EFFECT OF CYCLOHEXIMIDE (ACTI-DIONE) ON THE GERMINATION OF SPORES OF SEVERAL PHYTOPATHOGENIC FUNGI

By

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A THESIS

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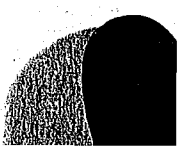

INTRODUCTION AND REVIEW OF LITERATURE

Cycloheximide, better known by the brand name Acti-dione,[1] is a by-product resulting from the process of Streptomycin production.

Acti-dione was first reported in the literature by Whiffen, Bohonos and Emerson in 1947 (48) as a substance found to exhibit antifungal but not antibacterial activity. Whiffen et al. conducted a number of tests with it and found that it inhibited a number of dermatophytes and several yeasts, but had absolutely no action against bacteria. Also in 1947, Leach, Ford and Whiffen (21) reported on the chemical formula $C_{27}H_{42}N_2O_7$. They found this substance to have a melting point of 115° to 116° C., a molecular weight of 507 and is most active in the acid range being rapidly inactivated by dilute alkalies at room temperatures.

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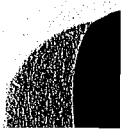
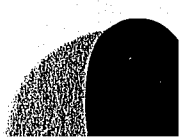
[1] Acti-dione is a patented name by the Upjohn Company of Kalamazoo, Michigan, and all of the material used in these studies was supplied by the Upjohn Company.

Early in 1948 Felber and Hamner (8) of the Department of Horticulture at Michigan State College, started a series of experimental studies involving the use of Cycloheximide (Acti-dione) in order to determine its growth effects upon higher plants. During their investigation they found Acti-dione to be toxic, to young bean plants and oat seedlings, in concentrations higher than 100 p.p.m. (parts per million). In the process of these investigations they observed a rather severe infection of powdery mildew Erysiphe polygoni D. C., on their older Red Kidney bean plants. This was quickly eradicated, within 48 hours by spraying the infected plants with a 10 p.p.m. solution of Acti-dione. The results of this investigation indicate that the substance may be effectively used as a fungicide for powdery mildew of beans. These findings served as a stimulus to the initiation of many new studies involving the application of this antibiotic in controlling plant diseases. Additional investigations were started with the object of studying the various physiological effects of this substance on a number of pathogenic fungi and also the cytological effects of this substance on the cells of several plants (11, 51).



Late in 1948, Vaughn and his co-workers (45) conducted a series of experiments to determine the effect of Acti-dione on various plant tissues and on the vegetative growth of several fungi. Their conclusions were that Acti-dione controls mildew on beans and roses, under greenhouse conditions. Mildew on beans was controlled at 10 p.p.m. with no apparent phytotoxic results. Mildew on roses was controlled with concentrations less than 5 p.p.m. They also state that injury varied between varieties, but that younger foliage was more susceptible than older foliage. In addition to this, they also found that "growth of pathogenic fungi on a culture medium containing actidione indicates strong inhibition of 10 different pathogenes at concentrations of 100 p.p.m. of actidione." In 1949, Vaughn and Hamner (46) reported on the effect of Actidione as a fungicide in controlling several common diseases, and concluded that the Acti-dione is worthy of further extensive testing on various crops against fungus diseases. Also in 1949, de Zeeuw (6) and Vaughn reported that Acti-dione produced the best control of cucumber scab, Cladosporium cucumerinum. From Richmond, Indiana, came reports, via correspondence to Vaughn (42), that greenhouse tests with this material

have given satisfactory control of powdery mildew of roses when the latter were sprayed with concentrations below 5 p.p.m. Klomparens (19) conducted an investigation to determine the efficiency of this substance as a wood preservative. He found that Acti-dione was very toxic to all of the wood-rotting basidiomycetes, which he used in his studies, even at very low concentrations. His conclusion was that Acti-dione was adsorbed by the tissues of the wooden block samples and his attempts to leach out the toxin, resulted in a very low recovery. In 1950, Whiffen (48) reported on experiments in which she studied the activity of Acti-dione in vitro on 33 species and strains of phytopathogenic fungi. These were inhibited by Acti-dione varying in range of concentration from 0.125 to 100.0 micrograms per milliliter. Petersen and Cation (38) reported their experimental results on the use of Acti-dione for the control of Peach Brown Rot (Monilinia fructicola), and Cherry Leaf Spot (Higginsia hyemalis). Their conclusion with respect to the brown rot is that in view of the fact that severe injury to near-ripe fruit was evident, Acti-dione is of doubtful value as a peach brown rot spray. On the other hand, in the case of cherry leaf spot, exceptionally good results were obtained as



an eradicant with no apparent injury to the foliage. In 1950 Gottlieb et al. (10) reported on experiments with Acti-dione as a plant protectant. They tested it at various concentrations against 6 organisms and found that all except Sclerotinia fructicola were controlled at 100 p.p.m. All 6 organisms, however, were controlled at 1,000 p.p.m. In addition they tested 5 economic plants in order to determine possible toxicity. Their results show injury to all of the plants except strawberries, at 100 p.p.m. and 1,000 p.p.m. In private conversation, R. H. Fulton, Department of Botany and Plant Pathology, Michigan State College, has told this author that his experiments with Acti-dione on strawberry plants have resulted in injury even at concentrations as low as 2 p.p.m. Early in 1951, Henry et al. (12) reported that "Acti-dione proved promising, particularly for the treatment of oats for the control of smut caused by Ustilago kolleri Wille." At the 42nd annual meeting of the American Phytopathological Society, Vaughn (47), reporting on his experiments with Acti-dione on turf diseases, stated that "Dollar Spot" Sclerotinia homeocarpa was consistently less prevalent in cycloheximide-treated plots than in those treated with common turf fungicides; and that "Melting-

out" (Helminthosporium sp.) was completely checked by cycloheximide. At the same meeting, Klomparens (20) reported on the toxicity of cycloheximide to certain wood-rotting fungi, and on his preliminary studies on the absorption of this antibiotic by tissues.

At the present time a number of investigators are conducting tests on various crops and diseases in attempts to learn more about this product which has until now created much widespread interest among pathologists and even some growers of fruit and vegetable crops.

Currently, studies are being made at Michigan State College by Vaughn and Klomparens on turf diseases, deZeeuw on cucumber scab and Cation and Fulton on fruit diseases. In addition to these, other turf and fruit investigations, using Actidione are being conducted in various experiment stations throughout the United States.

Spore germination tests have been used to assay fungicides for many years. According to Horsfall (17), Prevost was one of the earliest researchers to use this method of fungicide assay. Many other investigators have since used it and published their results with mixed opinions on the efficacy of the

method. Nevertheless, the method is well established, with many modifications. McCallan and Wilcoxon of the Boyce Thompson Institute have used it extensively as evidenced by their many publications on spore germination studies.

Since no previous investigation had been made of the effects of Acti-dione on pathogenic fungous spore germination, it was considered that such a study would be interesting and of possible value from a practical standpoint. It is the hope of the author that at some future time, these results may prove of value in the ever-constant struggle of controlling plant diseases. Perhaps some investigator who may wish to study the effects of Acti-dione on any one or more of the many diseases caused by the organisms used in this study will have access to some information regarding the action of Acti-dione upon the spores of the organism he is about to study.

An investigation was started by the writer in September, 1949, with the objective of studying the effects of Acti-dione on the germination of spores from several pathogenic fungi, under the influence of several environmental factors.

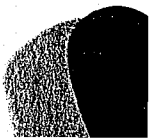
MATERIALS AND METHODS

Pure cultures of each organism used in this investigation were obtained from the stock collection of fungi of the Department of Botany and Plant Pathology at Michigan State College, except for the culture of Botrytis cinerea, which was isolated from naturally-infected Premier strawberries by Mr. Robert H. Fulton. Monosporous isolation of these organisms was rather difficult, so single hyphal tips were transferred from Petri dish cultures to agar slants in test tubes.

The conidial stages of these organisms were needed and all efforts were directed toward obtaining the most desirable media for conidial production. Monilinia fructicola was grown on an agar medium of the following formula:

Malt extract	5 grams
Yeast extract	1 gram
Sorbose	5 grams
Glycine	1 gram
Bacto-Agar (Difco) . . .	18 grams
Distilled water	1000 milliliters

This formula was given to the author by Dr. H. L. Barnett, of West Virginia University. Cladosporium cucumerinum, Ramularia

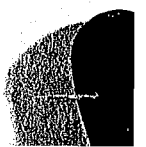


sp. and Botrytis cinerea were cultured on agar slants of potato-dextrose agar prepared in accordance with the following formula:

Liquid potato extract from 200 grams of potatoes
Dextrose, C. P. . . . . 5 grams
Bacto-Agar (Difco) . . . 17 grams
Distilled water . . . . . 1000 milliliters

The pH of these media was determined by a Beckman pH meter (model H2). Medium X, which refers to the medium used for Monilinia fructicola, had a pH value of 5.6. The potato-dextrose agar had a pH of 5.9 after sterilization. These media were used for producing the conidia which were later used in the experiments described in the latter portion of this section.

In preliminary tests sterile distilled water was used as the spore suspension medium and as the medium in which the spores would be allowed to germinate. After several tests with each organism it was found that the spores, from the organisms used in these tests, did not show a high percentage of germination. Stimulants were in order, and as recommended by McCallan (31) a number of them were tried. Difco nutrient broth was decided upon as the most suitable since it gave good



results, was readily available, and was always constant. This liquid nutrient broth was prepared by dissolving 8 grams of the dehydrated broth into 1,000 ml. of distilled water and autoclaving at 121° C. Following sterilization it was stored in the laboratory refrigerators until used. For comparison, at different intervals, sterile double-distilled water and sometimes sterile tap water was used. The water, however, proved very unsatisfactory since low germination was noted every time and its use was discontinued.

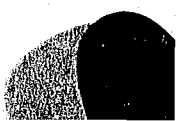
The slides used in this study were prepared according to the method described by P. D. Peterson (37). Clean glass slides were lightly smeared with a very thin layer of Vaseline by use of the index finger. Onto this surface were affixed 12-mm. circular cover glasses which had previously been cleaned in hot water containing Dreft, a good detergent for this purpose. The glasses were then stored in 70% alcohol until used. Each cover glass, before use, was quickly flamed and pressed into place, i.e., on the Vaseline surface of the slide. The slight pressure applied to the cover glass, resulted in a ridge of Vaseline forming around the edge of the cover glass. This edge rim served to restrict the spore suspension within

the boundaries of the cover glass and thus prevented running-off or coalescing of the spore suspension drops.

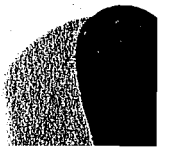
This method was chosen because, as Peterson mentions, it not only is quick and inexpensive in materials, but also provides a definite surface area over which the spore suspensions were placed and thus a more or less definite volume of liquid. Furthermore each and every replicate was duplicated in that each covered the same surface area. The volume of liquid spore suspension was quite constant in each replicate as an attempt was made to place only two equal size drops on each cover glass. Each drop was approximately 1/20 of a ml. as measured by a fairly accurate pipette.

Before these slides were used each was placed in a clean sterile Petri dish lined with 2 filter paper disks and then were exposed to ultraviolet irradiation by placing them under an ultraviolet lamp unit consisting of 2 Slimline lamps, 6 inches from the lower surface of the lamps. Each and every slide was exposed for a period of 10 minutes. There was almost no contamination under these conditions.

The spore suspensions used in the germination studies were prepared as follows: 7 ml. of each sterile nutrient



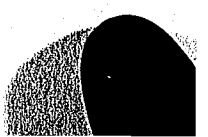
broth-Acti-dione concentration were poured, aseptically, into an agar slant culture, which was sporulating abundatly, and each test tube was rolled vigorously between the palms of the hands for approximately 100 times. This produced a good suspension every time. The suspension was then transferred immediately to sterile centrifuge tubes by filtering through several layers of sterile cheesecloth which had been previously placed in the centrifuge tubes. All suspensions were then centrifuged for approximately 2 minutes followed by decanting of the liquid with care so as to keep the clumped spores at the bottom of the centrifuge tubes. The spores were then resuspended in fresh sterile nutrient broth and centrifuged again for about 2 minutes. The liquid medium was again decanted and the spores were again resuspended in nutrient broth. Each centrifuge tube was then rolled between the palms of the hands to cause an even suspension of the spores and break any clumps that may have formed during centrifugation. To further insure this, a clean sterile 2-ml. pipette was placed into the liquid spore suspension, and by drawing some of the liquid into the pipette and allowing it to flow back into the tube, further dispersion of the spores was accomplished. Using the same pipette a



drop of the suspension was quickly placed upon a Howard Mold Counting Chamber slide and the suspension was standardized so as to contain approximately 30,000 spores per ml. of liquid, as recommended by the American Phytopathological Society Committee on Standardization of Fungicidal Tests (1).

After each suspension was standardized two drops were placed quickly on each of the two cover glass surfaces borne on the surface of the specially prepared slides previously described (Plate 1). Each slide was then quickly covered by placing the lid on each Petri dish, thus reducing the chances for contaminants to gain entrance. To each Petri dish 2.5 ml. of sterile distilled water was added to moisten the filter paper disk lining it, so as to produce and maintain a humidity sufficiently high so that the spore suspensions would not dry out (Plate 1).

Each experiment consisted of 7 Petri dishes containing one slide each and each slide contained one concentration of Acti-dione. The concentrations of Acti-dione used for this investigation were 0.5 part per million, 2.5 p.p.m., 5.0 p.p.m., 10.0 p.p.m., 25.0 p.p.m., 50.0 p.p.m., 75.0 p.p.m. and 100 p.p.m.



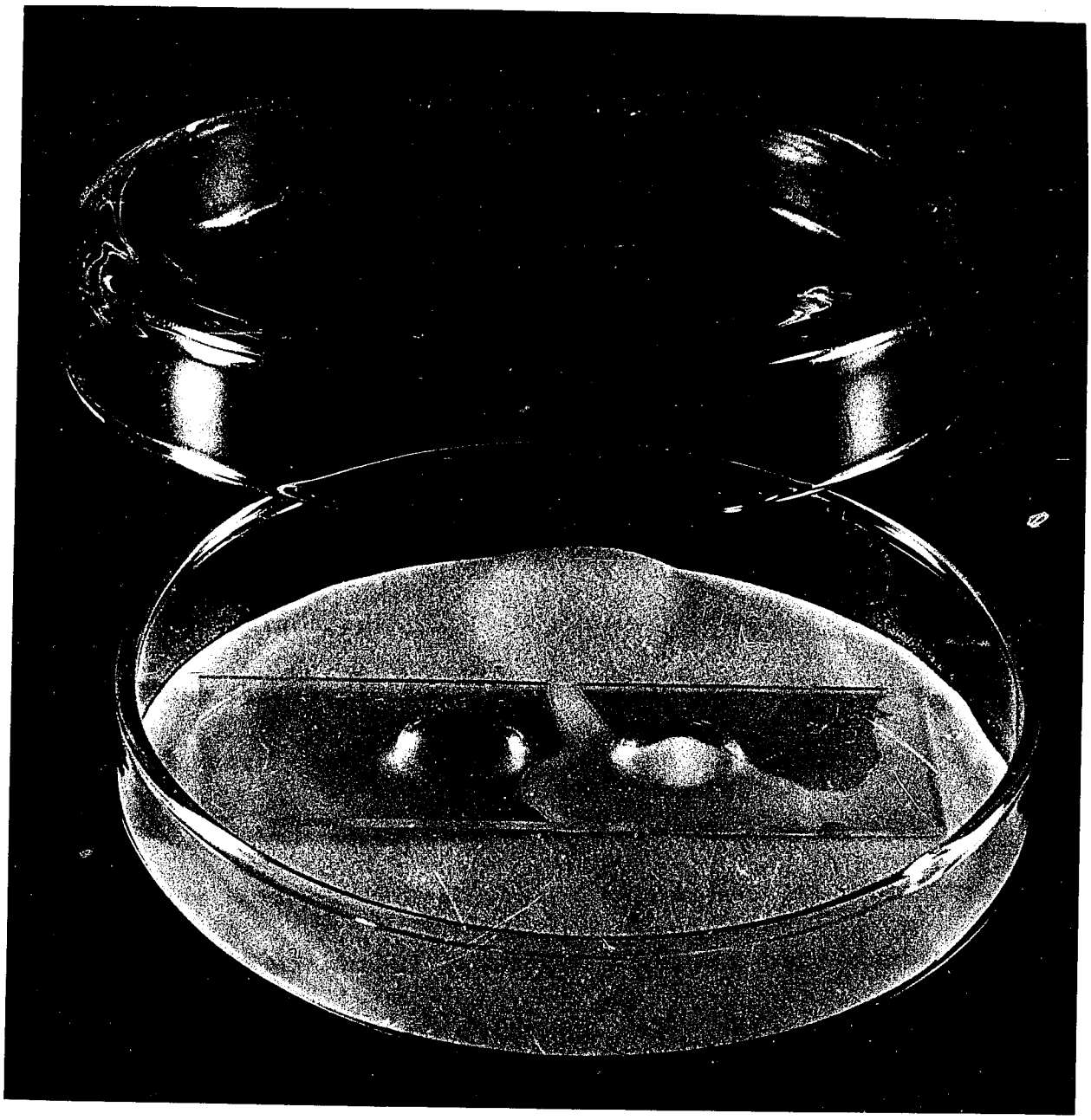


Plate 1

The latter two concentrations were used only in the experiments with Botrytis cinerea and Ramularia sp.

The Acti-dione concentrations were prepared by carefully dissolving 100.00 mg. of crystalline Acti-dione into 1,000 ml. of nutrient broth (Difco) in a 1,000-ml. volumetric flask. This solution contained 100 p.p.m. of Acti-dione. Using this as a stock solution, by a series of dilutions, the other concentrations were prepared.

After the sterile distilled water had been added to each Petri dish, each of the latter were marked and quickly placed along with the control, either in one of the several incubators used in this study, or left at room temperature. At room temperature, the Petri dishes containing the slides were incubated in large finger bowls to which enough water was added to maintain a high relative humidity (Plate 2). The temperatures used in this study were: Room temperature, 22° C., 24° C., 26° C., 28° C. and 30° C.

Method and Time Intervals Used in Making Counts

In the case of Cladosporium cucumerinum the suspensions were checked for germination as follows: The first count

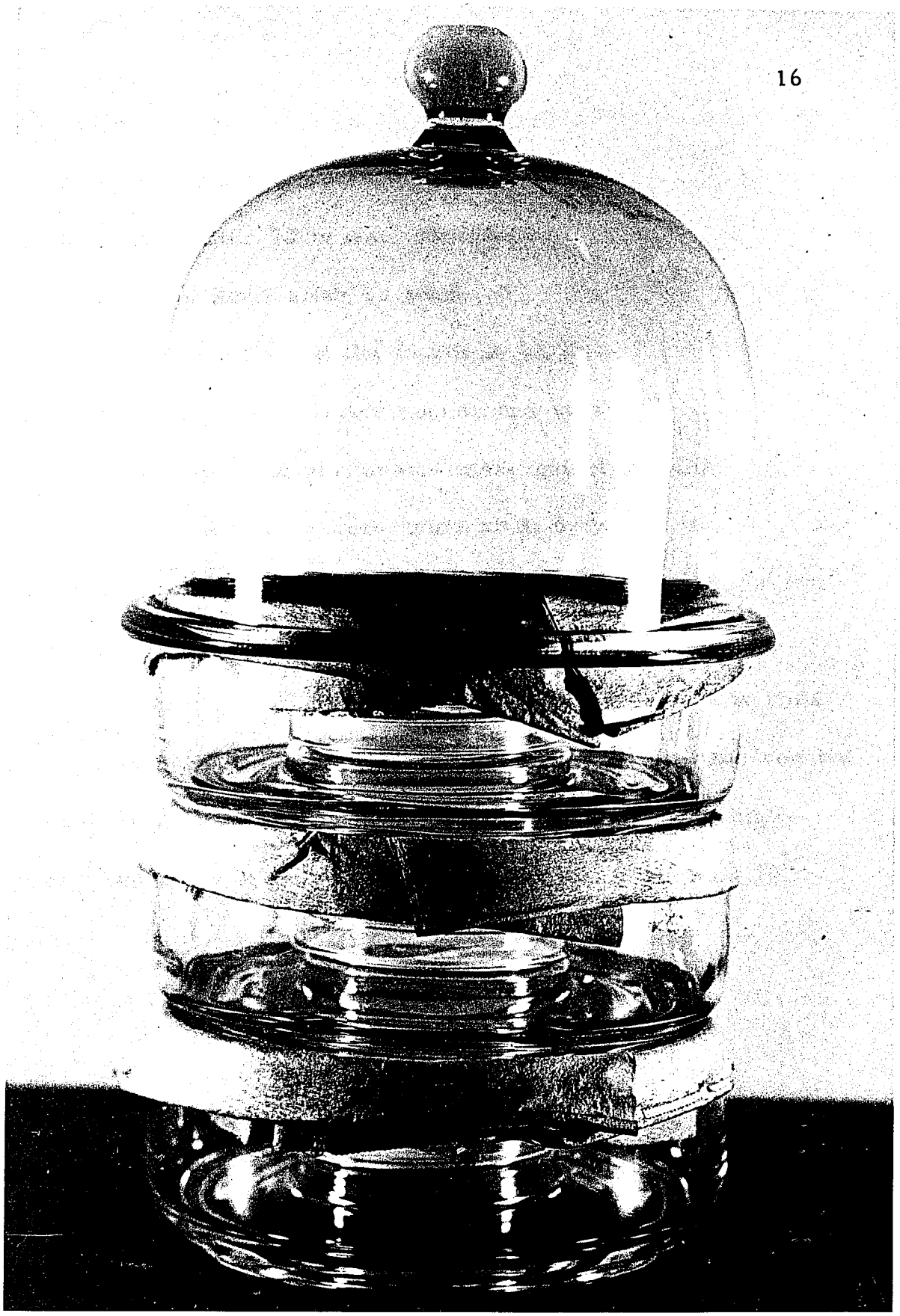


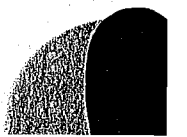
Plate 2

was made 12 hours after the start of the experiment, the second count was made after 24 hours, the third after 48 hours and the fourth after 72 hours. For Monilinia fructicola the first count was made after 12 hours, the second in 24 hours, the third in 48 hours and the fourth in 72 hours. The counts for Ramularia sp. were made sooner and at shorter intervals because this organism germinated more quickly than the previous ones. The counts were made at 3, 6, 9, 12, and 48 hours. The counts for Botrytis cinerea were made after 9, 18, 36 and 72 hours.

To facilitate counting, a special grid eyepiece was used in the microscope. A total of 25 high-power fields were counted in each of the two drops per slide giving a total of 50 high-power fields per slide. Each experiment was replicated 10 times.

Humidity Tests

Spore suspensions were prepared as previously mentioned but instead of being placed upon the special slide in liquid form as in the previous experiments, each suspension was sprayed onto the clean sterile slide by means of a small hand-operated



De Vilbis No. 15 atomizer, commonly used for nasal sprays (Plate 3). An attempt was made to spray each slide uniformly by spraying each 5 times, holding the nozzle 6 inches from the slide surface. This resulted in what appeared to be a uniform spray with a good distribution of the spores on each cover glass. The slides were then allowed to air dry following which they were placed in an air-tight glass-covered chamber (Plate 3), the lid of which was sealed with masking tape. To maintain each of the various humidities accurately, a specific chemical was placed in each chamber and the chamber was placed in an incubator of the right temperature. The results of these experiments were checked as in the previous experiments, namely, counts were made of the total number of spores germinated per high-power field as compared to the total ungerminated spores: The counts were made at the same time intervals mentioned previously.

Washing Experiments

Recalling the procedure of preparing each slide with its spore suspensions it will be noted that not all of the liquid spore suspension was used; most of it remained in the centrifuge

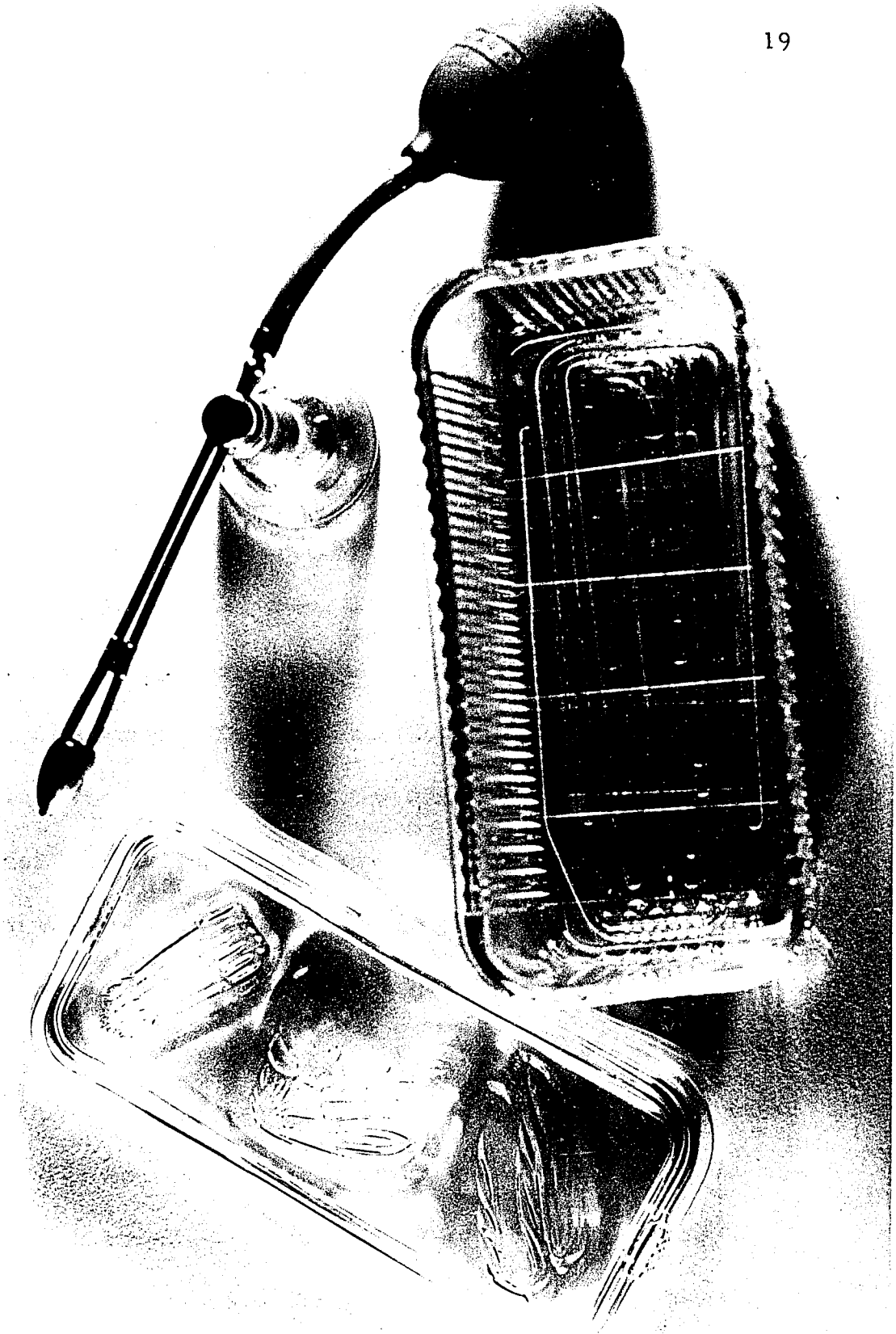
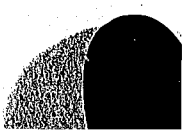


Plate 3

tubes. Each centrifuge tube was carefully marked and placed in the same incubator with the slides. After the final counts were made, the lowest concentration of Acti-dione which completely inhibited germination, plus the next lower concentration, was selected for the washing experiments. The proper centrifuge tubes containing the liquid spore suspensions that were inhibited by the Acti-dione, were removed from the incubator and each suspension was checked microscopically for evidence of germination, then if the evidence correlated with the results obtained from the slides the spore suspensions were carefully decanted. To each centrifuge tube 5 ml. of sterile distilled water was added and the tubes were rolled in the palms of the hands as in the previous experiments. This was done to disperse the spores through the water so that as much of the Acti-dione could be washed off as possible. The tubes were again centrifuged for 2 minutes, the liquid was decanted and the process was repeated 5 times; each time using fresh water. The spores were then resuspended in 5 ml. of the nutrient broth stimulant and placed on clean slides, in sterile Petri dish chambers and then placed back into the incubator from which they were originally taken. These were then checked



for germination as in the previous experiments and at the same intervals.

pH Experiments

A number of experiments were conducted involving a study to determine the effect that Acti-dione would have on the germination of spores, if the liquid medium in which the spores were suspended varied in hydrogen ion concentration. Five different pH values were arbitrarily chosen: pH 6.8, 6.0, 5.0, 4.0, and 3.0. Each experiment was prepared as previously described and conducted at each of the temperatures as stated previously. Prior to counting the results of germination on the slides, the pH was carefully measured by use of a Beckman pH meter, and any change in pH value of the medium was recorded.

EXPERIMENTAL RESULTS

In presenting the results of this investigation, both tables and graphs were used. The tables show the exact percentage of inhibition obtained when spore suspensions of each organism were exposed to the various concentrations of Acti-dione which had been adjusted to pH values of 6.8, 6.0, 5.0, 4.0 and 3.0 under the following temperatures: 22^{o}, 24^{o}, 26^{o}, 28^{o}, and 30^{o} C. Each table was then presented in a graph in order to demonstrate more clearly the entire picture for each organism as it was affected by Acti-dione under the factors mentioned above. In addition to this, data obtained from the humidity experiments, are presented in tabular and graphic form.

It will be noted that a logarithmic scale graph paper was used for the concentrations of the Acti-dione, thus it became necessary to indicate the results of the controls in what may be considered an unorthodox method. The controls have been placed on the extreme left side of each chart, next to the ordinate. This was done so that a comparison could be easily made between the controls and each concentration of Acti-dione

used. The concentrations of Acti-dione are expressed in parts per million and are hereafter designated by the abbreviation p.p.m.

It will be noted that the results of three environmental factors are presented in this thesis. A number of other environmental factors were studied and results were obtained as stated under the heading Materials and Methods. These are not presented here. The other environmental factors could not easily be controlled in the field and would thus be of no direct practical value. In view of this fact it was decided to mention these results briefly here and perhaps publish them as a separate paper at a later date.

Direct Immersion Experiments

Cladosporium Cucumerinum

In Table I are shown the results obtained with spores of Cladosporium cucumerinum exposed to solutions of Acti-dione previously adjusted to the following pH concentrations: 6.8, 6.0, 5.0, 4.0 and 3.0. These pH values in each concentration of Acti-dione were exposed to each of the following temperatures:

TABLE I

INHIBITION OF SPORE GERMINATION OF CLADOSPORIUM CUCUMERINUM BY DIFFERENT CONCENTRATIONS OF ACTI-DIONE AT FIVE DIFFERENT TEMPERATURES AND FIVE HYDROGEN ION CONCENTRATIONS

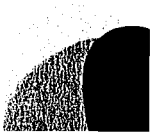
Temperatures (° C.)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
22	0.0	No Germination	62.55	33.93	5.22	4.65
	0.5		74.02	50.00	23.16	8.00
	2.5		89.41	60.18	28.01	16.33
	5.0		95.04	65.18	36.31	19.64
	10.0		100.00	82.00	79.98	75.56
	25.0		100.00	100.00	100.00	100.00
	50.0		100.00	100.00	100.00	100.00
24	0.0	No Germination	70.32	53.10	18.00	17.83
	0.5		61.09	44.77	12.03	10.90
	2.5		77.33	40.35	27.14	24.96
	5.0		88.81	48.70	54.01	61.21
	10.0		100.00	76.58	72.11	81.92
	25.0		100.00	98.20	100.00	100.00
	50.0		100.00	100.00	100.00	100.00
26	0.0	No Germination	43.10	28.83	10.10	8.59
	0.5		55.00	47.32	16.18	12.69
	2.5		62.13	66.96	42.00	34.38
	5.0		75.13	72.07	61.22	58.78
	10.0		84.10	87.39	89.42	91.72
	25.0		96.12	96.46	98.12	100.00
	50.0		100.00	100.00	100.00	100.00

TABLE I (Continued)

Temperatures (° C.)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
28	0.0	No Germination	87.00	56.88	38.00	45.23
	0.5		89.23	57.80	47.00	48.01
	2.5		95.16	79.13	62.65	57.32
	5.0		98.02	86.36	73.51	68.76
	10.0		100.00	100.00	100.00	100.00
	25.0		100.00	100.00	100.00	100.00
	50.0		100.00	100.00	100.00	100.00
30	0.0	No Germination	97.13	86.24	51.21	60.31
	0.5		91.06	74.55	62.14	66.56
	2.5		98.71	78.57	74.31	73.33
	5.0		100.00	94.55	88.91	85.15
	10.0		100.00	100.00	100.00	100.00
	25.0		100.00	100.00	100.00	100.00
	50.0		100.00	100.00	100.00	100.00

22° C., 24° C., 26° C., and 30° C. The results of Table I have been plotted on semi-logarithmic graph paper (Figures 1, 2, 3, 4).

It must be pointed out that in a number of cases the concentration of Acti-dione which prevented germination of spores after 12 hours was insufficient to maintain that inhibition after 24 hours and in some cases after 48 hours. No change was noted, however, after 72 hours in any case. It also may be noted that complete inhibition was obtained at 50.0 p.p.m. in all cases, regardless of temperature or pH concentration. In certain cases it appears that both temperature and pH were influential in the complete inhibition of spore germination at lower concentrations of Acti-dione. At pH 6.8 (Figure 1) inhibition was greatest at 30° C. at all concentrations of Acti-dione and progressively lessened with the drop in temperature. An exception to this is observed at only two points with temperatures of 24° C. and 26° C. It should also be pointed out that a definite stimulation was observed in 0.5 p.p.m. at 24° C. as compared to the control. At pH 6.0 (Figure 2) the same pattern of inhibition is observed as in the previous case except for the fact that a higher percent of



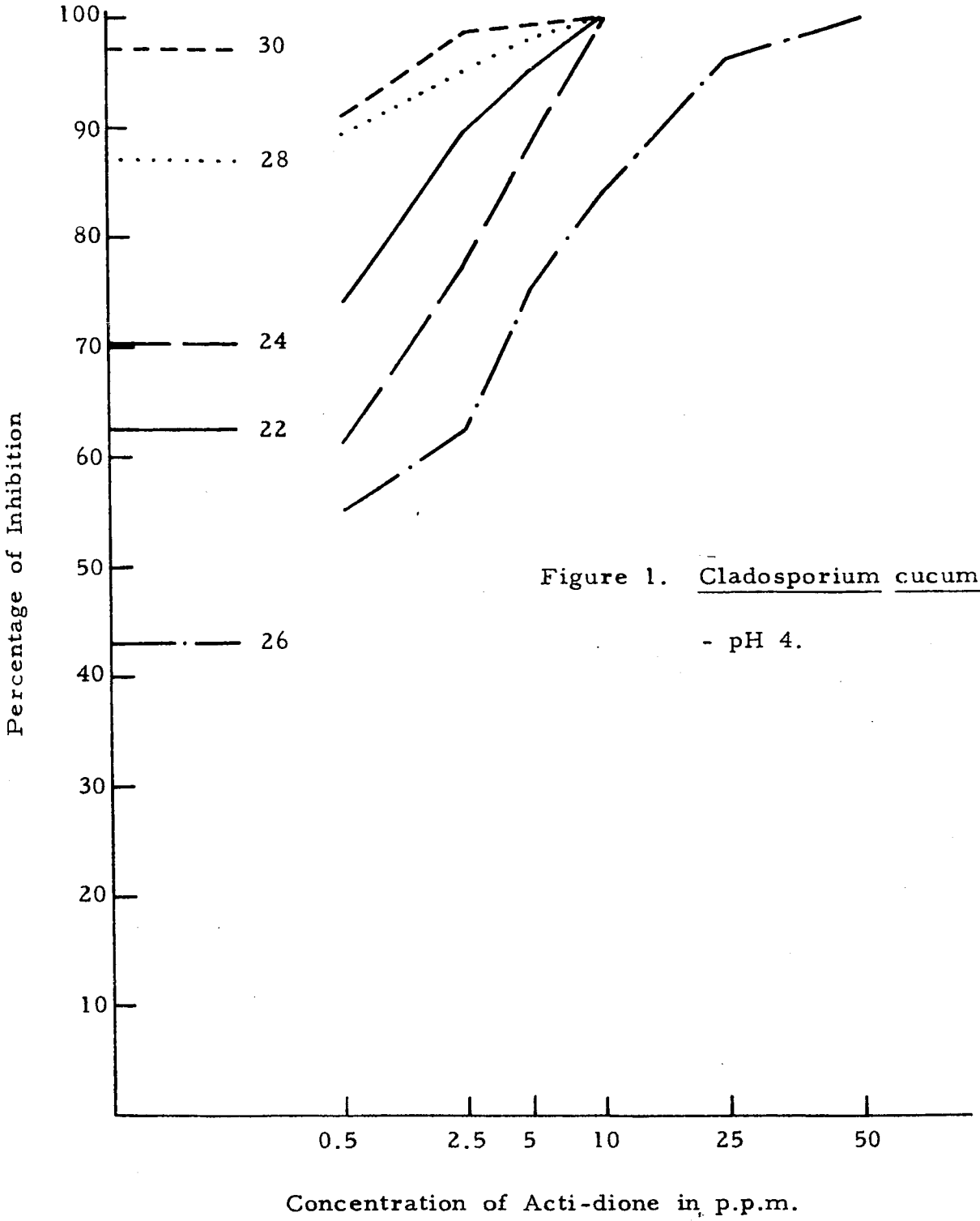


Figure 1. Cladosporium cucumerinum - pH 4.

