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DEVELOPMENT OF A PROTOCOL FOR TESTING EFFECTS OF TOXIC SUBSTANCES ON PLANTS

by

C. Ray Thompson, Gerrit Kats,
Philip Dawson and Denise Doyle
Statewide Air Pollution Research Center
University of California
Riverside, California 92521

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Project Officer

David T. Tingey
Plant Physiologist
Corvallis Environmental Research Laboratory
Office of Research and Development
U. S. Environmental Protection Agency
Corvallis, Oregon 97330

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16. ABSTRACT This study was designed to devise a rapid, simple, reproducible bioassay procedure to determine effects of so-called "toxic substances in the environment" on vegetation and provide a standardized procedure for evaluation and comparison of effects of diverse compounds. Eight different plant species were grown and evaluated for speed of growth, e.g., rapid production of leaf tissue, uniformity within the particular cultivar, plant habitus, e.g., structural characteristics that make it suitable for this particular application, and the potential for high ethylene production when exposed to mild stress. Of the varieties grown in the growth chambers, pink kidney beans and cucumbers were selected as most suitable.

Seven compounds were tested by the procedure devised: two organic herbicides: Paraquat and Endothall; three inorganic plant toxicants: Phytar, sodium fluoride and sodium chlorate; and two insecticides: Orthene and Diasinon. The statistical parameters, slope intercept and correlation coefficient were recorded. Reproducibility of the method was tested with two successive runs with Endothall. The slopes were 143.6 and 136.6 with correlation coefficient of 0.91 and 0.96 respectively. Analysis of covariance showed there was no significant difference between these slopes at the 95% confidence interval.

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory.

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine systems; the behavior, effects and control of pollutants in lakes and streams, and the development of predictive models on the movement of pollutants in the biosphere.

This report describes the development of a protocol for testing the effects of toxic substances on plants and details the kind of equipment required for foliar application of toxicants, cultural conditions for raising uniform test plants, environmental parameters for insuring reproducible responses which is the evolution of stress ethylene and statistical evaluation of data to yield results which will provide a relative evaluation of the toxicity of a given substance to plants.

T. A. Murphy
Director

ABSTRACT

The purpose of this study was to devise a rapid, simple and reproducible bioassay procedure to determine effects of so-called "Toxic Substances in the Environment" on vegetation and provide a standardized procedure for evaluation and comparison of effects of diverse compounds.

Eight different plant species were grown and evaluated for speed of growth, e.g., rapid production of leaf tissue, uniformity within the particular cultivar, plant habitus, e.g., structural characteristics that make it suitable for this particular application, and the potential for high ethylene production when exposed to mild stress.

Banana Squash (UCR selection), Corn (Early Sunglow), Cucumber (Pickling SMR-58), Bush Bean (Blue Lake), and Kidney Bean (Pink), Radish (Scarlet Globe), Spinach (Thickleaved Nobel), and Sunflower (Mammoth 307) were grown in growth chambers. Pink kidney beans and cucumbers were selected as most suitable.

The plants were grown in a growth chamber in small plastic pots in a commercial potting mix; beans for 9-10 days and cucumbers for 14 days prior to spraying. A photoperiod of 12 hrs was shown to produce plants which evolved the most stress ethylene. The plants were sprayed with a modified pendulum sprayer equipped to spray a single plant placed beneath the center of its arc of swing. Prior to spraying the plants were exposed to light for 2.0 hrs. Thirty minutes after spraying the plants were encapsulated under one-half gal glass jars with a water seal and were incubated for 24 hours in a dark chamber at 24°C. Preliminary range tests with 4 dosages of test compounds on 8 replicates each were used to establish suitable dosages for final evaluation. When the range of dosages was found where small increases in levels caused maximum changes in stress ethylene evolution, five dosages were used with 8 replicates for the final evaluation.

Ethylene samples were removed from the jars with a syringe having a bent needle and concentrations of ethylene were determined with a calibrated Aerograph 1520 gas chromatograph. The stress ethylene evolved from plants was plotted by computer vs the amount of compound applied from the equation: Log_e (ethylene concentration) = Log_e A + B (concentration of the toxicant).

Seven compounds were tested by the above procedure: two organic herbicides: Paraquat and Endothall; three inorganic plant toxicants: Phytar, sodium fluoride and sodium chlorate; and two insecticides: Orthene and Diazinon. The statistical parameters, slope, intercept and correlation coefficient were recorded.

Reproducibility of the method was tested with two successive runs with Endothall. The slopes were 143.6 and 136.6 with correlation coefficient of 0.91 and 0.96 respectively. Analysis of covariance showed there was no significant difference between these slopes at the 95% confidence interval.

The protocol as devised was prepared for publication in the Bulletin of Environmental Contamination and Toxicology.

This report was submitted in fulfillment of Grant R-806270-01 by the Statewide Air Pollution Research Center, University of California, Riverside under the sponsorship of the Corvallis Environmental Research Laboratory of the Environmental Protection Agency. This report covers the period of 8/15/78 to 8/14/80 and work was completed on 10/6/80.

