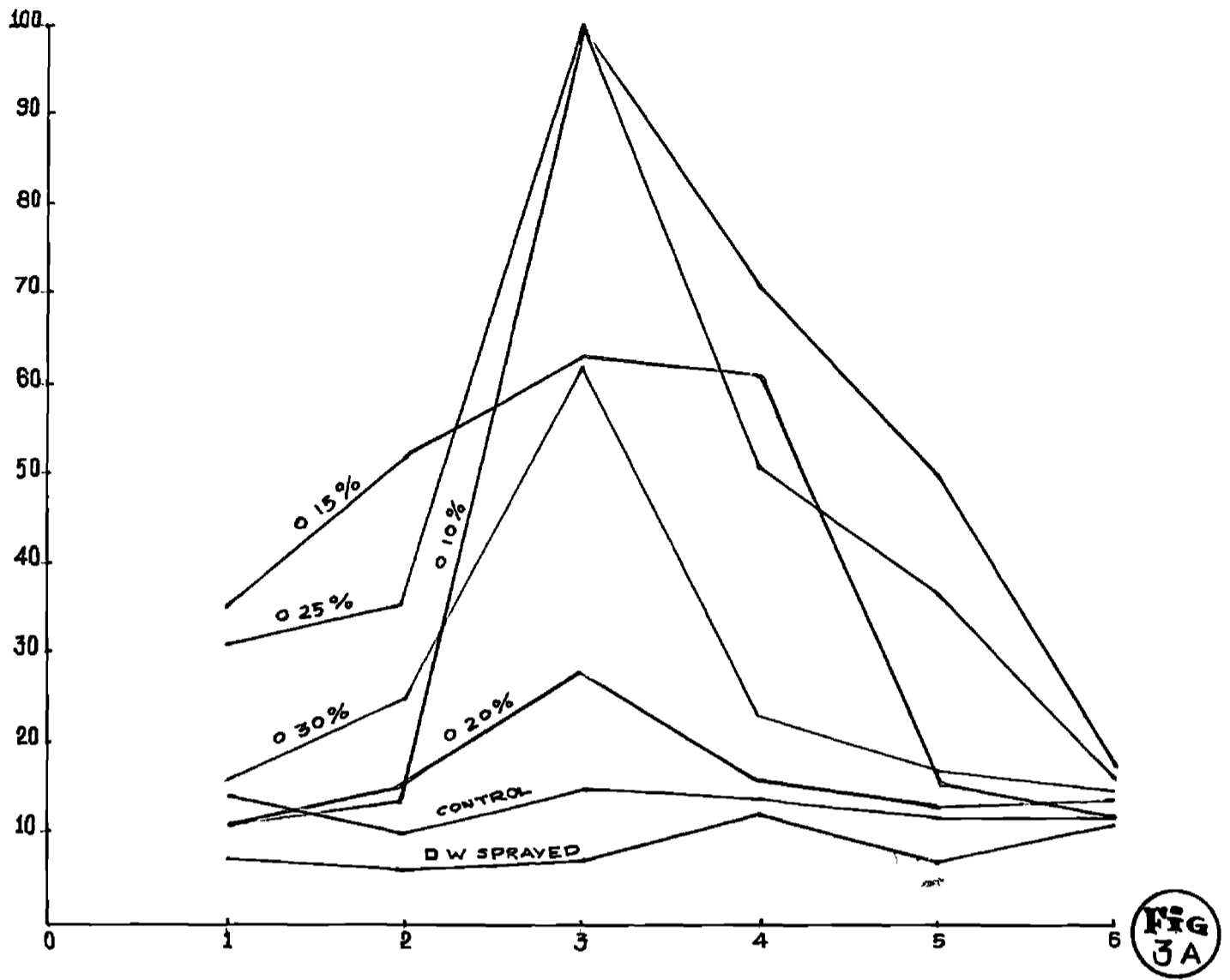


FIGURE IV

**Graph showing the percentage of pollen sterility by acetocarmine staining
technique in the second stage**

A. F, W-450

B. MH

**Note:- Observations were made 5 days after spraying in 5 days intervals,
until the restoration of fertility as in control.**

1 - 6 represents the intervals

C - Control.