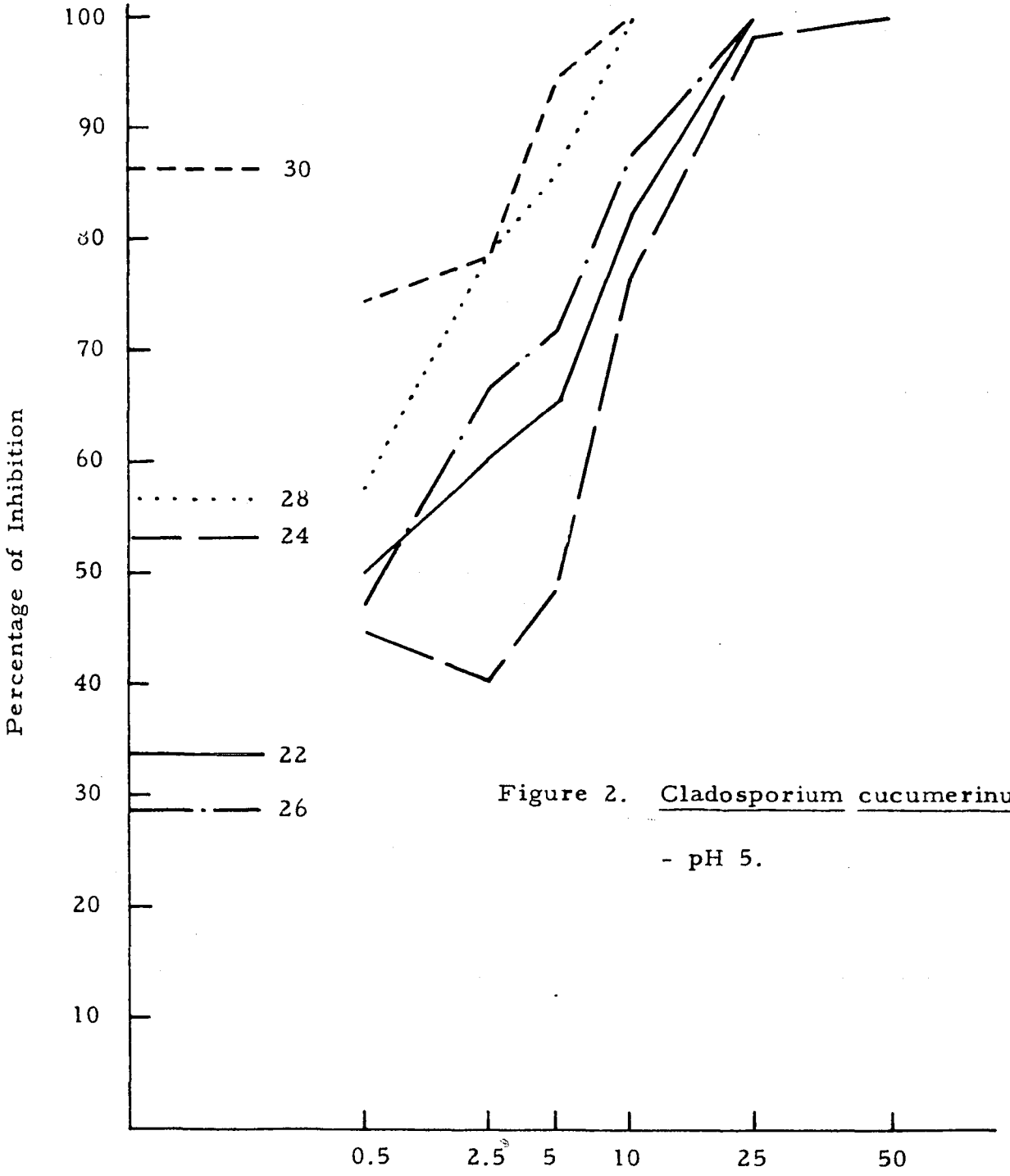


Figure 2. Cladosporium cucumerinum - pH 5.

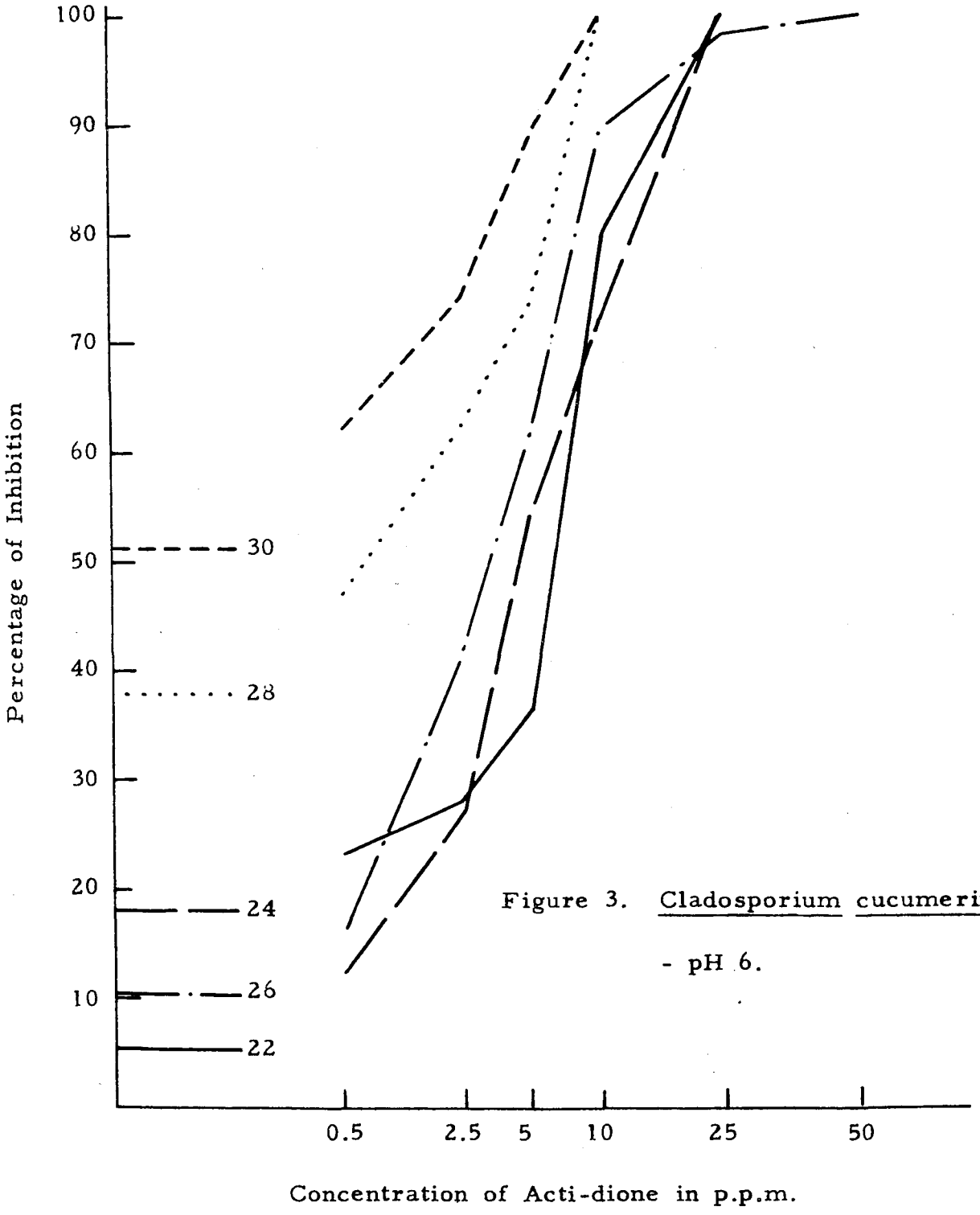


Figure 3. Cladosporium cucumerinum - pH 6.

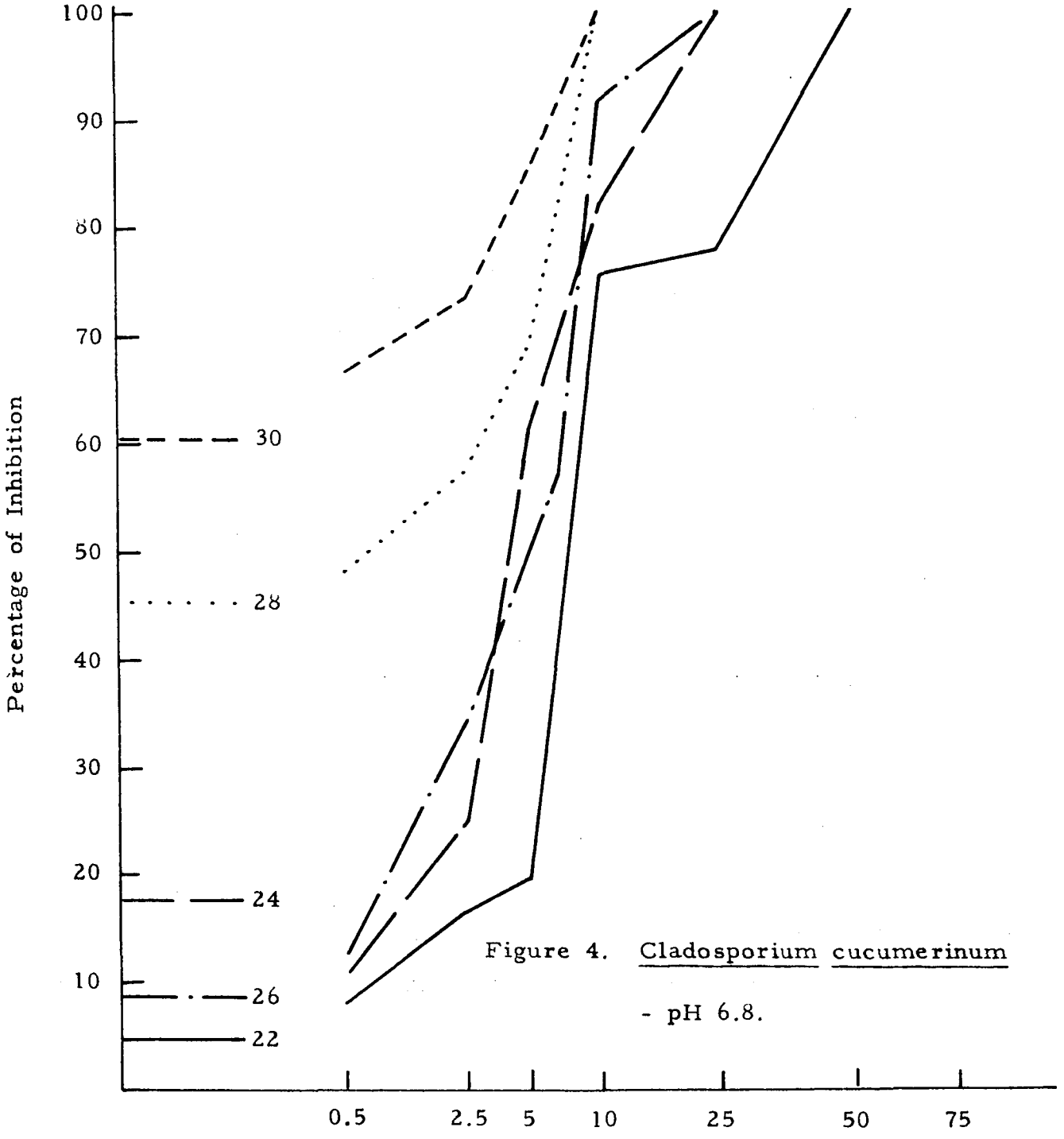


Figure 4. Cladosporium cucumerinum - pH 6.8.

inhibition was obtained at 0.5 p.p.m. at 22° C. than at 24° C. and 26° C. as was the case at pH 6.8. Also an anomaly to the general pattern of progressively lowered inhibition as the temperature is lowered may be observed between temperatures 22° C., 24° C. and 26° C. instead of 24° C. and 26° C. as in the previous case--pH 6.8. As in the previous case here again stimulation occurred in 0.5 p.p.m. at 25° C. as compared to the control. At pH 5.0 (Figure 3) Acti-dione was more effective at 22° C. than at 24° C. Also, a slight stimulation was observed in 0.5 p.p.m. and 2.5 p.p.m. at 25° C., and in 0.5 p.p.m. at 30° C. as compared to the control. At pH 4.0 (Figure 4) it will be noted that inhibition at 22° C. was greater than at 24° C. or 26° C. A stimulation was observed in 0.5 p.p.m. at 30° C. and 24° C. and at the latter it was greater than at 26° C. In summation it may be said that as the pH concentration was lowered, 22° C. and 24° C. became more effective temperatures as a factor in inhibition. No germination occurred at pH 3.0 at any treatment including the control, thus no results could be presented.

In Figures 5, 6, 7, 8, and 9 data are presented to show the relationship between the pH concentrations in relation to constant temperatures.

At 22° C. the general pattern appears to be that inhibition progressively increases with the lowering of pH concentration. At 24° C. the same general pattern prevails except that a slight stimulation in germination is noted in 0.5 p.p.m. at all pH concentrations. A further stimulation of germination is noted in 2.5 p.p.m. at pH 5.0. Also at higher concentrations than 2.5 p.p.m. more inhibition is noted at pH 6.8 than 6.0 or 5.0. As we proceed to 26° C. it is noted that pH 6.8 not only supercedes pH 6.0 and 5.0 as in the previous case, but pH 4.0 as well. pH 4.0 and 5.0 exchange positions in degrees of inhibition several times between 0.5 p.p.m. and 50.0 p.p.m. Otherwise, here also, we note the general pattern described previously. At 28° C. there appears no significant difference from the previous figures except for the absence of stimulation in germination and a definite tendency toward a higher degree of inhibition in all concentrations of Acti-dione and at all pH levels. Also to be noticed is the lack of position exchange in degree of inhibition by the various graph lines of

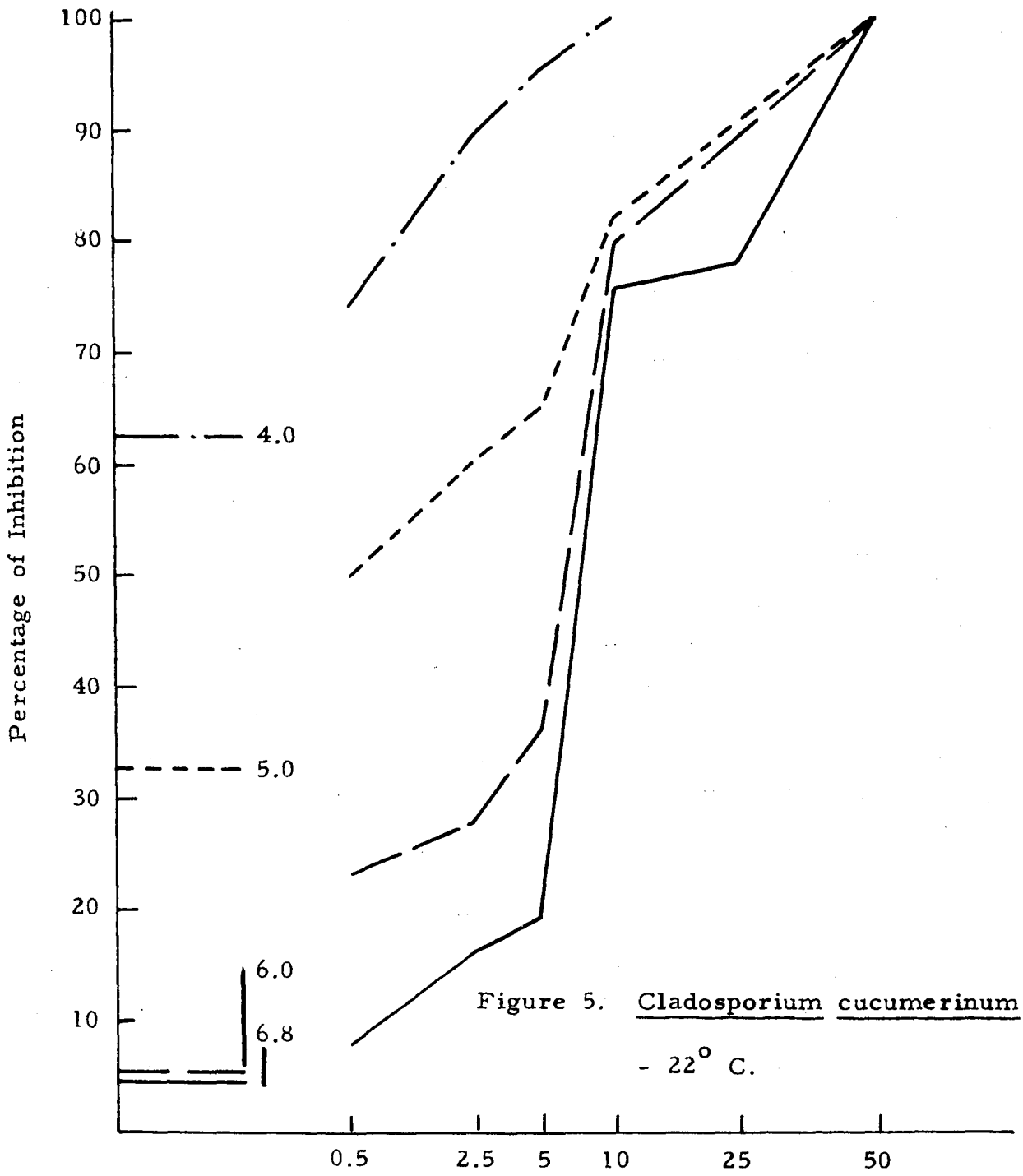


Figure 5. Cladosporium cucumerinum - 22° C.

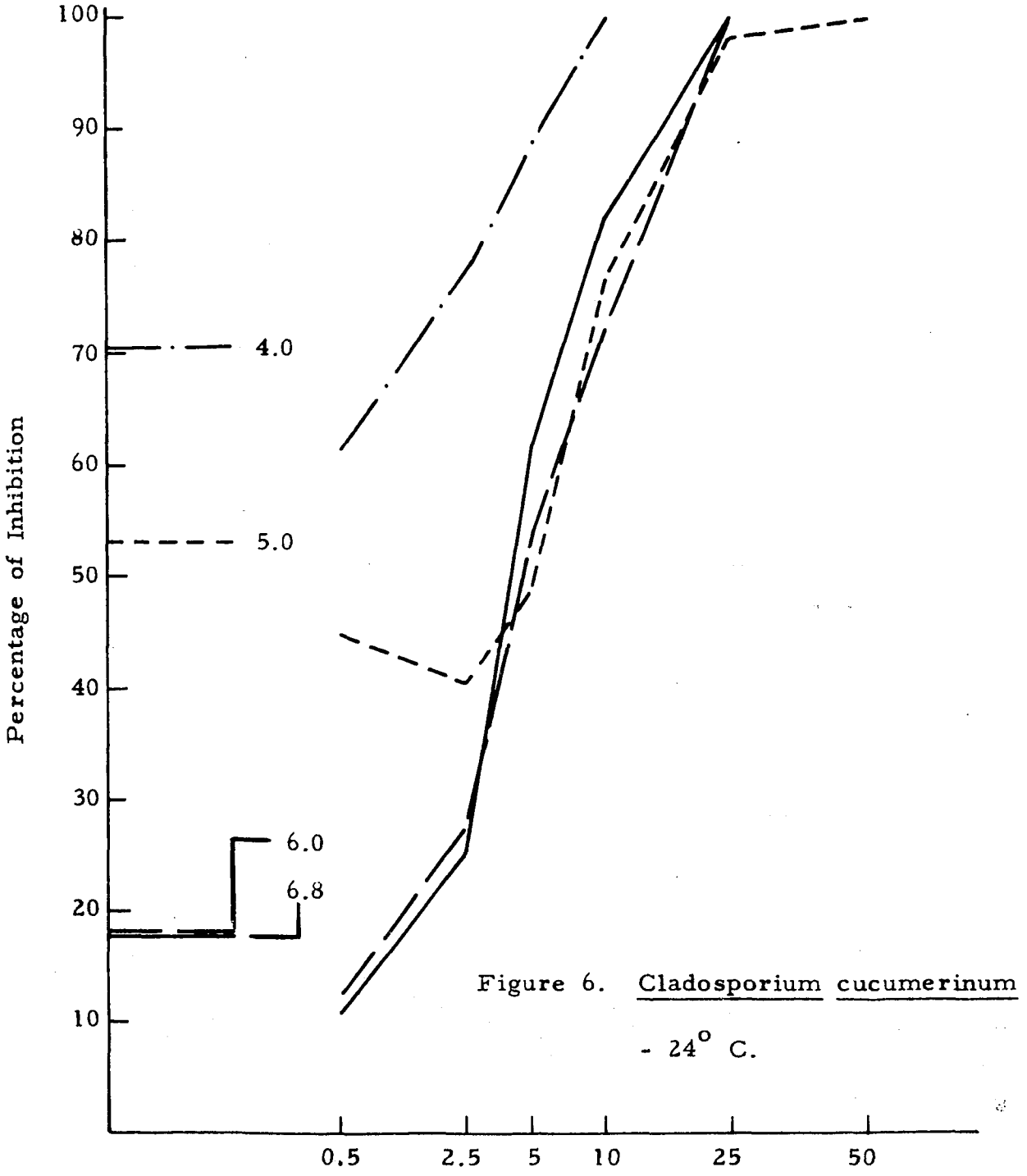


Figure 6. Cladosporium cucumerinum - 24° C.

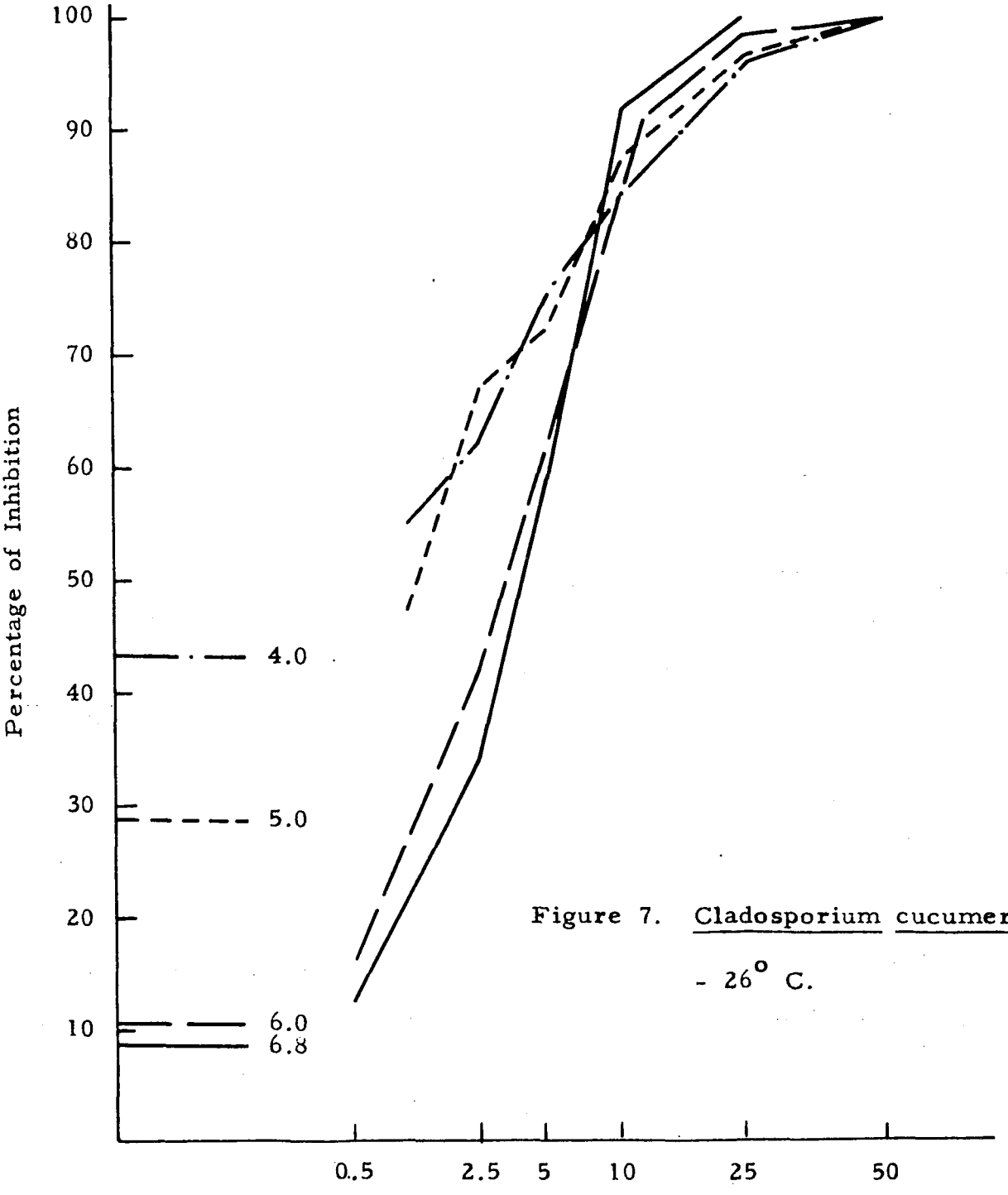


Figure 7. Cladosporium cucumerinum - 26° C.

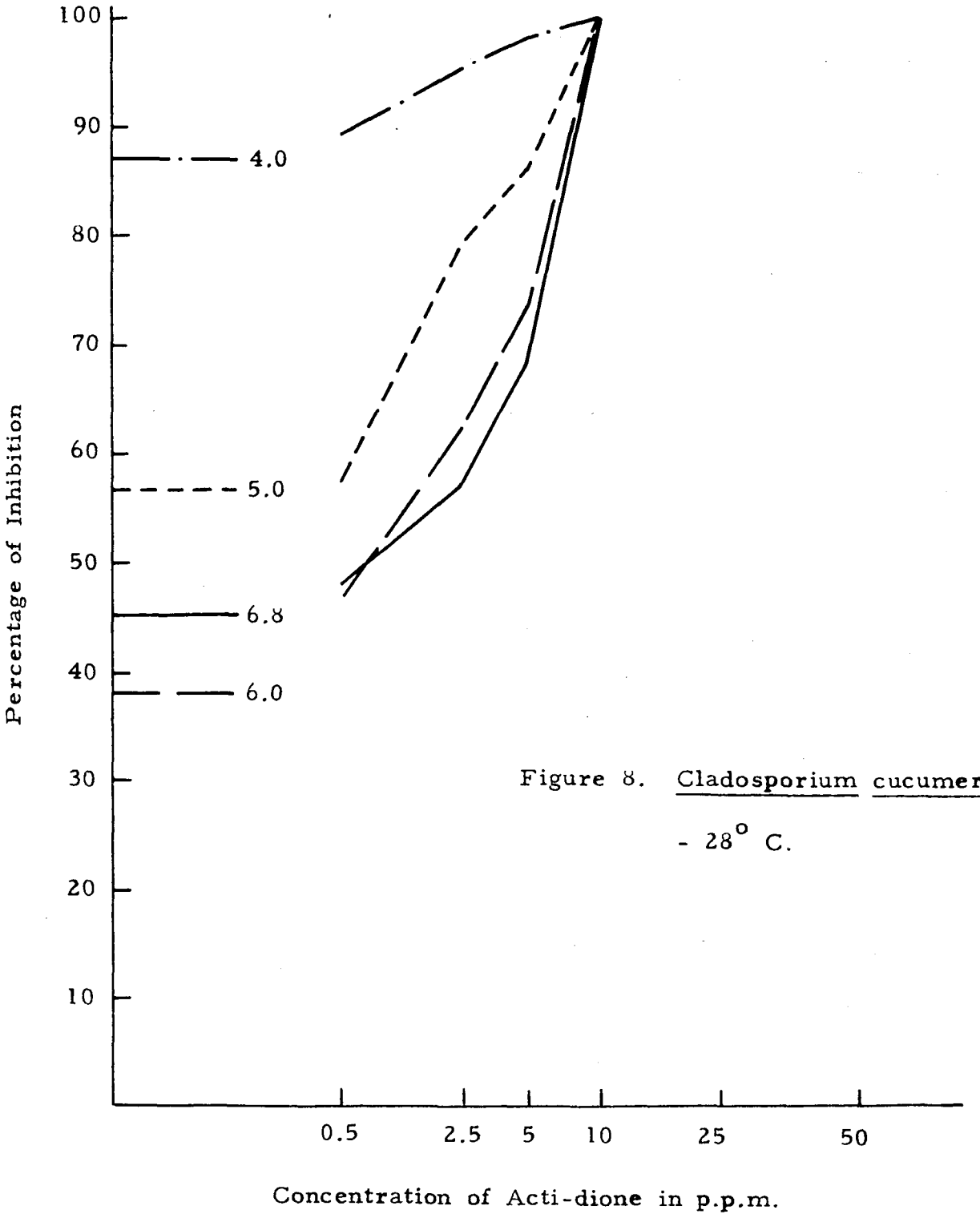


Figure 8. Cladosporium cucumerinum - 28° C.

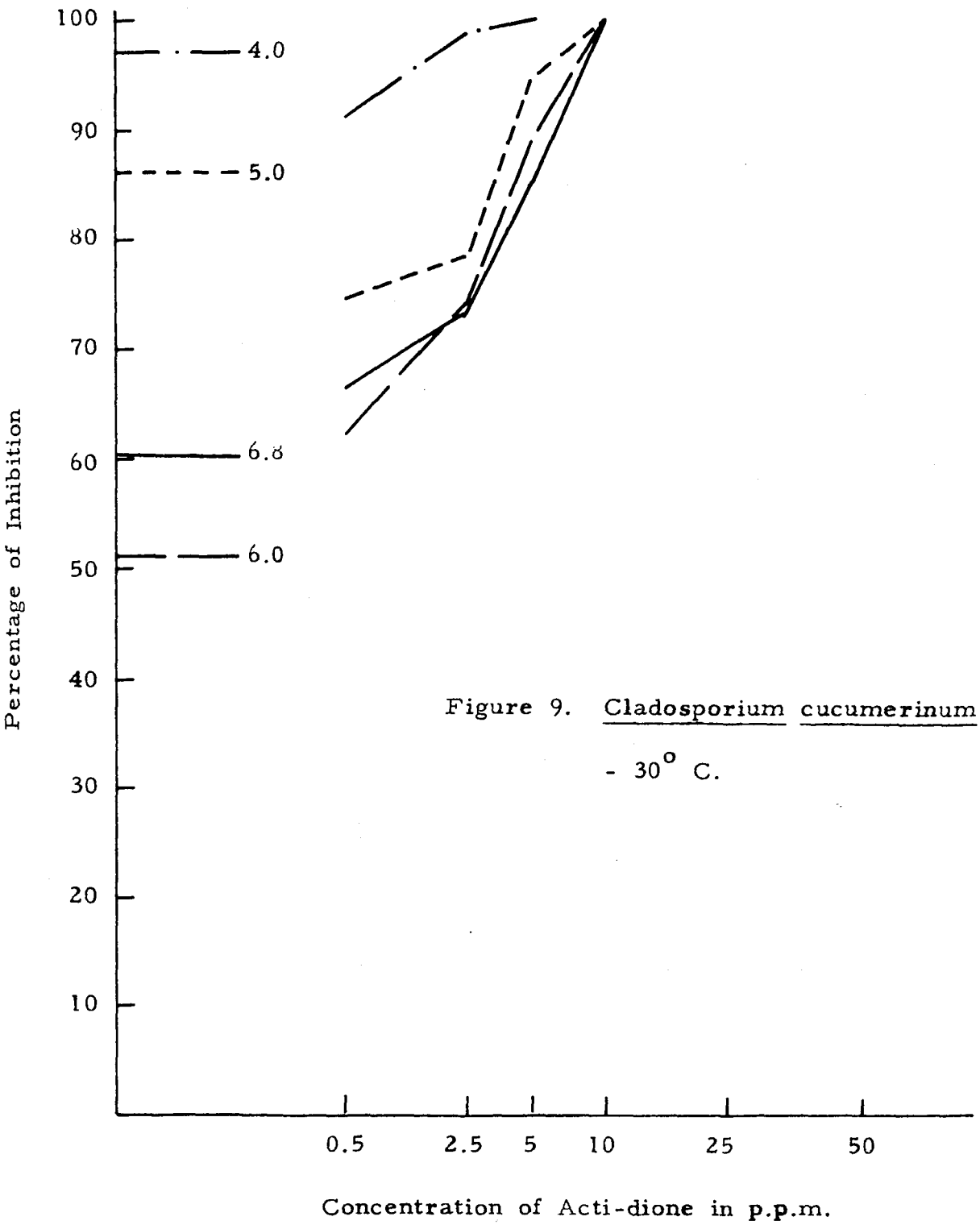


Figure 9. Cladosporium cucumerinum - 30° C.

the pH values, except for a minor anomaly occurring in 0.5 p.p.m. between pH 6.8 and pH 6.0. At 30° C. the highest temperature in this series, the general pattern prevails except in 0.5 p.p.m. where pH 6.8 results in higher inhibition than pH 6.0. It must also be mentioned that here Acti-dione has attained its greatest effect in this series of experiments. Note, that total inhibition of germination has taken place at 10 p.p.m. for all pH values except pH 4.0 whose maximum lethal effect is attained in 5.0 p.p.m. of Acti-dione.

Botrytis Cinerea

The experimental results obtained with this organism are quite variable from one temperature to another and between pH values. There seems to be no general pattern that could be easily described, except that Acti-dione definitely stimulates the germination of Botrytis spores at certain concentrations, temperatures and pH. The results of this investigation show stimulation in every single experiment. As was the case with M. fructicola, what appeared to be complete inhibition at a given concentration after 12 hours turned out to be only temporary inhibition. Usually at the second count, after 24 hours, there

was anywhere from trace to considerable germination which continued as long as 72 hours. No further change was noted after 72 hours under any circumstances.

pH 3.0. At this very low hydrogen ion concentration Acti-dione was most effective at 30° C. and least effective at 26° C. (Figure 10). At 0.5 p.p.m. at 30° C. it was less effective than at any of the other temperatures. However, its efficacy at 30° C. was superior to the other temperatures at 5.0 p.p.m. At 10.0 p.p.m., however, there was a strong reduction of inhibition of germination. The next most effective temperature at the lower concentrations of Acti-dione was that which resulted in the poorest inhibition at the higher concentrations, 26° C. At the lower concentrations, i.e., between 0.5 p.p.m. and 25.0 p.p.m. the next temperature in descending order of inhibition is 22° C. which becomes inferior to 28° C. at 2.5 p.p.m. but again superior to it at 25.0 p.p.m. and remains in this position up to 75.0 p.p.m. At 24° C. Acti-dione was superior to both 22° C. and 28° C. although only slightly. All graph lines meet at 75.0 p.p.m. except 26° C. whose peak performance is at 100.0 p.p.m. No definite pattern is evident in the results obtained from the control experiments.

