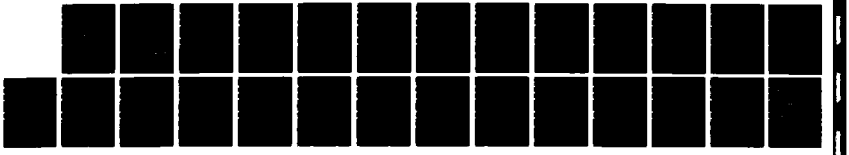
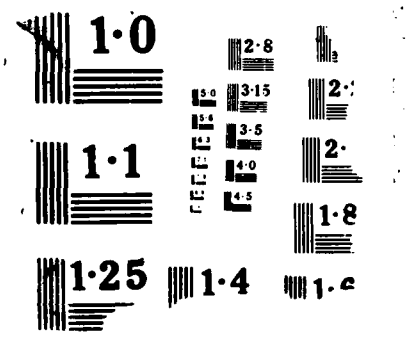
AD-A195 076 FURTHER STUDIES ON TOXIN DETECTION BASED ON THE CO2 PRODUCTION BY YEAST(U) NAVAL RESEARCH LAB WASHINGTON DC P J HANNAN ET AL 13 MAY 88 NRL-MR-6197 1/1

UNCLASSIFIED F/G 6/11 NL




1·0
1·1
1·25
1·4
2·8
3·15
3·5
4·0
4·5
2·
1·8

Naval Research Laboratory
Washington, DC 20375-5000

NRL Memorandum Report 6197

Further Studies on Toxin Detection Based On the CO_2 Production by Yeast

PATRICK J. HANNAN AND SEKETHIA L. SMITH

Navy Technology Center for Safety and Survivability Branch
Chemistry Division

May 13, 1988

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NRL Memorandum Report 6197	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Naval Research Laboratory 6b. OFFICE SYMBOL (*If applicable*) Code 6180	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (*City, State, and ZIP Code*) Washington, DC 20375-5000	7b. ADDRESS (*City, State, and ZIP Code*)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION HQ US Army Medical R&D CD 8b. OFFICE SYMBOL (*If applicable*) SGRD-RMC	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (*City, State, and ZIP Code*) Fort Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO.: 63750A PROJECT NO.: 84PP4813 TASK NO.: SC62608 WORK UNIT ACCESSION NO.

11. TITLE (*Include Security Classification*)

Further Studies on Toxin Detection Based on the CO_2 Production by Yeast

12. PERSONAL AUTHOR(S)
Hannan, Patrick J., and Smith, Sekethia L.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (*Year, Month, Day*)	15. PAGE COUNT
Final	FROM 2/86 TO 8/87	1988 May 13	26

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (*Continue on reverse if necessary and identify by block number*)
FIELD	GROUP	SUB-GROUP	Trichothecene toxins, T-2 [illegible], Roridin, Verrucarin, Detection methods, Yeast Respiration

19. ABSTRACT (*Continue on reverse if necessary and identify by block number*)

Previous studies had shown that certain trichothecene toxins could be detected, in microgram amounts, with baker's yeast as a test organism in a procedure which provided measurements of the CO_2 production rates of the test cultures. The present report concerns refinements of the method and the use of a different yeast, *Kluyveromyces fragilis*, to provide a more sensitive and more rapid detection method for these toxins. The gas exchange method has been modified to include a much higher CO_2-stripping rate which greatly decreases the time required to detect the toxins.

With the new method the minimum detectable amounts of T-2, roridin, and verrucarin are 0.2, 0.25, and 0.06 micrograms, respectively. The times required for detecting these minimal amounts are somewhat less than an hour; however, for amounts just slightly higher than these the detection times are only 3-5 minutes. Because of the all-or-nothing effects of trichothecenes on the CO_2 production rates of yeast, the method is not suitable for making a dose response curve. Its positive aspect is the speed with which is shows the presence of a toxin.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL Patrick J. Hannan	22b. TELEPHONE (*Include Area Code*) (202) 767-2002
	22c. OFFICE SYMBOL Code 6180

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

CONTENTS

FURTHER STUDIES ON TOXIN DETECTION BASED ON THE CO_2 PRODUCTION BY YEAST

INTRODUCTION

The need for a method to provide rapid detection of toxins in the field was demonstrated in the controversy surrounding the use of Yellow Rain in Southeast Asia and Afghanistan. Charges by the U.S. State Department that Yellow Rain (compounds known chemically as trichothecenes) had been used against villagers in Laos and Kampuchea were disputed by many governments and by scientists who were unable to detect the toxins in concentrations other than those to be expected at ambient levels. Undoubtedly a factor in this dilemma was the often substantial time lapse between the alleged attack and the subsequent analyses of the samples. In areas where the monthly rainfall is often 10", it should be expected that substantial leaching of the toxins from equipment or vegetation would have taken place, making detection of the compounds extremely difficult. Obviously, if there were a method which could be used easily in the field it could serve a useful purpose.

An approach to this problem has been described in an earlier report (1) concerning the effect of toxins on the $C0_2$ production rate of baker's yeast. It was shown that 2 micrograms of verrucarin, for example, could be detected in a test requiring only a half hour. The conventional method for toxin detection based on the growth of yeast requires an overnight incubation period since it depends on a comparison of the biomass of a control vs a treated sample. By measuring rates of $C0_2$ production, a comparison between two cultures can be made in a matter of minutes. Multiple examples of gas exchange studies are contained in the report by Hannan and Jones (2).

The earlier report on toxin detection (1) was based on only one yeast, viz baker's yeast which is available in all grocery stores. One advantage of such a test organism is its universal availability, and in all the previous experiments conducted with it the starting point was a freshly prepared culture containing a known weight of this dry yeast. It was shown to be sensitive to several trichothecenes and also to other toxins including patulin. PR, ochratoxin, and rubratoxin. During the course of that study it came to our attention that another yeast, Kluyveromyces fragilis, was particularly sensitive to trichothecenes (3). Researchers at the University of Saskatchewan, in Saskatoon, Canada, had found this organism to be the most sensitive of the 72 yeast species they had assayed with trichothecenes. Following correspondence by mail and phone, one of us (PJH) was invited to spend several weeks in Saskatoon to study the merits of the gas exchange approach with K. fragilis as the test organism. It is a pleasure to acknowledge here the extreme

Manuscript approved January 28, 1988.

cordiality of Dr. George Khachatourians and graduate student, Heather Khoshinsky, during this stay in Saskatoon.

This report describes the results obtained with K. fragilis including the detectable limits of several trichothecenes in subsequent work at NRL. A report of the results obtained during the visit to Saskatoon is being submitted to a technical journal. Included in the current study are the results obtained with a new culture chamber permitting high gas flow rates through the culture, the change in sensitivity of the method when the toxin in question is on a leaf sample vs direct addition to the culture, and improvements in sensitivity obtained with Breathing Air as the gas used to scrub the $C0_2$ from the test culture.

MATERIALS AND METHODS

Organisms:

Most of the experiments described in this report were performed with Kluyveromyces fragilis derived from the culture supplied by the researchers at Saskatoon. Agar slants were inoculated under sterile conditions and maintained in the refrigerator until use. Other experiments were performed with baker's yeast (Red Star Double Acting) purchased from the grocery store.

Culture Media:

Two media, differing only in the presence or absence of peptone, were used in the preparation of the test cultures. The full medium had the following composition:

Yeast extract	25 ml of a 4% stock solution
Glucose	2 grams
Peptone	1 "
Distilled water	75 ml

An inoculum of K. fragilis was placed in the above medium and cultured overnight to produce what will be referred to hereafter as the overnight culture. The same medium, but without peptone, was used for preparing what will be referred to as the test culture. Peptone was omitted from the latter because of the problems associated with foaming resulting from the fast bubbling used in the test.