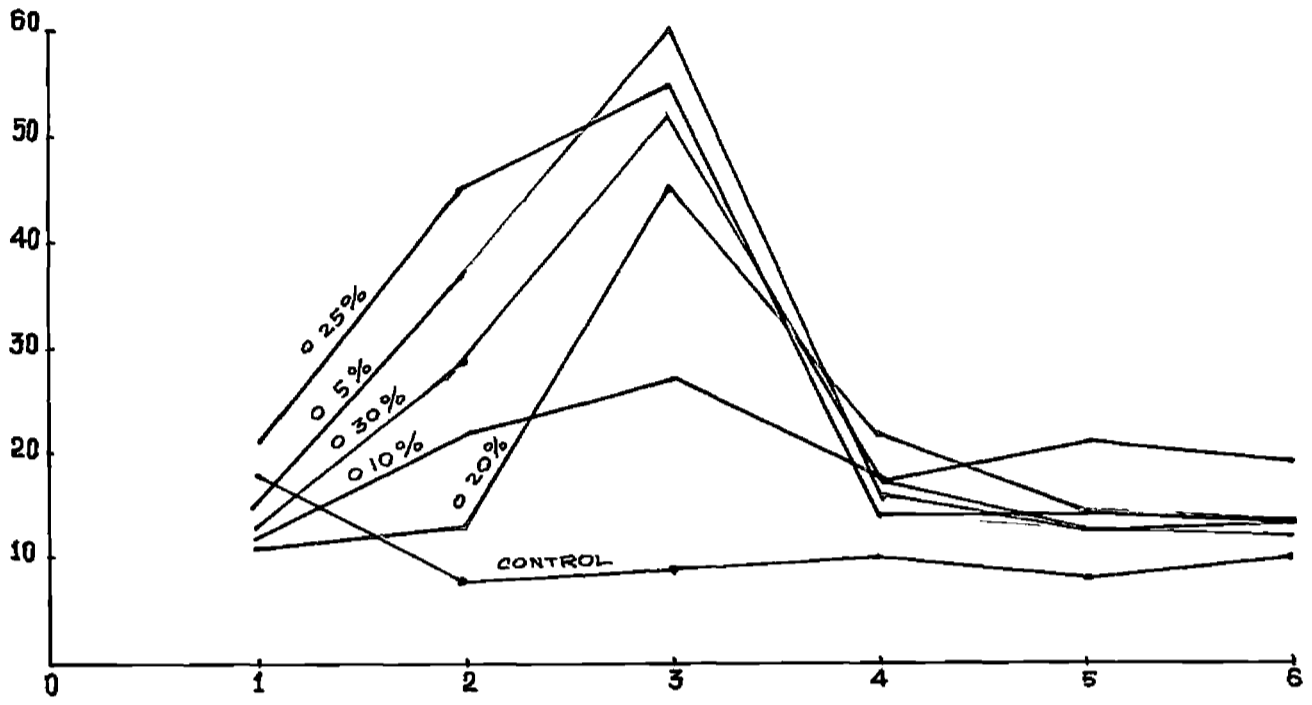


Fig
4 A

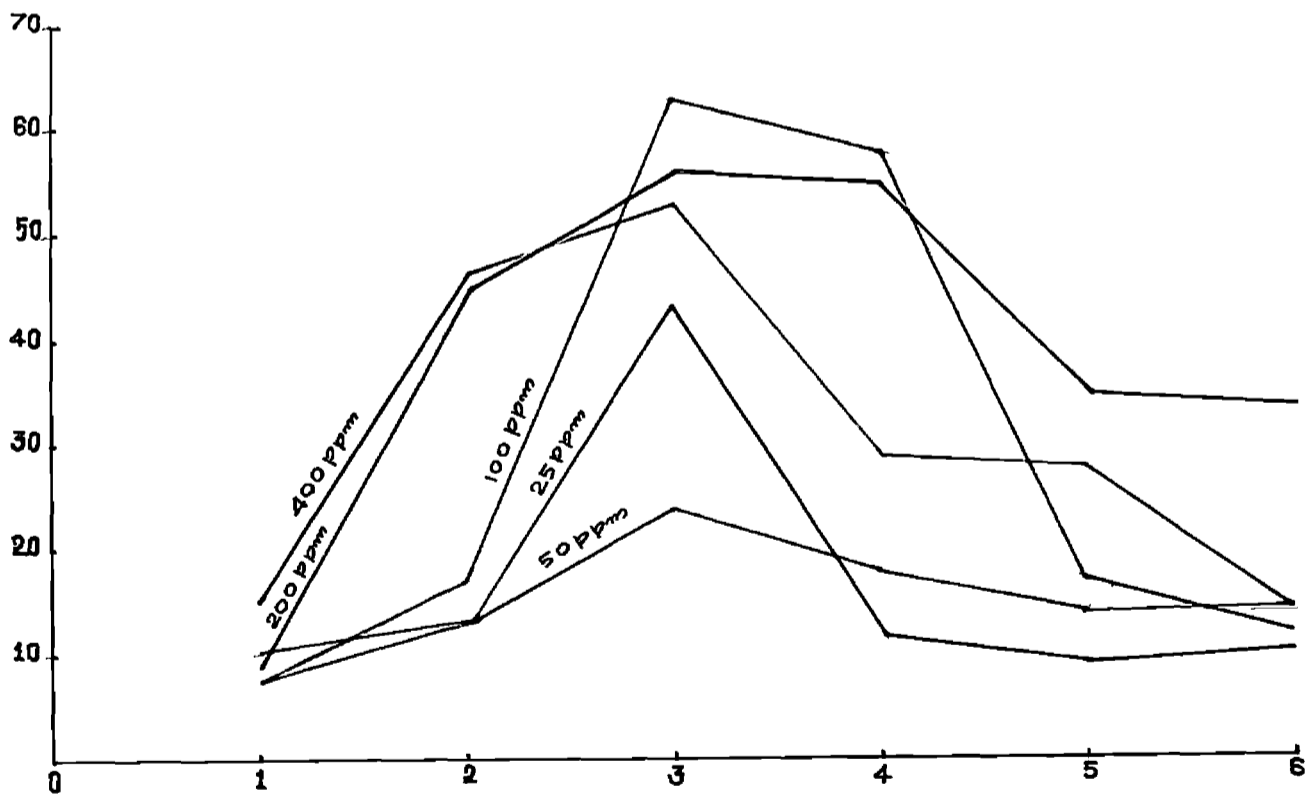


Fig
4 B

FIGURE V

**Graph showing the percentage of pollen sterility by acetocarmine staining
technique in the third stage**

A. F,W-450

B. MH

**Note:- Observations were made 5 days after spraying in 5 days intervals,
until the restoration of fertility as in control.**

1 - 6 represents the intervals

C - Control.