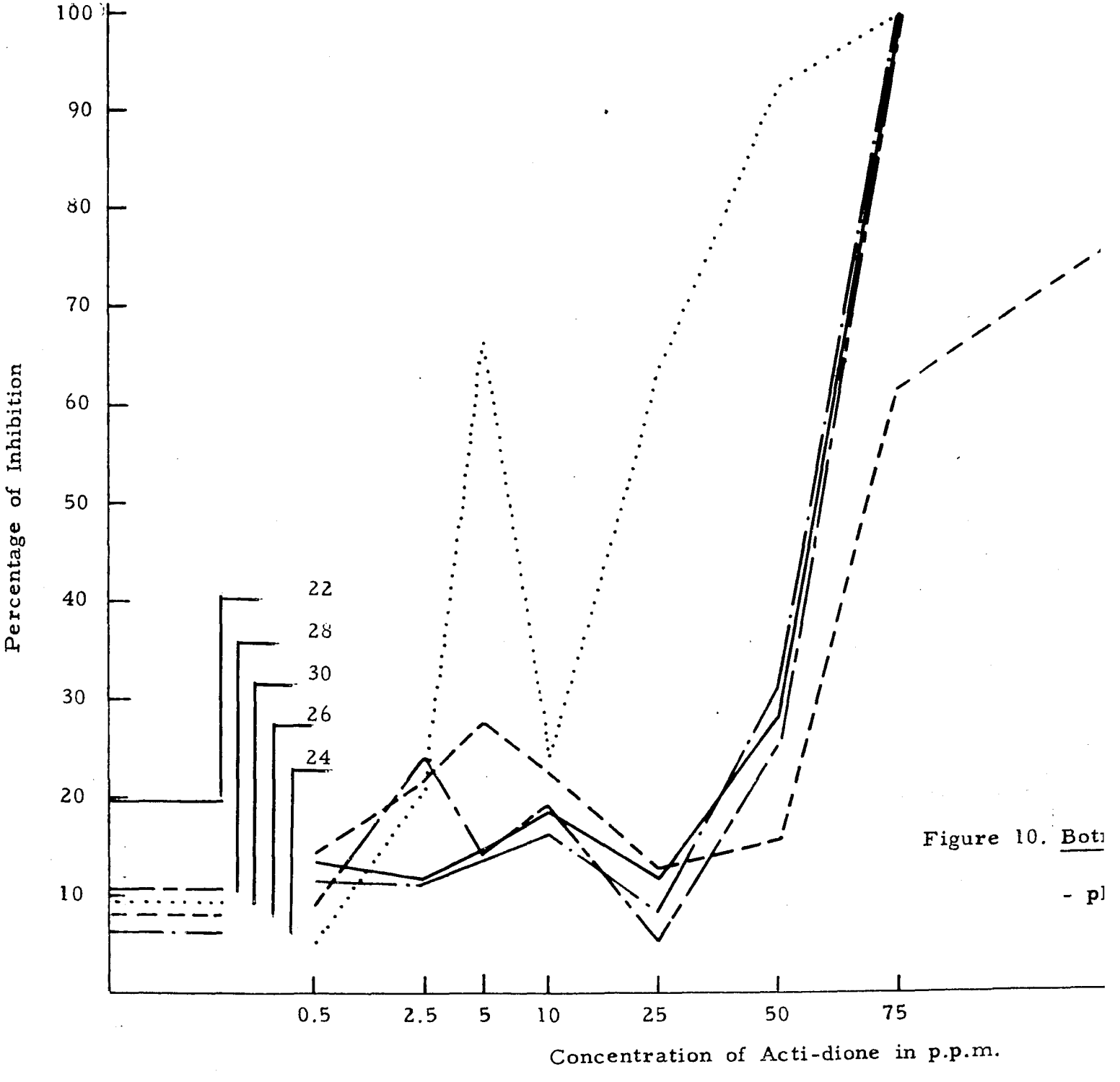


Figure 10. Both

- pl

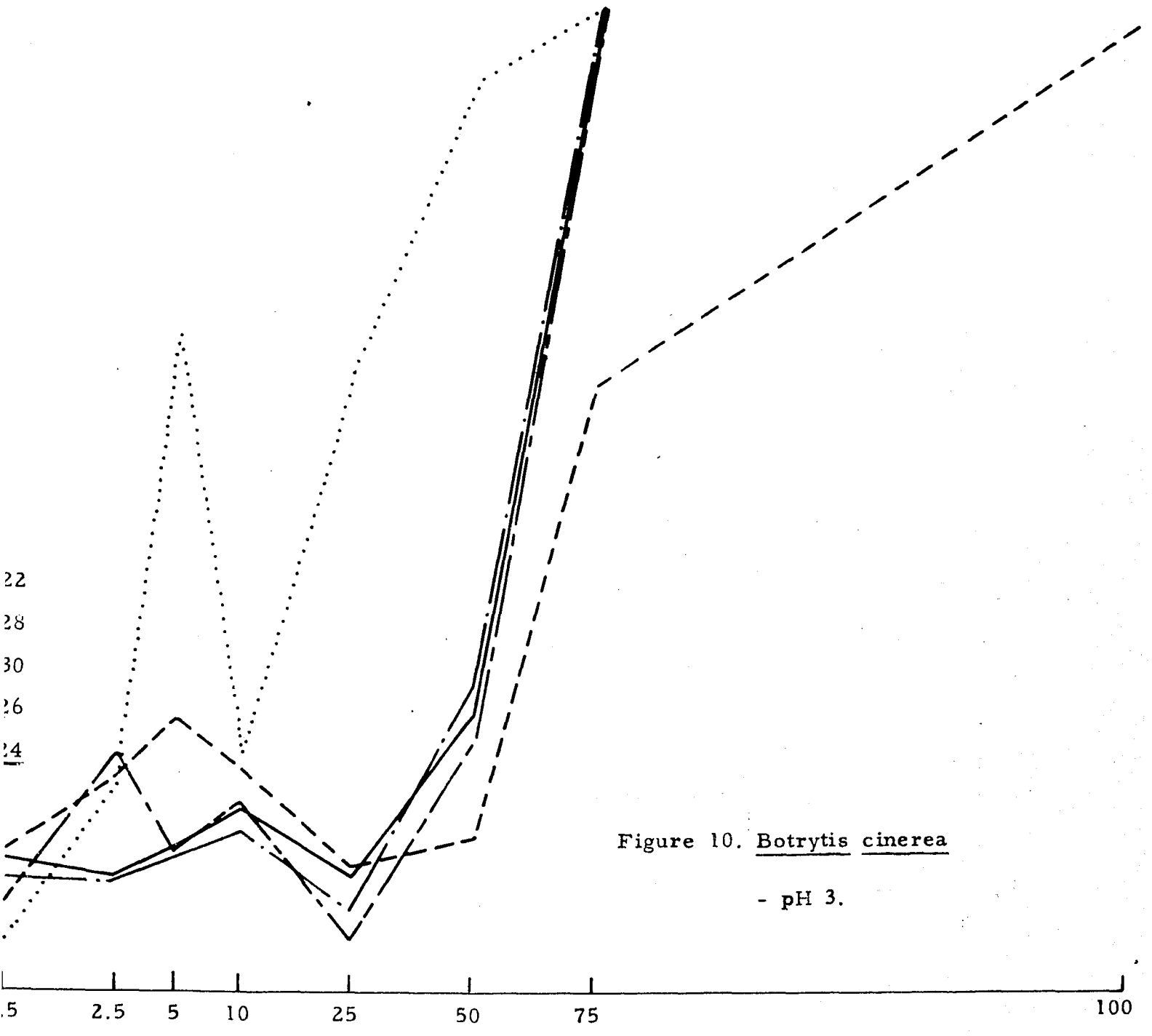


Figure 10. Botrytis cinerea

- pH 3.

As was mentioned above and with M. fructicola, no concentration was sufficient to completely inhibit the germination of the Botrytis spores except 100.0 p.p.m. At 10.0 p.p.m. there was no germination after 12 hours but definite germination did occur before the 24-hour count and continued to increase up to 48 hours.

Note the slight stimulatory effect of Acti-dione at 0.5 p.p.m. over the control, at 22° C., 28° C. and 30° C. and the slight stimulation at 2.5 p.p.m. at 22° C. A more pronounced stimulation occurs at 5.0 p.p.m. at 28° C. while the most pronounced occurred at 25.0 p.p.m. at 28° C.

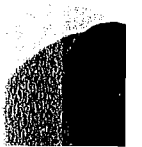
Spores of this organism, immersed in concentrations of Acti-dione which were too toxic to allow germination, after 48 hours appeared very shrunken, and somewhat dirty-yellow in color.

Also of interest was the appearance of the germ tubes in concentrations allowing germination. After 48 hours a large percentage of them would become discolored and extremely granular, taking on a somewhat dark yellowish-brown appearance. If allowed to remain in the Acti-dione solution after 92 hours most of these would shrink and curl in a "zig-zag" form.

pH 4.0. Complete inhibition was attained at 75.0 p.p.m. with all temperatures except 26° C., at the latter, complete inhibition was at 100.0 p.p.m. (Figure 11). There was stimulation at 0.5 p.p.m. at temperatures of 22°, 24°, 28° and 30° C. as compared to the controls. Also note the absence of the very marked stimulatory effects as exhibited in the previous case (Figure 10). The greatest amount of stimulation was at 0.5 p.p.m. at 30° C., followed by 24° C. at 5.0 p.p.m.

Of noteworthy witherest is the fact that as in the previous pH, the ungerminated spores appeared very distorted and considerably shrunken in size as compared to the ungerminated spores in the controls. The germ tubes also showed the same pattern of changes as previously described.

pH 5.0. The most significant difference at this hydrogen ion concentration was the fact that all of the temperatures exerted their greatest influence at 75.0 p.p.m. (Figure 12), and there are no strong stimulatory effects as previously. Slight stimulation, however, was present in the case of every temperature though each may have varied as to the Acti-dione concentration at which the stimulation was noted. At 0.5 p.p.m. Actidione exhibited stimulations of germination at all temperatures.



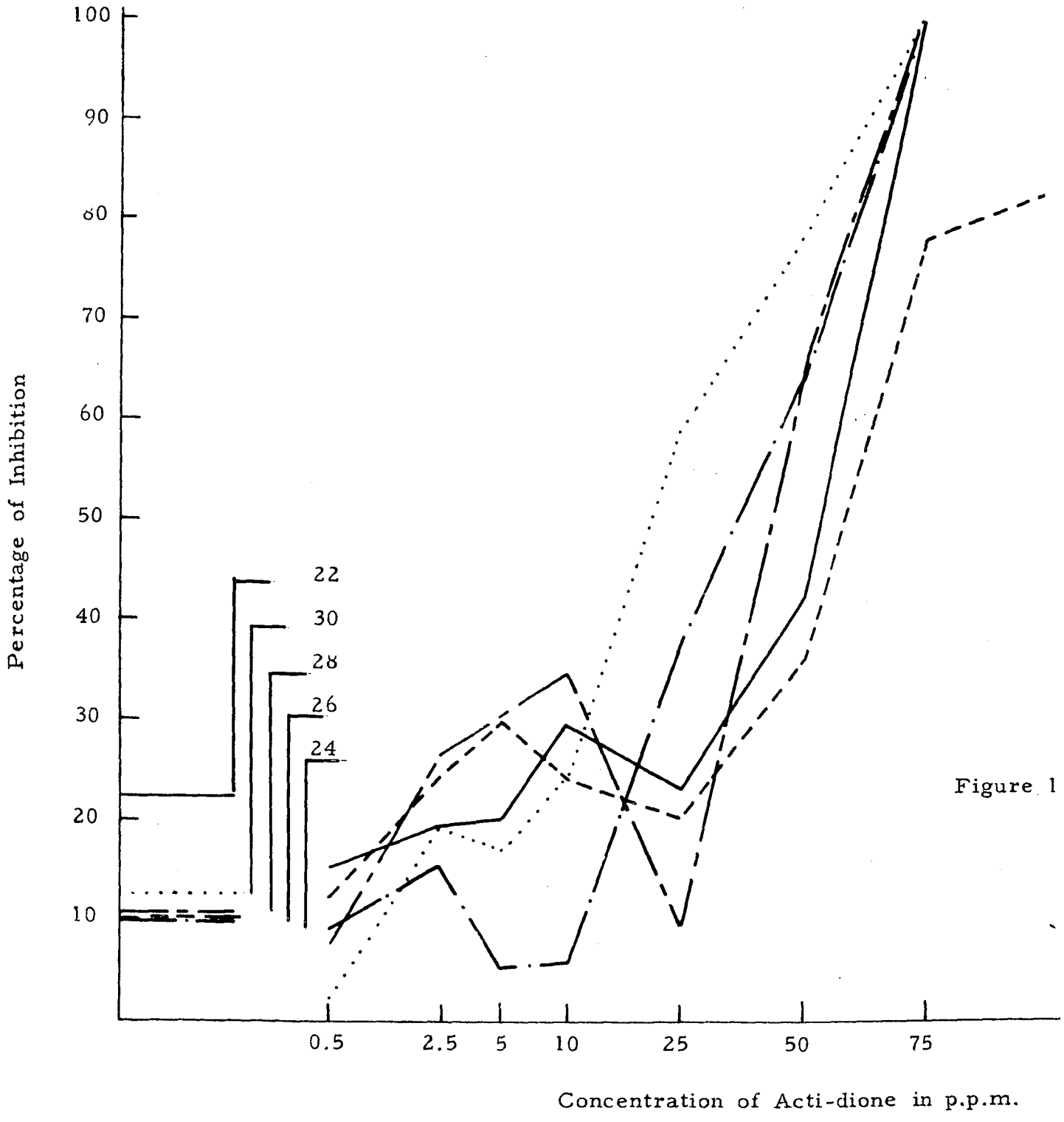

100
90
80
70
60
50
40
30
20
10
Percentage of Inhibition
22
30
28
26
24
0.5
2.5
5
10
25
50
75
Concentration of Acti-dione in p.p.m.

Figure 1

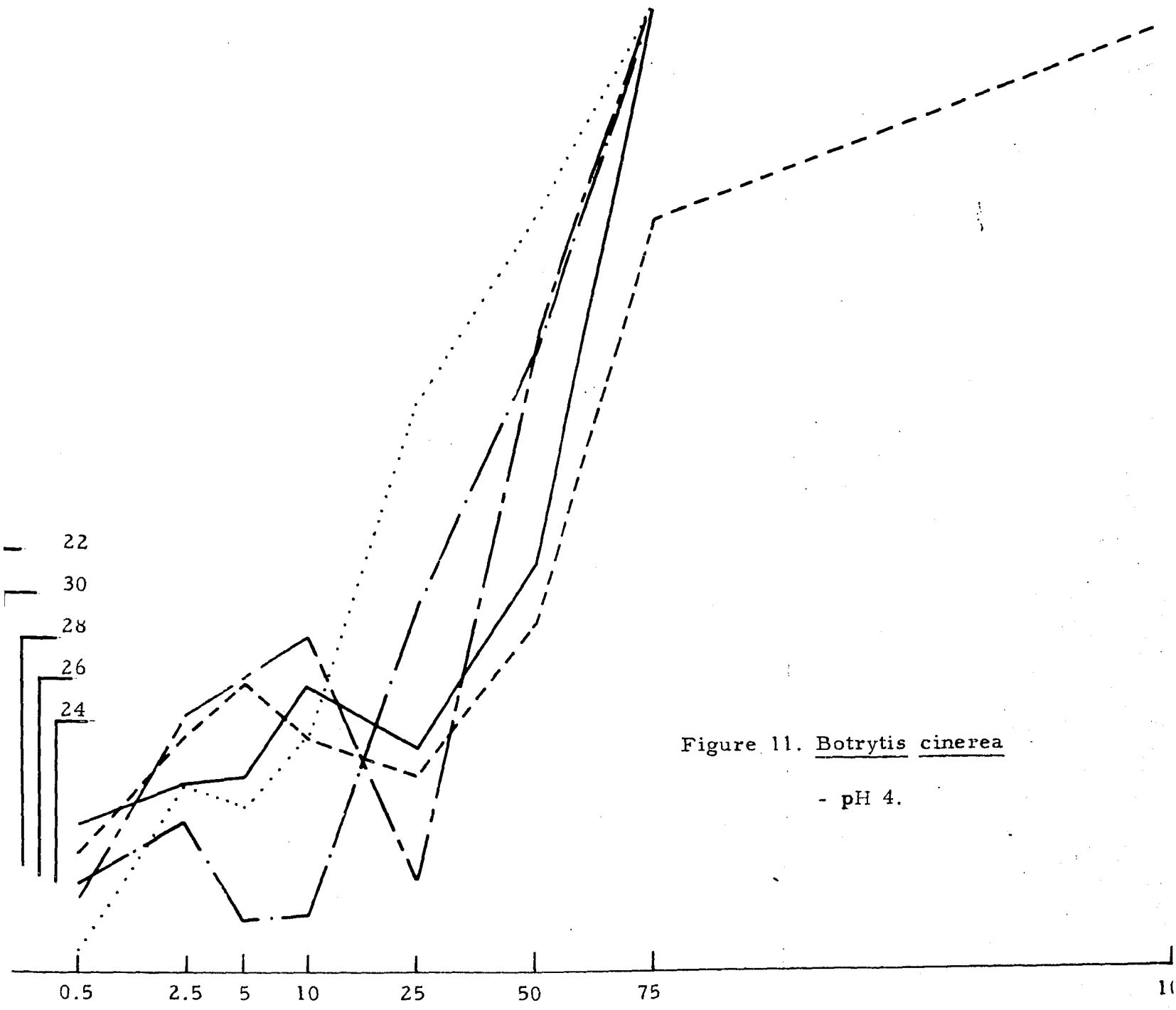
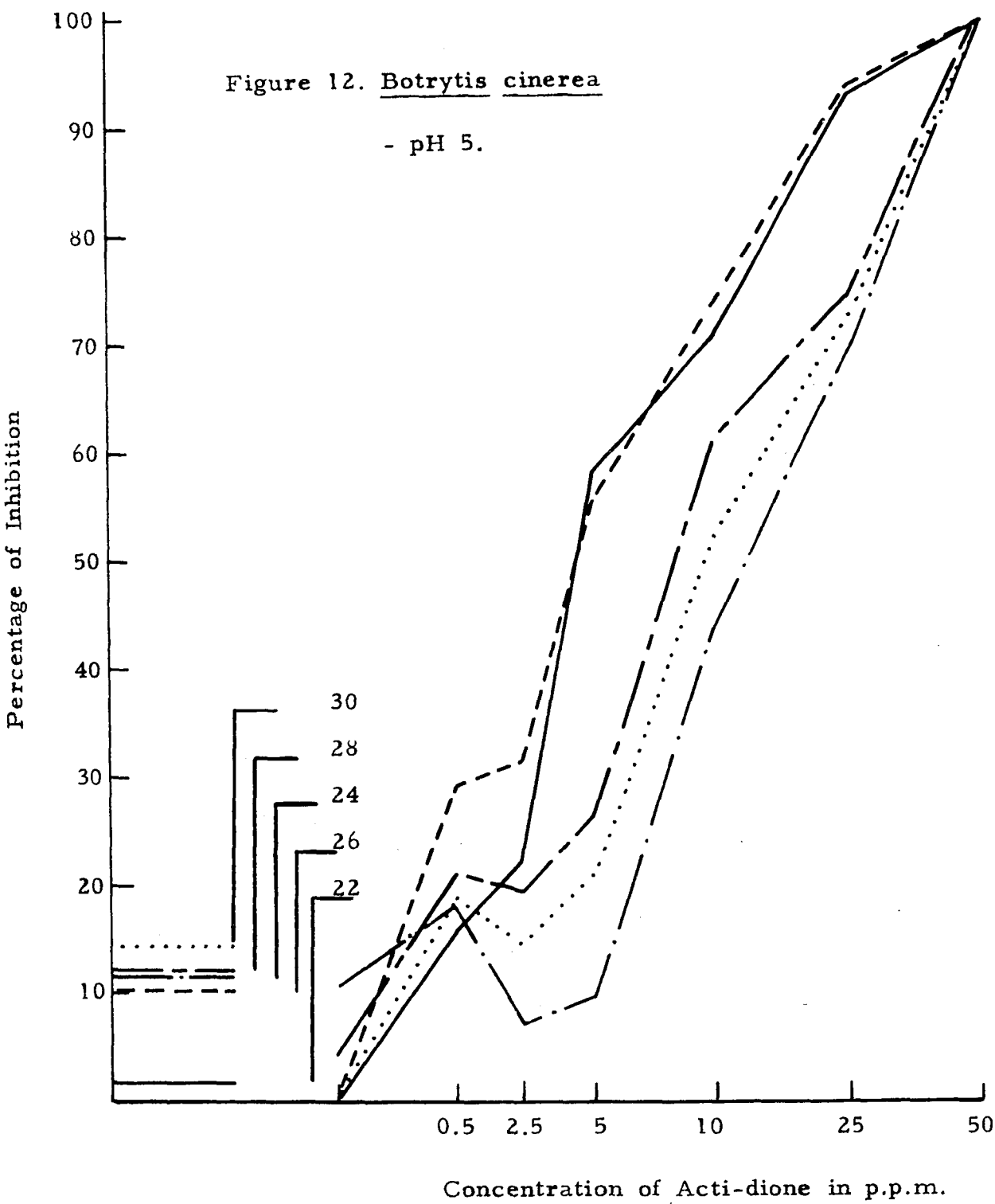


Figure 11. Botrytis cinerea

- pH 4.

Figure 12. Botrytis cinerea

- pH 5.



Stimulation also occurred at 5.0 p.p.m. except in the case of 22° C., 26° C., 28° C. and 30° C. Another difference from the previous pH was that 26° C. was superior, in its inhibition, to all of the other temperatures, instead of 30° C. as in the previous two cases. An additional item of interest was to be found in the control results. At this pH the highest percentage of germination was at 22° C. and the lowest at 30° C. whereas in the previous controls 24° C. gave the best germination and 22° C. the poorest. It is interesting to point out that although in the control, 24° C. and 30° C. were quite poor in germination, so were the treatments at those temperatures poorest in inhibiting germination.

As described previously, the spores in the concentrations too high to allow germination behaved in the same pattern. The breakdown of germ tubes occurred here after 92 hours but not at 72 hours. The only difference noted here was that the amount of breakdown was somewhat lower. No actual count was made of this, only a general estimate by an over-all inspection of 25 or more microscopic fields.

pH 6.0. The principal difference at this pH from all previous concentrations lies in the fact that most effective concentration of Acti-dione for the majority of the temperatures used in this study was 100 p.p.m. Thirty degrees centigrade was as in previous tests, one of the most effective temperatures, with pH 6.0 it was inferior only to 28° C. and then only at the higher concentrations of their range. Stimulation at 0.5 p.p.m. occurred only at 24° C. and 28° C.

As in all previous experiments, distortion of ungerminated spores was evident in concentrations of Acti-dione too toxic to allow germination (Table II). The amount of distortion, however, was even less than in the previous case.

pH 6.8. At this, the highest hydrogen ion concentration used in this study, there seems to be the least amount of anomaly in the pattern of behavior between pH values as exhibited by the results (Figure 14). Stimulation of germination at 0.5 p.p.m. occurred at 22° C., 24° C., and 26° C. A marked amount of stimulation occurred in 2.5 p.p.m. at 26° C. and a slight amount at 24° C. Furthermore, a definite reduction of inhibition occurred in 25.0 p.p.m. at 22° C. and 30° C. One principal difference between this pH and all of the previous

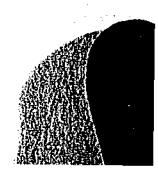


TABLE II

INHIBITION OF SPORE GERMINATION OF BOTRYTIS CINEREA BY DIFFERENT CONCENTRATIONS OF ACTI-DIONE AT FIVE DIFFERENT TEMPERATURES AND FIVE HYDROGEN ION CONCENTRATIONS

Temperatures (° C.)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
22	Control	19.81	22.03	1.77	11.11	20.22
	0.5	13.42	15.11	0.00	13.02	0.55
	2.5	11.73	19.20	15.74	26.50	7.01
	5.0	14.83	20.02	22.12	11.93	35.00
	10.0	18.61	29.50	32.48	42.10	57.84
	25.0	11.75	23.12	30.00	38.00	43.32
	50.0	28.20	42.48	58.02	69.98	78.00
	75.0	100.00	100.00	70.50	84.00	86.00
	100.0	100.00	100.00	100.00	100.00	100.00
24	Control	6.36	10.00	11.71	9.01	26.22
	0.5	11.43	9.13	10.62	5.18	12.61
	2.5	11.01	15.43	18.00	8.00	10.53
	5.0	15.05	5.26	7.00	18.73	17.72
	10.0	16.04	5.93	9.52	36.72	53.03
	25.0	8.11	37.01	43.97	52.00	39.04
	50.0	29.91	64.51	69.81	61.34	76.33
	75.0	100.00	100.00	100.00	82.20	83.01
	100.0	100.00	100.00	100.00	100.00	100.00

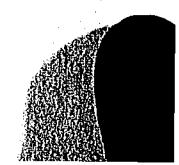


TABLE II (Continued)

Temperatures (° C.)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
26	Control	8.02	10.00	10.03	24.12	30.73
	0.5	14.42	12.05	0.00	30.01	26.61
	2.5	21.86	24.32	29.13	38.00	13.76
	5.0	27.92	29.85	31.45	41.33	26.59
	10.0	22.87	24.13	55.33	55.50	46.69
	25.0	12.85	20.09	74.00	67.02	66.59
	50.0	15.57	36.22	93.99	74.00	91.22
	75.0	62.00	78.31	100.00	81.11	95.10
	100.0	100.00	100.00	100.00	100.00	100.00
28	Control	10.71	10.50	12.00	25.04	30.98
	0.5	9.43	7.85	4.04	22.22	41.26
	2.5	24.30	26.51	21.12	21.93	35.17
	5.0	14.41	20.93	19.47	32.00	29.51
	10.0	19.27	34.77	26.55	41.59	43.43
	25.0	5.22	9.14	61.13	57.16	68.00
	50.0	25.66	65.66	74.53	83.05	84.22
	75.0	100.00	100.00	100.00	100.00	100.00
	100.0	100.00	100.00	100.00	100.00	100.00
30	Control	9.48	12.52	14.41	27.00	27.03
	0.5	5.01	2.28	0.00	34.24	60.71
	2.5	21.01	19.17	18.02	32.01	50.88
	5.0	66.04	17.00	14.79	43.96	40.09
	10.0	24.32	24.30	21.03	58.75	62.86
	25.0	63.11	59.00	52.16	69.13	78.38
	50.0	92.79	78.41	72.89	70.44	100.00
	75.0	100.00	100.00	100.00	100.00	100.00
	100.0	100.00	100.00	100.00	100.00	100.00

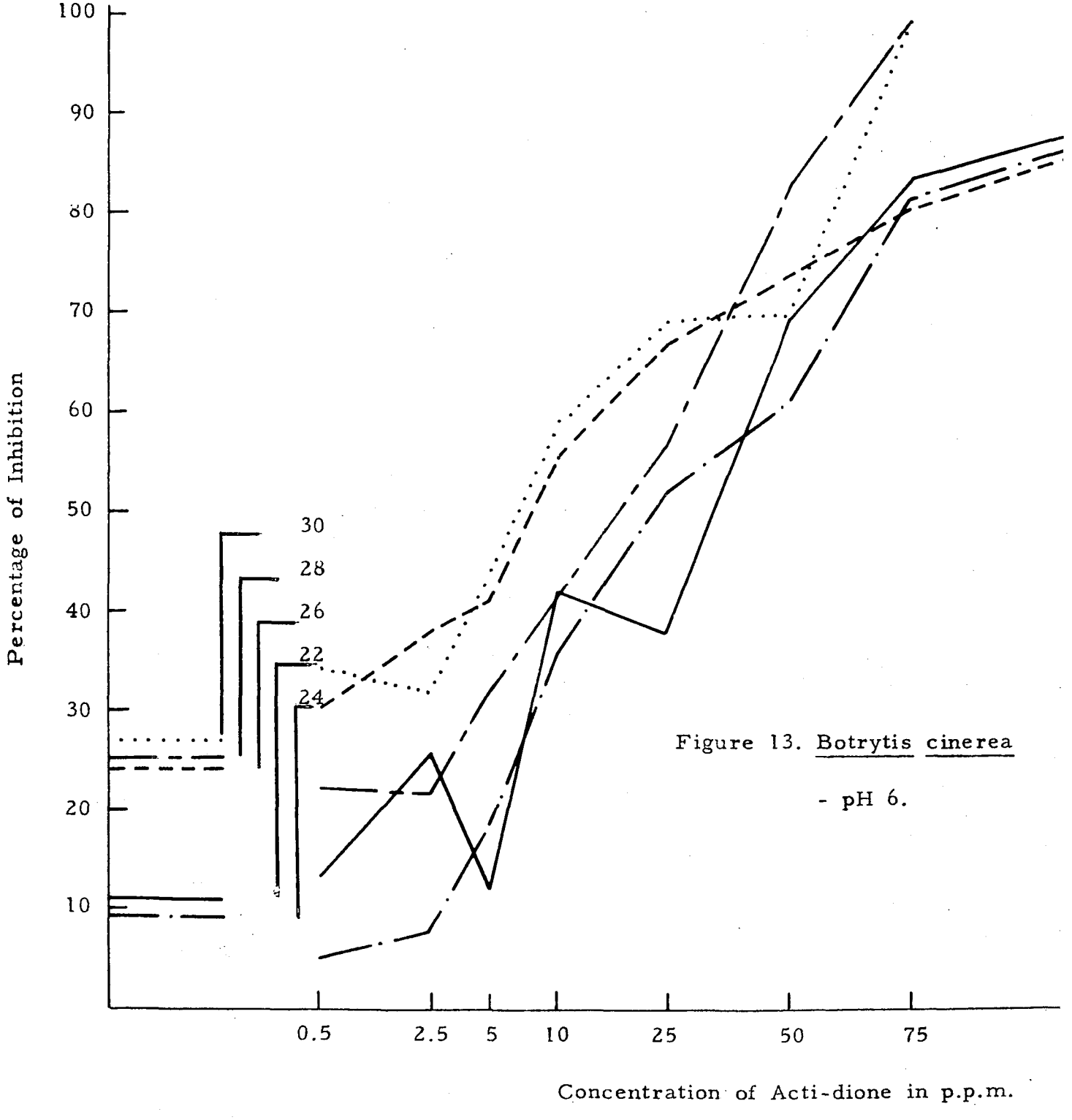


Figure 13. Botrytis cinerea

- pH 6.

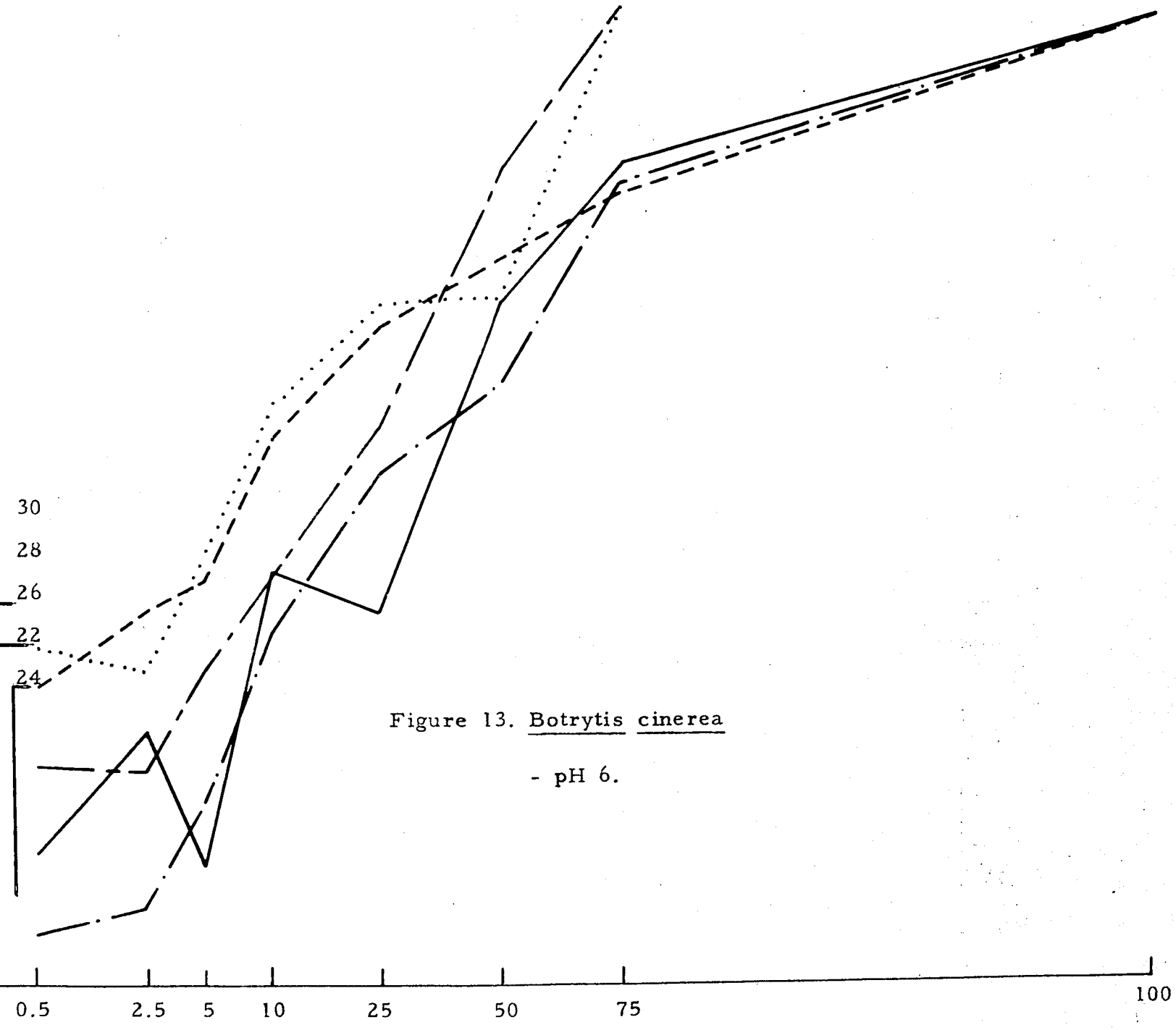


Figure 13. Botrytis cinerea

- pH 6.

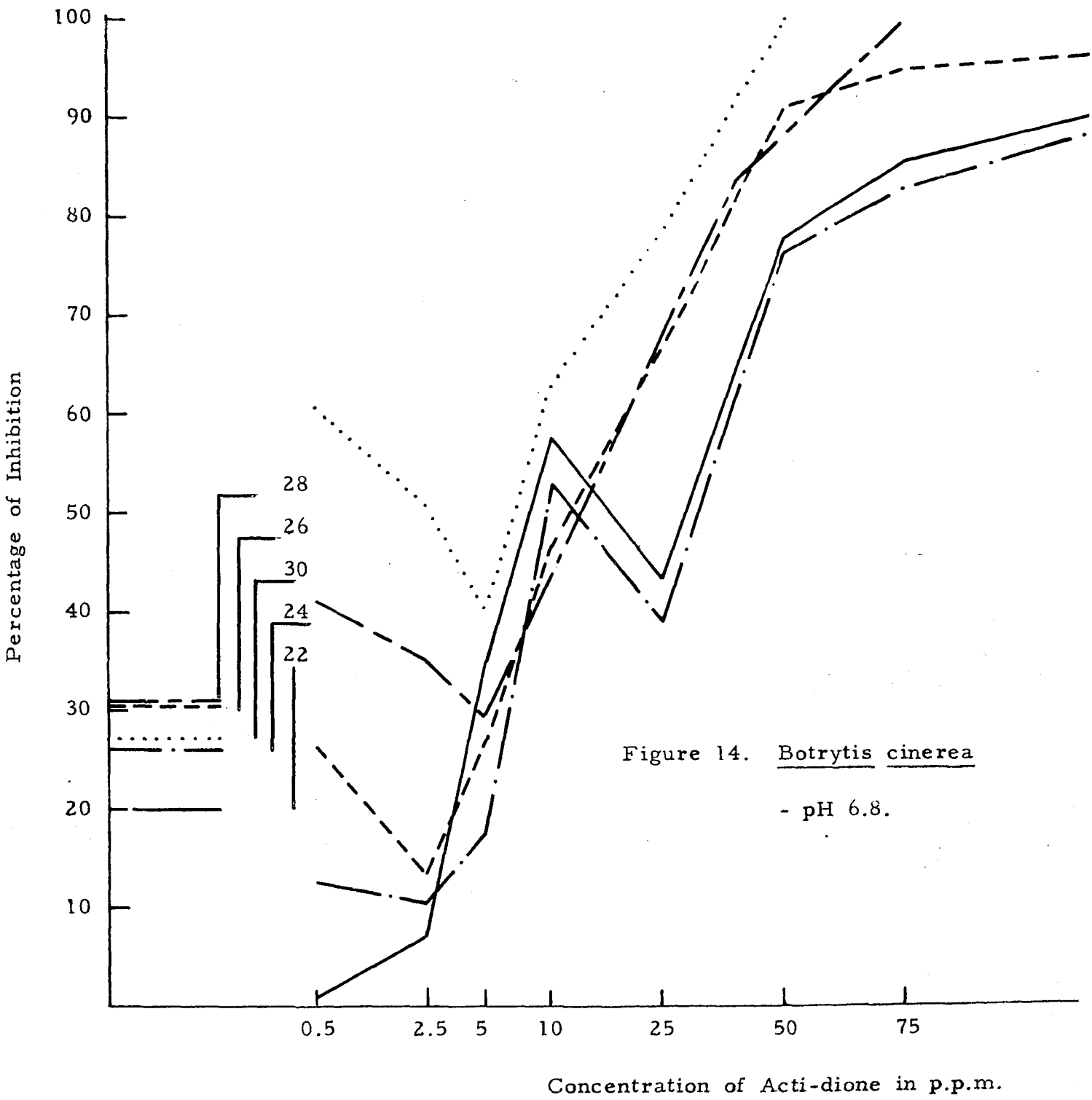


Figure 14. Botrytis cinerea

- pH 6.8.