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Introduction

A rapid, simple, inexpensive and reproducible bioassay procedure was needed to determine the deleterious effects of so-called "Toxic Substances in the Environment" on vegetation and provide a standardized procedure whereby laboratories could test these diverse substances under standard conditions. This would allow direct comparison of results between laboratories and provide information to local, state and federal agencies as to the hazards involved in the use of these substances. Where necessary, suitable controls and regulations would then be developed.

To develop this protocol it was decided to use ethylene evolution plus visible injury symptoms, as indices of toxicity. Stress induced ethylene production by plants has been shown to be an indication of injury which occurs following very mild trauma or unfavorable growing conditions. It was first observed by Williamson (1950) and occurs after very mildly adverse conditions such as chemical treatment (Cooper et al. 1968), insect injury (Galil 1968), temperature extremes (Vines et al. 1968), drought (McMicheal et al. 1972), γ irradiation (Williamson 1950), disease (Vines et al. 1968), mechanical effects such as wounding (Hall 1951) (Saltveit and Dilley 1978), pressure (Goeschl et al. 1966), or abrasion (Cooper et al. 1968).

Air pollutants such as ozone and Cl_2 (Abeles and Abeles 1972; Tingey et al. 1978) have been shown to induce stress ethylene production in several plants and have been suggested as a measure of ozone injury on plants (Tingey et al. 1976). Bressan et al. (1978) have also suggested the use of ethylene and ethane production to measure SO_2 injury.

It was proposed that a spectrum of plant species from diverse families be tested under standardized conditions with precise levels of toxic substances and the amount of stress ethylene produced measured. Correlations of dosage and level of ethylene produced would be made by suitable statistical procedures.

DEVELOPMENT OF A PROTOCOL FOR TESTING EFFECTS OF TOXIC SUBSTANCES ON PLANTS

I. Summary of Progress

A. Assembly of Equipment

1. Refurbish growth chambers and define growth conditions

Two growth chambers from Controlled Environments, Inc., Model E15P were rebuilt and made operational. These chambers control temperature, light and humidity.

2. Gas Chromatograph. An Aerograph 1520 gas chromatograph equipped with a 2 ml. sample loop and flame ionization detector was used for ethylene measurement. The instrument was installed in a controlled temperature room. A column of Poropak N* gave good separation of ethylene and ethane. After considerable experimentation the following flow rates and temperatures were selected to optimize ethylene measurement: N_2 57 ml/min, H_2 45 ml/min, O_2 300 ml/min, and a column temperature at 75°C, detector at 80°C and injector at 175°C. A standard tank of 1 ppm ethylene was used for calibration.

3. Method of Spray Application. To assure reproducibility and ease of operation a pendulum sprayer as described by Day et al. (1963) was modified for this purpose. It consists of a miniature compressed air sprayer mounted on a 1.8 m pendulum equipped to automatically spray a plant placed beneath the center of its arc of swing (Figure 1).

Character of the deposit and deposition rate are determined by: pressure at the nozzle tip, nozzle type and size, height above the leaf surface and the speed with which the nozzle passes over the plant. The objective was a generous wetting of the leaf surface without runoff through manipulation of these variables. The following conditions gave reproducible results. The pendulum was released 60 cm from the bottom of the arc of swing as measured at the nozzle tip. Pressure at the nozzle was 30 psi. Nozzle height above the leaf surface was 30 cm. The nozzle was a**Tee-Jet flat spray tip no. 4001. The delivery rate was determined by spraying preweighed paper towels or filter paper and weighing afterwards. One spray application consisted of two passes of the pendulum. The results showed an average liquid deposit from 5 applications to be 2.69 mg/cm^2 with a standard deviation of .06 mg/cm^2. See representative data in Table 1.

4. Collection of Ethylene and Sampling Procedure. Several different methods of enclosing treated plants and sampling the amount of ethylene produced were tried. Initially plants were encapsulated by placing them inside polyethylene or mylar bags, supported by wire frames (Figure 2) but these materials were permeable to ethylene. Tests with single film polyethylene or mylar bags showed aloss of 85.5% and 80.1%, respectively of 800 ppb of ethylene during 24 hrs while glass jars with a water seal lost 4.5%. Other

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*Alltech Associates
343 2nd Street
Los Altos, CA 94022

**Spraying Systems Company
North Avenue at Schmale Road
Wheaton, Illinois 60187

investigators found a multilayer "Cryovac B-620"** bag to lose about 15%. The final procedure devised is to cover the treated plant with a 2 qt wide-mouth glass Mason Jar. This is done by placing a small aluminum weighing dish in the bottom of a 6" plant saucer. *** The pot containing the treated plant is then placed in the aluminum dish and covered by the inverted jar. The plant saucer is filled with water to seal the opening (Figure 3). A gas tight syringe equipped with a bent hypodermic needle is inserted beneath the jar and is used to withdraw ethylene samples. Solubility of ethylene in water is 1-2% and is ignored as a systematic error.