Culture Conditions:

Most of the experiments in this series were performed with K. fragilis and began with the inoculation of a 50 ml volume of sterile culture medium in a 125 ml Erlenmeyer flask shaken overnight at 35 C. Unless stated otherwise there was no aeration of the flask during this time. On the following day about 1.5 ml of this overnight culture was added to 15 ml of fresh medium to give an optical density of approximately 0.26 at 610 nm. This suspension was then placed in a 50 ml graduate cylinder and N_2 bubbles from a glass frit were passed through rapidly for exactly three minutes to remove the previously formed $C0_2$. Immediately thereafter 2.0 ml portions were transferred by pipette to each of four test tubes, 18 x 148 mm, which were fitted with rubber stoppers drilled out to accommodate two sections of 3 mm OD glass tubing, one as an inlet and the other an outlet. To obtain fine bubbles for scrubbing out the gases dissolved in the culture, the bottom portion of a Pasteur pipette was attached with rubber tubing to the 3 mm glass inlet tube. The

flexibility afforded by the rubber connecting the two pieces of tubing minimized any problem of breakage of the glass tubing. Four bubblers were positioned in a bath thermostatted at 33° C and were connected by rubber tubing to needle valves mounted in a pipe manifold which could be connected to a laboratory compressed air system or to cylinders of either N_2 or Breathing Air (Air Products Co.). The $C0_2$ content of the Breathing Air was 3 ppm. Rubber tubing from each outlet could be connected to the inlet of a Beckman $C0_2$ analyzer; a flowmeter was connected to the outlet of the analyzer to provide a measure of the flow rate through each culture, usually 180 cc/minute. Sequential measurements of the $C0_2$ content of each of the four tubes could be made every three minutes so that in a typical experiment it was possible to plot the $C0_2$ production of each of four culture tubes with a data point for each one every 12 minutes. Momentary changes in gas flow rate required a delay in making a reading of the $C0_2$ analyzer since the $C0_2$ analysis is relevant only if it represents an equilibrium between the culture and the gas passing through it.

For the several experiments performed with baker's yeast the conditions used in each experiment will be described in the test.

$C0_2$ Analyses:

The $C0_2$ analyses were performed with a Beckman Model 864 Infrared Analyzer. This instrument provides a real time measurement of the $C0_2$ content of a gas stream passing through it. Three sensitivity ranges are provided, the most sensitive being 0-500 ppm; however, for the purpose of these experiments the instrument was adjusted to read 250 ppm full scale. To do this the intermediate sensitivity setting was used initially and the span adjusted so that a given concentration of $C0_2$ gave a reading equivalent to double that amount, following which the sensitivity setting was returned to the original. Standard gases supplied by Air Products Corp. were used to check the accuracy of this procedure and the agreement was within 5%. Such a discrepancy is not serious because the principal object was to determine differences between the $C0_2$ concentrations of gases from cultures in a given test. Absolute $C0_2$ concentrations were less important.

For optimum performance of the $C0_2$ analyzer all readings should be made at a constant gas flow rate. Therefore, even when the instrument was not in use air was passed through it at the 180 cc/minute rate used during the actual CO_2 measurements. The $C0_2$ production rates shown in this report are a product of the gas flow rate times the increase in $C0_2$ concentration of the gas as a result of its passage through the culture.

Toxins:

All the toxins were purchased from Sigma Chemical Co.. St Louis, Mo.. Stock solutions containing 1 mg/ml in methanol were stored in the refrigerator, and when dilutions were required they were made immediately before use to minimize adsorption of the toxin to the surfaces of the container.

RESULTS

Since the initial purpose of this study was to demonstrate the sensitivity of K. fragilis to trichothecenes, the first results described will be those obtained with

this organism under the standard conditions. Ultimately the research was extended to include different conditions which enhanced the sensitivity of the test and in that phase several experiments with baker's yeast will be included.

Sensitivity of K. fragilis to T-2, Roridin, and Verrucarin:

With baker's yeast as the test organism the minimum detectable amounts of T-2, roridin, and verrucarin were 3-, 3-, and 2-micrograms respectively. With K. fragilis the detectable limits were considerably less as shown in the first three figures. T-2 was assayed at 0.2-, 0.3-, and 0.4-micrograms with the results shown in Figure 1. The effect of 0.4 microgram could be seen in the first CO_2 production measurement, at 20 minutes, while a longer time was necessary to ensure that inhibition was also taking place with 0.2 and 0.3 micrograms. The difference between 0.2 microgram and the control was too slight to be a certainty until after 40 minutes.

Roridin had approximately the same toxicity as T-2. After some preliminary experiments it was decided to test the effects of 0.125-, 0.250-, and 0.375 micrograms with the results shown in Figure 2. With the first measurement made of the culture containing 0.375 micrograms at 14 minutes, it was obvious that there was inhibition. With 0.250 microgram the difference between it and the control was slight for an appreciable period but by the end of an hour the pattern seemed to be clear. If the test had been extended longer there may have been a real difference between the 0.125 microgram culture and the control, but without further refinements it would not be justified to set the minimum detectable limit for roridin at less than 0.250 microgram.

Verrucarin is consistently the most toxic trichothecene in these studies, and Figure 3 represents the data obtained with 0.063-, 0.125-, and 0.188 micrograms. *Even the culture containing only 0.063 microgram was inhibited.*

Each of the experiments summarized in the first three figures was conducted with different dosages than might be used in typical efforts to determine minimum detectable amounts. This was because corollary experiments were underway to determine minimum detectable amounts when the toxins were placed on specimens of Ficus leaves. The background for this is that Ficus is found often in Southeast Asia and, since the purpose of this investigation had been to develop a procedure useful for detecting toxins quickly in the field, there was interest in knowing how much needed to be present on a leaf in order to be detected. The experiments with diacetoxyscirpenol, described next, provide an insight into the typical differences to be found when toxins are applied directly to a yeast culture compared to when they are on a leaf.

Toxicity of Trichothecenes When on a Leaf and When Added to the Culture:

Applications of trichothecenes to leaf samples were performed in the following manner. A white adhesive disc, 8.5 mm OD, was placed on the under side of a Ficus leaf after which the leaf was placed on a lighted surface so that the limits of the disc could be seen readily; then 2- or 3-microliter portions of the toxin solution to be tested were spread over the area defined by the disc. After the solvent had evaporated, a hole puncher was used to cut out a 6 mm diameter section of leaf. Figure 4 contains plots showing the effects of DAS added directly to the culture and when added in the form of a treated leaf. The plot on the left shows that the effect of 3 micrograms is apparent when added directly to the culture; the right hand plot shows that double that amount was needed to be detectable on a leaf.

However, because the surface area of the 6 mm portion of the disc is about half that over which the toxin was spread, the activity of the toxin on the leaf is about the same as that when added directly to the culture. Figure 5 represents the inhibition of K. fragilis by leaf specimens coated with various amounts of T-2, roridin, and verrucarin. It shows that the leaf coated with 0.6 microgram T-2 was not inhibitory whereas in earlier work (Figure 1), 0.2 microgram provided inhibition when added to the culture as a solution of methanol. It is interesting to note that the leaf specimens containing roridin and verrucarin showed the same inhibition of $C0_2$ production. More will be said of this in the Discussion.

Effects of Aerobic Environment on CO_2 Production by K. fragilis

The standard procedure used in growing the yeast culture overnight was to place an inoculated flask in a shaker maintained at 35°C; when that culture was then made into a test culture having the required optical density, nitrogen was the carrier gas bubbled through the culture to scrub out its $C0_2$. There were reasons for using these procedures which need not be detailed here but it seemed desirable to learn whether there might be benefit in using other conditions. Presumably, during the overnight culturing there would be a lessening of the dissolved 0_2 content in the culture, and during the experiment involving $C0_2$ production measurements the use of N_2 bubbles would reduce the dissolved 0_2 to near zero. A series of experiments was performed in which K. fragilis cultures were subjected to different conditions. Cultures were either aerated overnight with Breathing Air or else unaerated; subsequently the $C0_2$ scrubbing of the test cultures was done either with N_2 or Breathing Air. Each experiment included a control culture and a culture containing a leaf specimen coated with 0.188 microgram of verrucarin. In the experiments designated in Figure 6 by A and B, Breathing Air was bubbled through the overnight culture; with A the carrier gas used for the $C0_2$ production measurements was N_2 and for B it was Breathing Air. In both cases there was a detectable difference between the control and the toxin treated culture, but the $C0_2$ production rates of the controls were not enhanced by the use of Breathing Air overnight. In the experiments designated as C and D there was no aeration overnight; with C the carrier gas was N_2 and with D it was Breathing Air. The combination of no overnight aeration followed by Breathing Air as the carrier gas produced by far the highest $C0_2$ production rate of the control. In each case the $C0_2$ production of the verrucarin-treated culture was rather constant, but the difference between it and the control in Plot D was so great as to be detectable immediately.

Figure 7 shows the results of another study on the effects of overnight culture conditions. Toxin treated leaves were assayed in cultures prepared from overnight cultures that were either un-aerated (A) or aerated with N_2 (B). In both cases Breathing Air was the carrier gas. Once again the $C0_2$ production rate was higher with the un-aerated culture, and any toxic effects (in this case with roridin and verrucarin) were apparent almost immediately.