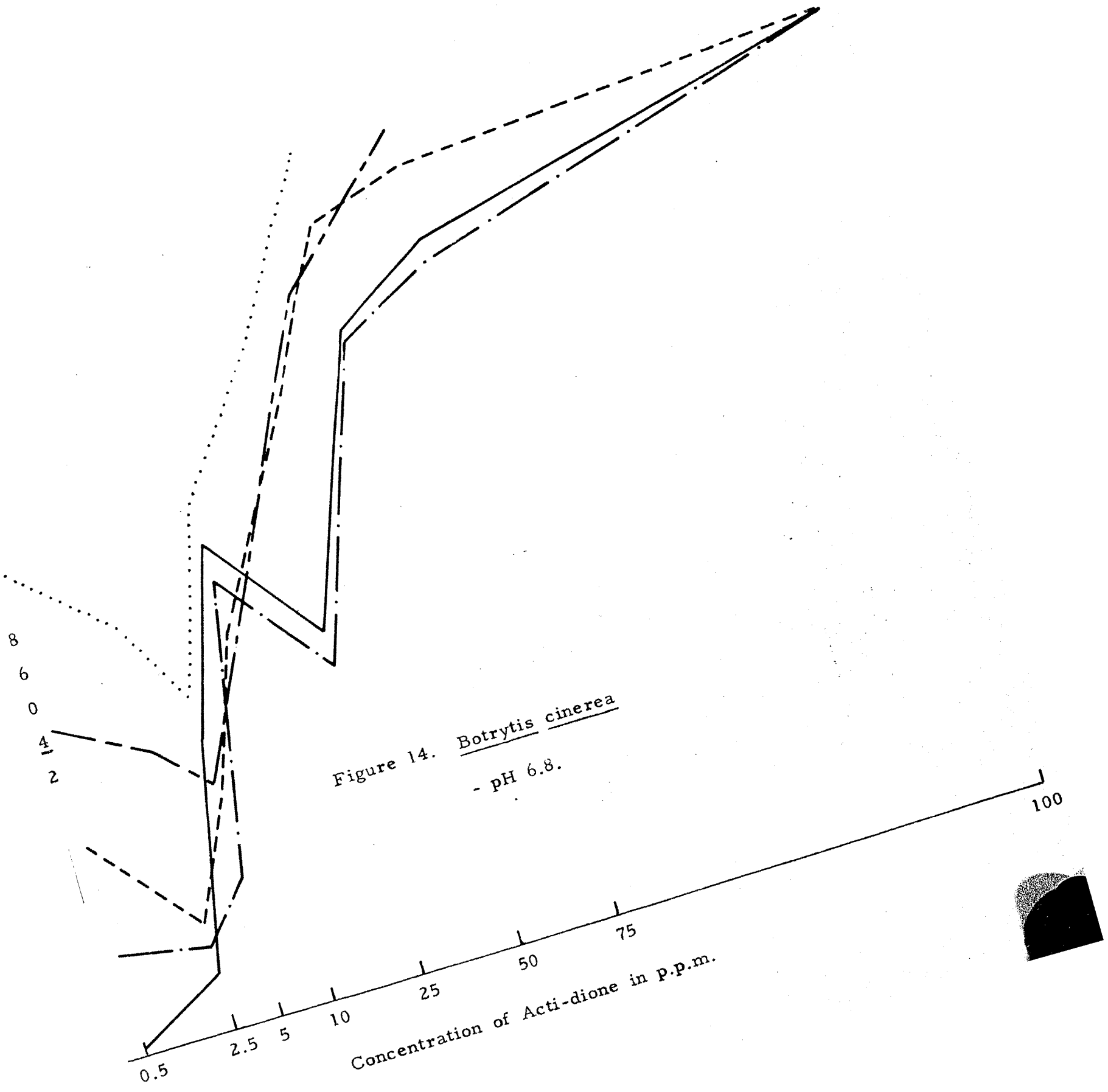


Figure 14. Botrytis cinerea - pH 6.8.

H-ion concentrations was that the maximum amount of inhibition was at 4 different concentrations of Acti-dione instead of 1 and 2 different concentrations. At 30° C. the maximum amount of inhibition was at 50.0 p.p.m., the maximum at 28° C. was at 75.0 p.p.m. and all other temperatures require 100.0 p.p.m. to completely inhibit the germination of Botrytis spores.

Distortion of spores and disintegration of germ tube cytoplasm also occurred here but in the least amount when compared with the other pH treatments. Germination continued to increase up to the 72-hour count (Table II).

Figures 15, 16, 17, 18 and 19 present the same data discussed above but in a different light. The attempt is made to show the relationships and differences between each pH value at each of the temperatures used in this study.

Temperature, 22° C. At this temperature there was a stimulatory effect of Acti-dione in 0.5 p.p.m. at every pH except 6.0. The largest amount of stimulation was in 0.5 p.p.m. at pH 5 followed by pH 4.0. Also to be noted is the stimulation of germination in 25 p.p.m. at pH 3.0. From 5.0 p.p.m. and higher the amount of inhibition at hydrogen ion concentrations of 6.8, 6.0 and 5.0 almost parallel each other until at

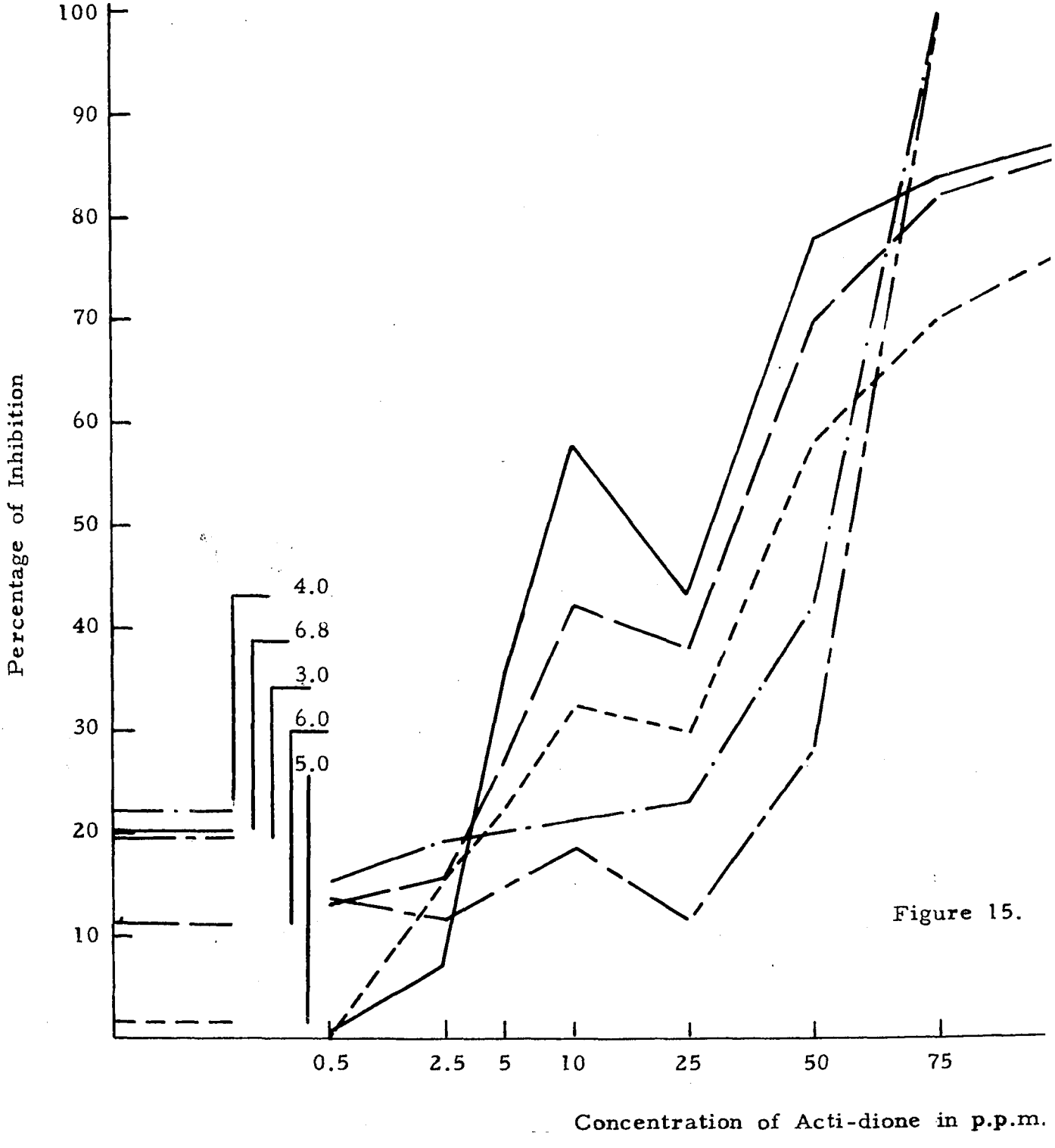

Percentage of Inhibition
100
90
80
70
60
50
40
30
20
10
4.0
6.8
3.0
6.0
5.0
0.5
2.5
5
10
25
50
75
Concentration of Acti-dione in p.p.m.

Figure 15.

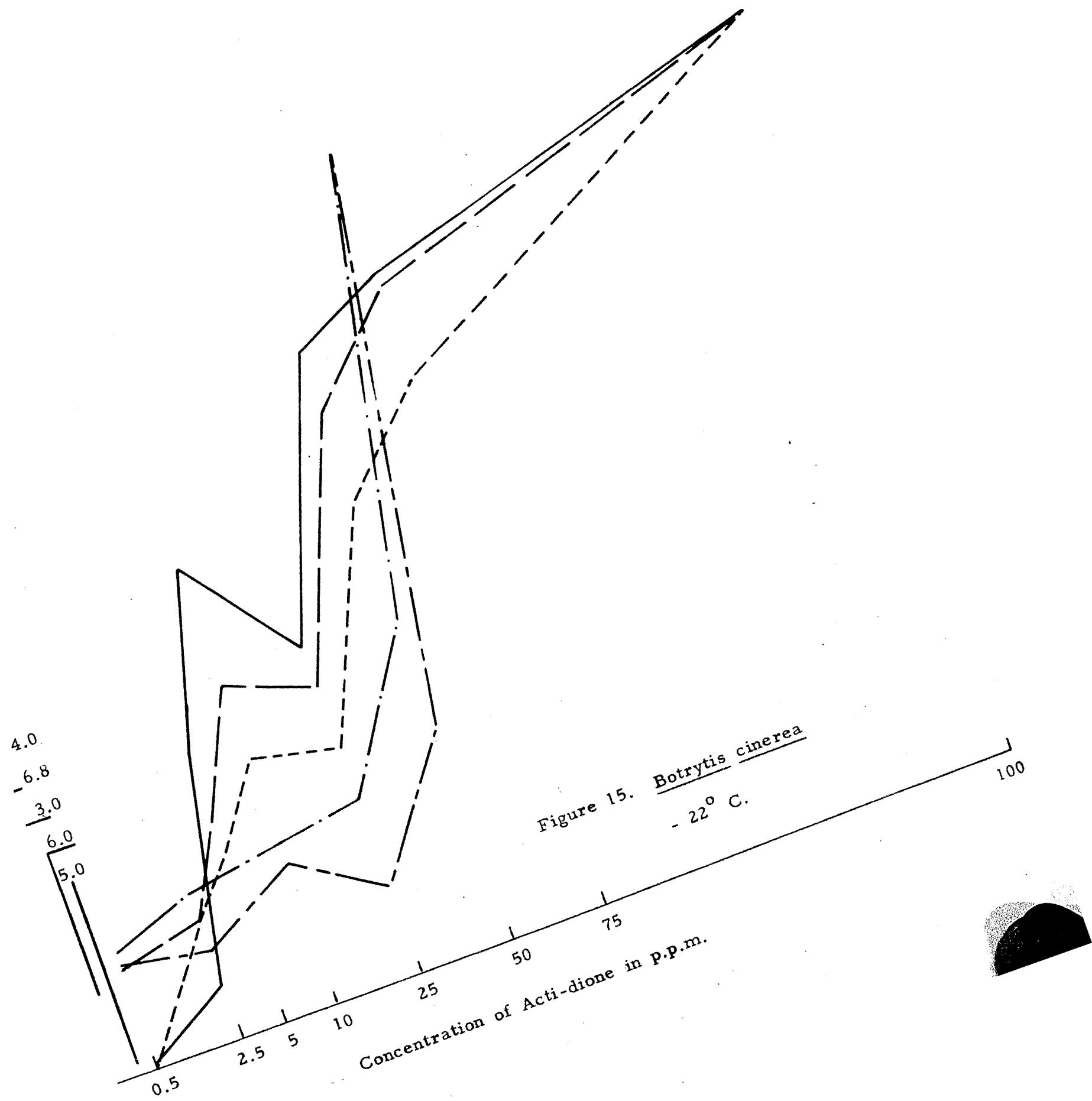


Figure 15. Botrytis cinerea - 22° C.

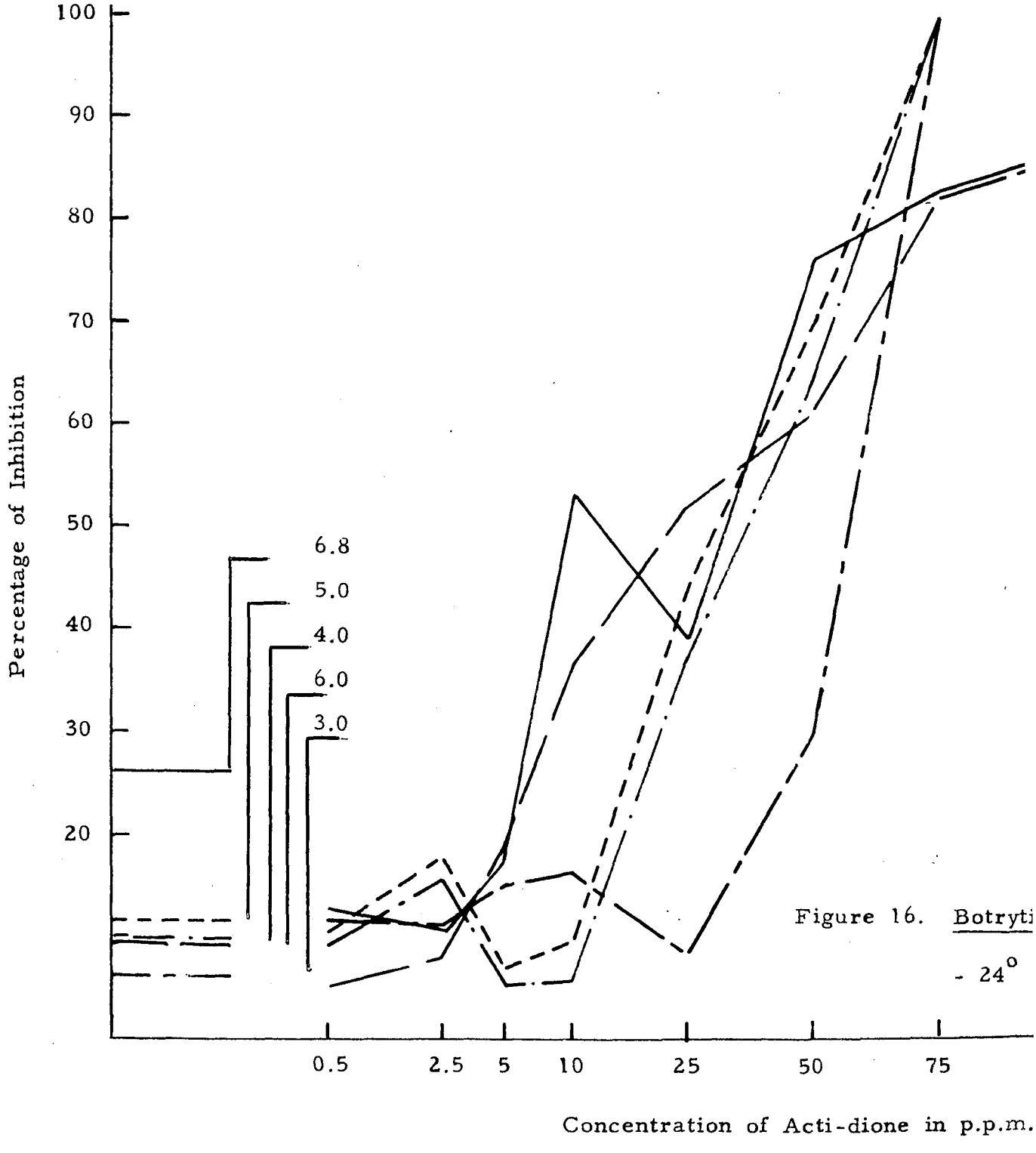


Figure 16. Botryti

- 24°

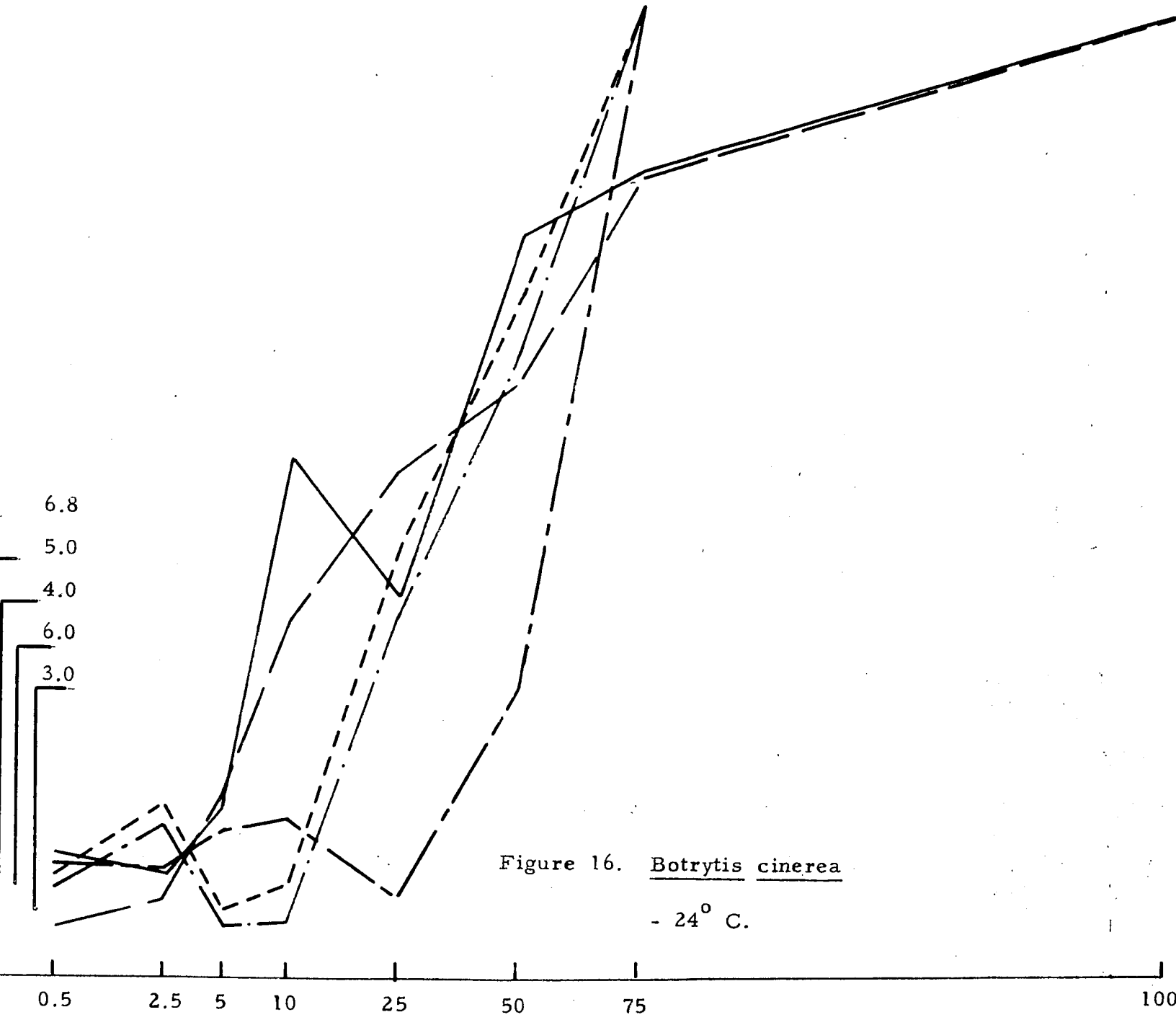


Figure 16. Botrytis cinerea - 24° C.

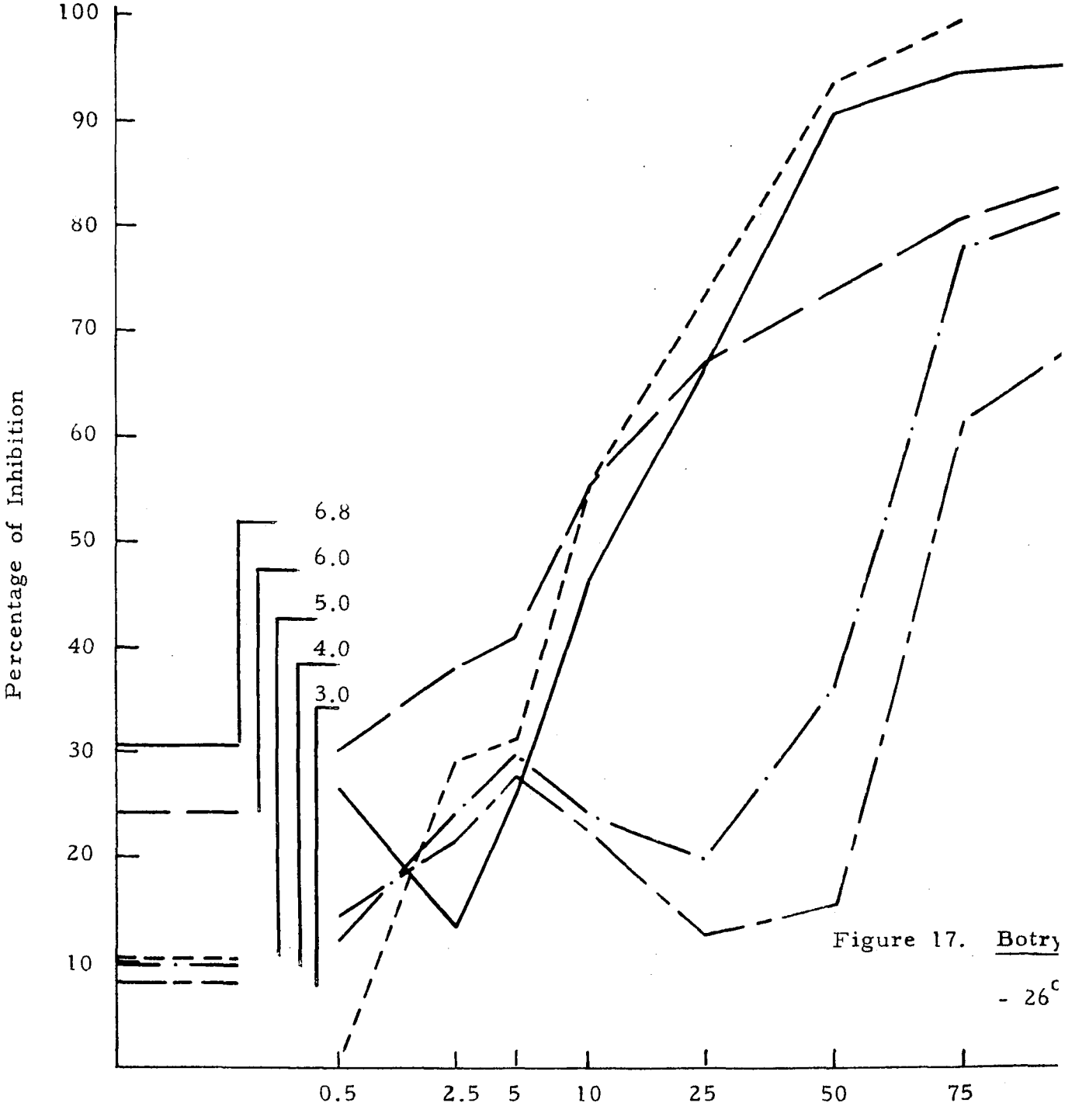


Figure 17. Botry

- 26°

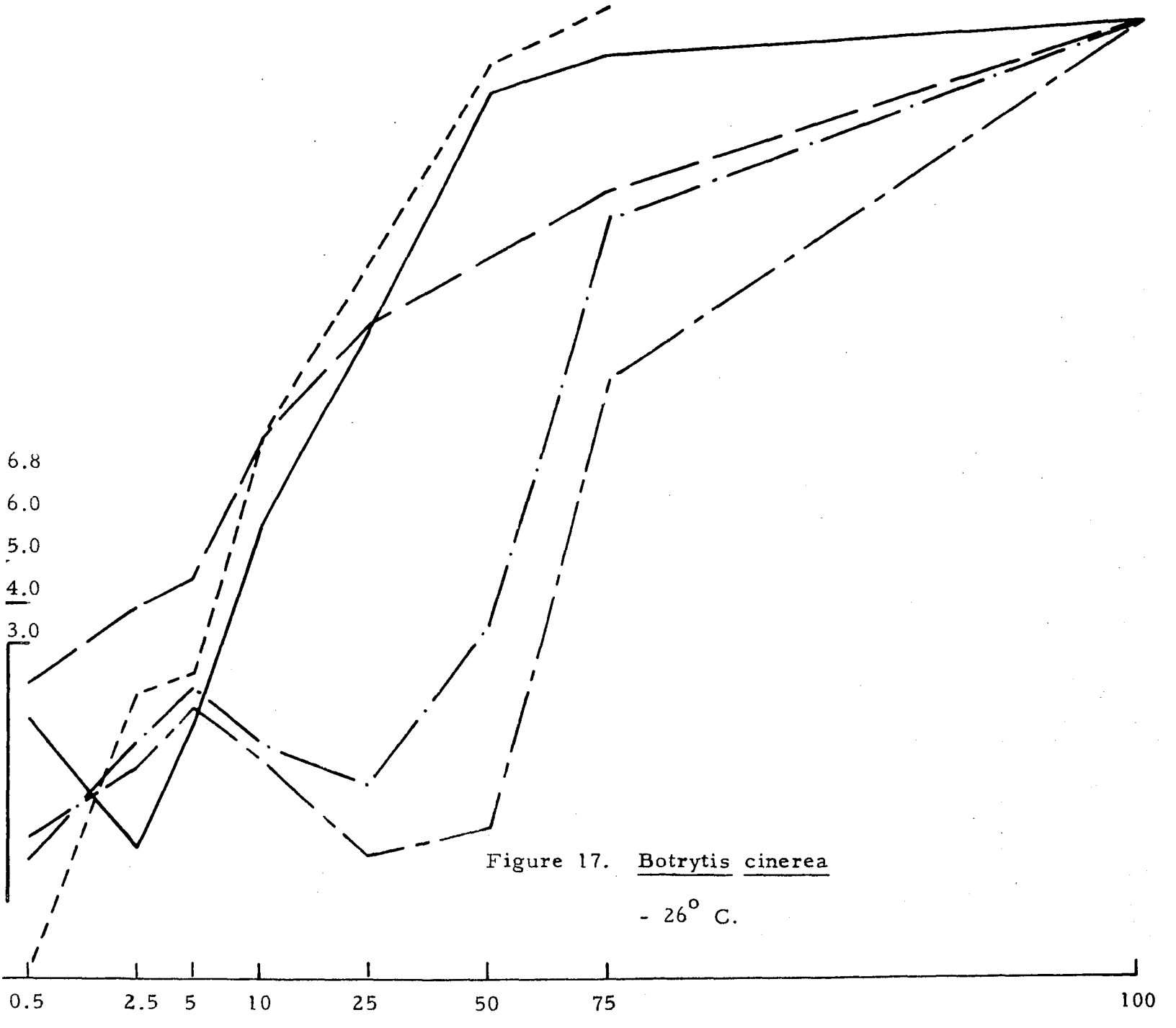


Figure 17. Botrytis cinerea - 26° C.

Figure 18. Botrytis cinerea - 28° C.

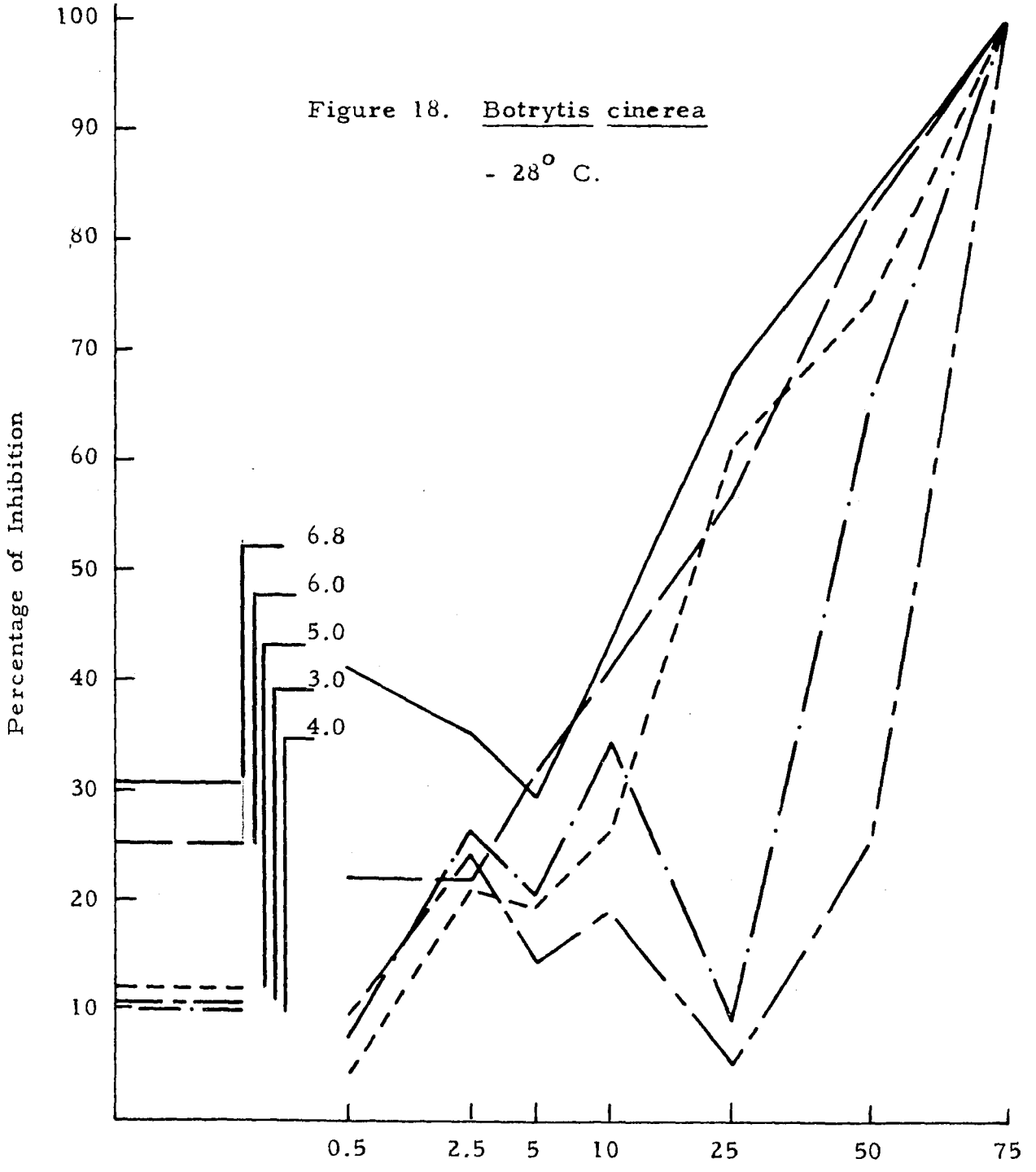
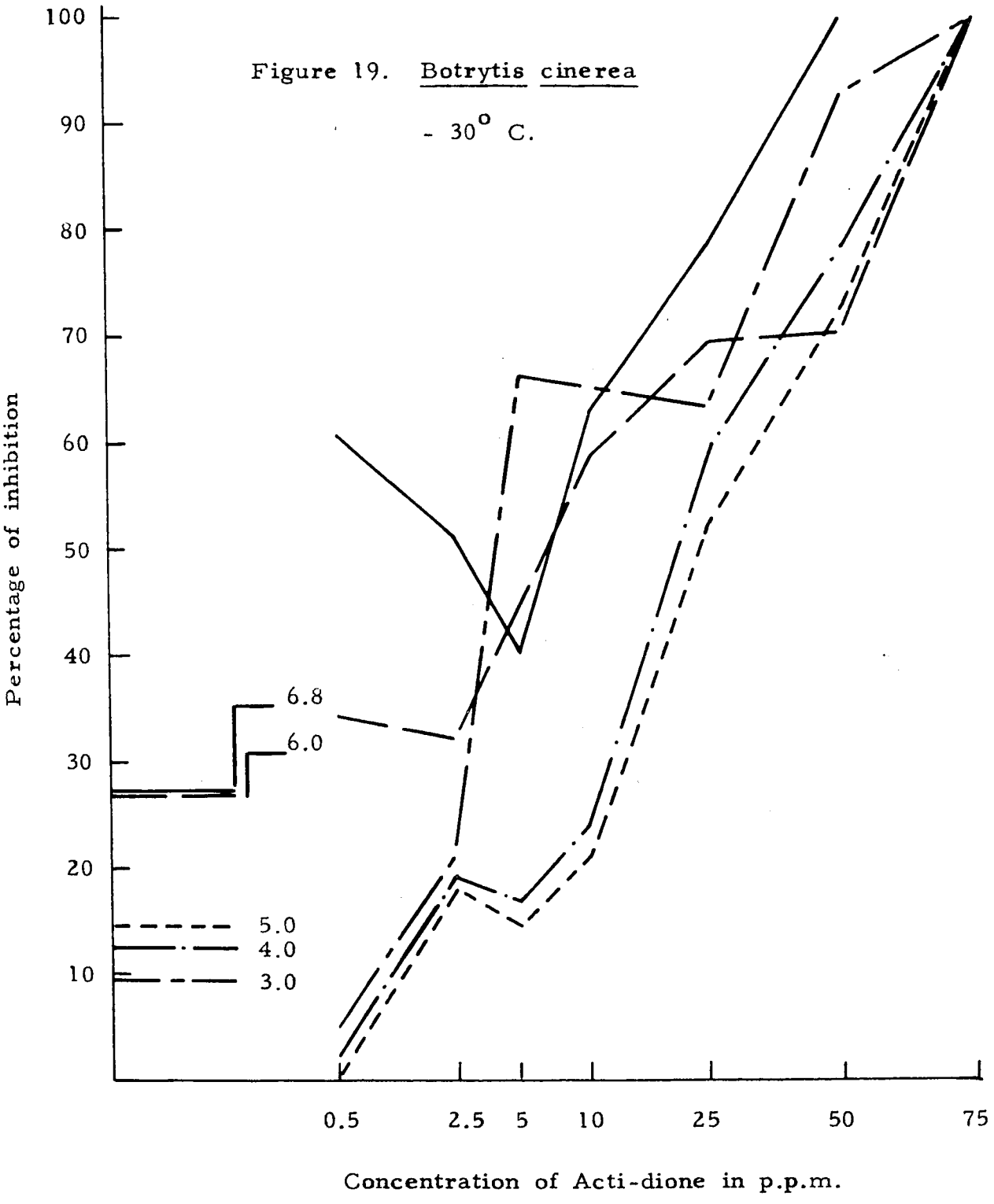


Figure 19. Botrytis cinerea - 30° C.



100.0 p.p.m. total inhibition was the result, for all three. Total inhibition of germination was at 75.0 p.p.m. at pH 4.0 and 3.0.

Temperature 24° C. The best results in inhibiting the germination of Botrytis spores were at pH 6.8 at almost every concentration of Acti-dione up to 50.0 p.p.m. (Figure 16). The next best pH value, according to the results, was pH 5.0. Three of the lines representing hydrogen ion concentrations meet at 75.0 p.p.m. and the other two meet at 100.0 p.p.m. indicating the maximum amount of inhibition.

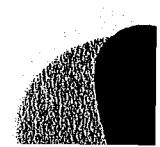
Definite stimulation occurred in 0,5 p.p.m. at every pH except 3.0. At pH 6.8 there was evident a very marked stimulation at 0.5 p.p.m. of Acti-dione. There were the slight reductions of inhibitory effect in 2.5 p.p.m. at pH 6.8, and in 5.0 p.p.m. at pH 4.0 and 5.0. Also to be noted is the complete absence of stimulation at pH 6.0.

Temperature 26° C. The greatest difference at this temperature is the wide variation in concentrations of Acti-dione required in effecting 30% or more inhibition, e.g., at pH 6.0 only 0.5 p.p.m. of Acti-dione were required, to inhibit 30%

of the spores as compared to approximately 62.0 p.p.m. required at pH 3.0.

A slight stimulation occurred at pH 6.8 in 0.5 p.p.m. and a marked stimulation at pH 5.0 in the same concentration of Acti-dione. In fact the line representing pH 5.0 (Figure 17), indicated 0% inhibition or total germination. Also a significant amount of reduction of inhibition in 25.0 p.p.m. of Acti-dione at the pH concentrations of 4.0 and 3.0. All but one of the pH graph lines terminate at 100 p.p.m.; pH 6.0 is the only line terminating at 75.0 p.p.m. of Acti-dione concentration.

Temperature 28° C. The principal difference between this and the previous temperature is that all pH graph lines terminate at 75.0 p.p.m. (Figure 18). Also a higher percentage of inhibition was found at pH 6.8 than previously in 0.5 p.p.m. The stimulation of germination in 0.5 p.p.m. of Acti-dione were at the hydrogen ion concentration of 3.0, 4.0, 5.0, and 6.0. In addition there was a marked reduction of inhibition occurring at 5.0 p.p.m. at every pH but one and there was stimulation at 25.0 p.p.m. at pH 4.0 and pH 3.0. Acti-dione was most effective here at pH 6.8 as in the previous results.