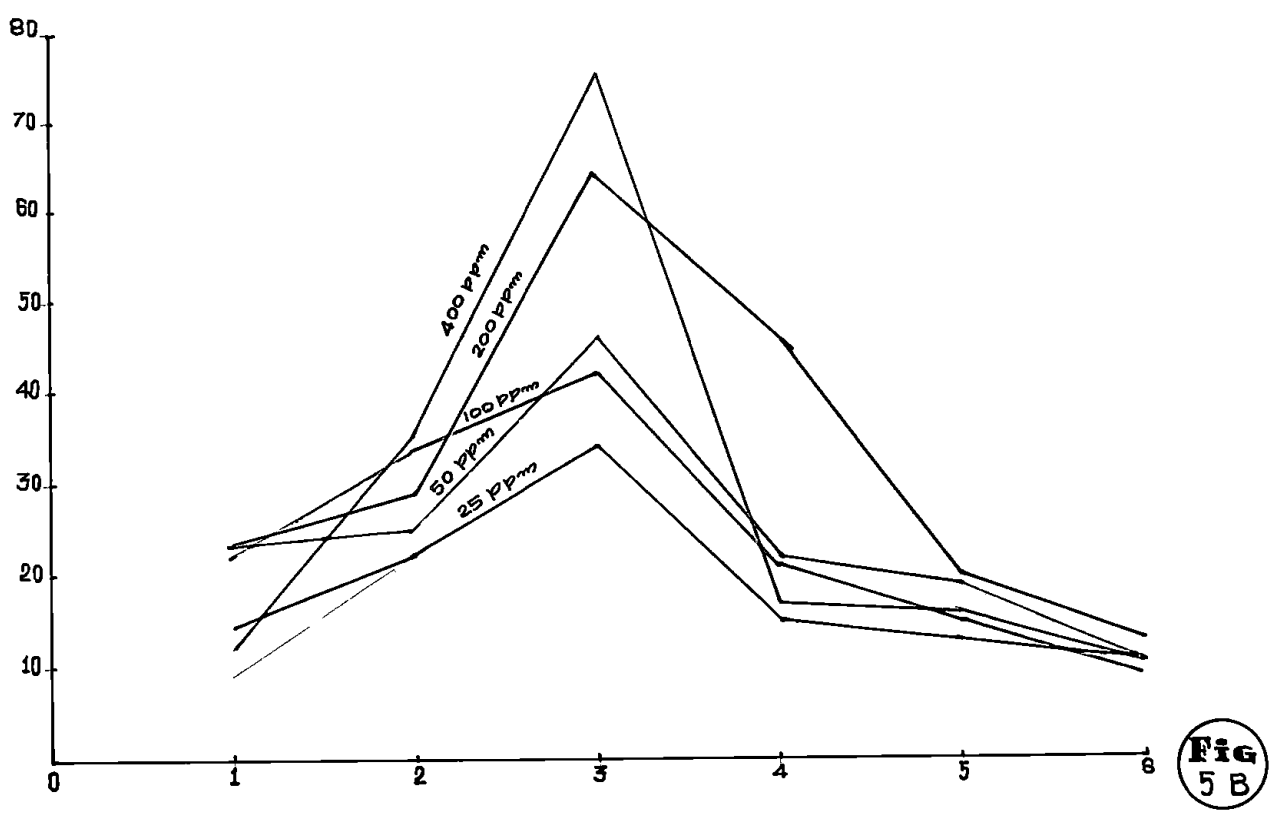
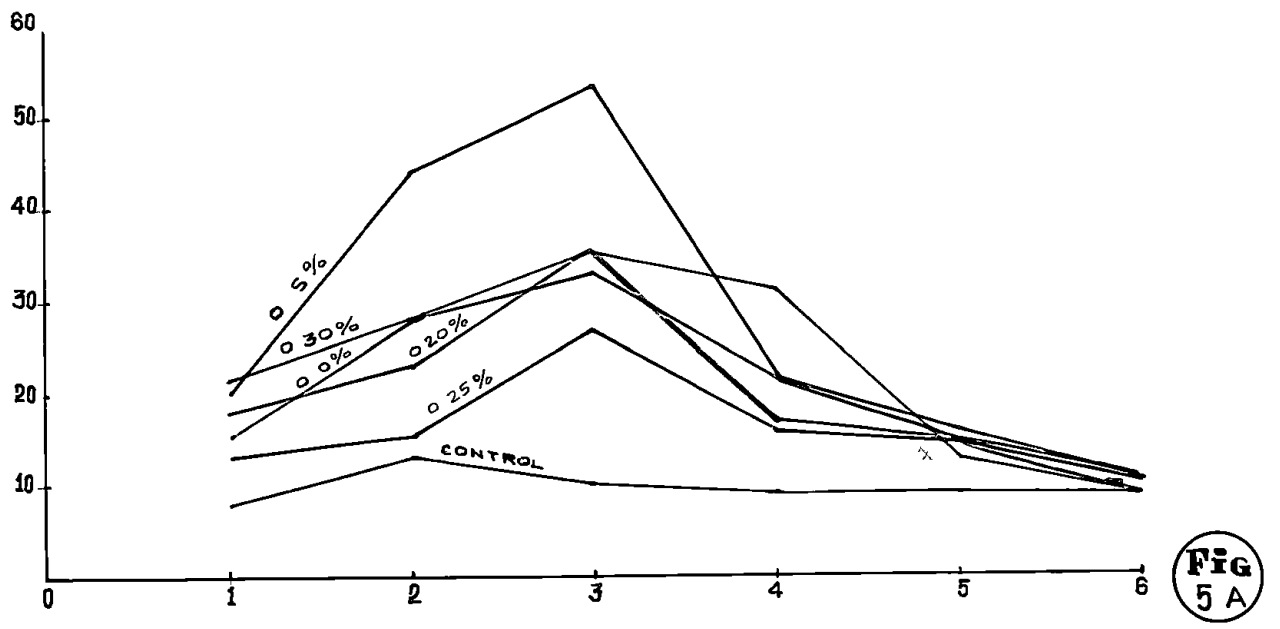