Verrucarin had always been the most toxic compound in these studies. To determine what the detectable limit would be under the most favorable circumstances, the toxin was added directly to a culture prepared from one which had not been aerated overnight and then scrubbed with Breathing Air for the $C0_2$ production study. The results (Figure 8) indicate that as little as 0.03 microgram could be detected in 35 minutes or less, but 0.01 microgram was not detected in more than an hour.

Studies with Baker's Yeast

The earliest experiments in this study, reported in Ref. 1, were based on the use of baker's yeast as the test organism since the emphasis at that time was on the development of a field method where the availability of a test organism was paramount. In all of those experiments the yeast suspensions were made with dry yeast, the concentration being 0.25 mg/0.8 ml suspension. By contrast all of the experiments with K. fragilis began with yeast suspensions that had been cultured overnight. What would happen if the baker's yeast were first cultured overnight before being made into a test culture? A comparison was made, therefore, between two cultures of baker's yeast, both having an optical density of 0.26 but one made with dry yeast and the other from an unaerated overnight culture. Each test included a culture with 2 micrograms of verrucarin since that had been considered the minimum detectable amount with baker's yeast as the test organism. Figure 9 compares the results obtained with the culture prepared from the one that had been grown overnight (A) with one prepared from dry yeast (B). The conclusions were as follows:

a. The $C0_2$ production of the control is enhanced greatly with the use of a culture that has first been grown overnight.

b. The toxic effect of the verrucarin is apparent much sooner with the culture derived from the previously grown culture than with the dry yeast.

c. The actual $C0_2$ production rates of the verrucarin treated cultures are rather similar in both cases but the rate is decreasing in one and increasing in the other.

To determine the minimum detectable amount of verrucarin required to inhibit baker's yeast derived from an overnight culture, another experiment was performed with 0.5 to 1.5 micrograms of verrucarin (Figure 10). Clearly, even the 0.5 microgram culture inhibited $C0_2$ production though it did not become apparent until after about 50 minutes. That was the same time required for 2 micrograms to show an effect when the test culture was prepared from dry yeast.

DISCUSSION

The beauty of the gas exchange approach in monitoring the growth of microorganisms is that it provides an almost instantaneous measure of a culture's performance. No other method can give such a continuous measure of growth. For the maximum effectiveness of the method one should provide a fast stream of small bubbles rising through a culture so that each bubble is in contact with the liquid for an appreciable time; the smaller the bubble. the higher the surface/volume ratio, therefore the greater the efficiency in scrubbing out dissolved gases. With yeast cultures which have a tendency to foam, it is not practical to aerate a long column of liquid because the yeast would be entrained by the bubbles and removed from the system. The use of 18 x 184 mm test tubes containing only 2 ml of culture, with bubbles supplied through the tip of a Pasteur pipette, is a good compromise in that a high gas flow rate can be used without the loss of any culture. Each bubbler has its own flow characteristics, and with Pasteur pipettes it was possible to maintain steady gas flow rates of about 180 cc/minute with a back pressure of 8 psi. At this flow rate only two minutes were required for the $C0_2$ analyzer to give a reading which was representative of the gas composition within the bubbler being aerated.

With a high gas flow rate the advantage is that the time delay between a change in metabolic rate of the culture and its reflection in the reading of the $C0_2$ analyzer is minimal. The disadvantage could be that the difference in $C0_2$ content between the inlet and exit gases might be undetectable. With the conditions used here that was not the case because even with the minimum $C0_2$ production rates in this study the change in reading of the $C0_2$ analyzer was 15% of the full scale.

These experiments demonstrated, as expected, the extreme sensitivity of the yeast, K. fragilis, to the trichothecenes. Any listing of minimum detectable amounts must be related to the length of time one is willing to run a test; with K. fragilis as the test organism and with Breathing Air as the carrier gas during the scrubbing of $C0_2$ from the culture, the detectable amounts of toxins and the times required are as shown in Table 1. These minimum detectable values are approximately the same as those obtained in a typical overnight experiment involving treated discs placed on an inoculated agar. In the $C0_2$ production experiments, if the amount of toxin present is even just slightly more than the minimum detectable amount, then the time required for inhibition to be apparent is 10 minutes or less. This raises an interesting point, viz that with each of the yeasts used in this study there appears to be a basal $C0_2$ production which persists even in the presence of excessive amounts of toxin. In one experiment with baker's yeast, the same $C0_2$ production rate was obtained with 100 micrograms of verrucarin as there had been with just 5. For this reason the method does not permit the construction of a dose response curve relating inhibition to amount of toxin present. If the minimum detectable amount of toxin were known, of course, one could tell by the use of serial dilutions the amount of toxin present in a sample.

Trichothecenes were the only toxins used in this particular study. They are known to be powerful inhibitors of protein synthesis and, perhaps, this is the mechanism being affected in yeasts. At initiation of protein synthesis in eucaryotes, ribosomal subunits join the messenger RNA at the initiation region, involving a process which includes several initiation factors and peptidyl transferase, which is to form the first peptide bond. Elongation then occurs whereby amino acids are added sequentially to form a growing chain. It is possible, of course, that the trichothecenes have more than a single mechanism of action.

In the previous two paragraphs certain shortcomings of the method described here have been pointed out. Mention should be made also of a unique advantage, i.e. that there need not be any workup of a field sample before it is assayed by the yeast method. If a leaf or other form of vegetation is suspected of being coated with a toxin, then that sample can just be added to the test culture to see what effect it has. For screening purposes in the field, that could be very important. For the absolute identification of a toxin the use of mass spectrometry, or other analytical method, is preferred but would entail a considerable workup of the field samples prior to the analyses.

For detecting the difference between a toxin treated culture and a control, it is best that conditions be used to maximize the $C0_2$ production rate of each. This is illustrated in Figure 6 which summarizes the data from four experiments in which the overnight culture conditions and the use of Breathing Air or N_2 as the carrier gas were investigated. In all cases the same concentration of toxin was used, the effect being most noticeable when there was no aeration of the overnight culture followed by the use of Breathing Air as the carrier gas. At this writing, the reasons for this optimal set of conditions are unknown. It is known, however, that

CO_2 production of yeasts can be independent of whether the cultures are being scrubbed with N_2 or air so long as there is a surfeit of pre-formed sterols and fatty acids present in the yeast.

In interpreting any of the graphs shown in this report the determinant regarding the toxicity of a treated culture is the shape of its CO_2 production curve relative to that of the control. The absolute CO_2 production value at any time is so dependent on the bubble size of the gas used to scrub the culture that it should not be compared directly with another culture. It would be possible to have parallel plots, but differing considerably in magnitude, in cultures having exactly the same CO_2 production rates, the only difference being in the bubble distribution in the two plots. For any given culture it can be presumed that the bubble distribution is constant, therefore a reduction in CO_2 production could only be ascribed to a lowered rate.

Verrucarin is the most toxic compound used in this study, being detectable at only 0.06 microgram. Stated in other terms this would be 30 parts per billion or 6.4×10^{8} molar.

After spending several years on the development of the methodology described here, some comments on the pro's and con's are in order. Some of the advantages are:

1. Speed: Direct comparisons between a control culture and one containing a toxin can be definitive in just a few minutes if the amount of toxin present is appreciable. If the minimum detectable amount is all that is present, the test might require 30 minutes or more.

2. Organism availability: For laboratory work a single culture can be maintained in a refrigerator for several months. For field work, dry yeasts could be kept indefinitely but the sensitivity would suffer from the use of dry yeast rather than one which had been cultured overnight. Compared with other potential test organisms, such as guinea pigs or rabbits, the yeasts would be extremely convenient.

3. No workup required: For field use, vegetation samples can be added directly to the test culture. This would have been valuable in the situations in Laos and Kampuchea where there were long delays between collection of field samples and their analyses.

Some of the disadvantages are the following:

1. Dose response: The method does not lend itself to constructing a dose response curve. Either there is inhibition or there isn't, except in those situations where the minimum detectable amount is being tested, in which case the divergence in CO_2 production rate of a toxin-treated culture relative to a control might be slow.

2. Lack of specificity: With just one organism it is not possible to determine the nature of the toxin being tested. It is entirely conceivable that, with a spectrum of test organisms which provide a varied response for each toxin, there could be an identity of the toxin.

3. Need for trained personnel: It would be necessary for the operator to be familiar with the $C0_2$ analyzer so that it could be properly calibrated before use, that the gas flow rate be kept constant. etc.. However, this requirement would not be nearly so demanding as that for a mass spectrometer.