Temperature 30° C. As noted in the three previous experiments, the results of the controls parallel each other in arrangement on the graphs, i.e., the largest amount of germination was observed at pH 3.0 and the least at pH 6.8. The results of all other pH values followed in sequence.

Other than the similarity in the control results, the general pattern of behavior was similar at this pH concentration to the previous one. Another exception to be noted was the absence of the marked stimulations occurring in 25.0 p.p.m. of Acti-dione.

Monilinia Fructicola

The general tendency, in the case of this organism, was an increase of inhibition as the pH concentration of the Acti-dione was increased. Furthermore, with only one exception, pH 5.0, the most effective temperature was 30° C.

pH 3.0. At pH 3.0, Acti-dione appeared to be most effective at a concentration of 10.0 p.p.m. at 30° C. (Figure 20). (Figure 20). It was equally effective in its properties of inhibition at the temperatures used, but at a much higher concentration. Of considerable interest was the observation that

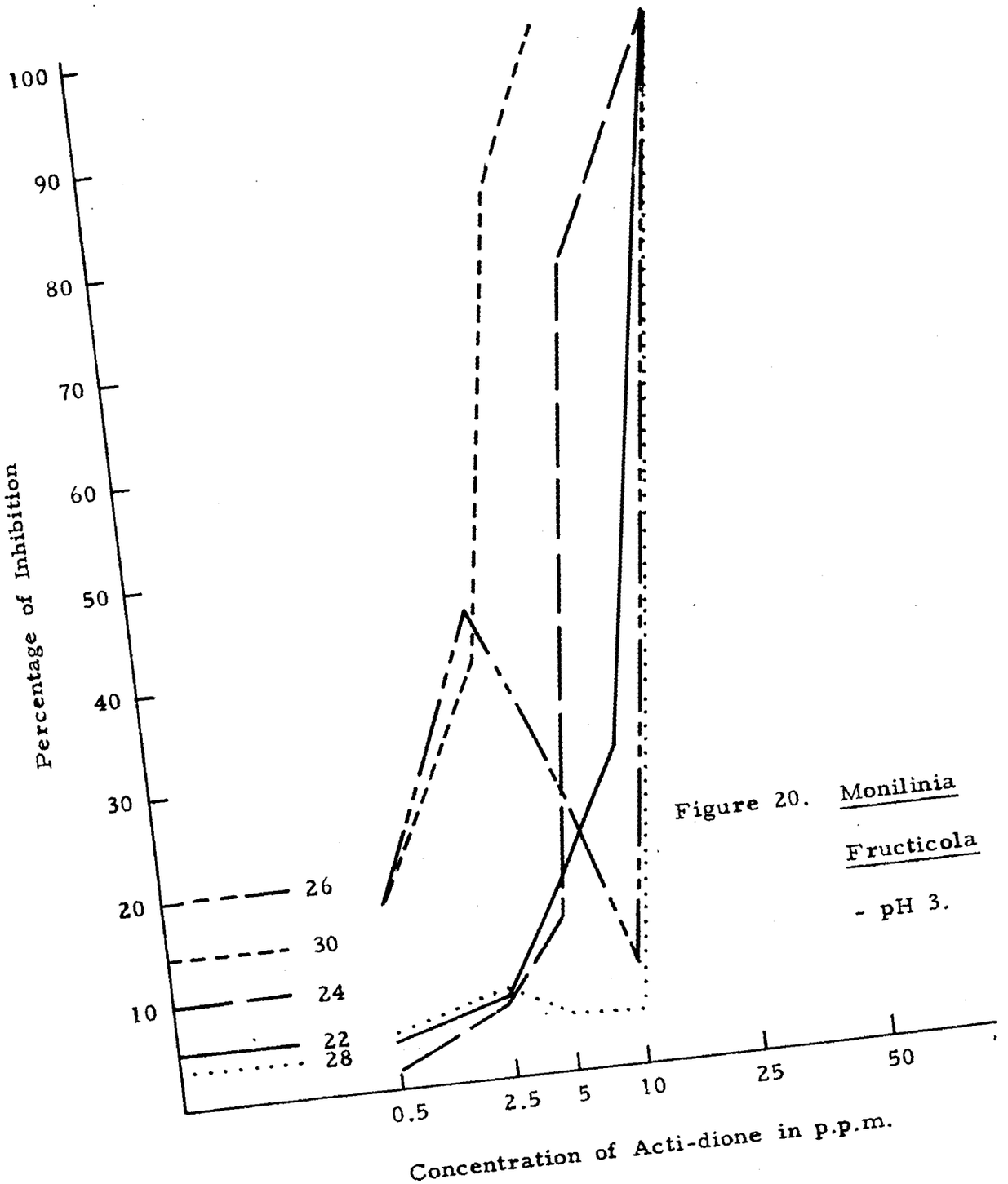


Figure 20. Monilinia Fructicola - pH 3.

the internal structure of all the germinated spores appeared to be disintegrated and unorganized. The cytoplasm within the spores and their germ tubes changed from a rather clear hyaline structure to a dirty, pale-yellow color and became extremely granular. The same morphological picture plus considerable shriveling was present in the ungerminated spores at the higher concentrations of Acti-dione where no germination was present at all. The walls of these spores appeared to have collapsed, resulting in a very rough outline of the spores. In addition to the above-mentioned cytoplasmic changes, considerable distortion was evident in the germ tubes. They were, in many cases, stunted, and somewhat blunt and thick. In others, the individual germ tube was curled in a helical manner. This disintegration was present only in spores incubated at 30° C. and in concentrations of Acti-dione of 2.5 p.p.m. and higher. Of additional interest was the stimulation occurring at 0.5 p.p.m. at every temperature except 28° C. At this temperature only a mild reduction of inhibition occurred in 5.0 and 10.0 p.p.m. at 26° C. a very marked stimulation was noted at 10.0 p.p.m.

At the time of the first count, after 12 hours, no germination had occurred in 5.0 p.p.m. appearing as though this concentration was too toxic to allow any germination to take place. The spores were left undisturbed, however, in their solution and at the proper temperature for as long as 72 hours. When the second count was made after 24 hours, definite germination had resulted (Figure 20). The percentage of germination continued to increase in most cases up to 48 hours, but no further increase was noted after 48 hours.

pH 4.0. The general pattern of behavior here (Figure 21), was similar to that at pH 3.0 except for the complete absence of the cytoplasmic breakdown. The distortion of the germ tubes which was described previously was present, however, to the same extent in the same concentrations at 30° C. No distortions were noted at any concentration of the lower temperatures. The germinated spores at concentrations higher than 2.5 p.p.m., however, had germ tubes that were very short as compared with the germinated spores of the controls. In the lower concentrations and the controls, the ungerminated spores especially in concentrations above 5.0 p.p.m. appeared considerably shrunken or shriveled. No germination had occurred in

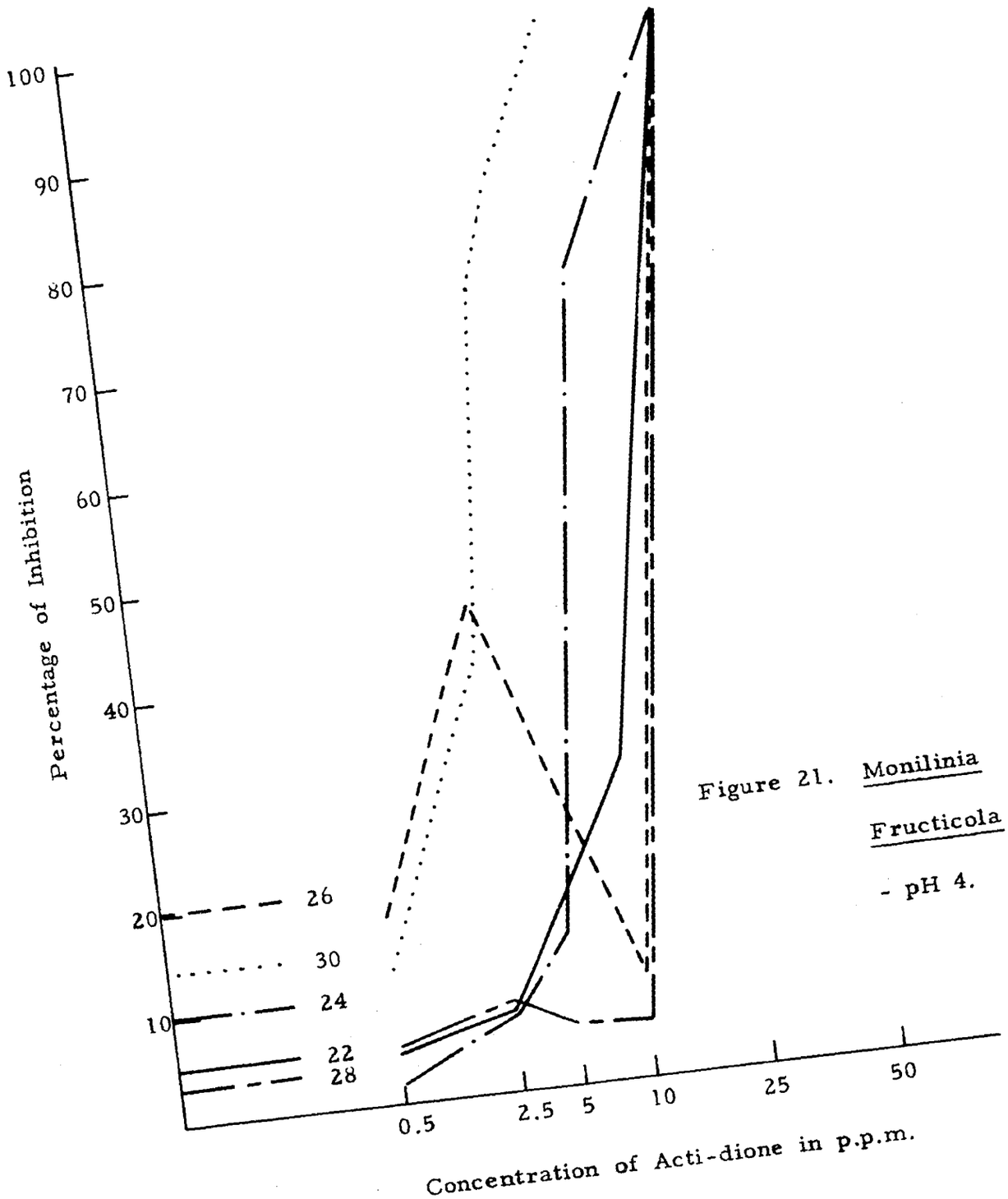


Figure 21. Monilinia Fructicola - pH 4.

5.0 p.p.m. after 12 hours but on further incubation some germination did occur, varying in extent according to the temperature.

pH 5.0. In this pH concentration there was a definite departure from the general pattern exhibited above in that Actidione was more inhibitory at the lower concentrations and the stimulation of germination which normally occurred at 0.5 p.p.m. in the previous two cases, was now found at 2.5 p.p.m. (Figure 22). Inhibition of germination was considerably higher in 0.5 p.p.m. than in the previous two cases. There was no stimulation in 0.5 p.p.m. over the controls. Furthermore, it appears that the most inhibition was at 28° C. in 10.0 p.p.m. instead of at 30° C. as previously. The mycelium present in Acti-dione concentrations above 2.5 p.p.m. were very short but not as short as at pH 4.0. The spores which failed to germinate in Acti-dione concentrations higher than 5.0 p.p.m. appeared slightly shriveled but not to the extent they were at pH 3.0 or pH 4.0.

When the first count was made, after 12 hours, no germination had appeared in 5.0 p.p.m. so it was believed that this concentration was too toxic for any germination. Twelve hours

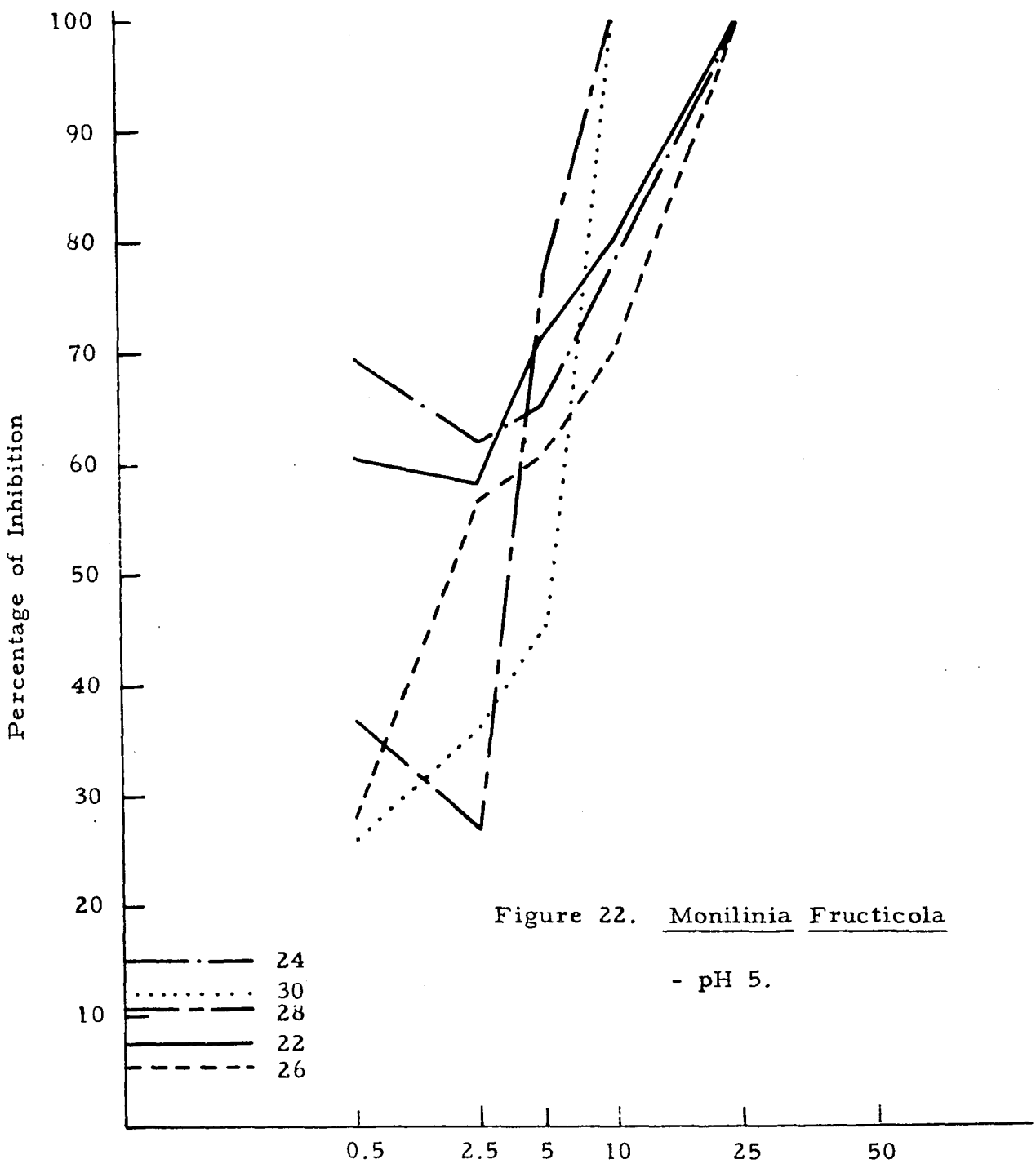
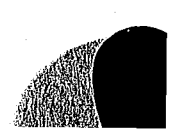


Figure 22. Monilinia Fructicola - pH 5.

later, however, definite germination after 12 and 24 hours continued until 48 hours. No further increase could be detected by counting after 48 hours.

pH 6.0. At this pH concentration, the general pattern described earlier still holds true. Considerably higher inhibition is attained at the lower concentrations of Acti-dione which at the lower pH values were not very effective. Inhibition of germination at 30° C. in 0.5 p.p.m. starts at 62.18% as compared to much lower percentages at the lower pH values. The same degree of inhibition holds true at the other temperatures. Note the slight reduction of inhibition which occurred at 2.5 p.p.m. at 22° C. and 24° C. Also to be mentioned is the fact that what has been said previously about the germination pattern at 5.0 p.p.m. holds true in this case. Distortion of germ tubes was absent but the stunting remained to lesser degree. In other words the germinated spores, in concentrations higher than 2.5 p.p.m., had short germ tubes but longer than those of the same Acti-dione concentration at lower pH values. Germination of spores continued to increase up to 48 hours in all concentrations and at every temperature. The twelve-hour count was zero at 5.0 p.p.m. but germination did occur later and continued



to increase in the pattern described previously. At 26° C. and 28° C. the results of the control were identical.

pH 6.8. Contrary to the prevailing concept, that Acti-dione is most effective at the lower hydrogen ion concentrations, we note, here at a pH value very close to neutrality that it is most effective in all temperatures and concentrations. Except for this, the pattern of behavior was very similar to that at pH 6.0.

Again there is absence of stimulation at 0.5 p.p.m. exhibited earlier and the definite reduction of inhibition at 2.5 p.p.m. at three different temperatures.

Figures 25, 26, 27, 28 and 29 present the same data discussed previously, but in a manner to show the relationship or difference in effect of the pH concentrations used in this investigation.

Temperature 22° C. Results shown in Figure 25 will readily substantiate the fact stated earlier that Acti-dione was most effective against the spores of M. fructicola at pH 6.8 followed by pH 6.0, 5.0, 4.0 and 3.0. To be noted is the slight stimulation at 2.5 p.p.m. at all pH levels except pH 3.0. However,

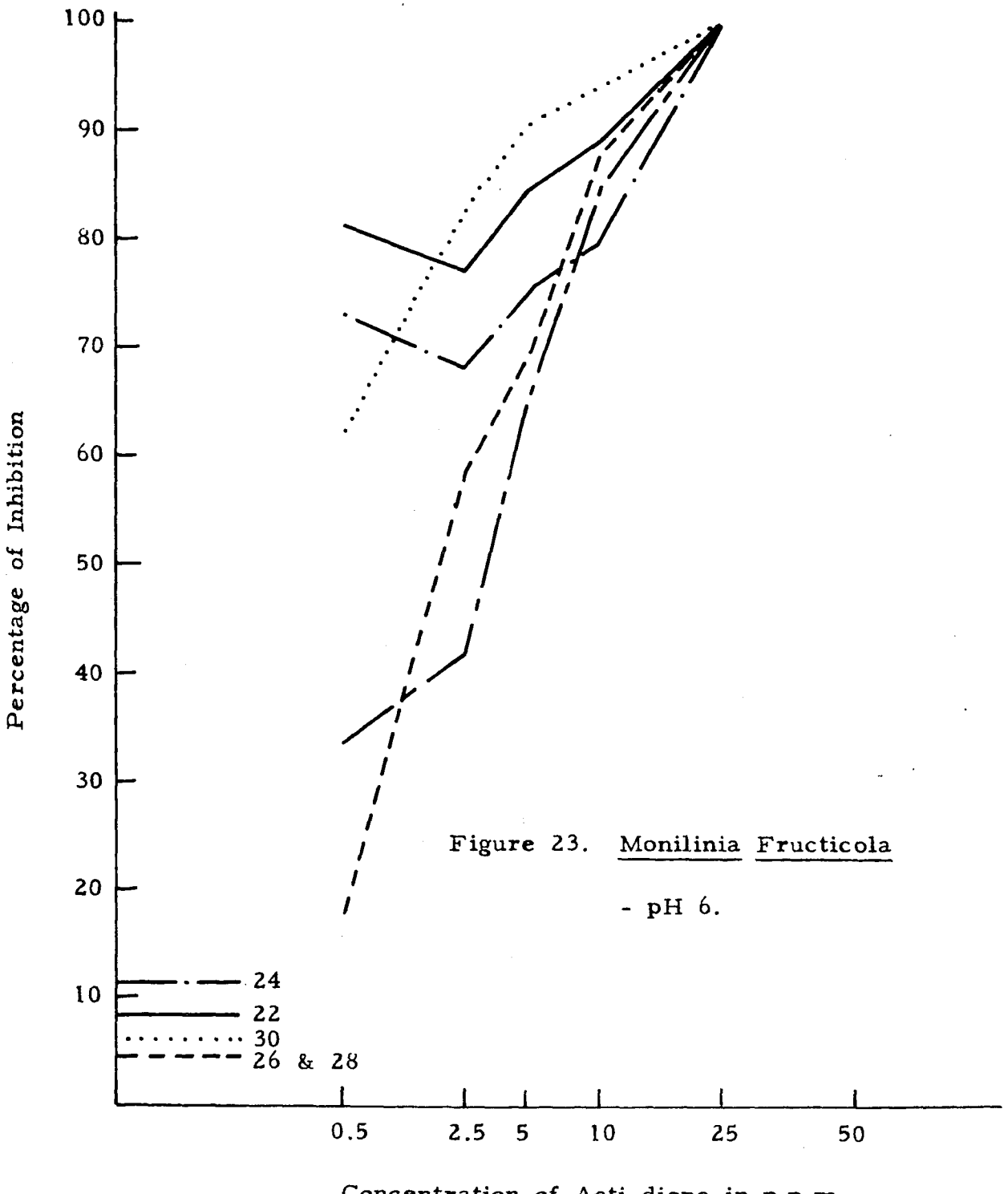


Figure 23. Monilinia Fructicola - pH 6.

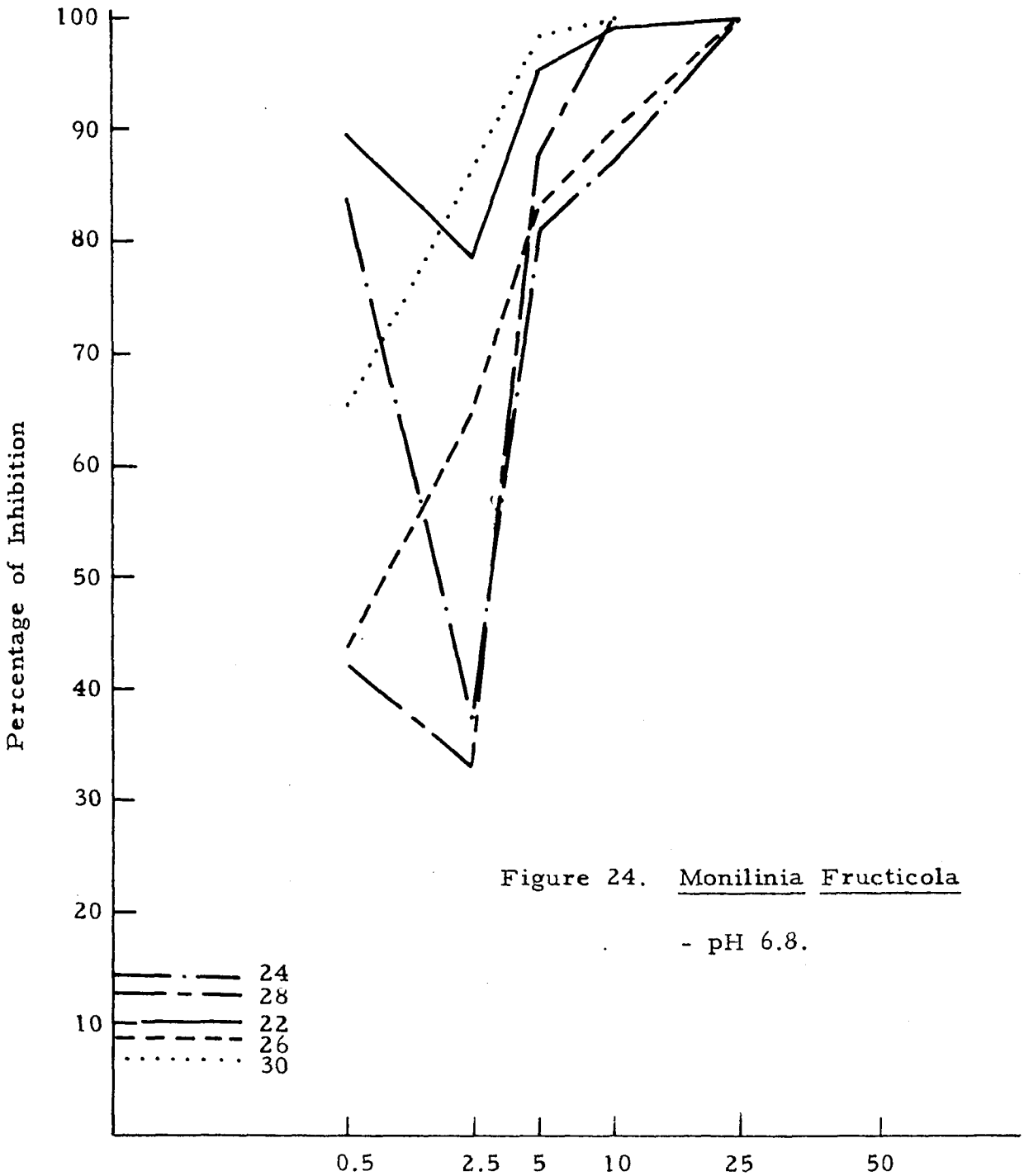


Figure 24. Monilinia Fructicola

- pH 6.8.

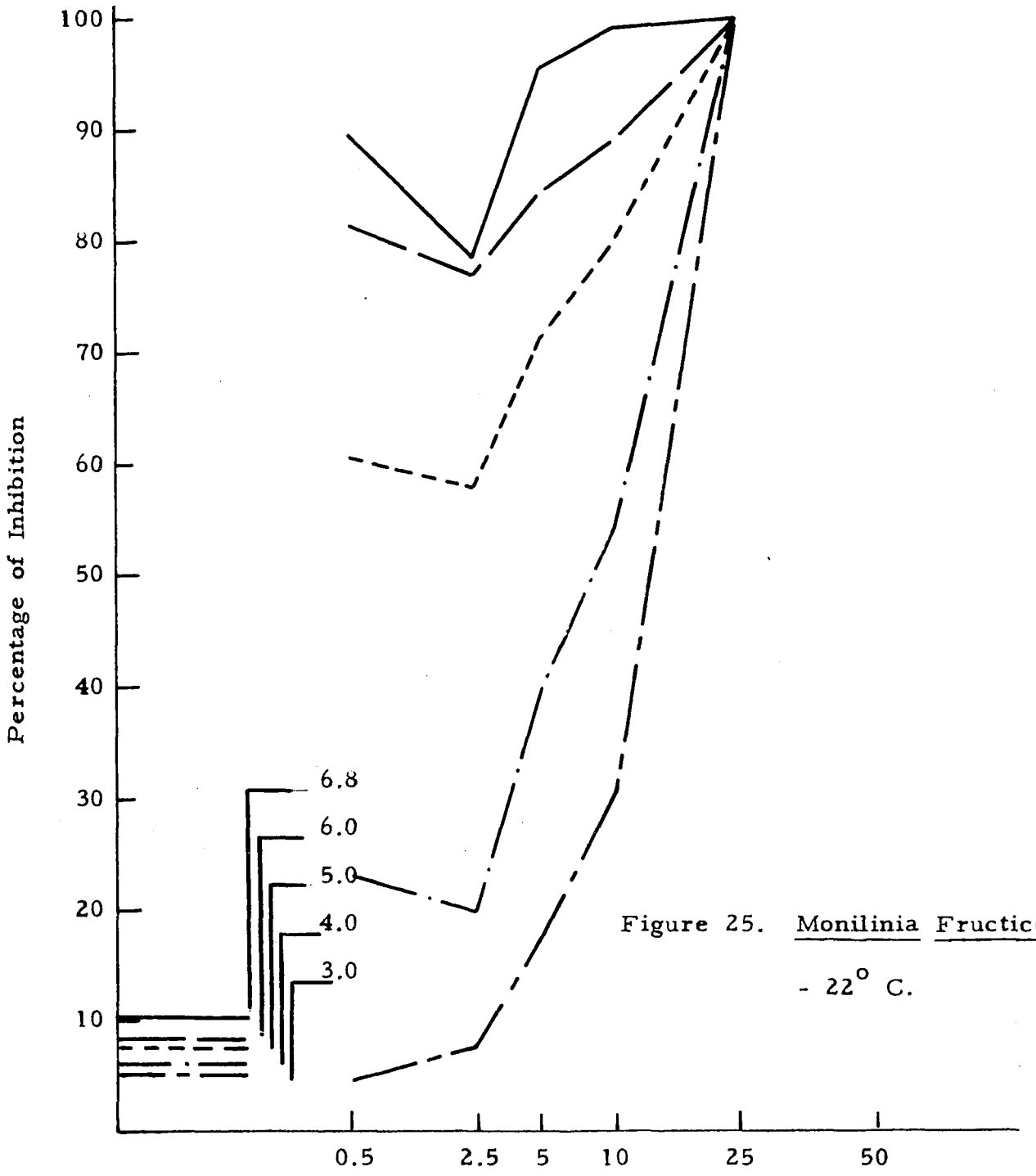


Figure 25. Monilinia Fructicola - 22° C.

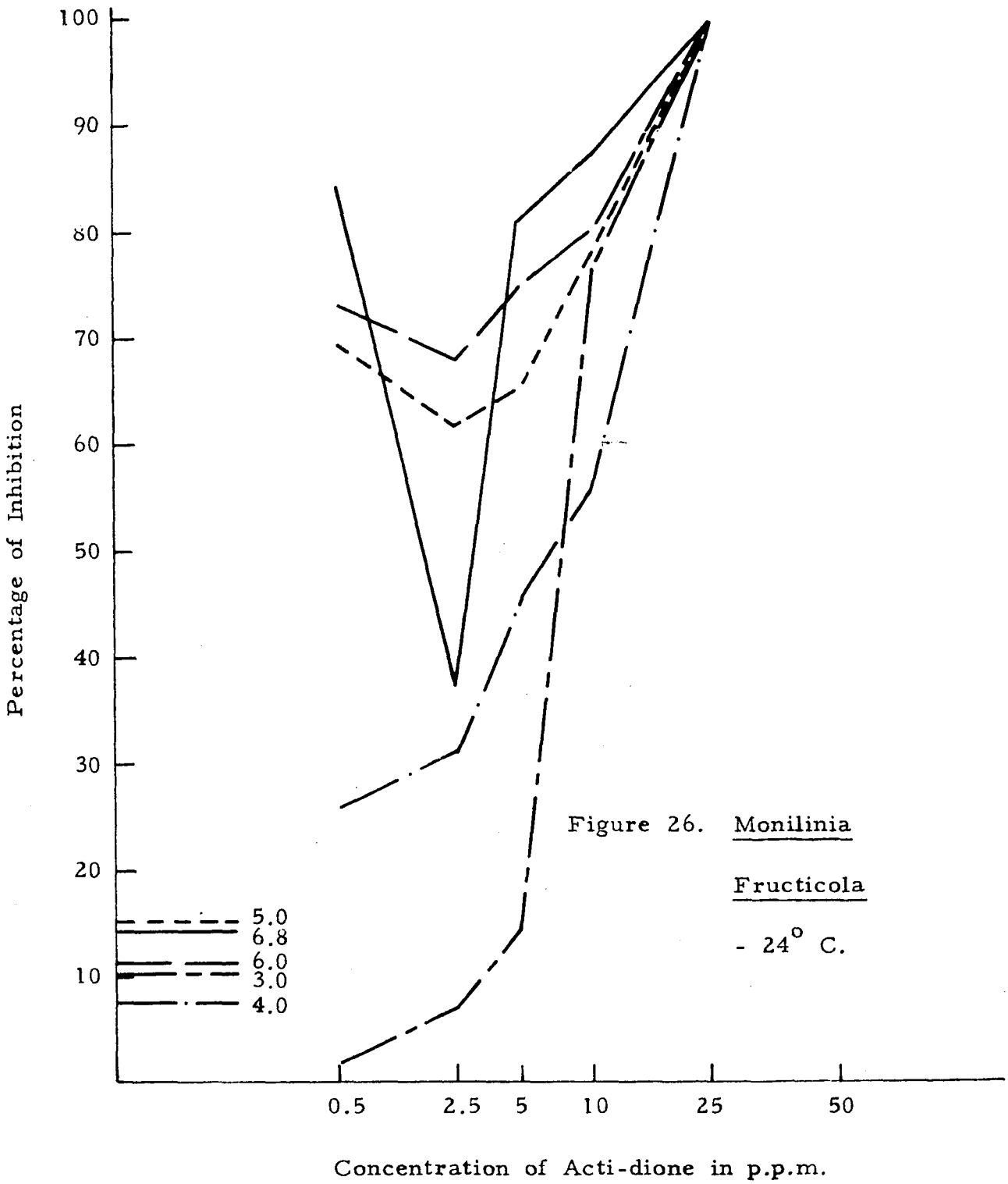


Figure 26. Monilinia Fructicola - 24° C.

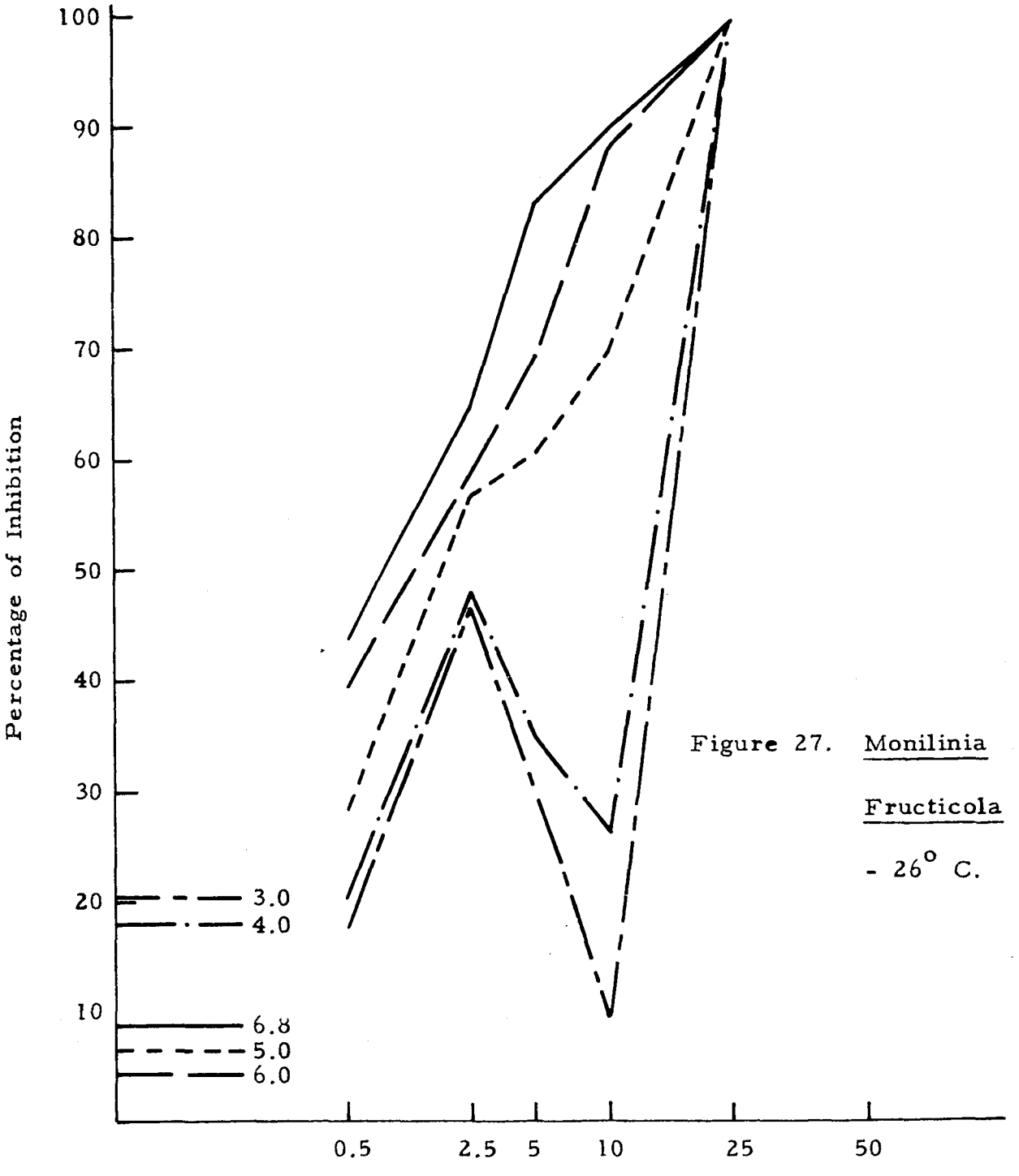


Figure 27. Monilinia Fructicola - 26° C.