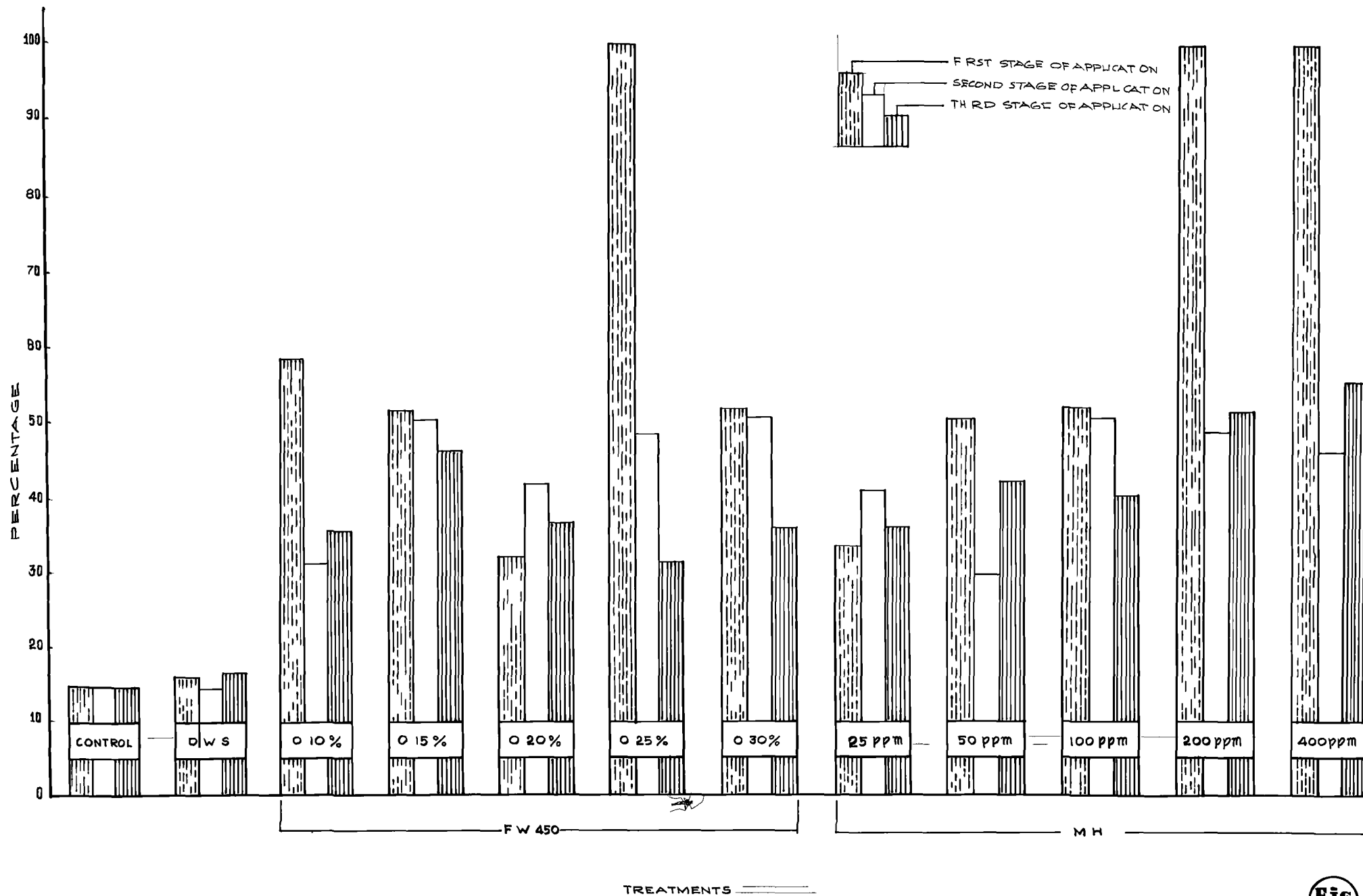