REFERENCES

1. Hannan, P.J., and Smith, S.L., "The Detection of Toxins by a New Method Based on the $C0_2$ Production by Yeast", NRL Memorandum Report 5864, Sept.. 1986.

2. Hannan, P.J., and Jones, D.S., "Advantages of the Gas Exchange Approach to Micro-biological Studies". NRL Memorandum Report 5744, April, 1986.

3. Sukroongreung, S., Schappert, K.T., and Khachatourians, G.G., "Survey of Sen-sitivity of Twelve Yeast Genera Toward T-2 Toxin, Appl. & Env. Microbiol., 48, 416, 1984.

ACKNOWLEDGEMENT

The authors wish to express their thanks to Dr. Homer Carhart for his continued interest and support in this study. They also wish to thank Dr. James Murday for his help in the publication of this report.

Table 1. Estimated Minimum Detectable Amounts, and Times Required, for Assays Based on Kluyveromyces fragilis

Toxin	Micrograms	Minutes
T-2	0.2	36
	0.3	9
	0.4	3
Roridin	0.125	90
	0.250	50
	0.375	25
Verrucarin	0.03	25
	0.06	20
	0.19	3
Diacetoxyscirpenol	3.0	35
	4.0	25

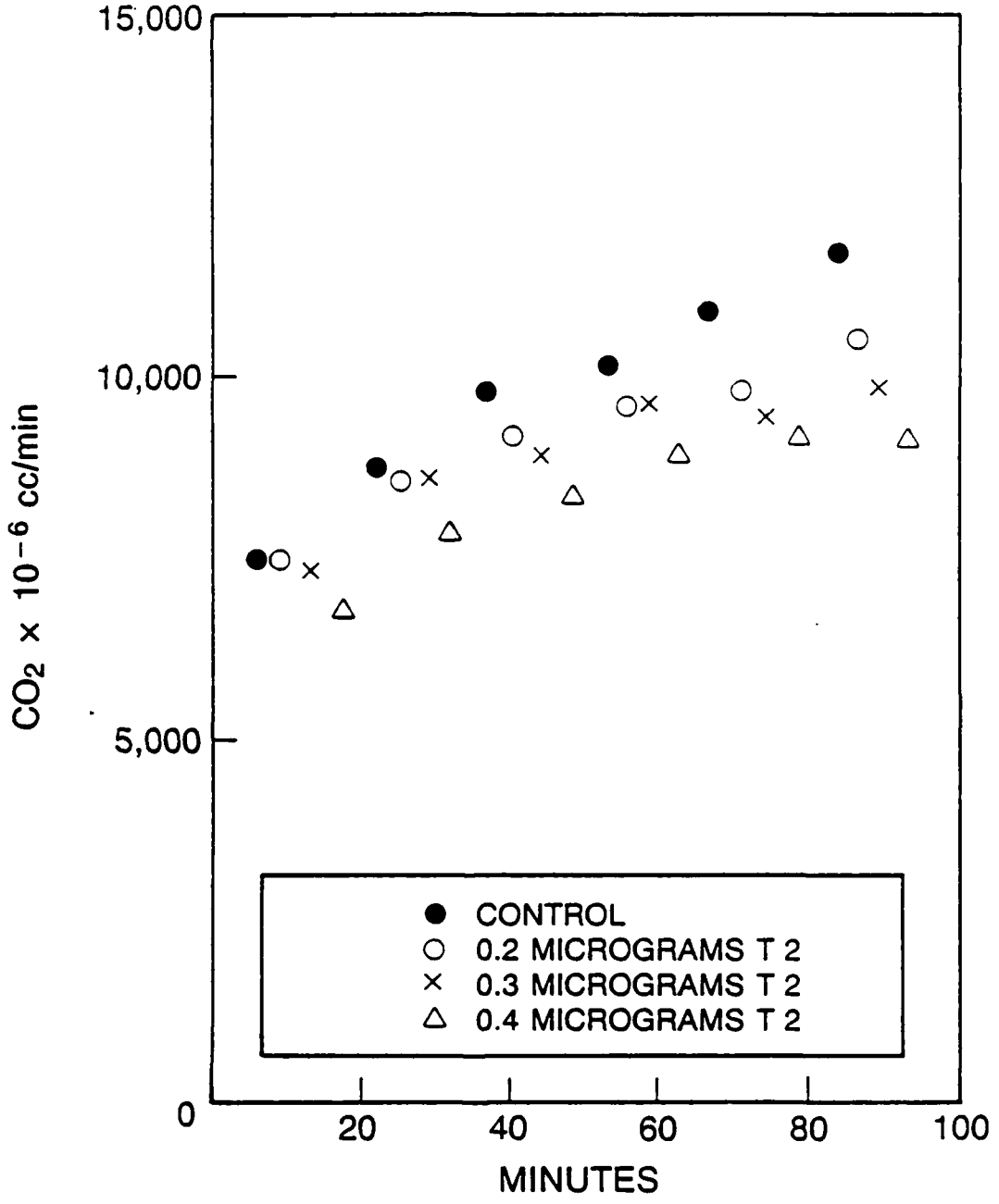


Figure 1. The effect of various amounts of T-2 on the CO_2 production rate of K. fragilis, with N_2 as the carrier gas. (●) Control, (o) 0.2 mcg, 0.3 mcg, (△) 0.4 mcg.

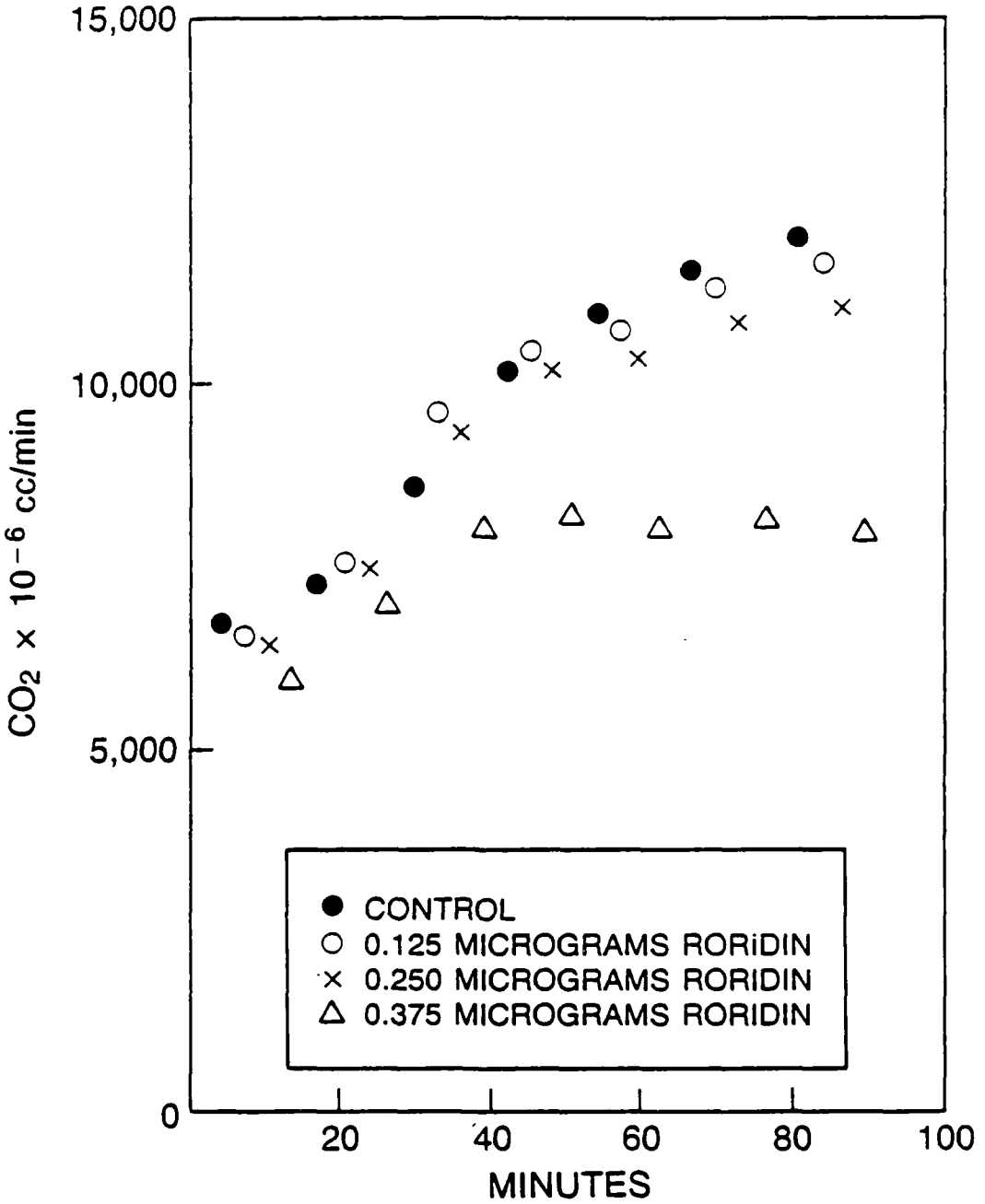


Figure 2. The effect of various amounts of roridin on the $C0_2$ production rate of K. fragilis, with N_2 as the carrier gas. (x) Control, (o) 0.125 mcg, (●) 0.250 mcg, (Δ) 0.375 mcg.