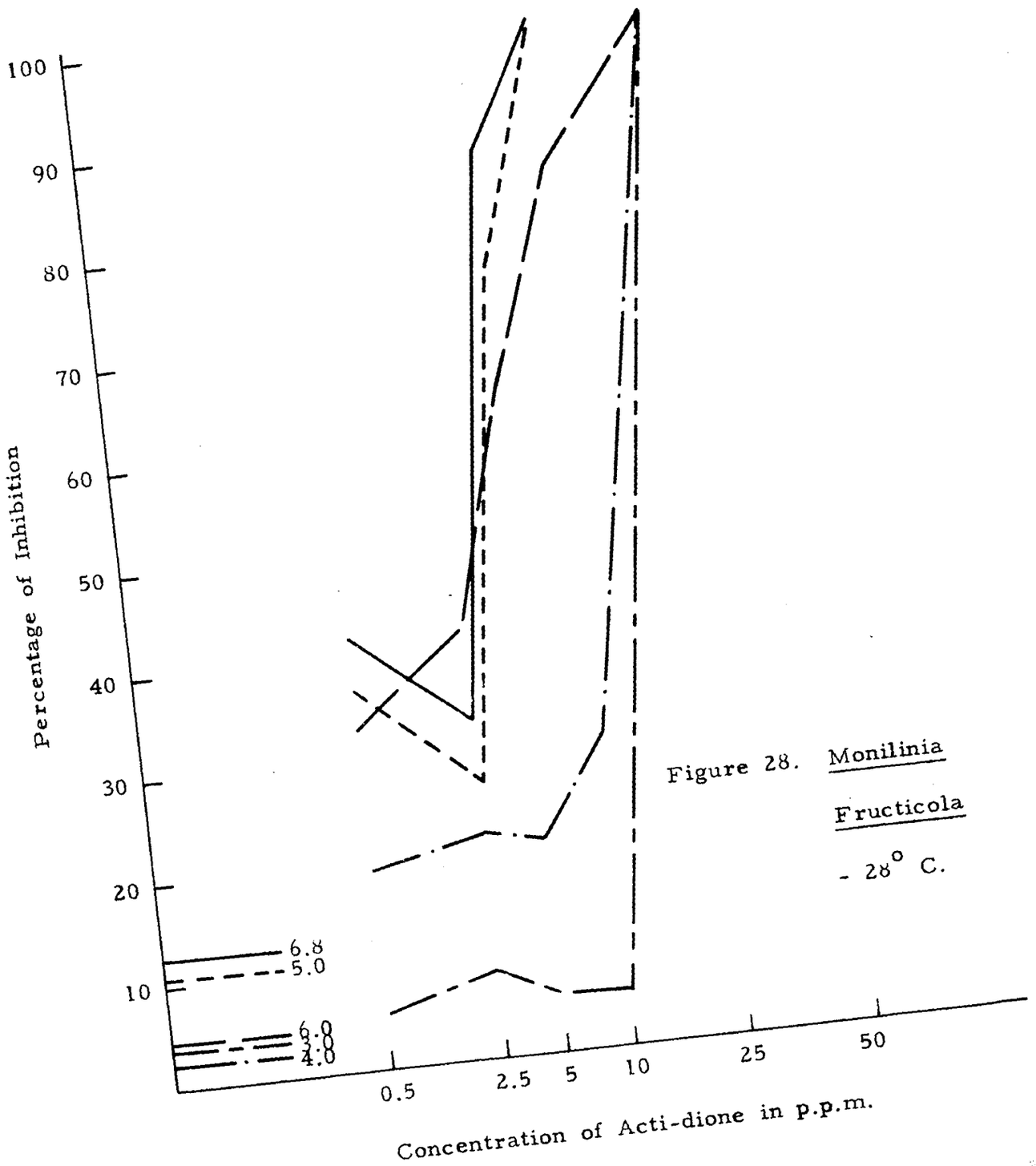


Figure 28. Monilinia Fructicola - 28° C.

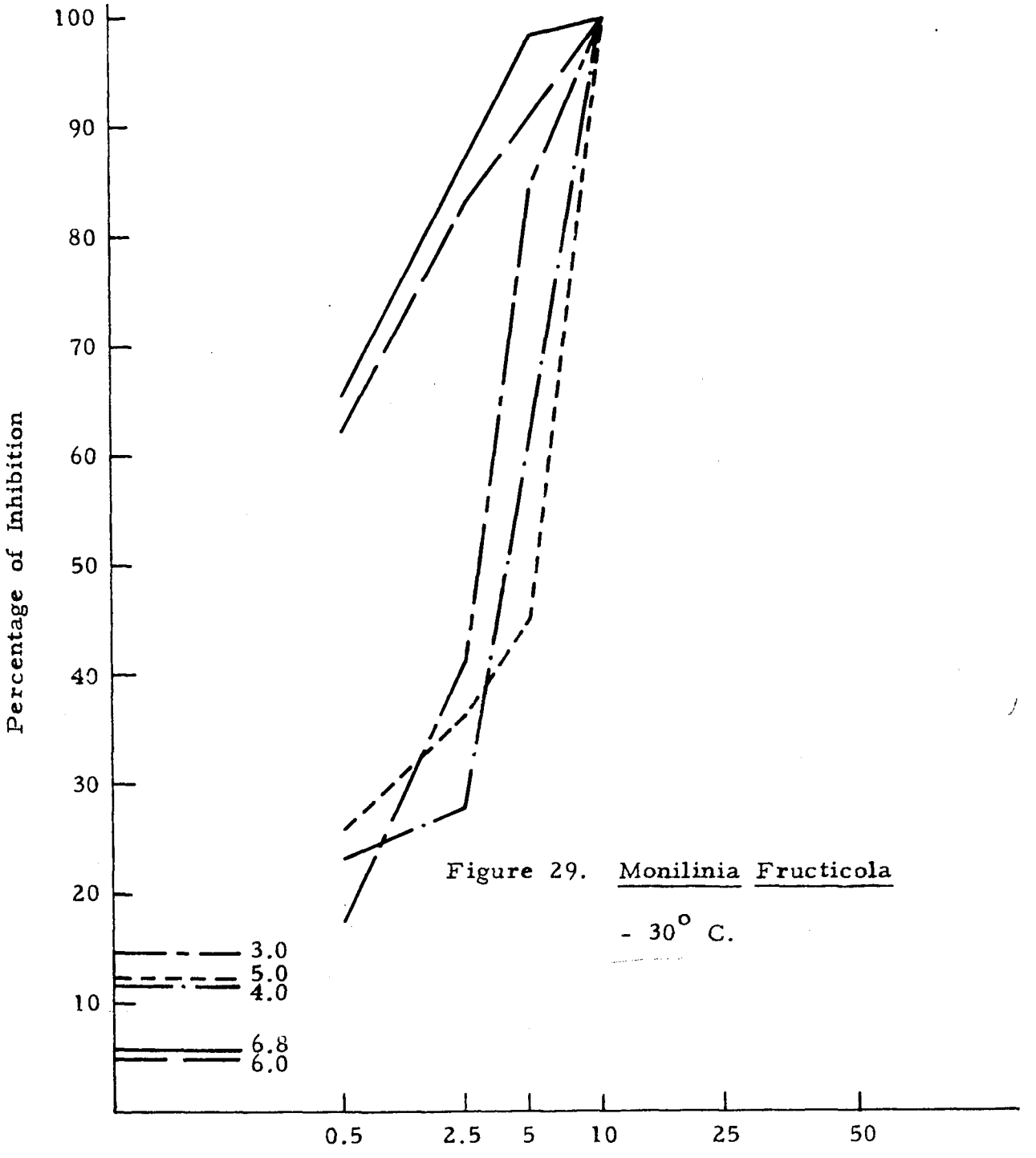


Figure 29. Monilinia Fructicola - 30° C.

that at this temperature, all pH values reached their maximum effect at 25.0 p.p.m. Also to be noted is the allignment of the control results as compared to the treatments.

Temperature 24° C. Again, Acti-dione is most effective at pH 6.8 (Figure 26). Except for the very marked stimulation of germination at 2.5 p.p.m. at pH 6.8 and the rearrangement of the control results the pattern of behavior was similar to the previous (Figure 25). All pH values converge at 25.0 p.p.m. indicating maximum inhibition. Also the line representing pH 6.8 crosses pH values 6.0 and 5.0 at two different points of concentration. The line representing pH 4.0 crosses pH 3.0 and indicates less effect or similar effect as pH 3.0 but at higher concentrations of Acti-dione.

Temperature 26° C. The pH value of 6.8 indicates maximum effect followed in sequence by pH 6.0, 5.0, 4.0, and 3.0 (Figure 27). As in the previous tests, the control results do not follow the pattern of the treatments. There was an absence of stimulation of germination at pH 6.8 and the presence of it at pH 4.0 and pH 3.0 at 10.0 p.p.m. There was also a very slight stimulation noted at 0.5 p.p.m. at pH 3.0., approximately

2.00%. All lines representing pH values converge at 25.0 p.p.m.

Temperature 28° C. The most striking difference noted here is that the graph lines do not all meet at 25.0 p.p.m. as in the three previous experiments. Furthermore it appears that at this temperature pH 5.0 reached its maximum at a lower concentration of Acti-dione than pH 6.0 as was the case previously. At lower concentrations (2.5 p.p.m.), however, pH 4.0 was superior in inhibition to both pH 6.8 and 5.0. At this temperature, some reduction of inhibition occurred at all pH values except pH 6.0. Also note that the stimulation occurred at a lower concentration of Acti-dione than at 26° C.

Temperature 30° C. The principle difference between this temperature and 28° C. was that all pH values reached their maximum effect at 10.0 p.p.m. (Figure 29). Another difference of interest was the fact that pH 3.0 was superior in inhibition to pH 5.0 and 4.0. Both of the latter, however, were superior to pH 3.0 at 0.5 p.p.m. Also of considerable interest was the complete exchange of positions by pH 6.8 and 3.0 in the control results.

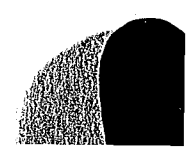


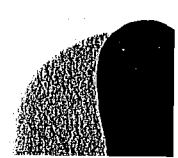
TABLE III

INHIBITION OF SPORE GERMINATION OF MONILINIA FRUCTICOLA BY DIFFERENT CONCENTRATIONS OF ACTI-DIONE AT FIVE DIFFERENT TEMPERATURES AND FIVE HYDROGEN ION CONCENTRATIONS

Temperatures (° C.)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
22	Control	5.26	6.12	7.85	8.33	10.28
	0.5	4.63	23.01	60.48	81.22	89.72
	2.5	7.84	19.89	58.23	77.00	78.57
	5.0	17.59	39.72	71.32	84.49	95.50
	10.0	30.63	54.49	80.00	89.12	99.11
	25.0	100.00	100.00	100.00	100.00	100.00
24	Control	10.10	7.51	15.15	11.05	14.29
	0.5	1.89	26.00	69.83	73.10	84.40
	2.5	7.21	31.40	62.00	68.14	37.27
	5.0	14.85	45.88	65.52	75.02	81.08
	10.0	76.99	56.42	78.22	80.12	87.39
	25.0	100.00	100.00	100.00	100.00	100.00
26	Control	20.19	18.00	6.50	4.51	8.77
	0.5	17.76	20.01	28.25	17.69	43.75
	2.5	46.30	48.19	56.98	58.62	64.55
	5.0	30.10	35.00	60.88	69.31	83.33
	10.0	9.48	26.33	70.00	88.12	90.00
	25.0	100.00	100.00	100.00	100.00	100.00

TABLE III (Continued)

Temperatures (° C.)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
28	Control	3.77	2.03	10.91	4.51	12.61
	0.5	5.41	19.50	37.11	33.40	42.20
	2.5	8.49	22.14	27.10	41.99	33.03
	5.0	5.94	21.00	76.21	65.00	87.96
	10.0	5.66	30.71	100.00	85.00	100.00
	25.0	100.00	100.00	100.00	100.00	100.00
30	Control	14.81	11.56	12.10	6.00	6.96
	0.5	17.92	23.26	26.00	62.18	65.49
	2.5	40.57	28.00	36.50	83.11	86.73
	5.0	84.40	60.10	45.23	91.00	98.18
	10.0	100.00	100.00	100.00	100.00	100.00
	25.0	100.00	100.00	100.00	100.00	100.00

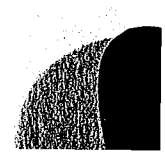


Ramularia sp.

The spores of this organism were prepared for each experiment, as previously described in the section entitled Materials and Methods. The counting, however, was made at shorter intervals than those used for the other organisms due to the fact that the spores germinated after 6 hours. Consequently counts were made after 6, 12, 24 and 36 hours. At the end of this period counting was discontinued.

It should be pointed out that no distortion or destruction of germ tubes or their cytoplasmic content occurred in the experiments involving this organism. In concentrations of 5.0 p.p.m. and higher, it was consistently observed that the germ tubes of the germinated spores were considerably shorter than those of the controls. Furthermore, they appeared shorter in each succeeding higher concentration.

pH 3.0. Three peaks of maximum inhibition are to be noted at this hydrogen ion concentration, the first being at 5.0 p.p.m. at a temperature of 22° C., the second at 10.0 p.p.m. at a temperature of 26° C., and the third at 50.0 p.p.m. by all of the temperatures used in this investigation (Figure 30). A



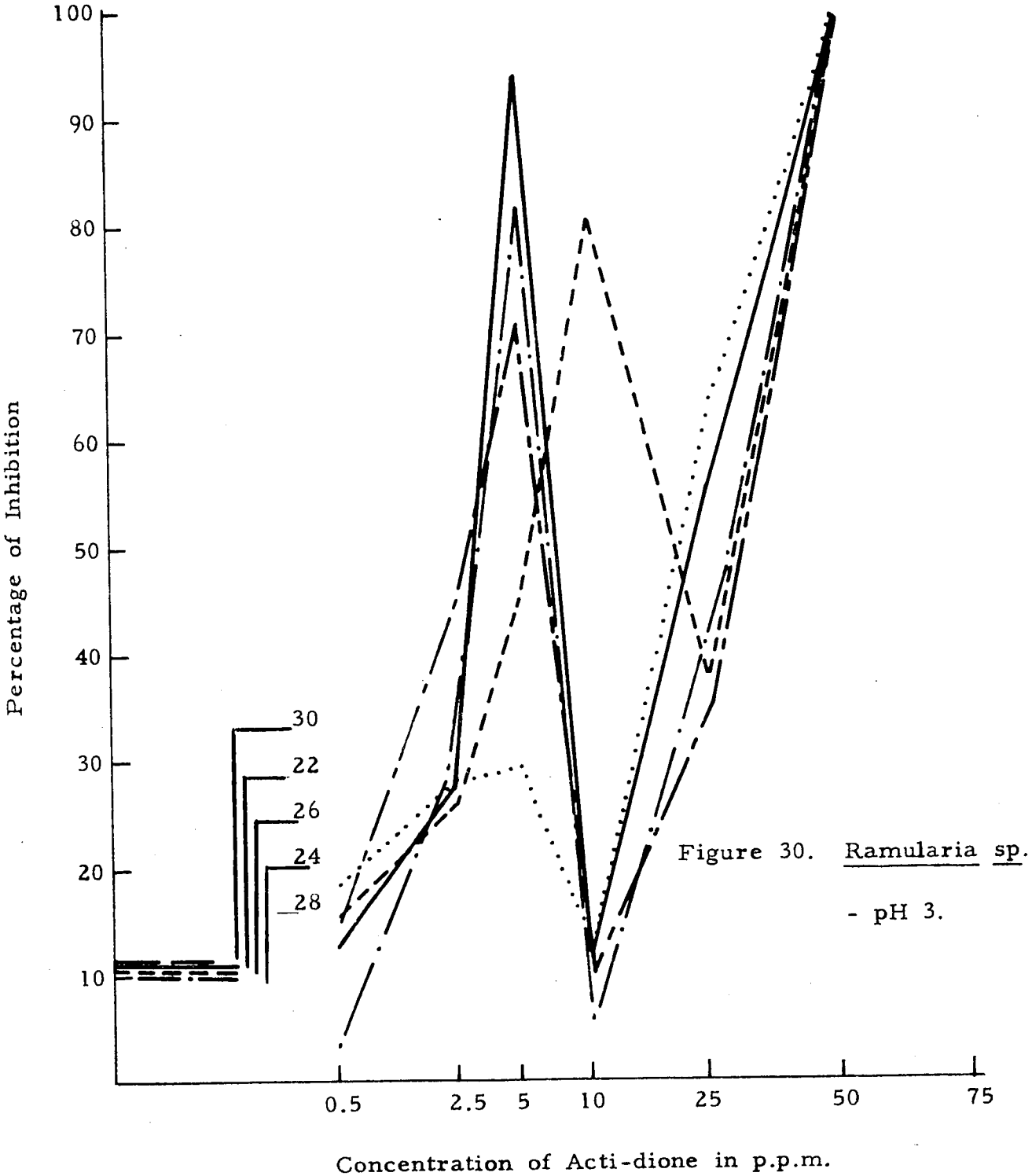
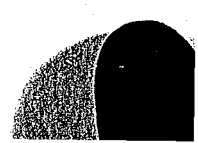


Figure 30. Ramularia sp. - pH 3.

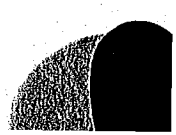
glance at Figure 30 will quickly reveal the anomalous behavior of this organism or the erratic effect of the Acti-dione upon the spores of this organism. In 0.5 p.p.m. the spores were most susceptible to the toxin at 30° C. but quickly the picture changed and 28° C. became the temperature which was most effective. This change, however, did not persist since at approximately 3.0 p.p.m., 22° C. took the lead and reached its maximum in 5.0 p.p.m. This marked reversal of inhibition may be interpreted as stimulation. On the other hand it may be a matter of the toxin having less effect at that particular concentration under the given conditions. As one proceeds up the scale of Acti-dione concentration, it becomes evident that the toxic effect is greatest at 30° C.

Here also are two instances of definite stimulation which occurred in 0.5 p.p.m. and 10.0 p.p.m. at 24° C. This was the only case of stimulation in this experiment. What has been described above regarding the various temperatures which were most effective at various concentrations of Acti-dione is also true in the case of other temperatures; they cross each other at several places. It is interesting to note that in the controls, the results at 24° C. and 28° C. are identical.



pH 4.0. As in the preceding experiment, there are here three peaks of maximum inhibition observed. The first was in 5.0 p.p.m. at 24° C., the second in 50.0 p.p.m. at 24° C. and 28° C.; for the latter temperature this represents total germination. The third peak represents total inhibition by all temperatures, except 28° C., in 75 p.p.m. The erratic behavior of the organism or as previously stated the action of the toxin upon the spores, is shown by Figure 31.

pH 5.0. The principal difference here as compared to the previous experiments is the fact that the maximum amount of inhibition was in 75.0 p.p.m. at all temperatures (Figure 32). The only peak of inhibition other than the above is in 5.0 p.p.m. at 22° C. which is followed by a sharp reduction of inhibitory action at 10.0 p.p.m. At this pH, Acti-dione was most effective at 24° C. and 30° C. The former temperature was most effective in 0.5 p.p.m. and 2.5 p.p.m. while the latter was most effective at all higher concentrations. Noteworthy is the stimulatory effect of the Acti-dione in 0.5 p.p.m. at 26° C., 28° C. and 30° C. No other stimulation is noted at any of the higher concentrations of Acti-dione.



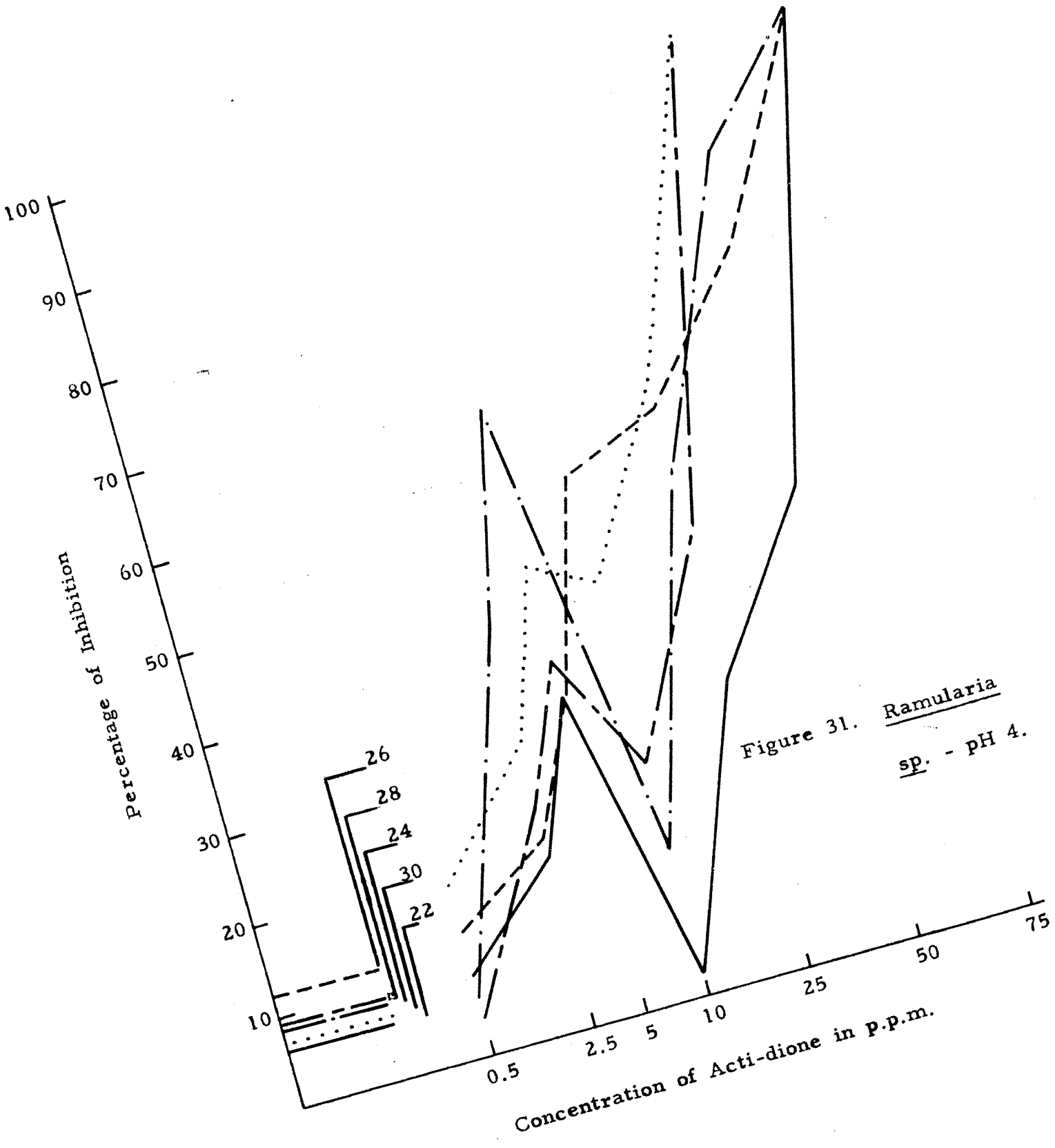


Figure 31. Ramularia sp. - pH 4.

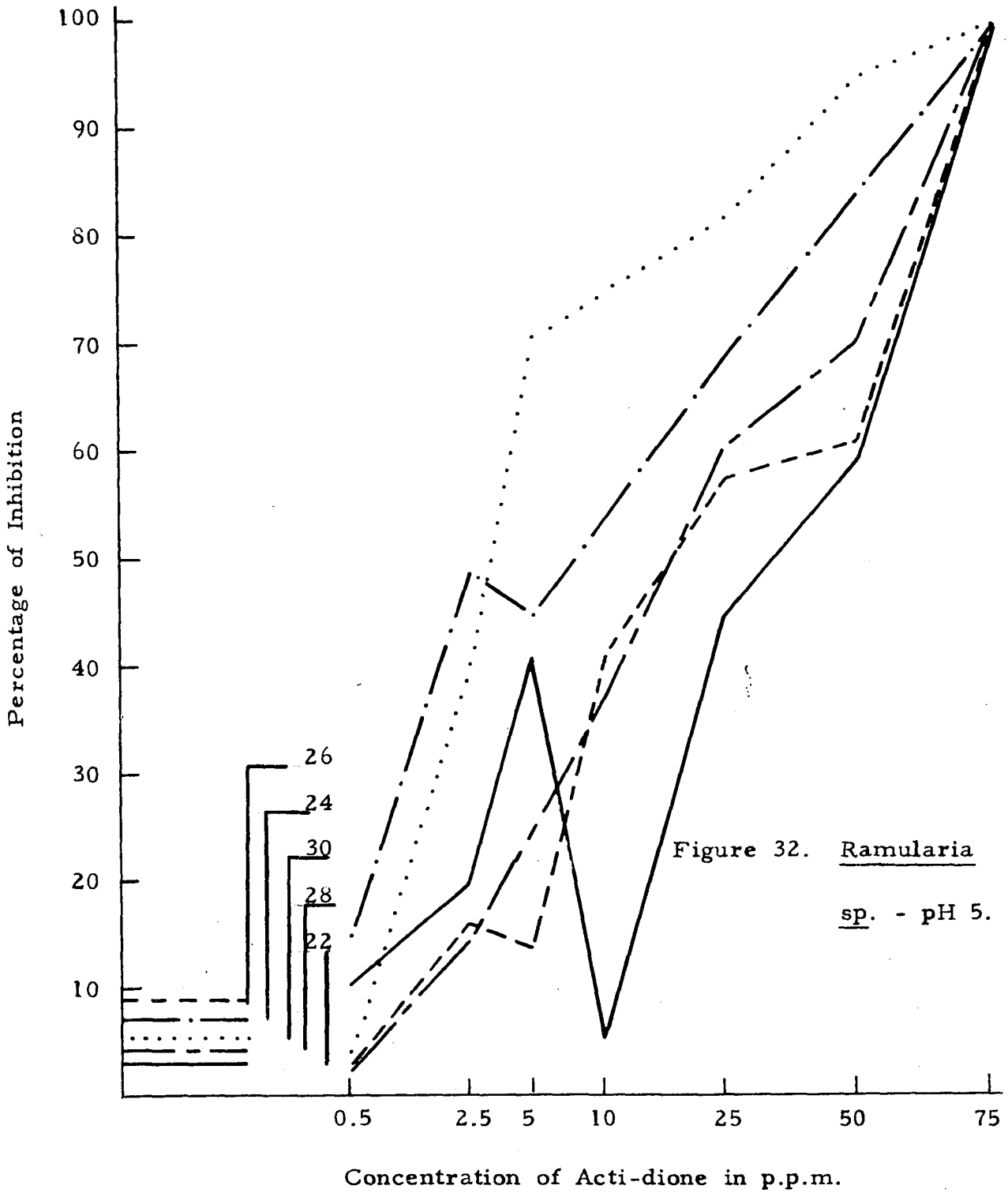


Figure 32. Ramularia sp. - pH 5.

pH 6.0. The most striking difference noted here is the very conspicuous solitary peak of inhibition at 22° C. in 2.5 p.p.m. and the fact that at 30° C. Acti-dione is the least effective (Figure 33). In connection with this observation it is interesting that in the control results the least amount of germination occurred at 30° C. and the most at 22° C. Noteworthy is the absence of the very erratic pattern observed in the previous experiments with this organism. The only stimulation of spore germination observed here was in 0.5 p.p.m. at 26° C. and 30° C.

pH 6.8. The most noteworthy observation in this series of experiments was the very high inhibitory action of Acti-dione at the low concentration of 2.5 p.p.m. and the immediate reduction of inhibition at the next higher concentration used--2.5 p.p.m. As in all previous experiments, the only stimulatory action occurred at 26° C. and 30° C. in 0.5 p.p.m. To be noted is the extreme influence of temperature upon the effect of Acti-dione in its inhibitory action as demonstrated by the graph line representing 22° C. and any of the other temperatures at any given concentration of Acti-dione.

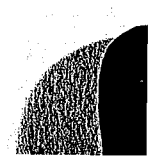
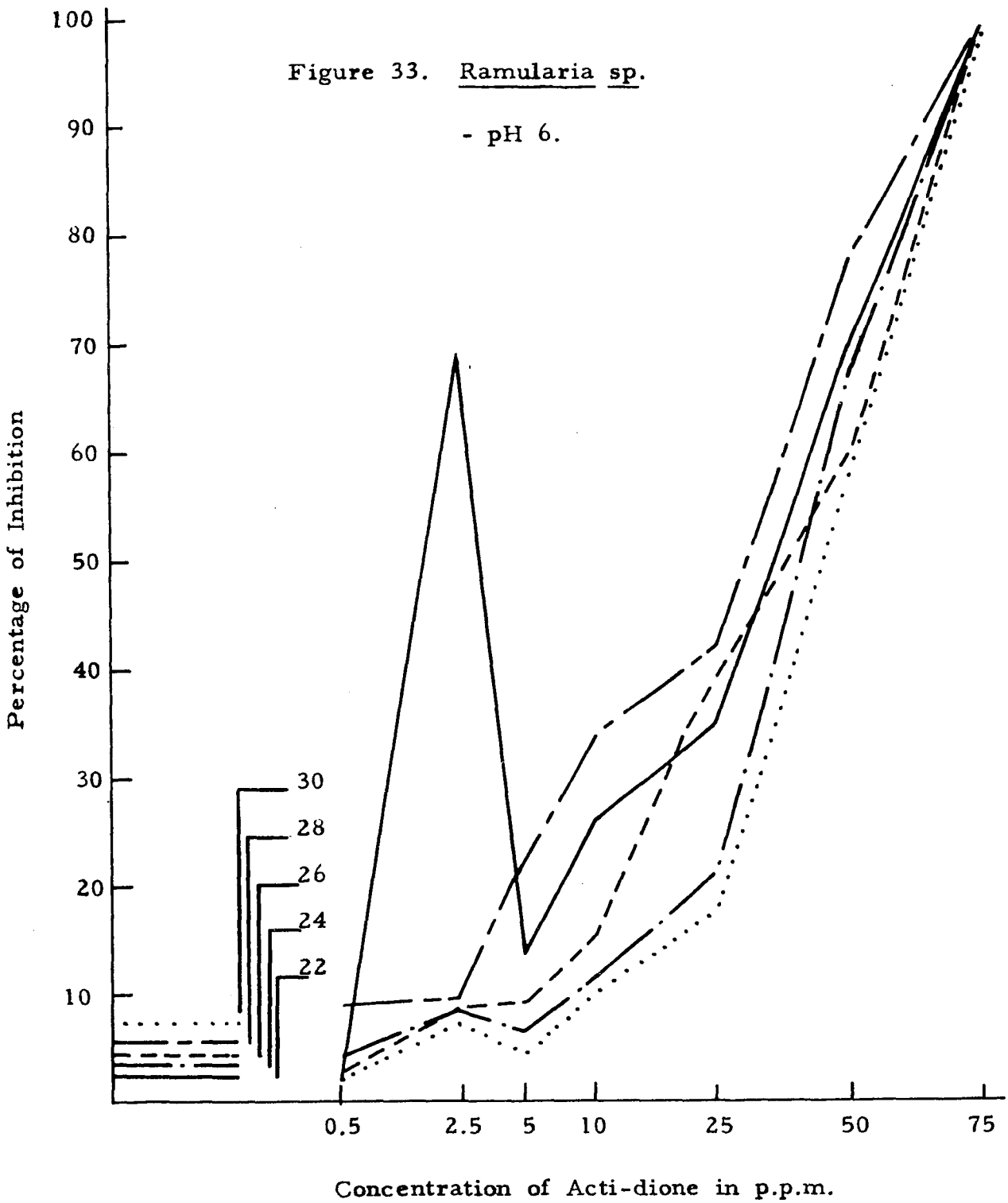


Figure 33. Ramularia sp.

- pH 6.



Figures 35, 36, 37, 38 and 39 present the same data discussed above but in a different manner. The attempt is made to show the relationships and differences between each pH value at each of the temperatures used in this study.

Temperature 22° C. Here there are stimulatory effects of Acti-dione in 0.5 p.p.m. at every hydrogen ion concentration except 6.0. The largest amount of stimulation is in 0.5 p.p.m. at pH 5 followed by pH 4.0. Also to be noted is the stimulation of germination in 25.0 p.p.m. at each and every hydrogen ion concentration. From 5.0 p.p.m. and higher the amount of inhibition at hydrogen ion concentrations of 6.8, 5.0 and 5.0 almost parallel each other until at 100.0 p.p.m. total inhibition are the results for all three. Total inhibition of germination was at 75.0 p.p.m. at pH 4.0 and 3.0.

Temperature 24° C. The best results in inhibiting the germination of spores were attained at pH 6.8 almost at every concentration of Acti-dione up to 50.0 p.p.m. (Figure 36). The next best pH value, according to the results, was pH 5.0. Three of the lines representing hydrogen ion concentrations meet at 75.0 p.p.m. to indicate the maximum amount of inhibition and the other two meet at 100.0 p.p.m.

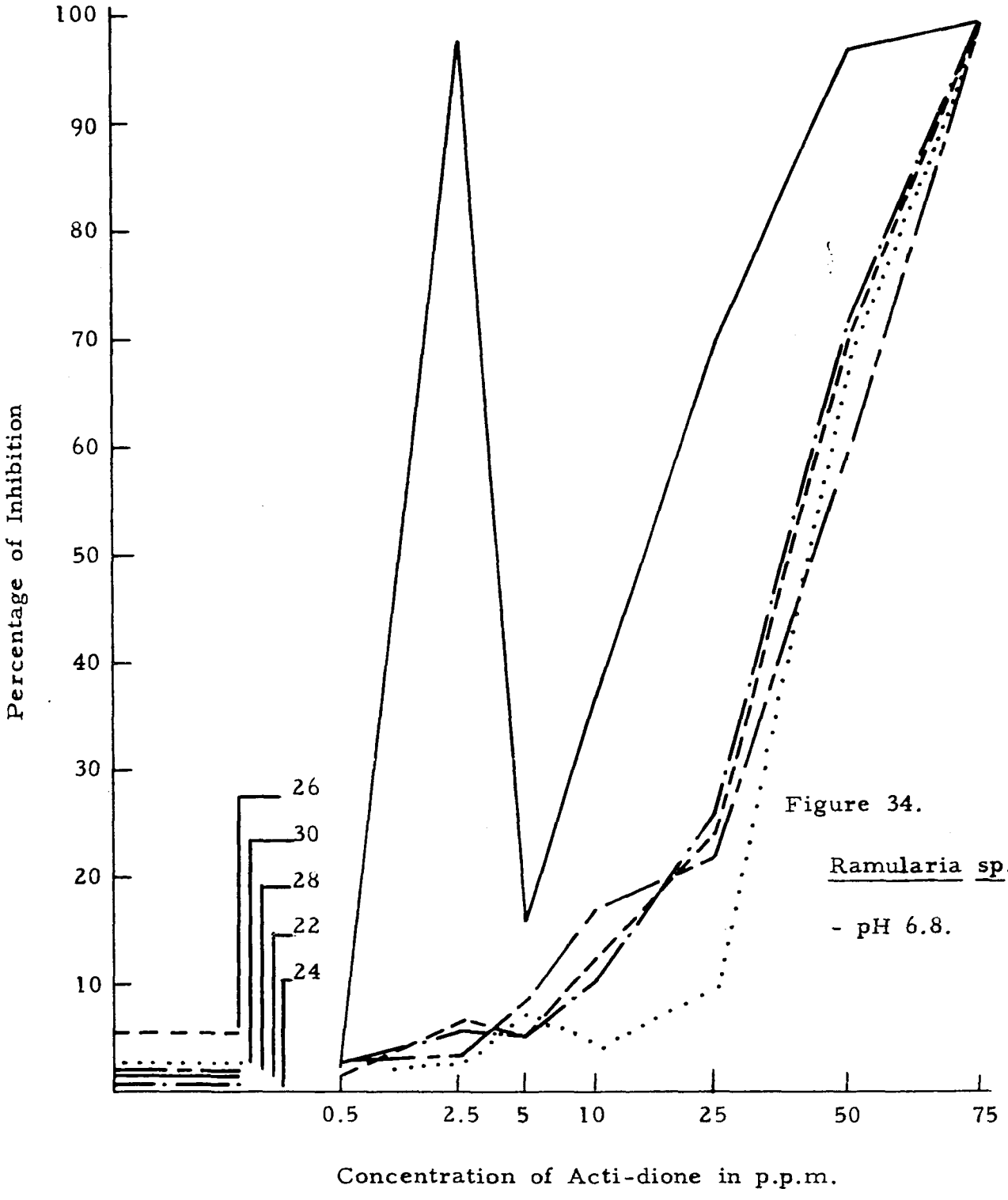


Figure 34.

Ramularia sp.

- pH 6.8.