FIGURE VI

Bar diagram showing the percentage of pollen sterility in
culture media.



F I G U R E VII

Bar diagram showing the length of pollen tube in u.

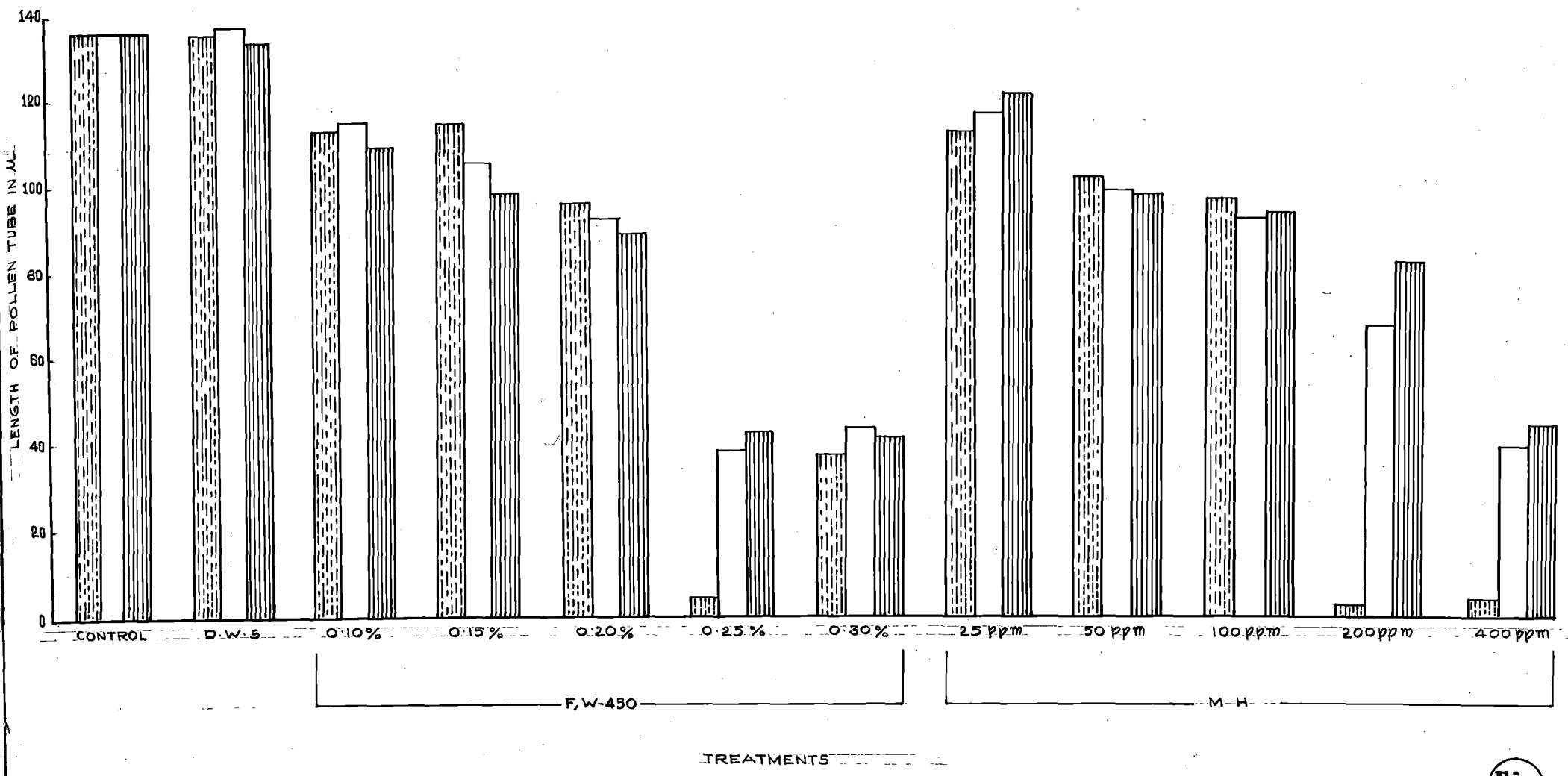
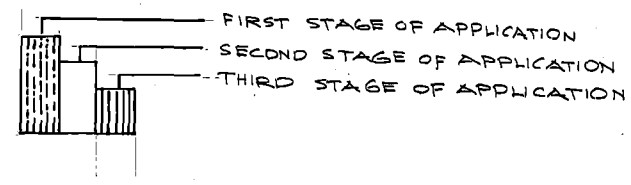


Fig 7

F I G U R E V I I I

Bar diagram showing the weight of fruits per plant in grams.