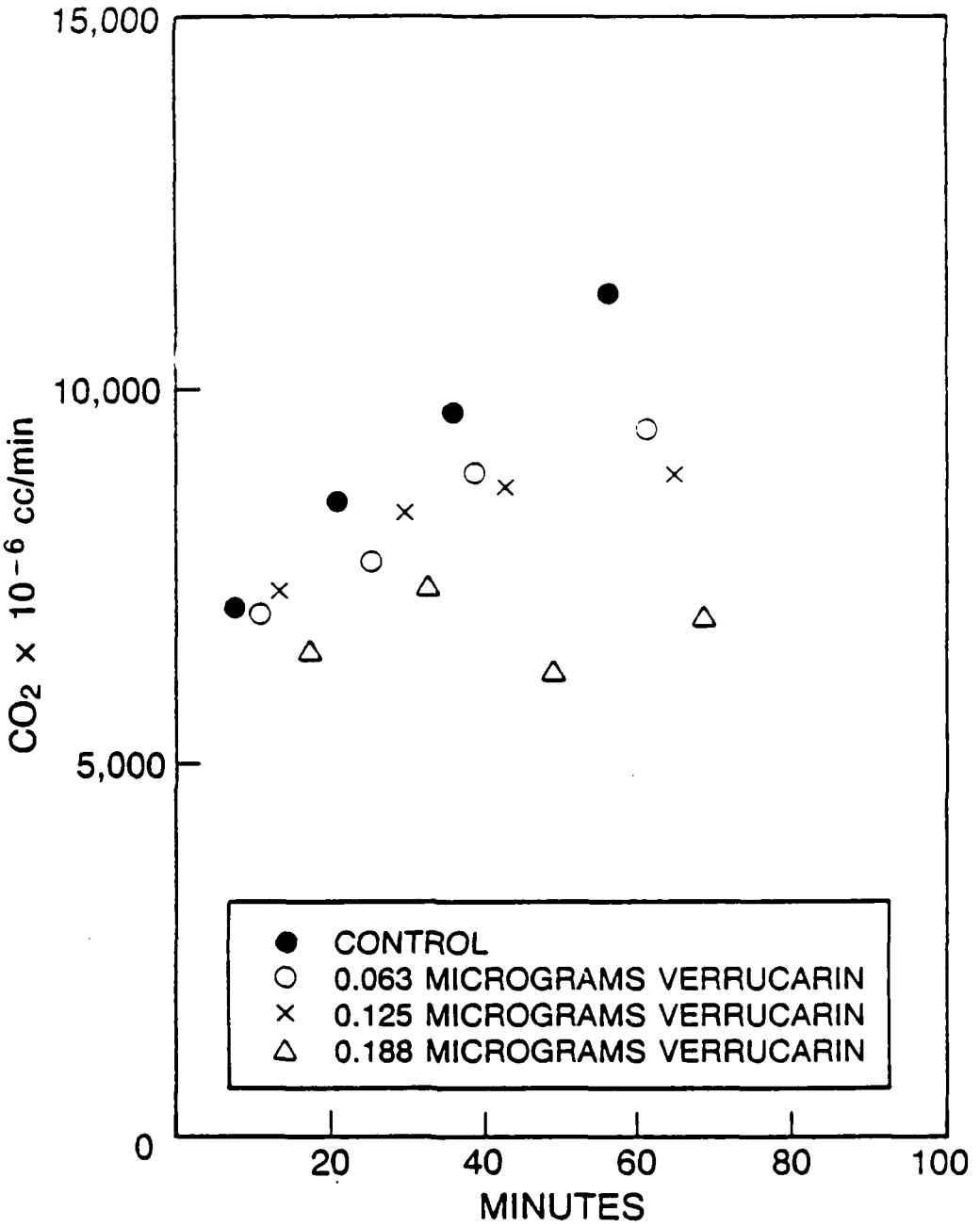


Figure 3. The effect of various amounts of verrucarin on the $C0_2$ production rate of K. fragilis, with N_2 as the carrier gas. (●) Control, (o) 0.063 mcg, (x) 0.125 mcg, (Δ) 0.188 mcg.

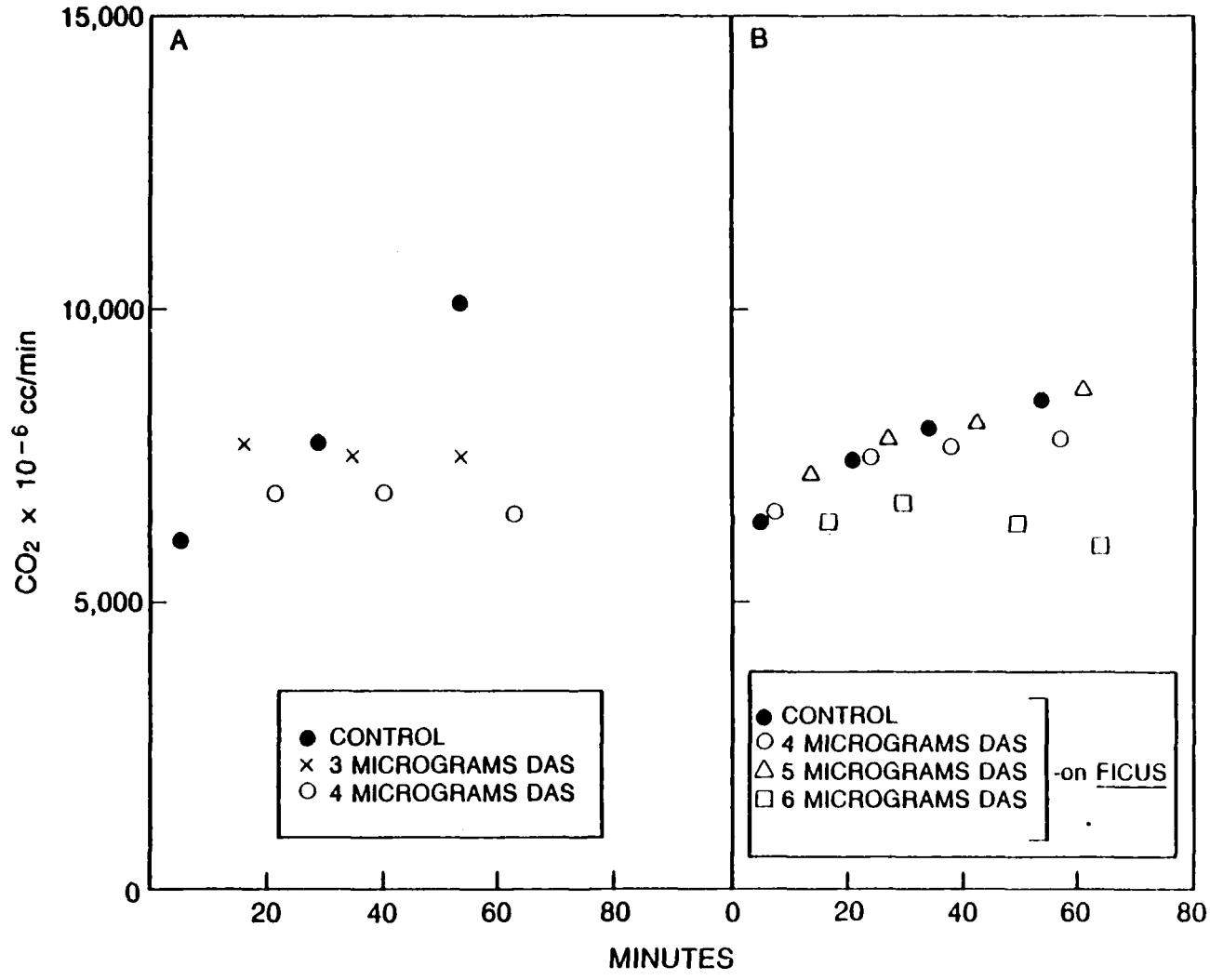


Figure 4. The effect of diacetoxyscirpenol (DAS) on the $C0_2$ production rate of K. fragilis, with N_2 as the carrier gas. A: DAS added directly to the culture, (●) Control, (x) 3 mcg, (o) 4 mcg. B: DAS applied to Ficus leaves, (●) Control, (o) 4 mcg, (△) 5 mcg. (●) 6 mcg.