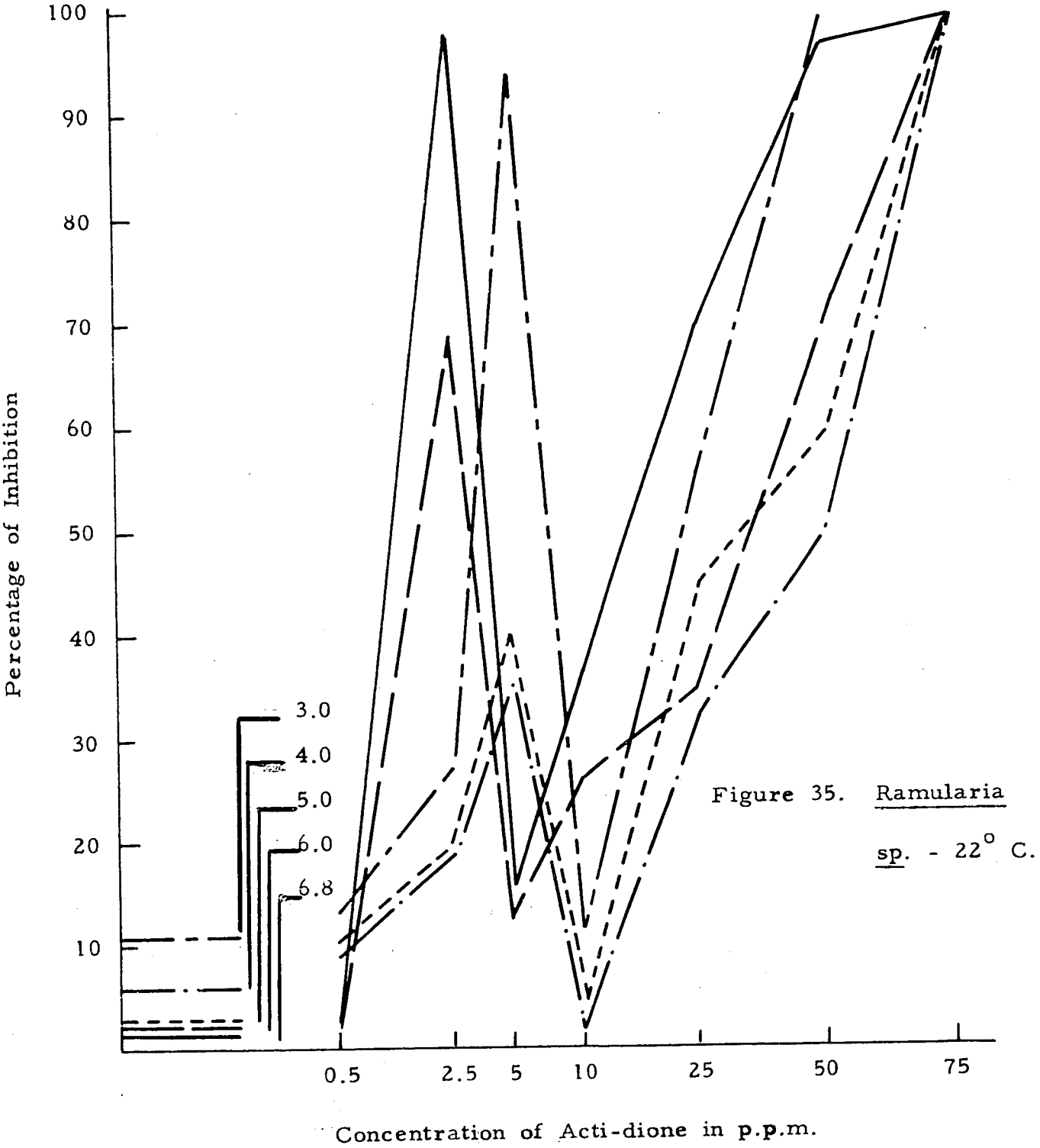


Figure 35. Ramularia sp. - 22° C.

Figure 36. Ramularia sp.
- 24° C.

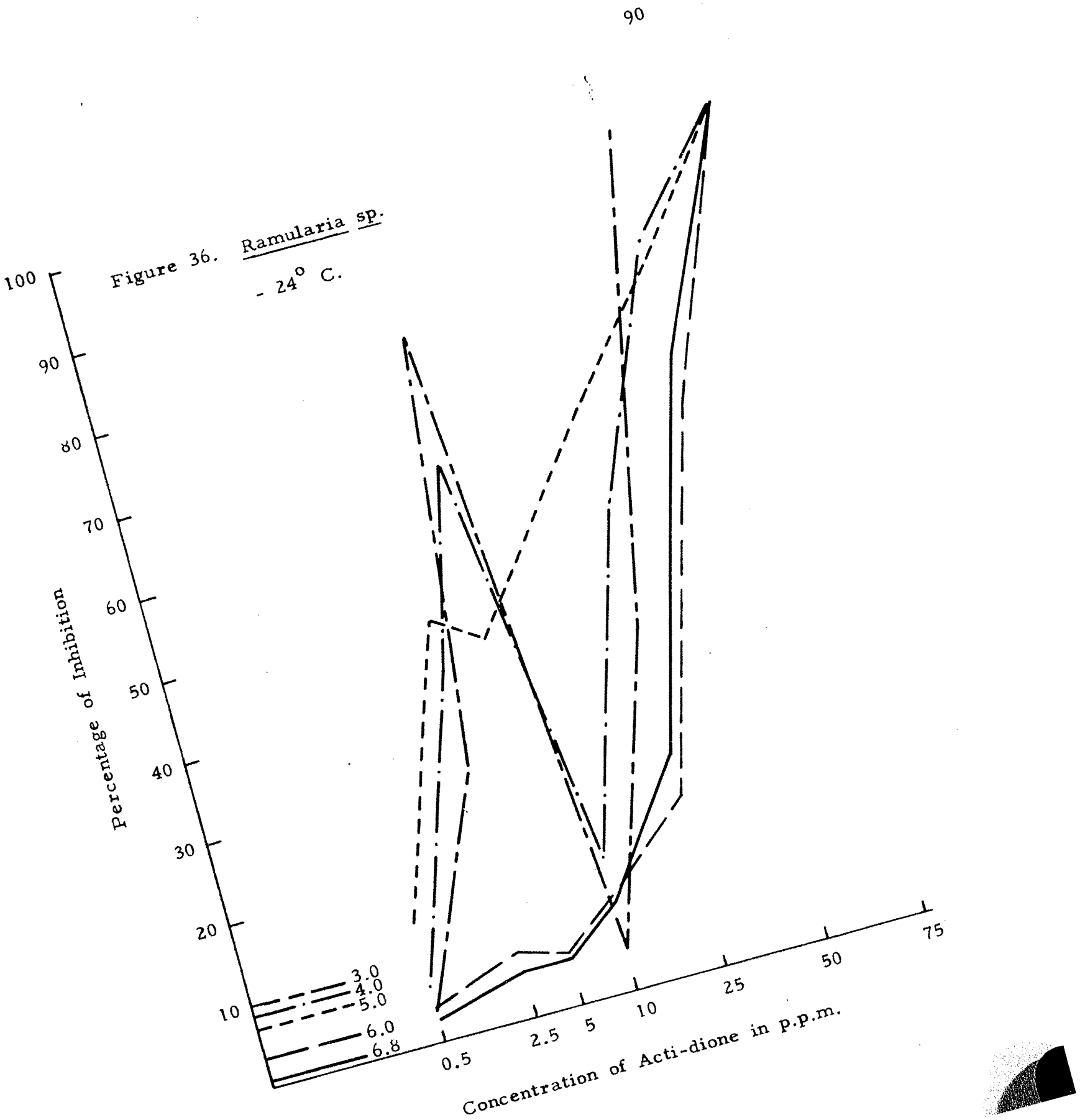


Figure 37. Ramularia sp.

- 26° C.

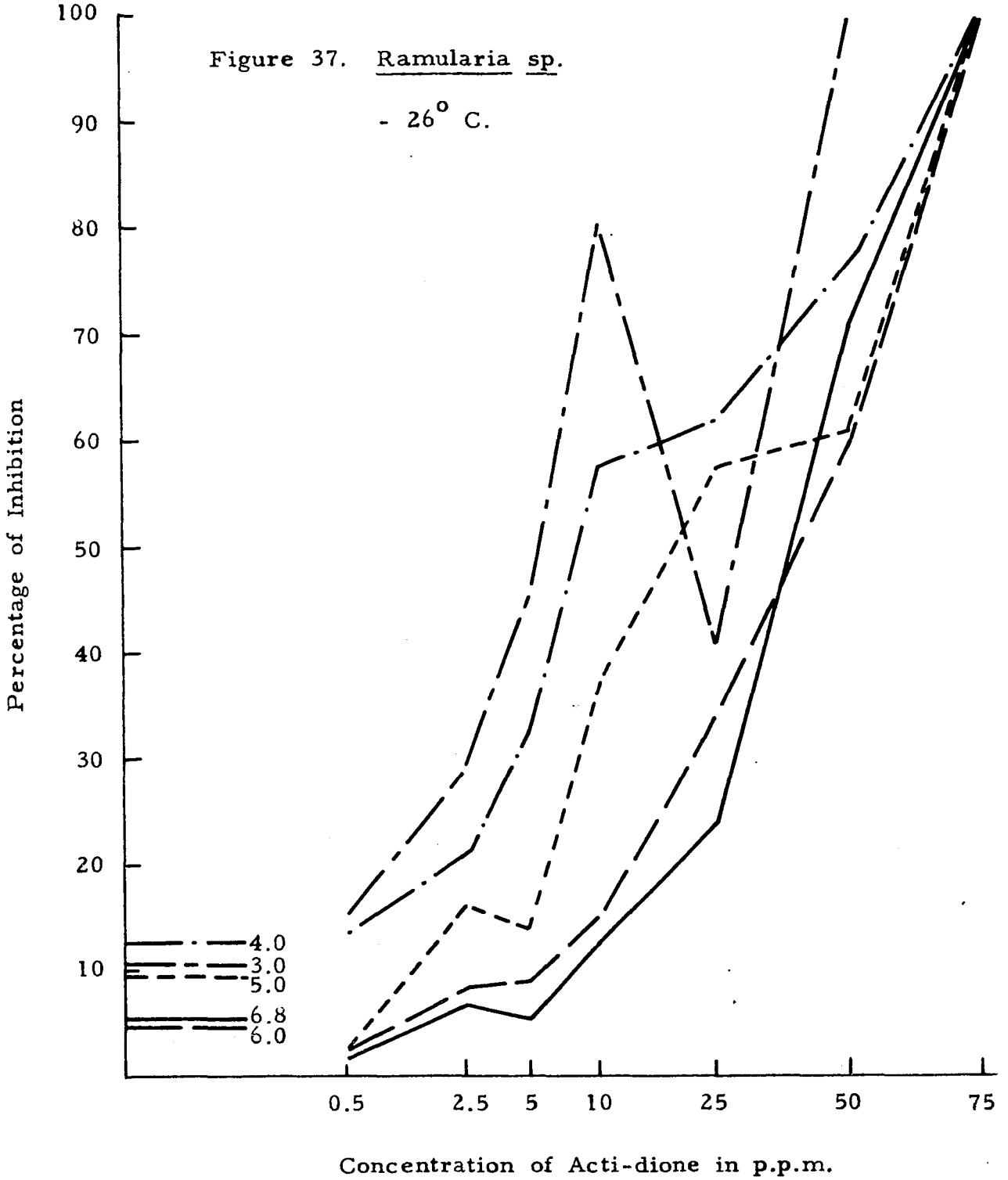


Figure 38. Ramularia sp.

- 28° C.

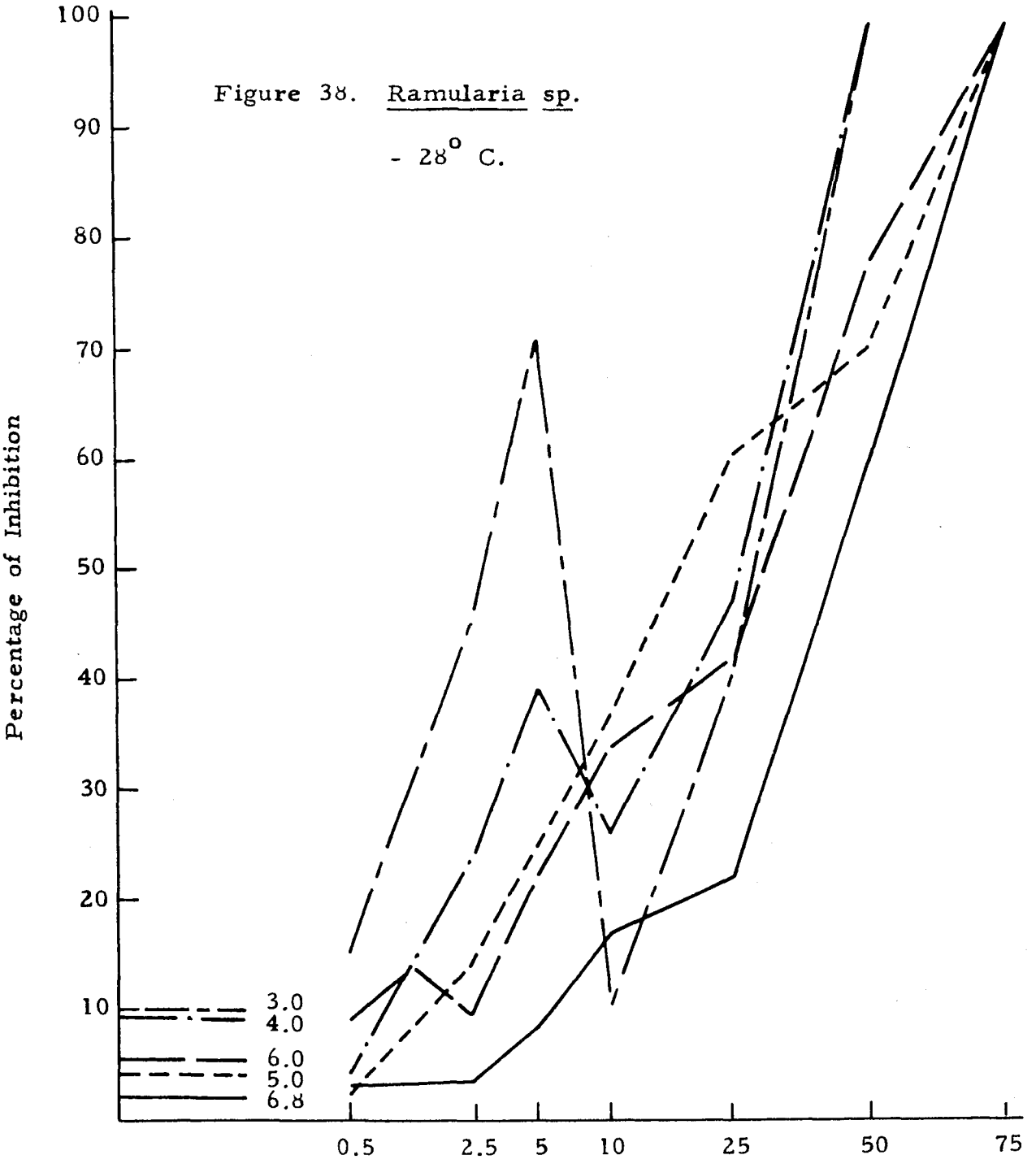
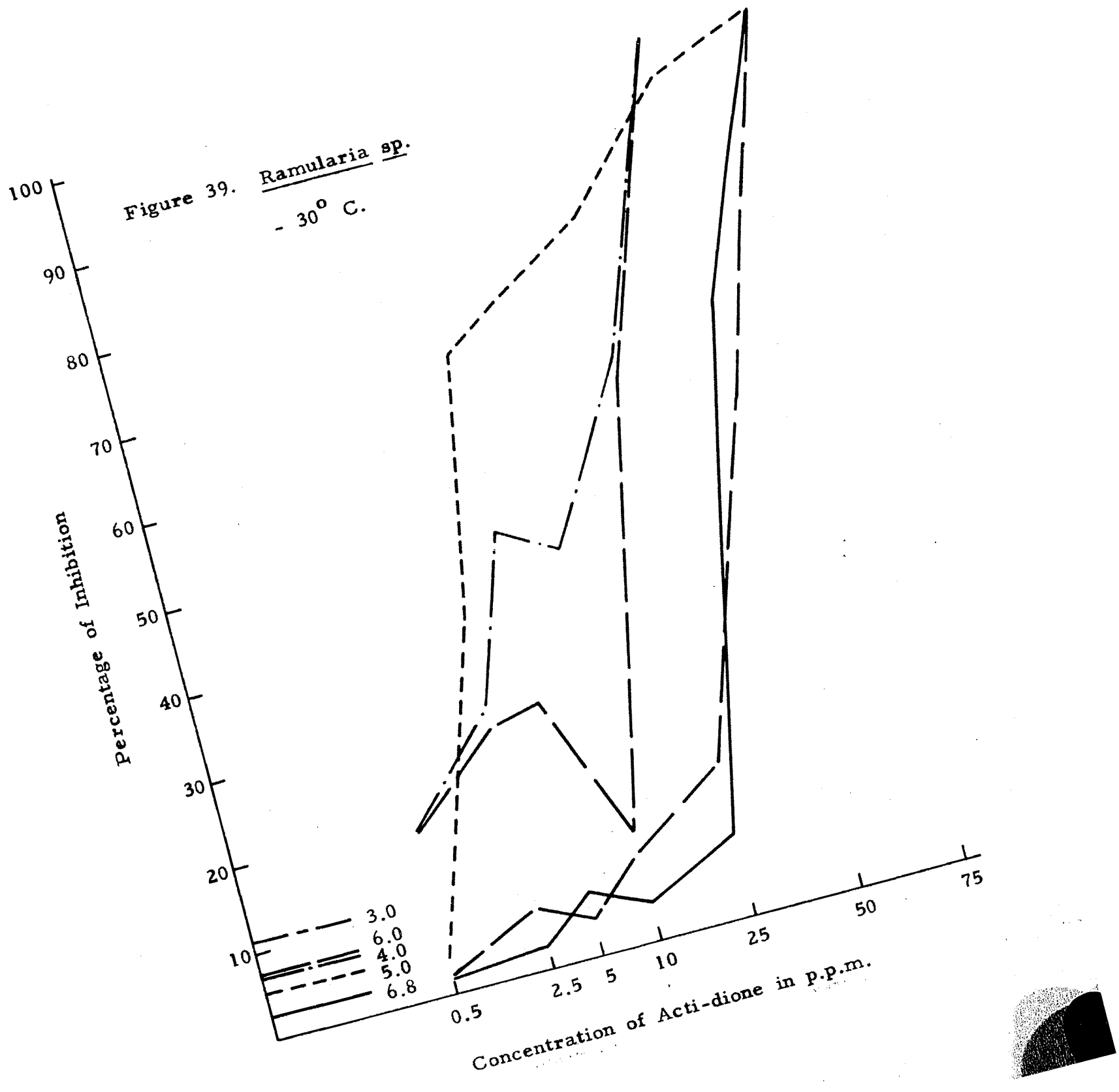


Figure 39. Ramularia sp. - 30° C.



Definite stimulation occurred in 0.5 p.p.m. at every pH except 3.0. At pH 6.8 there was evident a very marked stimulation in 0.5 p.p.m. of Acti-dione. Additional stimulations occurred at 2.5 p.p.m., 5.0 p.p.m. and 25.0 p.p.m. Here there is the complete absence of stimulation at pH 6.0.

Temperature 26° C. The most noteworthy difference at this temperature was the wide difference in concentration of Acti-dione required in effecting 30% or more inhibition, e.g., at pH 6.0 only 0.5 p.p.m. of Acti-dione was required at pH 3.0.

A very slight stimulation occurred at pH 6.8 in 0.5 p.p.m. and a very marked stimulation at pH 5.0 in the same concentration of Acti-dione. In fact the line representing pH 5.0 (Figure 37) indicated 0% inhibition or total germination. Also a rather significant amount of stimulation in 25.0 p.p.m. of Acti-dione at the pH concentrations of 4.0 and 3.0. To be noted is the fact that all but one of the pH graph lines terminate at 100.0 p.p.m.; pH 6.0 is the only line terminating at 75.0 p.p.m. of Acti-dione concentrations.

Temperature 28° C. The principal difference between this and the previous temperature is that all pH graph lines terminate at 75.0 p.p.m. (Figure 38). In addition a higher percentage of inhibition is attained at pH 6.8 than previously in 0.5 p.p.m. And there is stimulation of germination in 0.5 p.p.m. of Acti-dione at the pH concentrations of 3.0, 4.0, 5.0 and 6.0. Also there were marked stimulations occurring at 5.0 p.p.m. at every hydrogen ion concentration but one and at 25.0 p.p.m. at pH 4.0 and pH 3.0. Acti-dione was most effective here at pH 6.8 as in the previous experiments.

Temperature 30° C. As noted in the previous three tests the results of the controls parallel each other in arrangement on the graphs, i.e., the largest amount of germination was observed at pH 3.0 and the least at pH 6.8. The results of all other pH values followed in sequence.

Other than the similarity in the control results, the general pattern of behavior is similar at this hydrogen ion concentration as in the previous test. Another exception to be noted is the absence of the marked stimulations occurring in 25.0 p.p.m. of Acti-dione.

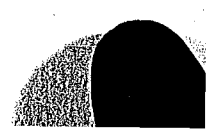


TABLE IV

INHIBITION OF SPORE GERMINATION OF RAMULARIA SP. BY DIFFERENT CONCENTRATIONS OF ACTI-DIONE AT FIVE DIFFERENT TEMPERATURES AND FIVE HYDROGEN ION CONCENTRATIONS

Temperatures (° C.)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
22	Control	11.01	6.06	3.25	2.50	1.82
	0.5	13.16	8.95	10.50	2.00	2.70
	2.5	27.83	19.00	20.00	69.46	98.00
	5.0	94.44	35.48	40.95	13.71	15.93
	10.0	11.71	2.05	5.26	26.21	36.94
	25.0	56.00	32.40	45.00	35.09	70.27
	50.0	100.00	50.25	59.51	70.25	97.25
	75.0	100.00	100.00	100.00	100.00	100.00
24	Control	10.00	8.92	7.05	3.51	0.92
	0.5	3.95	6.48	14.86	4.05	2.70
	2.5	30.63	42.55	49.21	8.12	5.61
	5.0	82.30	67.00	45.30	6.53	5.61
	10.0	5.36	16.37	54.10	11.56	10.71
	25.0	42.00	55.54	69.00	21.15	26.13
	50.0	100.00	86.12	83.99	67.28	72.07
	75.0	100.00	100.00	100.00	100.00	100.00
26	Control	10.71	12.55	9.12	4.68	5.50
	0.5	15.60	13.52	3.00	2.75	1.77
	2.5	26.09	21.10	16.41	8.56	6.96
	5.0	45.87	33.09	14.20	9.13	5.45
	10.0	80.91	57.81	37.28	15.17	12.73
	25.0	38.10	62.15	57.80	34.06	24.32
	50.0	100.00	77.00	61.00	60.24	70.91
	75.0	100.00	100.00	100.00	100.00	100.00

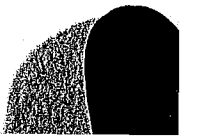


TABLE IV (Continued)

Temperatures (° C.)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
28	Control	10.00	9.30	4.16	5.51	2.05
	0.5	15.05	4.18	2.50	9.01	3.15
	2.5	45.19	24.50	14.67	22.51	3.50
	5.0	71.17	39.28	25.00	34.13	8.57
	10.0	10.19	26.14	37.28	42.27	17.13
	25.0	35.25	47.88	60.52	78.20	22.28
	50.0	100.00	100.00	70.34	100.00	60.10
	75.0	100.00	100.00	100.00	100.00	100.00
30	Control	11.11	7.12	5.41	7.25	2.70
	0.5	18.52	19.00	4.02	2.02	1.79
	2.5	28.18	31.00	40.28	7.13	2.70
	5.0	29.91	49.82	70.50	4.59	7.34
	10.0	12.84	46.22	75.03	10.32	4.42
	25.0	63.00	65.11	81.99	18.00	9.73
	50.0	100.00	100.00	95.21	57.45	68.42
	75.0	100.00	100.00	100.00	100.00	100.00

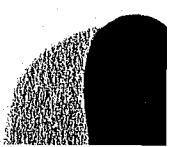
Humidity Experiments

Each organism was tested in three different relative humidities (98%, 95%, 93%) at the hydrogen ion concentrations used in the previous experiments. The spores failed to germinate in 93% relative humidity, so results were obtained in the 98% and 95% relative humidity tests.

In 98% humidity the germ tubes of all organisms were considerably shorter, considerably thinner and more irregular than those germinated directly in liquid. The germ tubes in 95% were even shorter and quite irregular. A considerable amount of plasmolysis was evident in both germ tubes and ungerminated spores after 24 hours. No disorganization of cytoplasm was noted at the lower concentrations of Acti-dione in any organism. Some breakdown could be observed at the higher concentrations.

Cladosporium Cucumerinum

Only the results from the 98% relative humidity test will be presented since no germination was observed at 95%. The best inhibition was found in 10.0 p.p.m. of Acti-dione at pH 4.0. No anomalies were observed as with some of the other



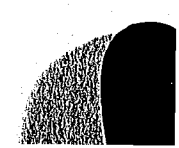
spores when immersed in solution (Figure 40). The poorest results were obtained at pH 6.8 and stimulation of germination occurred only at pH 4.0 in 0.5 p.p.m.

Botrytis Cinerea

98% relative humidity. The best inhibition was observed in 50.0 p.p.m. of Acti-dione and the next best in 10.0 p.p.m. at pH 6.8 (Figure 41). The poorest results were obtained at pH 5.0 in 0.5 p.p.m. and at pH 3.0 at the higher concentrations of Acti-dione. This pattern of behavior as compared to the Cladosporium, was somewhat anomalous. In addition there was some stimulation of germination in 0.5 p.p.m. at pH concentrations of 4.0, 5.0 and 6.8.

Abnormalities observed, compared with the controls, were confined to the germ tubes and ungerminated spores. The abnormalities were the same as listed previously for this organism. In addition to this, the germ tubes were very thin and quite short.

95% relative humidity. The principal difference between these results and at 98% relative humidity is that a more or



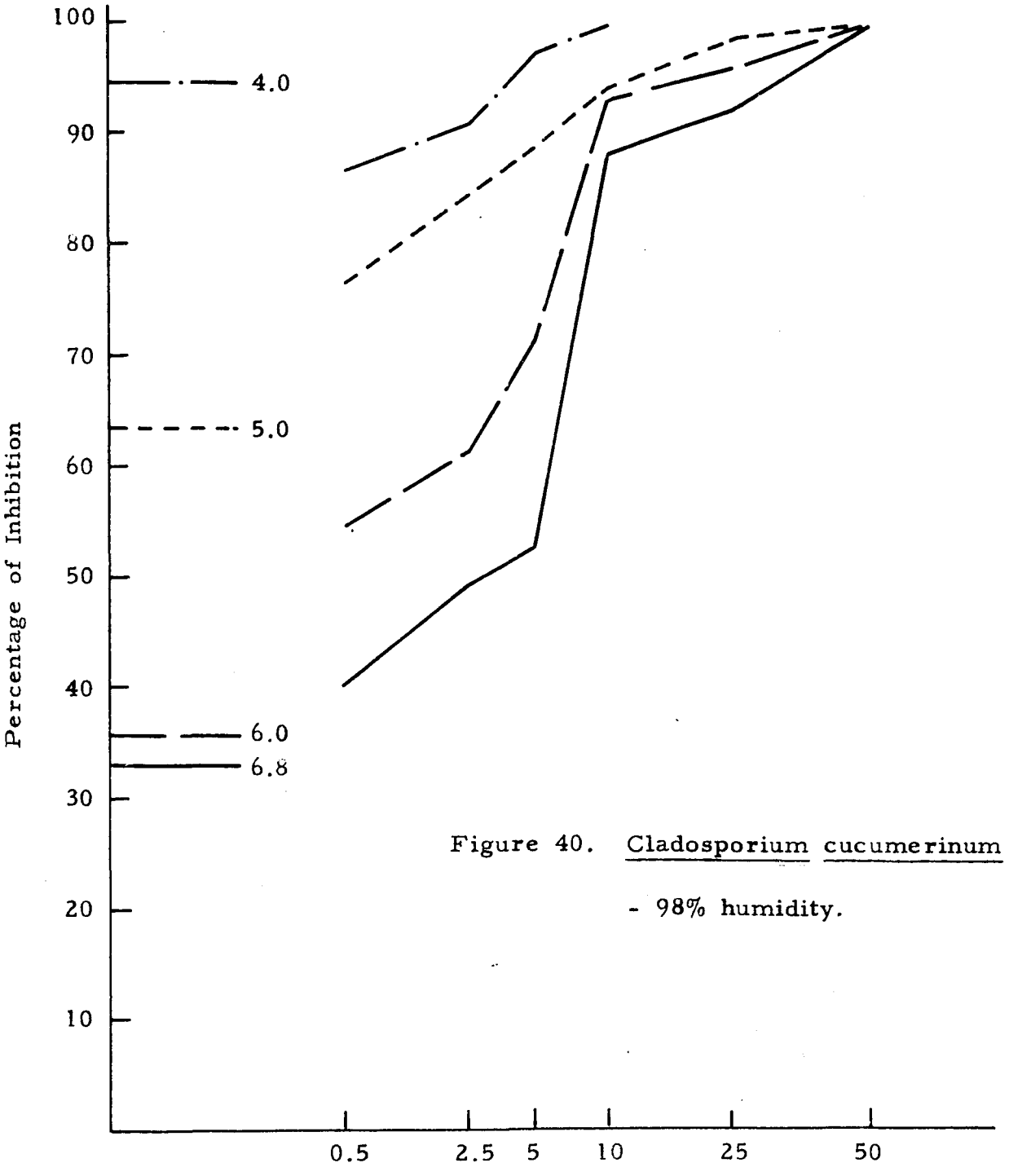


Figure 40. Cladosporium cucumerinum - 98% humidity.

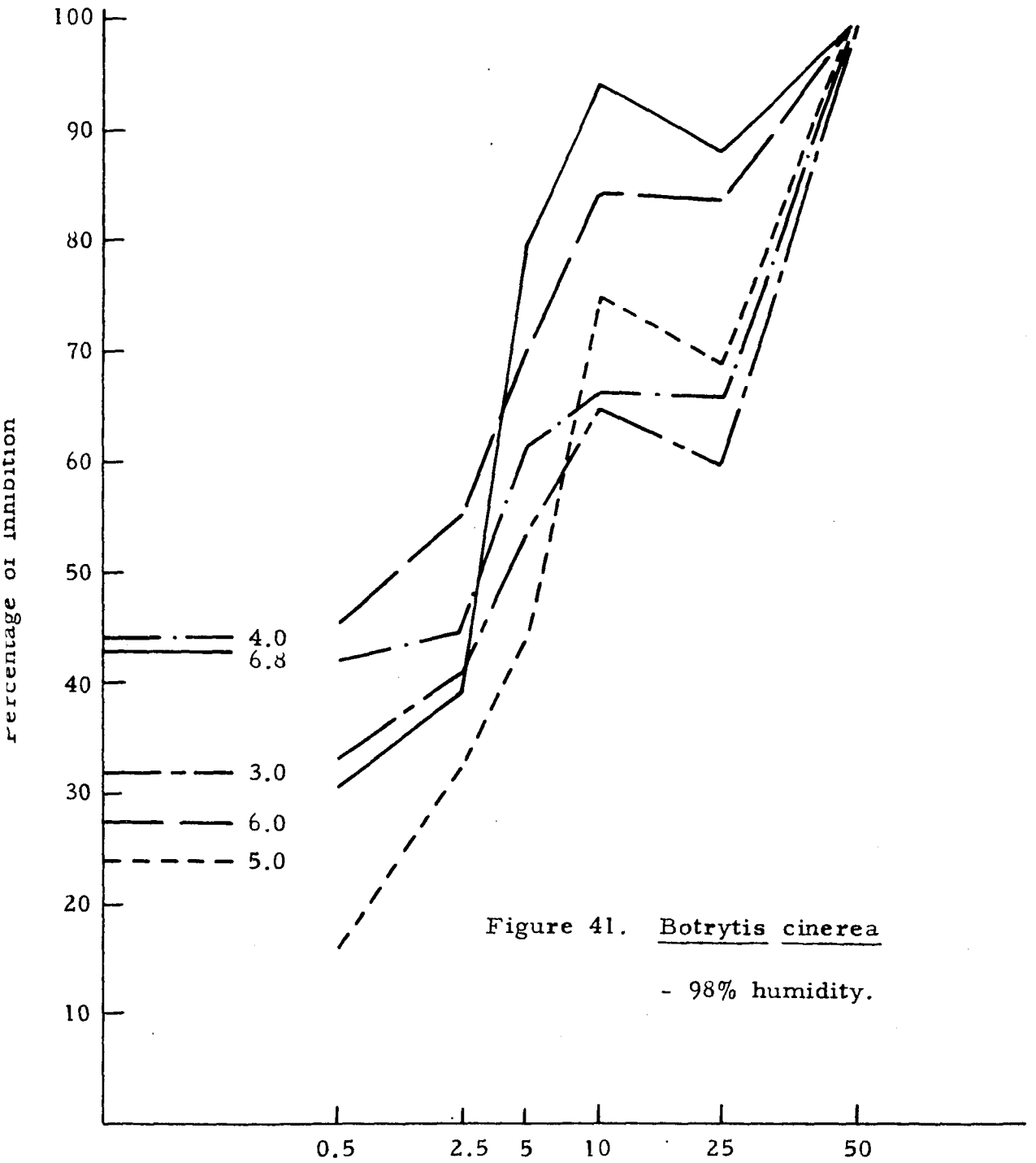


Figure 41. Botrytis cinerea

- 98% humidity.

less better inhibition resulted at a given concentration of Acti-dione at a given pH concentration (Figure 42).

Monilinia Fructicola

98% relative humidity. The results at this relative humidity are strikingly similar to those obtained where the spores were immersed directly in the liquid Acti-dione. The principal difference is that a higher per cent of inhibition was found in a given Acti-dione concentration and pH than in the direct immersion experiments. The distortion and other morphological abnormalities that were present in the direct immersion experiments were also present here, plus the very thin germ tubes that were observed in the case of the Botrytis organism.

The most favorable pH was 6.8 in 0.5 p.p.m. and 10.0 p.p.m. The poorest or most unfavorable pH was 3.0 (Figure 43).

95% relative humidity. The results at this pH are very similar to the above, except for a slight difference in amount of inhibition exhibited at 2.5 p.p.m. In the previous test the lowest inhibition was attained at pH 3.0 whereas here the lowest

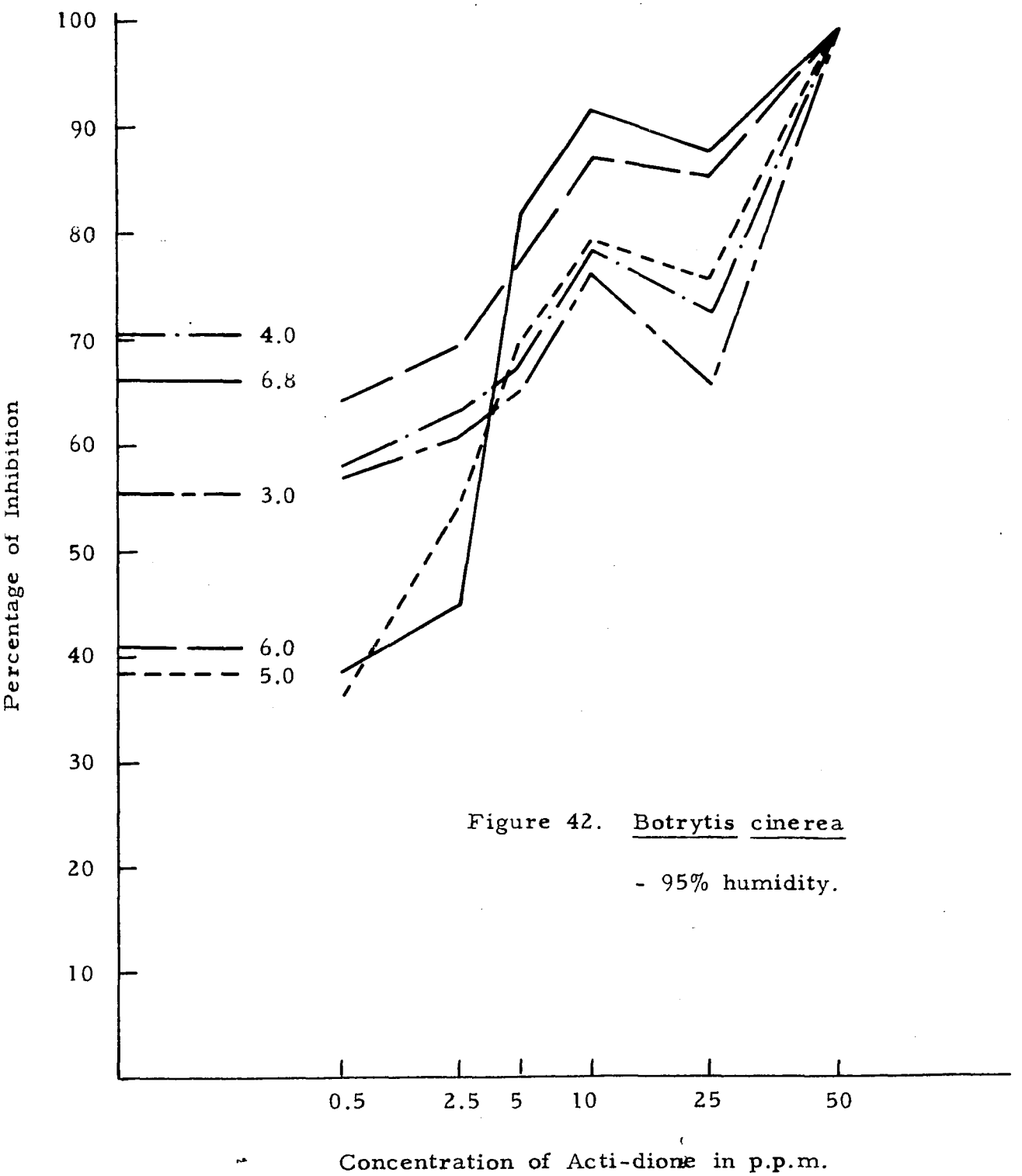


Figure 42. Botrytis cinerea

- 95% humidity.