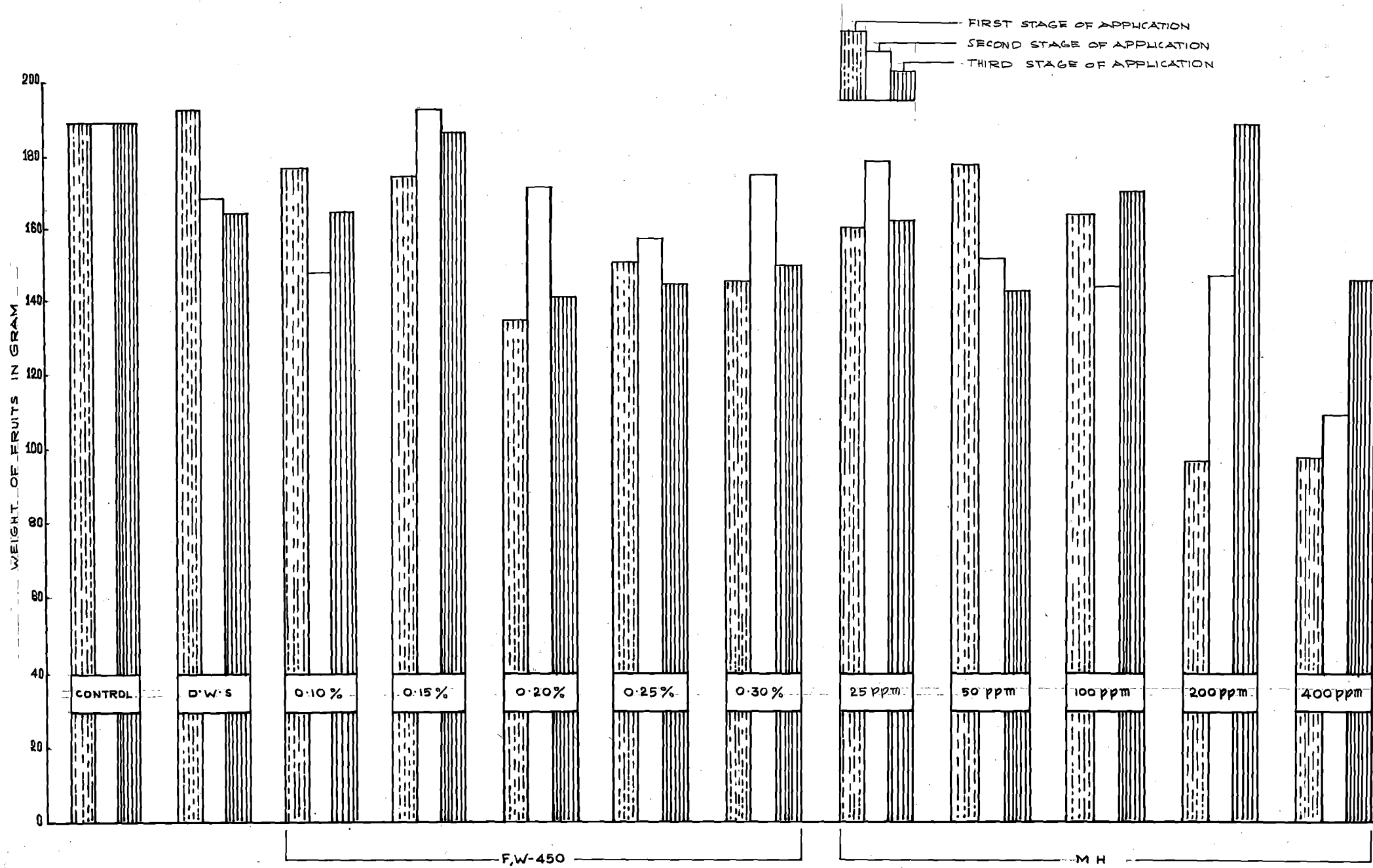


FIGURE IX

Bar diagram showing the number of seeds per selfed fruit.