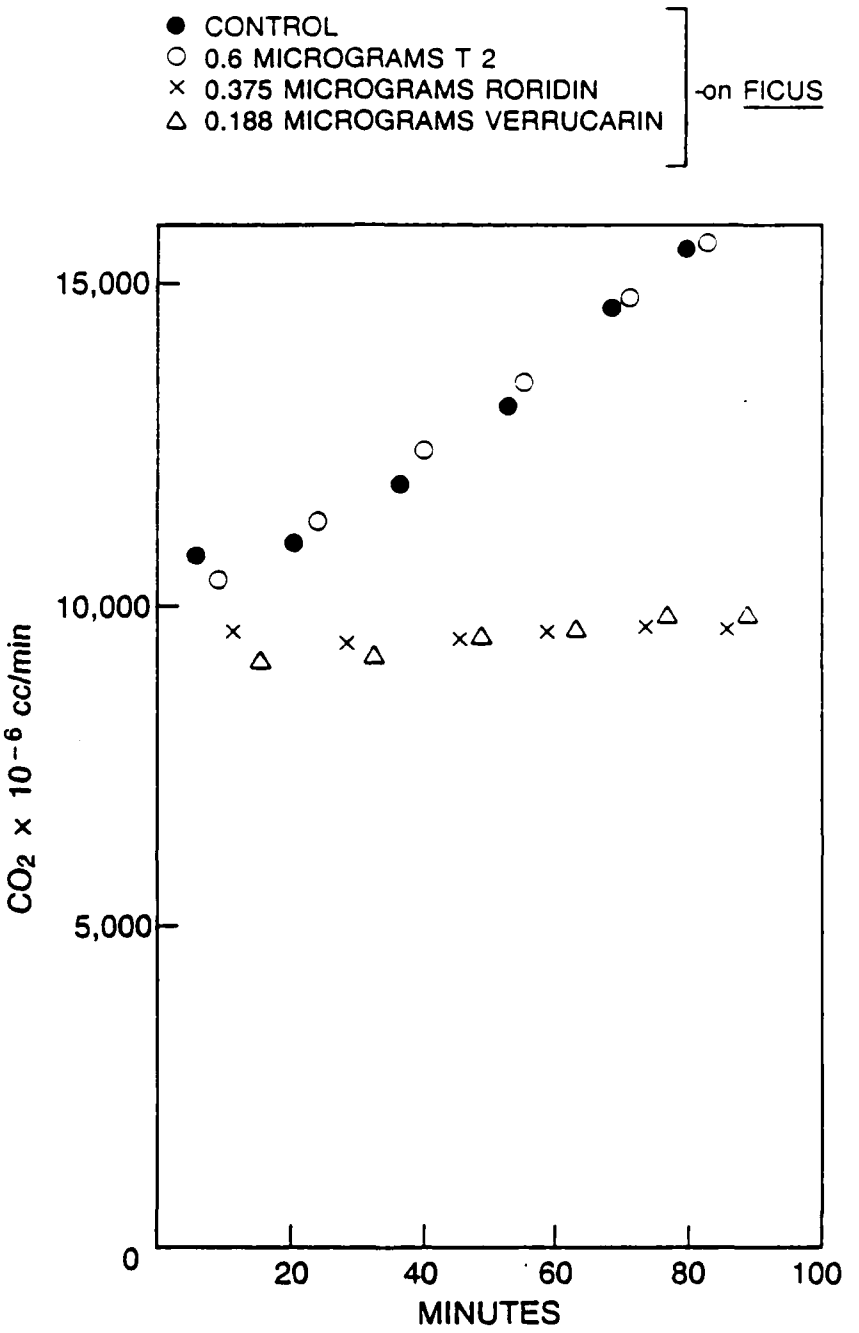


Figure 5. The effects of various amounts of trichothecenes, applied to Ficus leaves, on the C02 production rate of K. fragilis with N_2 as the carrier gas. (●) Control, (o) 0.6 mcg T 2, (x) 0.375 mcg roridin, (△) 0.188 mcg verrucarin.

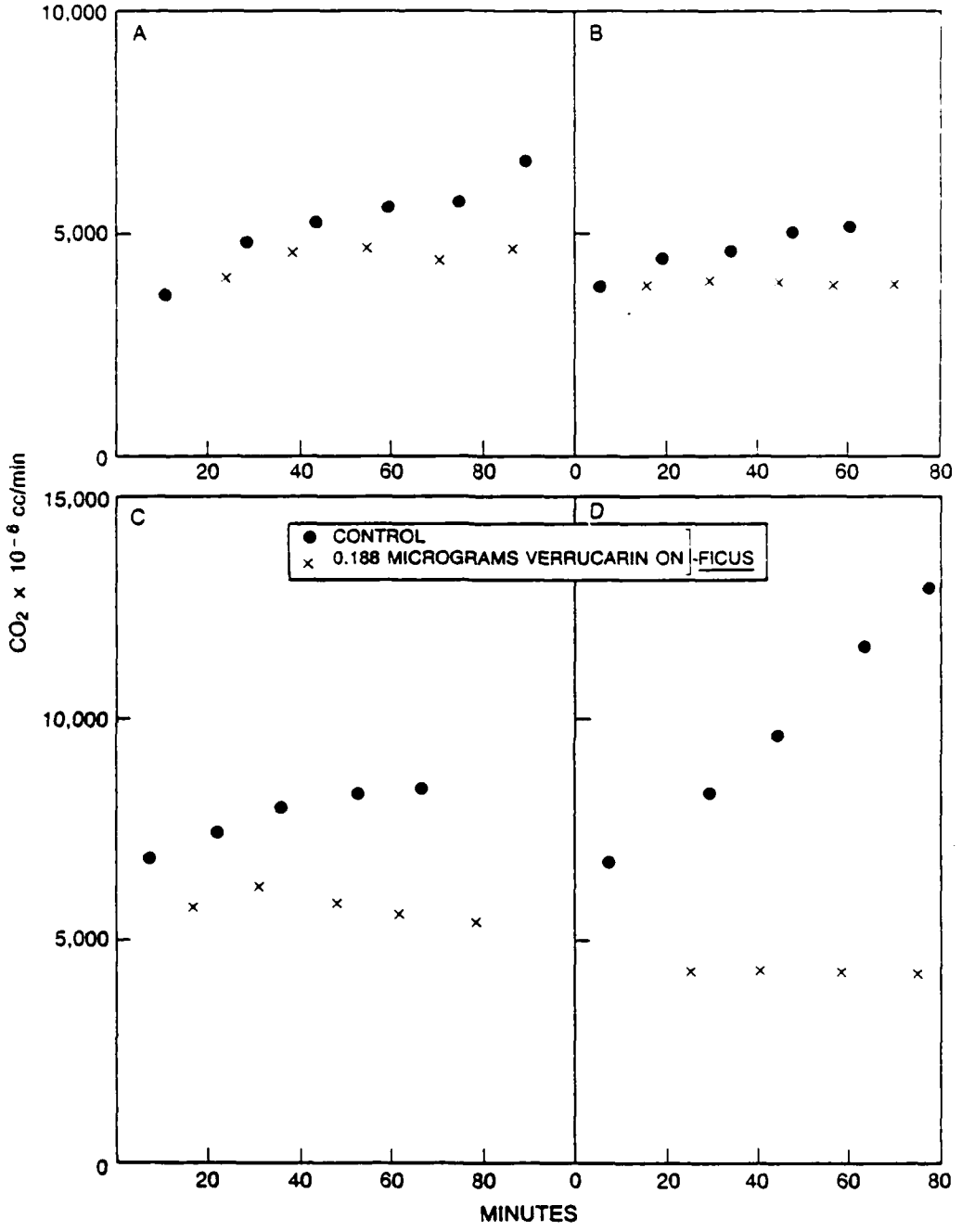


Figure 6. The effect of 0.188 mcg verrucarin, applied to Ficus leaves, on the CO_2 production rate of K. fragilis. A: Breathing Air bubbled through culture overnight, followed by N_2 as carrier gas. B: Breathing Air bubbled through culture overnight, followed by Breathing Air as carrier gas. C: No aeration overnight. followed by N_2 as carrier gas. D: No aeration overnight, followed by Breathing Air as carrier gas.