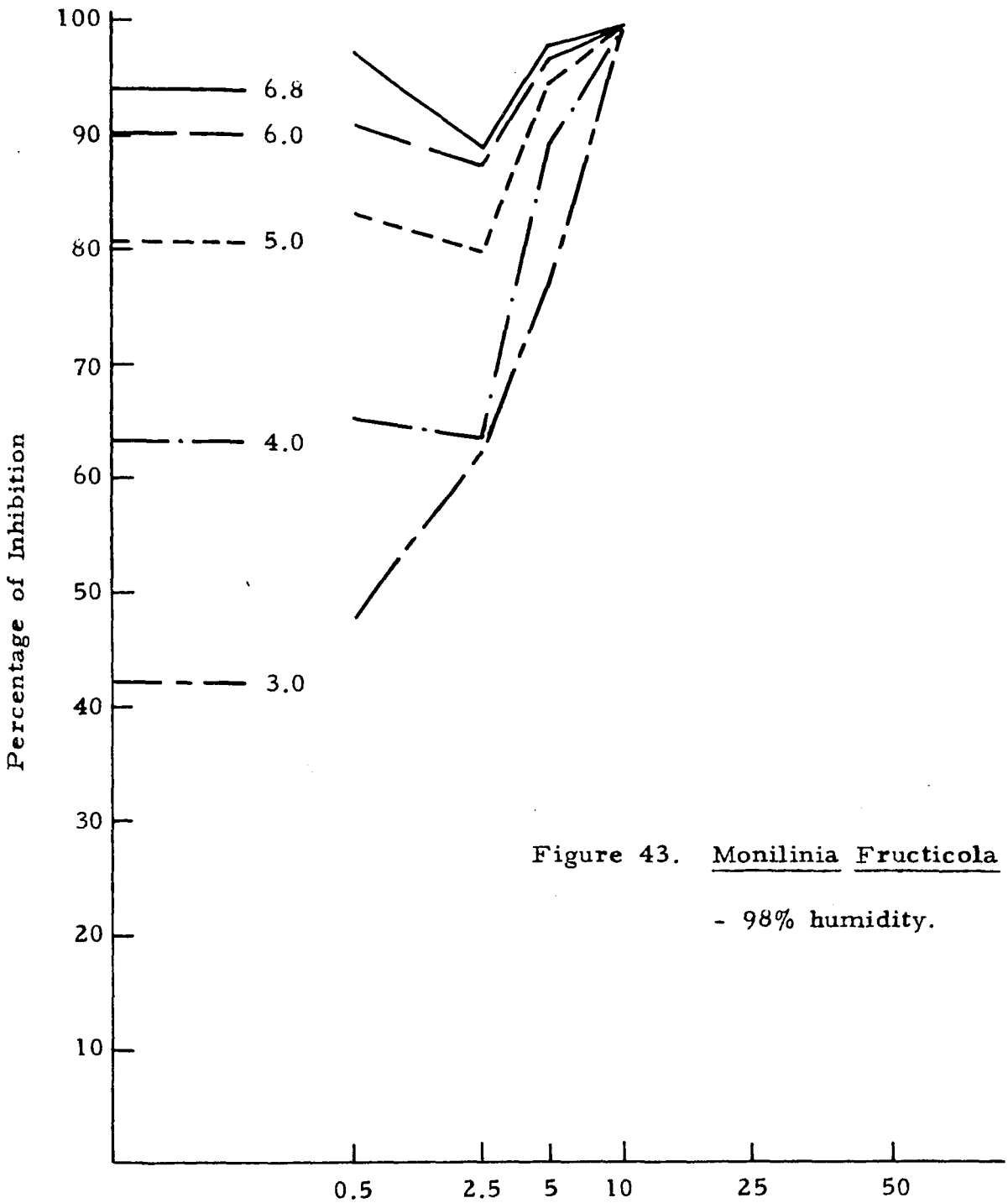


Figure 43. Monilinia Fructicola

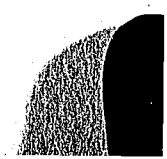
- 98% humidity.

is at pH 4.0 (Figure 44). Also to be noted here is the absence of germination at pH 6.8.

Ramularia Sp.

98% relative humidity. The highest per cent of inhibition at this relative humidity was observed in 2.5 p.p.m. at pH 6.8 and again at all hydrogen ion concentrations in 50 p.p.m. (Figure 45). No stimulations were observed in 0.5 p.p.m. as in previous experiments, but a considerable reduction of inhibition was observed in 5.0 and 10.0 p.p.m. of Acti-dione. Actually, there was a slight stimulation in 10.0 p.p.m. at pH 3.0 and 4.0.

93% relative humidity. The hydrogen-ion concentration most favorable in this relative humidity was 6.8 in the Acti-dione concentration of 2.5 p.p.m. (Figure 46). Total inhibition of germination occurred at the same pH but at 50 p.p.m. The next most favorable hydrogen ion concentration was 3.0 in the Acti-dione concentration of 5.0 p.p.m. The complex behavior pattern of this organism under the various treatments at this relative humidity was noticeable. There are a number of reductions of inhibition in concentrations where normally an increase of inhibition of spore germination was expected. These



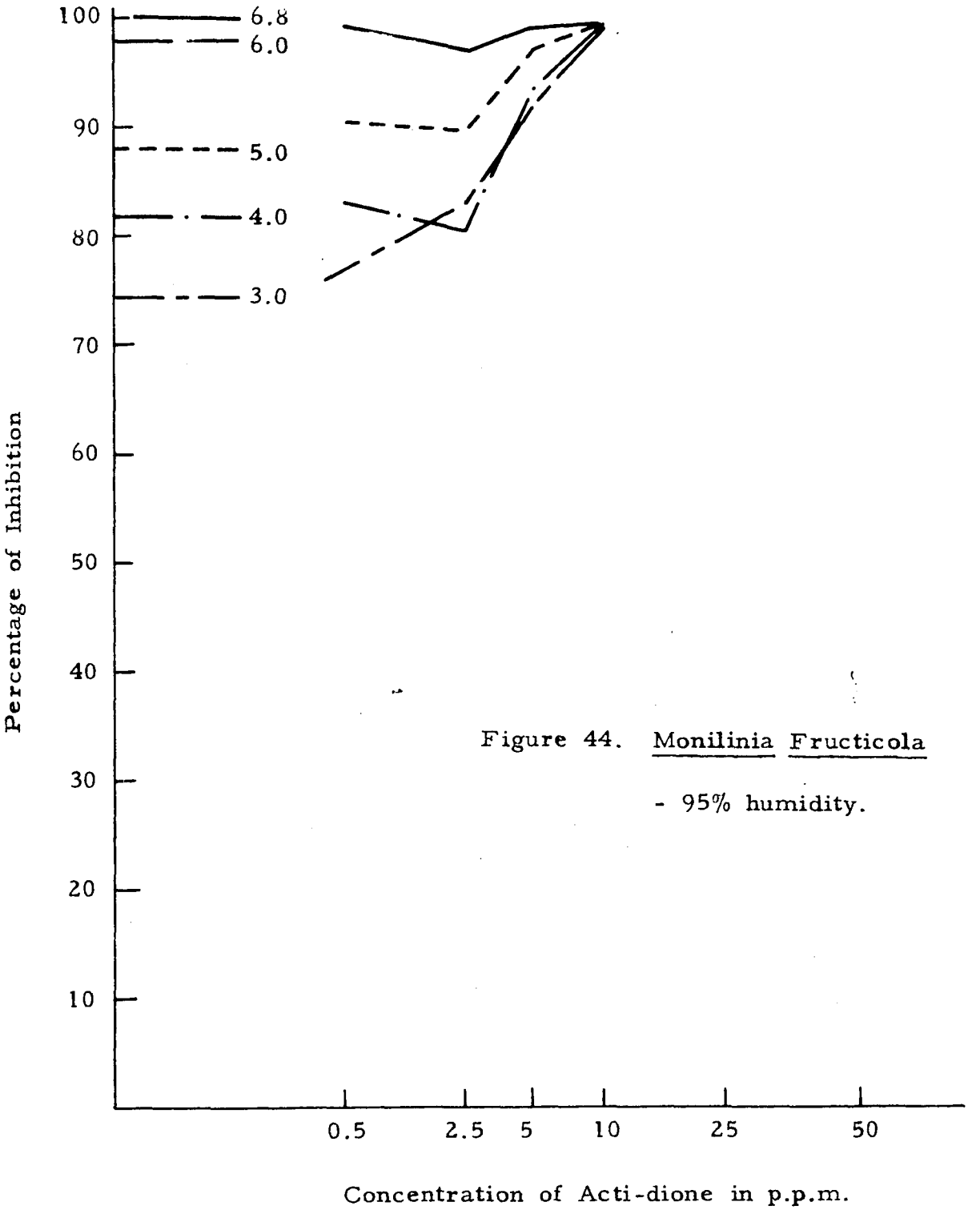


Figure 44. Monilinia Fructicola

- 95% humidity.

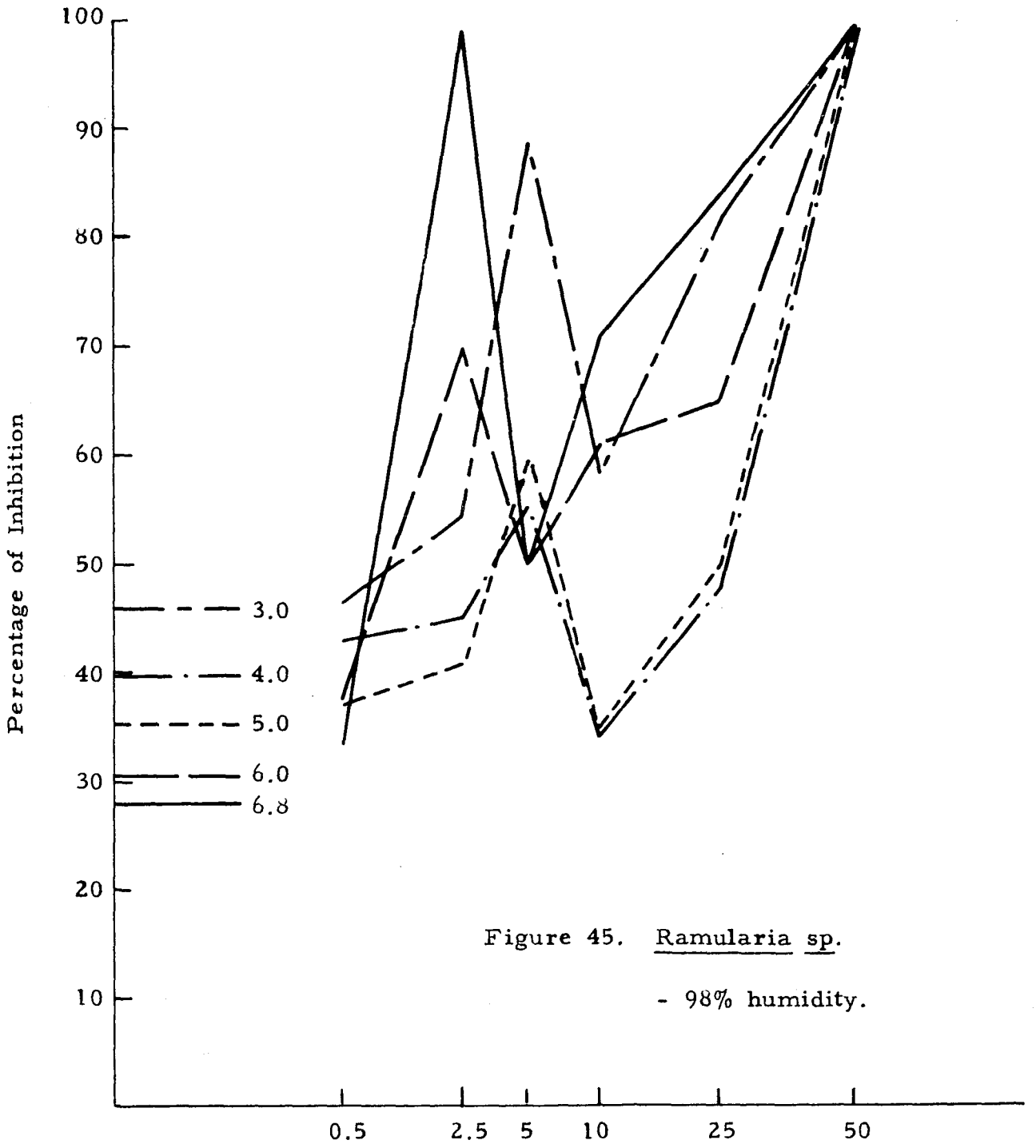


Figure 45. Ramularia sp.

- 98% humidity.

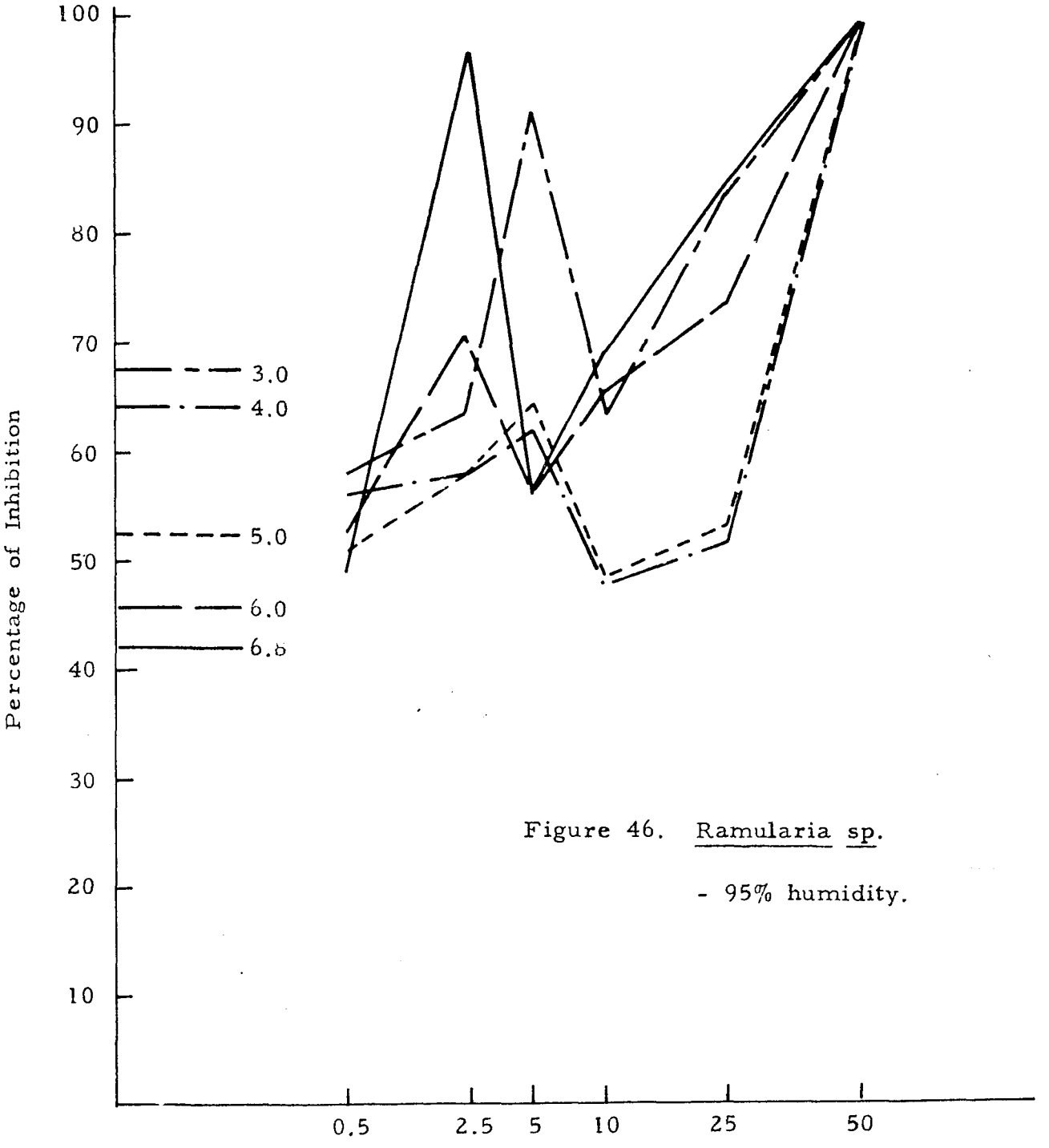


Figure 46. Ramularia sp.

- 95% humidity.

anomalies were observed at 5.0 and 10.0 p.p.m. concentrations. Stimulations of spore germination appear in 0.5 p.p.m. at pH 3.0, 4.0 and 5.0. Further stimulations were observed in 10.0 p.p.m. at pH 4.0 and 5.0.

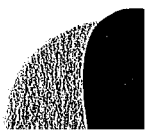


TABLE V

INHIBITION OF SPORE GERMINATION OF CLADOSPORIUM CUCUMERINUM BY DIFFERENT CONCENTRATIONS OF ACTI-DIONE AT FIVE DIFFERENT HYDROGEN ION CONCENTRATIONS AND TWO RELATIVE HUMIDITIES AT 20° C.

Relative Humidity (%)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
98	0.0	No Germination	94.51	63.45	35.84	33.00
	0.5		86.61	76.42	54.65	40.09
	2.5		90.84	84.49	61.30	49.31
	5.0		97.38	88.95	71.45	52.75
	10.0		100.00	94.28	93.28	88.32
	25.0		100.00	98.78	96.10	92.20
	50.0		100.00	100.00	100.00	100.00

TABLE VI

INHIBITION OF SPORE GERMINATION OF BOTRYTIS CINEREA BY DIFFERENT CONCENTRATIONS OF ACTI-DIONE AT FIVE DIFFERENT HYDROGEN ION CONCENTRATIONS AND TWO RELATIVE HUMIDITIES AT 20° C.

Relative Humidity (%)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
98	0.0	32.00	44.18	24.00	27.50	43.00
	0.5	33.26	42.35	11.00	45.32	30.52
	2.5	41.00	44.92	32.50	55.21	39.21
	5.0	53.85	61.65	44.35	70.30	79.99
	10.0	65.10	66.50	75.16	84.76	94.65
	25.0	60.12	66.00	69.12	84.00	83.60
	50.0	100.00	100.00	100.00	100.00	100.00
95	0.0	50.49	70.62	48.51	41.00	66.22
	0.5	57.00	58.13	36.23	64.39	38.60
	2.5	61.00	63.50	24.25	69.80	45.27
	10.0	76.78	78.90	78.85	87.78	92.00
	25.0	66.00	73.12	76.22	86.00	88.34
	50.0	100.00	100.00	100.00	100.00	100.00

TABLE VII

INHIBITION OF SPORE GERMINATION OF MONILINIA FRUCTICOLA BY DIFFERENT CONCENTRATIONS OF ACTI-DIONE AT FIVE DIFFERENT HYDROGEN ION CONCENTRATIONS AND TWO RELATIVE HUMIDITIES AT 20° C.

Relative Humidity (%)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
98	0.0	42.25	63.35	80.85	90.13	94.00
	0.5	47.87	65.18	83.46	91.00	97.35
	2.5	62.50	63.75	80.04	87.50	89.23
	5.0	77.20	89.82	84.90	97.00	98.19
	10.0	100.00	100.00	100.00	100.00	100.00
95	0.0	74.35	81.82	88.09	98.00	
	0.5	75.98	83.28	90.85	99.48	
	2.5	83.25	80.76	89.90	97.35	
	5.0	92.30	93.60	97.45	99.50	
	10.0	100.00	100.00	100.00	100.00	

TABLE VIII

INHIBITION OF SPORE GERMINATION OF RAMULARIA SP. BY DIFFERENT CONCENTRATIONS OF ACTI-DIONE AT FIVE DIFFERENT HYDROGEN ION CONCENTRATIONS AND TWO RELATIVE HUMIDITIES AT 20° C.

Relative Humidity (%)	Concentration of Acti-dione in p.p.m.	Percentage of Inhibition				
		pH 3.0	pH 4.0	pH 5.0	pH 6.0	pH 6.8
98	0.0	46.00	39.88	35.31	30.52	28.00
	0.5	46.55	43.10	37.22	38.20	33.25
	2.5	54.72	45.12	41.00	70.02	99.50
	5.0	89.00	55.49	59.71	50.15	50.01
	10.0	58.50	34.15	35.01	61.46	71.45
	25.0	82.25	48.11	50.18	65.12	84.50
	50.0	100.00	100.00	100.00	100.00	100.00
95	0.0	67.82	64.35	52.65	45.96	42.00
	0.5	58.01	56.15	51.00	52.18	49.33
	2.5	63.76	58.14	58.14	71.16	97.00
	5.00	91.51	62.18	64.93	56.49	57.21
	10.0	63.42	48.00	48.89	65.90	69.50
	25.0	84.15	52.00	53.50	74.00	85.00
	50.0	100.00	100.00	100.00	100.00	100.00

DISCUSSION

The observations made during this investigation indicate several patterns of behavior by the organisms in response to a number of treatments by Acti-dione under the influence of temperature, pH and moisture.

Acti-dione was used in concentrations from 0.5 p.p.m. to 100.00 p.p.m. At the lowest concentration, it was consistently observed that there was frequently a stimulatory rather than an inhibitory effect upon spore germination. This is quite anomalous to the general pattern of behavior, of the four organisms studied, which indicated the tendency toward a higher percentage of inhibition of germination as the concentration of the Acti-dione was increased. The stimulation occurring in 0.5 p.p.m. of Acti-dione ranged from 1.09% at pH 4.0 at 24° C. with Botrytis to 14.41% at pH 5.0 at 26° C. with the same organism. In the experiments with Cladosporium, it is interesting to note, that stimulation of germination occurred six times in 0.5 p.p.m., four of these were observed at every hydrogen ion concentration at 24° C. while the other two occurred only at pH 4.0 and 5.0 at 30° C. Stimulation in 2.5 p.p.m. was

considerable less prevalent; only two occurrences were observed, one at pH 5.0 at 24° C. and the other at pH 5.0 at 30° C. (Table I and Figure 1). From these results it is obvious that the majority of stimulations occurred at pH 5.0. These results may therefore indicate that the conidia of Cladosporium cucumerinum are prone to stimulation at this pH at two different temperatures, 24° C. and 30° C.

In the Botrytis experiments stimulation at 0.5 p.p.m. was considerably more pronounced than in tests with Cladosporium. A total of 38 stimulations were observed. Stimulation was observed at every temperature and at almost all hydrogen ion concentrations (Table II). A total of 17 stimulations occurred in 0.5 p.p.m. as compared to 6 with C. cucumerinum. Of further interest was the observation that some stimulatory effects occurred in Acti-dione concentrations as high as 25.0 p.p.m.; these, of course, were less prevalent. In contrast to C. cucumerinum, the hydrogen ion concentrations favoring stimulation were 3.0, 4.0 and 6.8 instead of pH 5.0 (Table II).

Additional stimulatory effects were observed in experiments with Monilinia fructicola and Ramularia sp. In the former

only 5 stimulations were observed while in the latter there were 15. All of the 5 occurring in M. fructicola were at pH 3.0 at 22°, 24° and 26° C. When compared with the other 3 organisms M. fructicola was the least susceptible to stimulation by exposure to Acti-dione. In the Ramularia sp. as in the Botrytis experiments, stimulation of germination occurred in every pH and at every temperature. The principal difference, however, is that with Ramularia the greatest stimulatory tendency was at pH 3.0, 4.0 and 6.8 whereas with Botrytis it was at pH 4.0, 5.0 and 6.0.

Of considerable interest, was the similarity to the results above in the relative humidity tests (Tables V, VI, and VII). Stimulation was observed 27 times with all four organisms in all humidities and hydrogen ion concentrations. Of this total, however, only 10 occurred at 0.5 p.p.m. and 10 at 2.5 p.p.m. of Acti-dione experiments, the others were found in Acti-dione concentrations up to 25.00 p.p.m.

Of additional interest is the fact that in the humidity tests, the Cladosporium spores were significantly less prone to stimulation than in the previous experiments. Also a slight stimulation was noted here at pH 4.0 in 2.5 p.p.m. that was

absent in the previous tests. Botrytis spores also responded to stimulation in a similar manner as in the previous tests but in a lesser amount. Most of the stimulation occurred at 0.5 p.p.m. In the humidity tests with this organism, there was an absence of any stimulation at pH 3.0 which was favorable to this action in the direct immersion experiments. In the humidity tests the M. fructicola spores were no more susceptible to stimulatory action than previously. It was interesting to observe that the stimulation which did occur was not in 0.5 p.p.m. or at pH 3.0, but at pH 4.0, 6.0 and 6.8 in 2.5 p.p.m. (Table VII). Most of the stimulation, in the direct immersion tests with Ramularia occurred at pH 6.0 (Table VIII), the complete absence of stimulation at this pH in the humidity tests is noteworthy. The stimulations which did occur were found mostly at pH 4.0 in 95% relative humidity.

In addition to the stimulatory effects observed, several other observations were of interest. All 4 organisms were exposed to Acti-dione adjusted to all of the hydrogen ion concentrations as described in the Materials and Methods section. Some germination occurred in every experiment except at pH 3.0 with the spores of C. cucumerinum.

The minimum lethal dose at which complete inhibition occurred varied between organisms and was definitely influenced to some extent by the various temperatures and H ion concentrations.

In C. cucumerinum the lethal dose varied with both the pH and the temperature, but in no case was it more than 50.00 p.p.m. (Table I). A glance at these results indicate that temperature and pH had what could be termed a synergistic effect, neither one being able to work independently in accomplishing the same effects. Generally the tendency indicated was that as the pH was lowered and the temperature was raised less Acti-dione was necessary to effect complete inhibition. The lethal dose varied from 50.0 p.p.m. to 5.0 p.p.m. There were definite exceptions to this as may be observed in Table I.

The fact that plasmolytic distortion of the spores and germ tubes was absent in this organism may be due to the several factors. It is possible that the cytoplasmic content of the spores and their germ tubes was in or near equilibrium with the Acti-dione solutions in which they were immersed. True, in the humidity tests there was no immersion, but a film of hygroscopic liquid which contained Acti-dione plus the

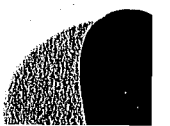
nutrient stimulant was present at all times, consequently the same condition may have prevailed. In addition, it is possible that the spore wall of this organism was of such a structure as to not allow the free passage outward of the cytoplasmic salts, thus preventing plasmolysis. A third factor which may be considered is the possibility that the cytoplasmic concentration, in both the spores and the germ tubes, was slightly less than the Acti-dione solution, so instead of plasmolysis the reverse was the result, but not enough to cause plasmoptysis. The reason for mentioning this factor is that the only abnormality observed in the results of this organism was a tendency for shorter, stouter germ tubes which increased with the increase in the concentration of Acti-dione.

The general pattern observed in the Botrytis tests was a tendency to increase toxicity with the increase in Acti-dione concentration and the lowering of the pH of the solution (Table II). An exception to this was observed at pH 6.8 at 30° C. where total inhibition resulted in 50.0 p.p.m. Also here, it is obvious that temperature and pH worked together to bring about the observed results. In the humidity tests with this organism it was observed that no stimulation occurred at pH 3.0 whereas

in the immersion tests this pH definitely favored stimulation. The reason for this is unknown.

Both distortion, and what appeared to be cytoplasmic disorganization, was observed in this and in the spores of M. fructicola. The reasons for this are possibly the reverse of those stated for C. cucumerinum which exhibited no plasmolytic distortion. The wall structure of the Botrytis and the Monilinia spores may be constructed to allow free passage of water from the cytoplasm out to a less dense liquid with which the spore wall may be surrounded. This, of course, presupposes the concentration of the water in the cytoplasm to be higher than that of the Acti-dione, otherwise plasmolysis would not take place. The most striking difference between these two organisms is the amount of stimulation (Tables I and II). In Botrytis not only did stimulation occur more frequently than it did in Monilinia but a number of sharp reductions in amount of germination were noted where normally none would be expected.

The results of Ramularia sp. resemble those of C. cucumerinum in that the ungerminated spores in higher concentrations of Acti-dione and the germinated in the lower concentrations were free from distortions and cytoplasmic disorganization.



On the other hand, Acti-dione was more effective at a higher pH against C. cucumerinum than Ramularia whose most effective response to the Acti-dione was in pH 3.0 (Table IV). Another difference between the two organisms is the amount of stimulation occurring in 0.5 p.p.m. of Acti-dione, it was considerably higher with the spores of Ramularia.

The pH which favored the most stimulations was pH 6.0, even though some stimulation, as with Botrytis, occurred with every pH and at every temperature.

The most noteworthy observation in the humidity tests is that although in many instances no change was noted in inhibition at a given Acti-dione concentration and at a given pH, the tendency was toward higher inhibition at 95% than at 98% relative humidity. This indicates the limiting influence of the humidity upon germination in general. The control results at 95% humidity also were generally higher than at 98%.

CONCLUSIONS

The results of this investigation have shown that Acti-dione is definitely toxic to the spores of the phytopathogenic fungi used in this study. The toxicity varied with the presence of three factors which had an influence upon the response of the organisms to the Acti-dione. The four factors were: concentration of Acti-dione, temperature, pH of the spore suspension medium and humidity. These factors probably exerted an influence independently of each other but also in combination with one another. As a result of this influence, certain trends were observed.

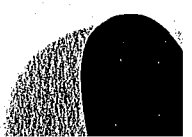
The general tendency was a greater inhibition as the concentration of Acti-dione was increased until a lethal concentration was reached. The concentration at which this was observed varied with the organism and the combination of the three factors present, but the range of this effect varied from 5.0 p.p.m. to 100.0 p.p.m. (see tables and graphs).

Another characteristic of Acti-dione was its stimulatory effect upon the germination of the spores, both in the immersion and the humidity experiments. In general, the greatest

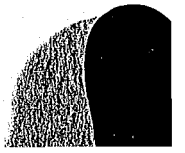
amount of stimulation occurred at 0.5 p.p.m. irrespective of the pH or temperature. The organisms most prone to stimulation at this concentration were Botrytis cinerea followed in order by Ramularia sp. The least amount of stimulation at this concentration of Acti-dione occurred with Monilinia fructicola and Cladosporium cucumerinum. Additional stimulations were observed at 2.5, 5.0, 10.0 and 25.0 p.p.m. primarily with the spores of B. cinerea and to a lesser extent with Ramularia sp.

An investigator desiring to study the effects of Acti-dione under field conditions would have some previous knowledge of its behavior under the influence of the four factors used in this study. He could thus accordingly plan his experiments more efficiently and possibly avoid some errors which might otherwise present themselves in the absence of this information.

If this investigation was to be continued it would be of real interest and of possible practical importance to conduct additional experiments of the same type on more spores of phytopathogenic fungi. The information obtained might prove of great value in combating any number of diseases for which at



present we lack suitable fungicides. Also, it would be not only of interest but of possible practical value to determine exactly how the Acti-dione inhibits the germination of susceptible spores. If this information was known, some adjustment of the influencing factors could be made so that it would become more effective over a wider range of organisms. This might mean addition of other chemicals to control effect of pH and the application of spray under suitable temperatures.

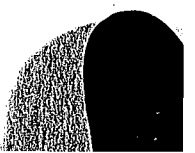


SUMMARY

1. The spores of Cladosporium cucumerinum, Botrytis cinerea, Monilinia fructicola and Ramularia sp. were subjected to a series of tests to determine their response to Acti-dione in various concentrations as influenced by several environmental conditions; temperatures, pH and humidity.

2. The temperatures used were 22°, 24°, 26°, 28° and 30° C. The hydrogen ion concentrations used were 3.0, 4.0, 5.0, 6.0 and 6.8. The relative humidities tested were 98%, 95% and 93%. The latter produced no results.

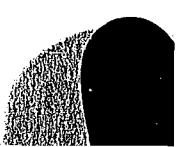
3. In general the lethal action increased along with the increase of Acti-dione concentration. Stimulation was prevalent in all temperatures and H-ion concentrations but varied with the organisms. Most stimulatory effects were observed at 0.5 p.p.m. and were especially prevalent with the spores of Botrytis and Ramularia sp., C. cucumerinum and M. fructicola were stimulated but not to the extent of the other two organisms.



4. Cladosporium cucumerinum was susceptible to Acti-dione in all of the concentrations; lethal results were obtained in concentrations from 5.0 p.p.m. to 50.0 p.p.m. depending upon the temperatures and pH. At 22° C. Acti-dione was more effective at pH 4.0 whereas at 30° C. it was more effective at pH 6.8. No distortion or cytoplasmic plasmolysis was noted even after 72 hours exposure.

5. The spores of B. cinerea were susceptible to the lethal action of Acti-dione but generally at a higher concentration than those of the other three organisms. The minimum lethal dose for total kill varied from 50.0 to 100.0 p.p.m. of Acti-dione. The lethal dose of 50.0 p.p.m. was at pH 6.8 at 30° C. whereas the 100.0 p.p.m. was observed throughout all of the other temperatures and by hydrogen ion concentrations.

Stimulations occurred frequently in several concentrations of Acti-dione at all hydrogen ion concentrations and temperatures. The concentration of Acti-dione at which most of the stimulations occurred was at 0.5 p.p.m. The most favorable hydrogen ion concentrations for the occurrence of stimulation were 4.0, 5.0 and 6.8. Considerable distortion of the



ungerminated and germinated spores and their germ tubes was evident.

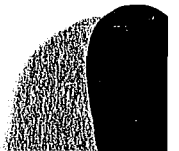
6. Monilinia fructicola was susceptible to lethal doses of Acti-dione varying from 10.0 to 25.0 p.p.m. Total kill at 10.0 p.p.m. was observed at 30° C. at all hydrogen ion concentrations, whereas at all other temperatures and hydrogen ion concentrations total kill was at 25.0 p.p.m. of Acti-dione.

Both distortion and plasmolysis were observed, in the higher concentrations, in the ungerminated and germinated spores and their germ tubes. Stimulation did occur but only at pH 3.0 and at 22°, 24° and 26° C.

7. The spores of Ramularia sp. were subject to inhibition and lethal action as those of the previous three organisms, but were free from distortion in the higher concentrations of Acti-dione. Total kill varied from 50.0 to 75.0 p.p.m. of Acti-dione. Stimulations occurred frequently but not to the extent they occurred with Botrytis. Most stimulations occurred at 0.5 p.p.m. but were also observed at other concentrations of Acti-dione. No one pH was more favorable than another in influencing stimulations.

8. In general the results of the humidity tests were correlated with the immersion tests. A somewhat higher percentage of inhibition was noted however, and it is believed that this was due to the lack of adequate moisture rather than the effect of the humidity upon the Acti-dione. A number of stimulations did occur with every organism usually at the same concentration of Acti-dione, pH and temperature as observed in the immersion tests. Some distortion did occur but was primarily a stunting of the germ tubes which appeared very thin and short.

9. The washing experiments in which treated spores of the organisms were washed after exposure to Acti-dione produced very poor results. Only a tract of the Acti-dione could be recovered from the treated spores. So small was the quantity of recovered Acti-dione that it was negligible.



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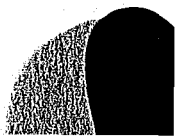

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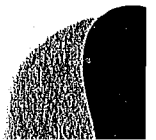

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EFFECT OF CYCLOHEXIMIDE (ACTI-DIONE) ON THE GERMINATION OF SPORES OF SEVERAL PHYTOPATHOGENIC FUNGI

By

Andrew Thanos

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

Year 1952

Approved John R. Vaughan

ANDREW THANOS

ABSTRACT

A number of in vitro tests were conducted in order to determine the effect of Acti-dione under various micro-environmental conditions. Spore suspensions, incorporating various concentrations of Acti-dione, were prepared and studied under a series of temperatures ranging from 22° C. to 30° C. Additional tests were made to determine the effect of pH in connection with each of the above temperatures. A series of washing experiments were made in an attempt to determine how much of the Acti-dione could be recovered from the treated spores. In addition to these, a series of humidity tests were conducted to determine the effect of Acti-dione upon the spores of the same organisms under varying humidities and with various hydrogen-ion concentrations.

The results indicate that in the case of Cladosporium cucumerinum, Acti-dione was most effective in inhibiting germination at 22° C. at pH 4.0 and at 30° C. at pH 6.8. No germination occurred at pH 3.0. Some stimulation of germination occurred at 0.5, 2.5 and 5.0 p.p.m. of Acti-dione at 24° C. and 30° C. No stimulation was observed at all of the other temperatures. No distortion was observed at any treatment.

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ABSTRACT

The spores of B. cinerea were killed at 50.0 and 100.0 p.p.m. depending upon the temperature and pH. Stimulation of germination occurred frequently at all temperatures and hydrogen ion concentrations but principally at 0.5 p.p.m. A considerable amount of distortion and cytoplasmic disorganization was noted in both germinated and ungerminated spores.

M. fructicola spores were completely inhibited at concentrations of Acti-dione varying from 10.0 to 25.0 p.p.m. The former concentration was effective at 28° and 30° C. whereas the latter was most effective at 22°, 24° and 26° C. Some distortion and disorganization was observed in the higher concentrations of the treatments used.

The spores of Ramularia sp. were free from any of the distortions observed with the other organisms. Some stimulations were observed at every temperature and hydrogen ion concentration. Total kill occurred at 50.0 and 100.0 p.p.m. depending upon the temperature and the pH of the solutions in which the spores were immersed.