FIRST STAGE OF APPLICATION
 SECOND STAGE OF APPLICATION
 THIRD STAGE OF APPLICATION

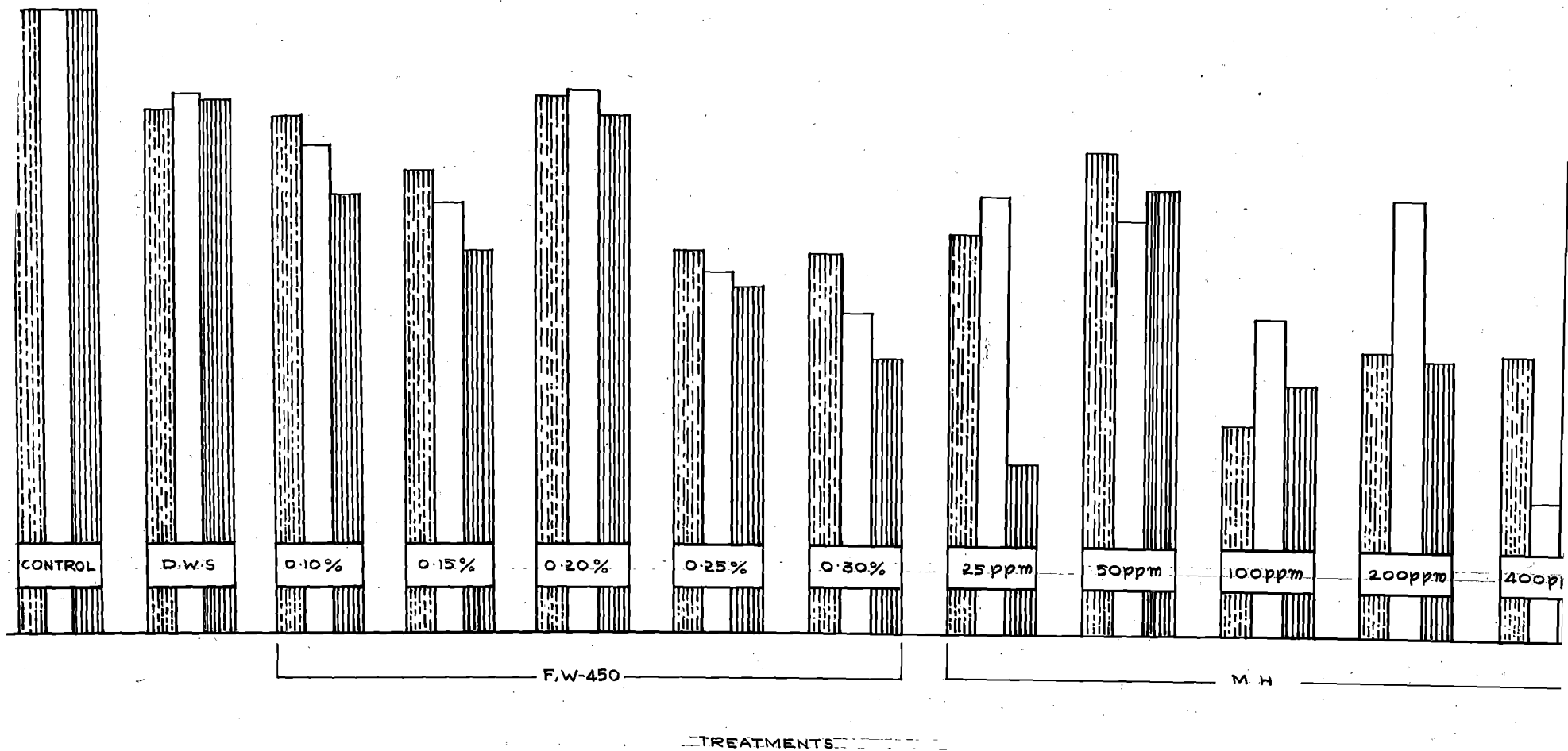


FIGURE I

**Bar diagram showing the percentage of germination of seeds in
selfed fruit.**

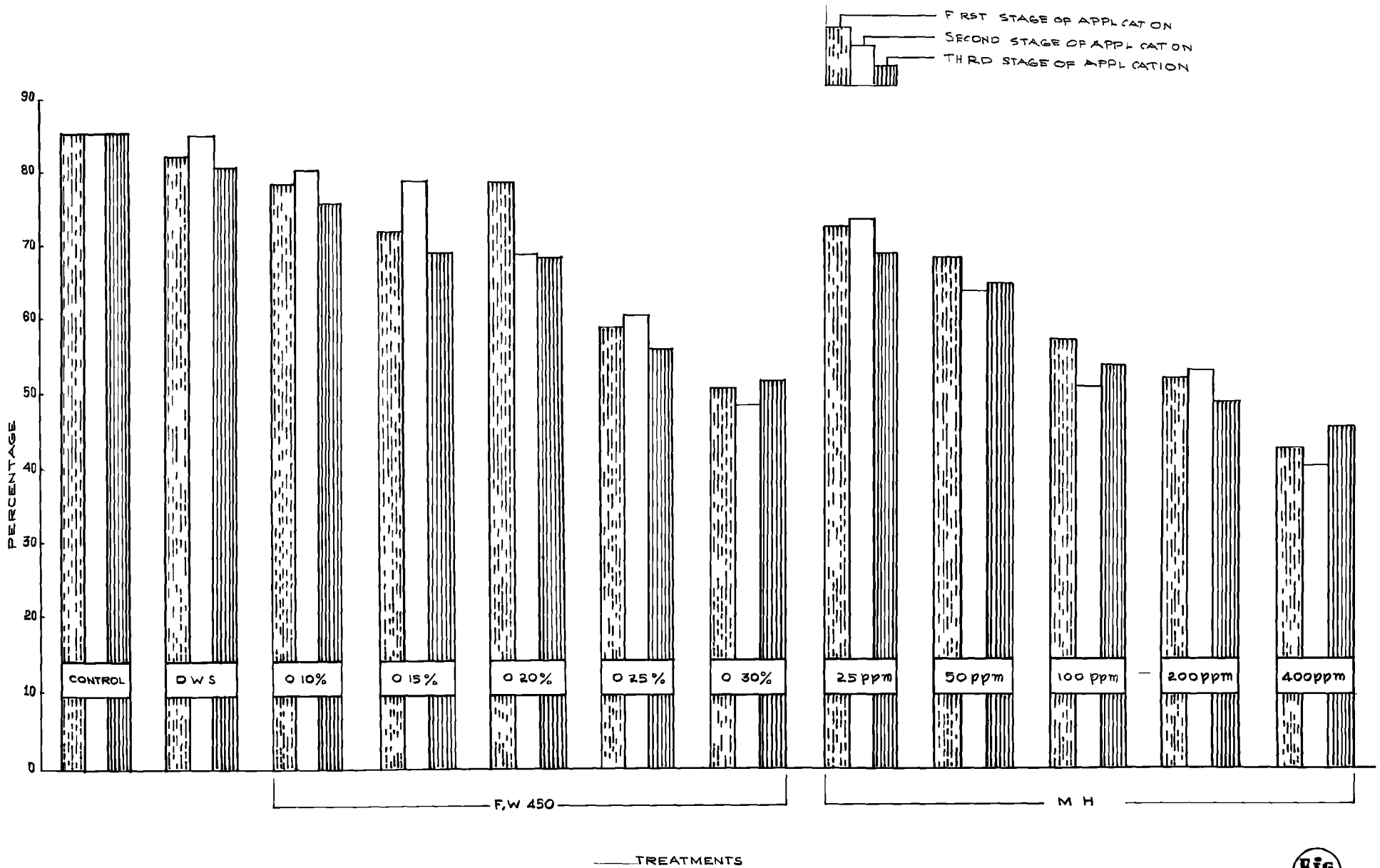
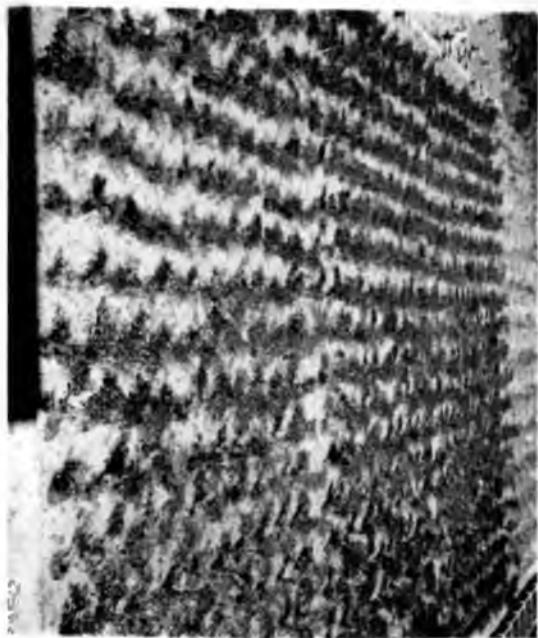


PLATE I

General layout of the field experiment.