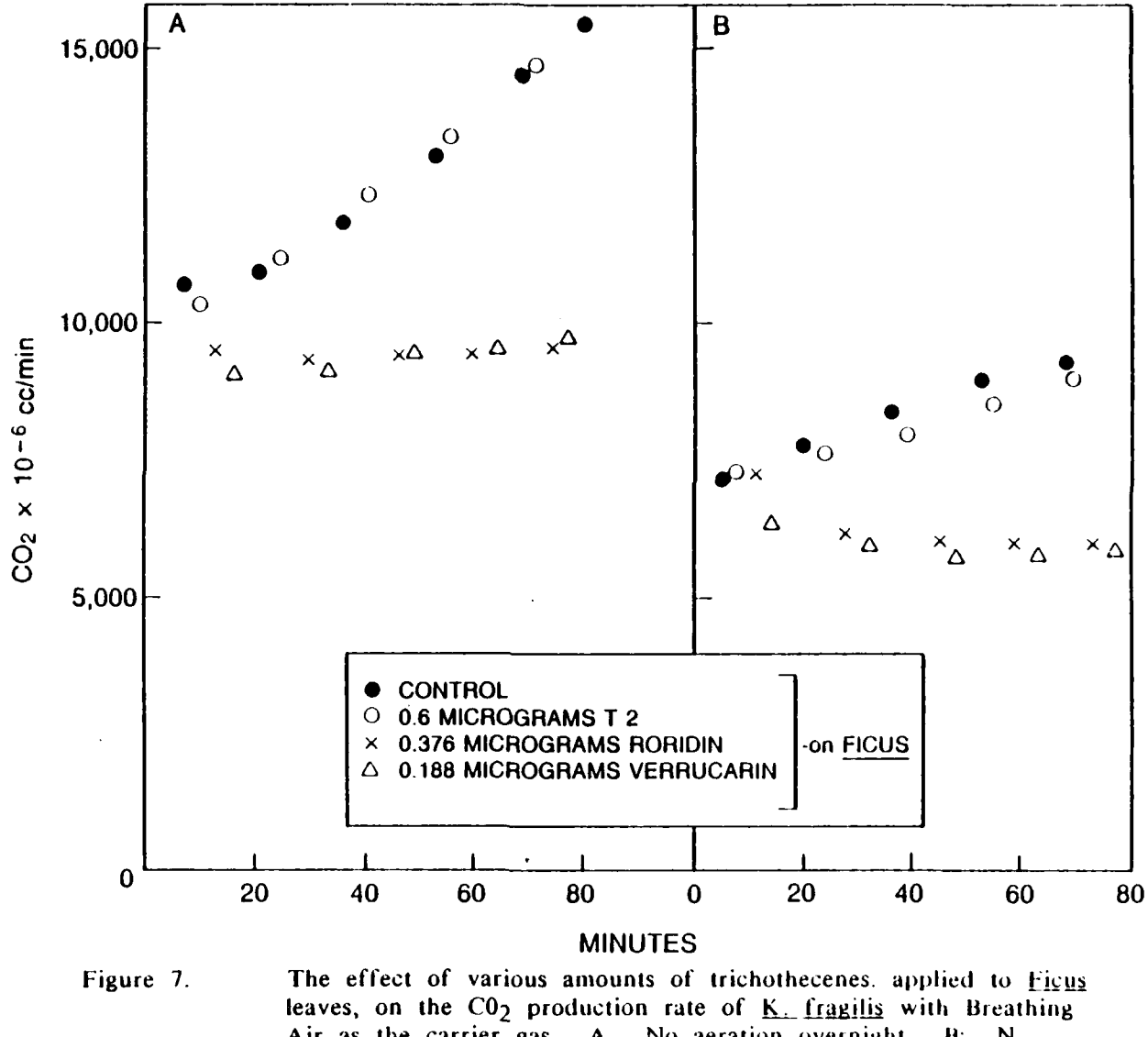


Figure 7. The effect of various amounts of trichothecenes, applied to Ficus leaves, on the $C0_2$ production rate of K. fragilis with Breathing Air as the carrier gas. A. No aeration overnight. B: N_2 bubbled through culture overnight. (●) Control, (o) 0.6 mcg T 2, (x) 0.375 mcg roridin, (Δ) 0.188 mcg verrucarin.

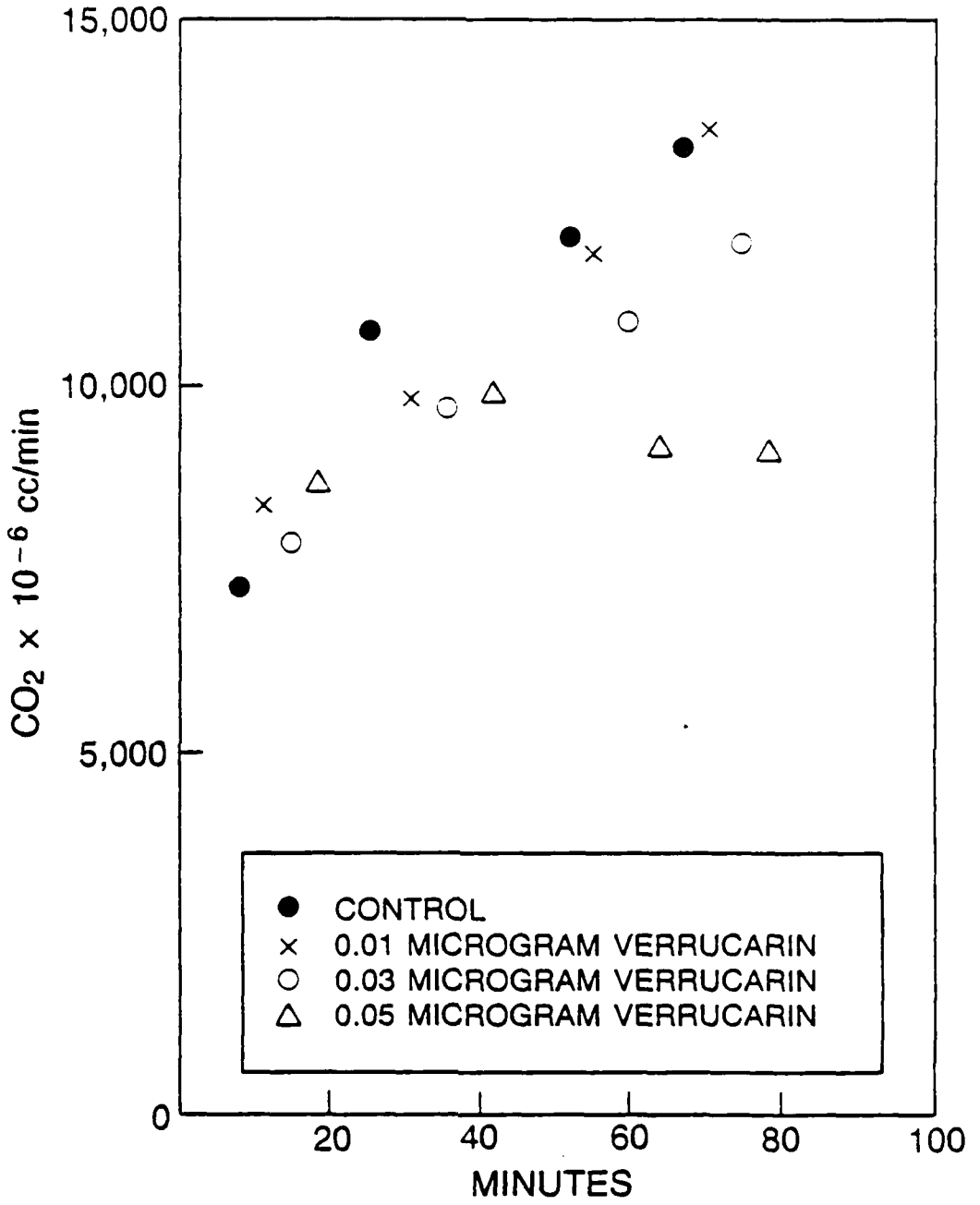


Figure 8. The effect of various amounts of verrucarin on the CO_2 production rate of K. fragilis; no aeration overnight, followed by Breathing Air as the carrier gas. (●) Control, (x) 0.01 mcg, (o) 0.03 mcg, (Δ) 0.05 mcg.

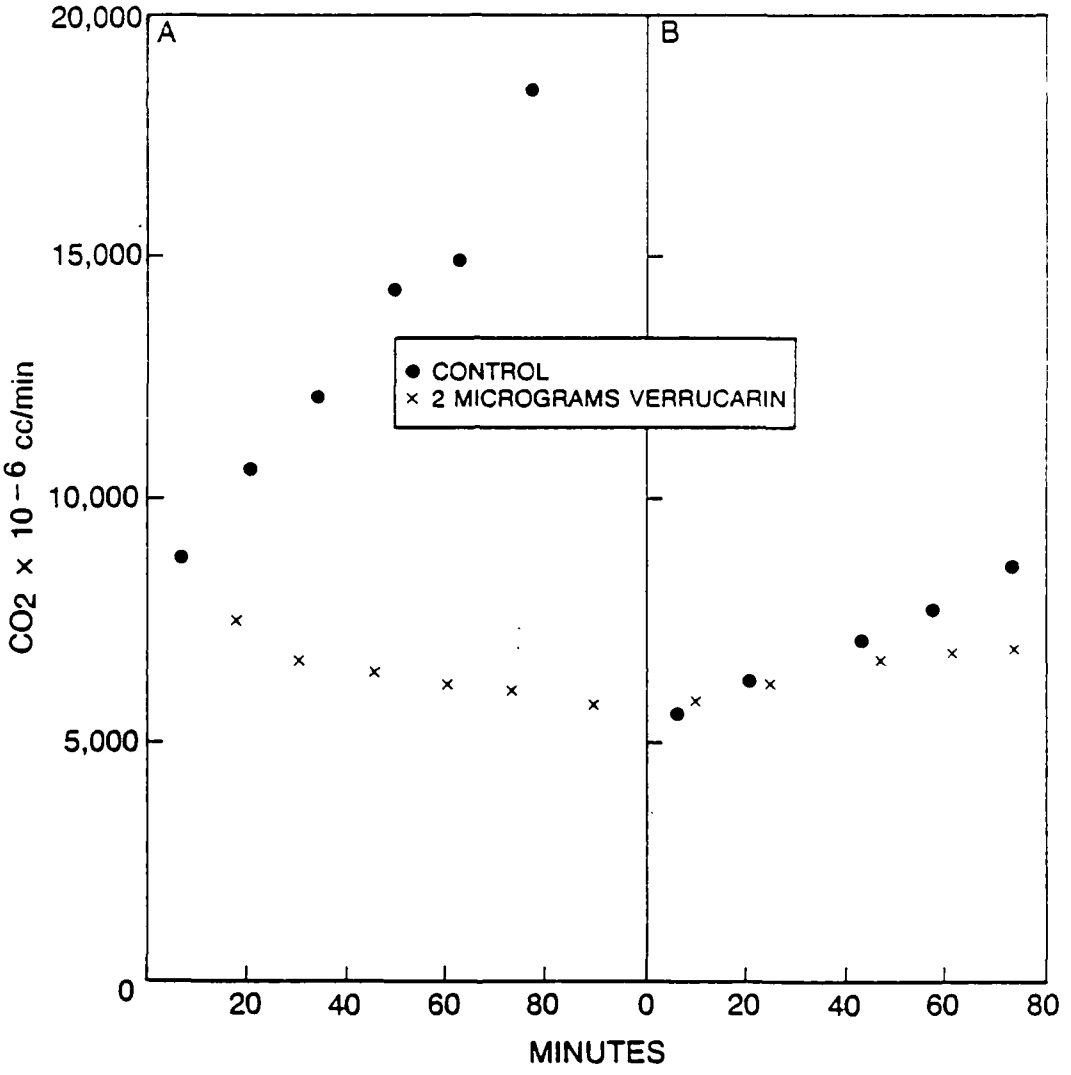


Figure 9. The effect of 2 mcg verrucarin on the rate of $C0_2$ production of baker's yeast (O.D. .260), with N_2 as the carrier gas. A: No aeration overnight, B: Culture prepared from dry yeast. (●) Control, (x) 2 mcg verrucarin.

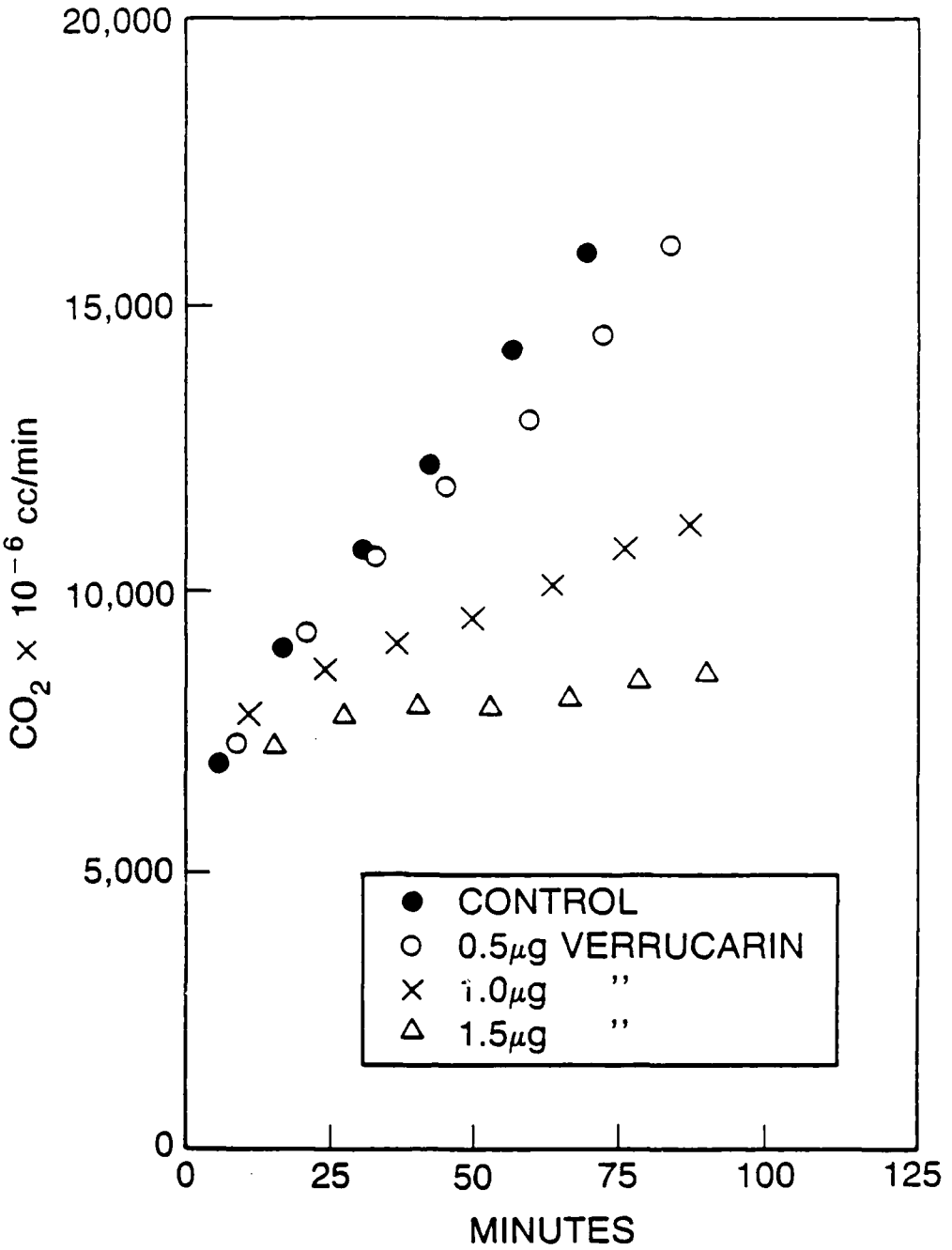


Figure 10. The effect of various amounts of verrucarin on the $C0_2$ production rate of baker's yeast, with N_2 as the carrier gas. (●) Control, (o) 0.5 mcg, (x) 1.0 mcg. (Δ) 1.5 mcg.

END
DATED
FILM
8-88
DTIC