PAES

P L A T E I I

Control plant on 45th day after planting

P L A T E I I I

**EM at 200 ppm treated plants, 15 days after the
first stage of application.**

III 8 J



II 8 J



P L A T E I V

**MI at 400 ppm treated plants, 15 days after the
first stage of application**

P L A T E V

**F, W-450 at 0.30% treated plants, 15 days after the
first stage of application.**



PLATE IV



PLATE V

P L A T E VI

**A comparative study of control, F,W-450 and MH treated flowers
at the first stage of application**

Notes:- G - Control

Upper row showing 25, 50, 100, 200 and 400 ppm of MH.

Lower row showing 0.10, 0.15, 0.20, 0.25 and 0.30% of F,W-450.

P L A T E VII

**A comparative study of control, F,W-450 and MH treated flowers
at the second stage of application**

Notes:- G - Control

Upper row showing 25, 50, 100, 200 and 400 ppm of MH.

Lower row showing 0.10, 0.15, 0.20, 0.25 and 0.30% of F,W-450.

IIA

★ ★ ★ ★ ★
★ ★ ★ ★ ★
★ ★ ★ ★ ★
★ ★ ★ ★ ★
★ ★ ★ ★ ★

IA

★ ★ ★ ★ ★
★ ★ ★ ★ ★
★ ★ ★ ★ ★
★ ★ ★ ★ ★
★ ★ ★ ★ ★

P L A T E VIII

Selfed fruits of control, F₂W-450 and MH treated plants

Notes:- C - Control

Upper row showing 25, 50, 100, 200 and 400 ppm of MH

Lower row showing 0.10, 0.15, 0.20, 0.25 and 0.50% of F₂W-450.

P L A T E IX

Crossed fruits of control, F₂W-450 and MH treated plants

Notes:- C - Control

Upper row showing 25, 50, 100, 200 and 400 ppm of MH

Lower row showing 0.10, 0.15, 0.20, 0.25 and 0.50% of F₂W-450.

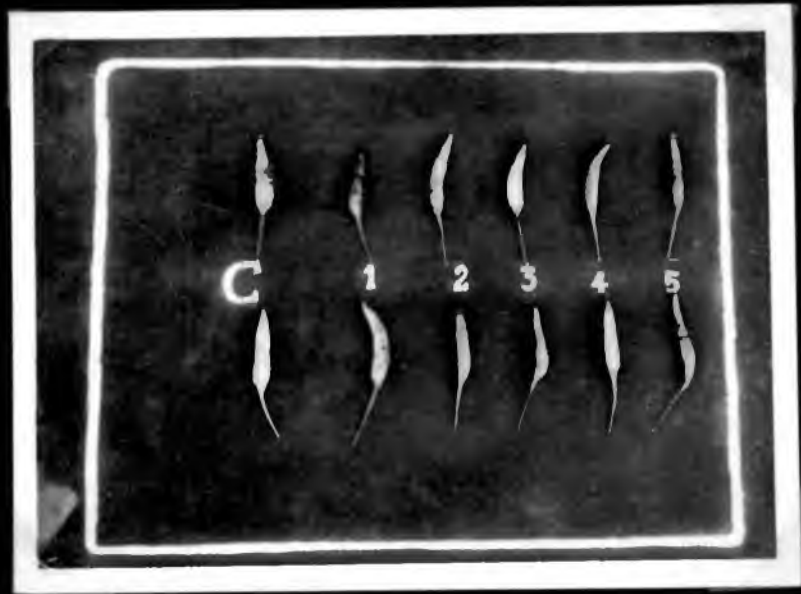


PLATE VIII

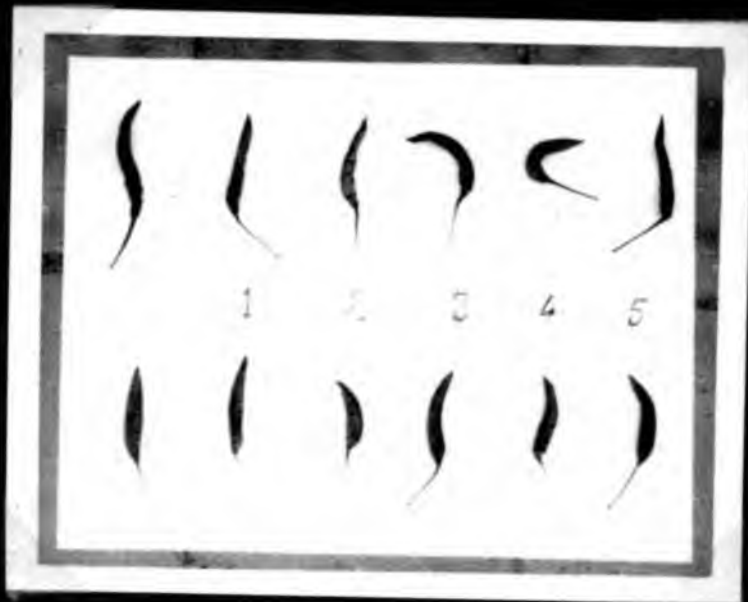


PLATE IX