Incubation temperature affected ethylene production causing higher production at increased temperatures. To control this variable a darkened incubation box was used. Warm air from the growth chamber held the temperature at about 24°C for the 24 hours of incubation (Table 7).

B. Test Plants

1. Species of Plants Tested. The criteria considered in choosing the test plants were: fast growth, e.g., rapid production of leaf tissue, uniformity within the particular cultivar, plant habitus, e.g., structural characteristics that make it suitable for this particular application, and the potential for high ethylene production when exposed to mild stress.

Banana Squash (UCR selection), Corn (Early Sunglow), Cucumber (Pickling SMR-58), Bush Bean (Blue Lake), and Kidney Bean (Pink), Radish (Scarlet Globe), Spinach (Thickleaved Nobel), and Sunflower (Mammoth 307) were grown in growth chambers. The latter three had a number of undesirable characteristics of which the relative slow growth was the most important. The other cultivars were tested after the first normal leaf was fully developed.

Phytar and Endothall, both weed killers and known to cause ethylene production, were applied as sprays at low concentrations. The ethylene accumulated during 24 hours after this application was measured (Table 2). The results show that the rate of ethylene production differs greatly depending upon the cultivar and the toxicant. Combining the requirement of fast growth and high ethylene production, cucumbers as well as the kidney beans showed superior qualities.

2. Growth Conditions. Environmental conditions proved to affect not only growth and development of the test plants, but also the ethylene response although not all environmental parameters were tested exhaustively. Kidney beans (pink) and cucumbers (pickling SMR-58) were grown in 6-oz styrofoam cups (180 ml.) provided with drain holes in the bottom. Initially a peat/sponge rock mix (2:1 vol:vol) was used, but a commercial product called Jiffy Mix† gave superior results with regard to uniformity of germination and saved preparation time. A single test comparing the rate of ethylene evolution of plants grown in two media, Jiffy Mix and Jiffy Mix plus sponge rock and sprayed with Phytar showed that beans or cucumbers grown in Jiffy Mix alone gave a marked improvement in uniformity of the amounts of ethylene evolved as well as the total amount (Table 3).

** Cyrovac Division
W. R. Grace and Co.
Duncan, SC 29334

*** Super Saucers
Childs and Associates
P.O. Box 807
Mill Valley, CA 94941

† Jiffy Products of America
250 Town Road
Chicago, IL 60185

The seeds were planted 2 cm deep in Jiffy Mix moistened with tap water. No watering was done on the top until the seeds had completely germinated. This prevented the seeds from rotting and improved uniformity considerably. Once germinated, the plants were irrigated with one-half strength NCSU phytotron nutrient solution (Downs and Bonaminio 1976). Light intensity in growth chambers was 322 μ Einsteins/m^{-2}/sec^{-1}. This was close to the maximum for these chambers. Although light intensity during growth may well have an effect on the rate of ethylene production after treatment, no tests were done to determine the optimum. A 12-hour photoperiod was compared with a 16-hr photoperiod and did not show a significant increase in growth rates or plant quality, but beans grown under the shorter photoperiod produced considerably more ethylene after spraying with Endothall and Phytar (Table 4 and Figure 4), but slightly less when treated with sodium flouride.

The day and night temperatures and relative humidity established from previous horticultural experience for beans were 27°C, 21°C, and 65%, respectively, and for cucumbers 30°C, 26°C, and 80%. Under these conditions both plant species grew most rapidly while maintaining the quality required for handling the plants during spraying and encapsulating. Kidney beans (pink) were considered ready for testing when the primary leaves had reached a maximum size which was typically 9-10 days after planting. Cucumbers were used when the first normal leaves reached maximum size which required 14 days.

3. Determination of Optimum Light Period Before Application of the Toxicant. Preliminary observations showed that application of the toxicant in the morning gave more ethylene evolution than in afternoon indicating that the length of the light period following darkness and prior to spray application affected this response of bean and cucumber plants greatly. A series of tests showed that there is an optimum light period for stress ethylene. This optimum might also be affected by the toxicant used and would require a large number of tests for precise determination. Our tests indicated that toxicant application between 1.5 and 2.5 hr would be an adequate guideline. It also has logistic advantages because it provides enough time for the spray application, drying, and encapsulation during which the plants are exposed to light. The test plants were grown in Jiffy Mix incubated in 2 qt jars, and the values shown in Table 5 represent mean values for ethylene evolution from 8-10 plants per treatment.

4. Effect of the light period after spraying on the rate of ethylene production of bean or cucumber plants. The effect of the light period after spraying was studied using bean and cucumber plants treated with Endothall, Phytar and sodium fluoride. The Endothall concentrations were .02 g/l, Phytar 0.37 g/l and NaF 10.59 g/l for beans. For cucumbers the concentrations of Endothall, Phytar and NaF were .040, 0.37 and 5.25 g/l, respectively. Eight plants per treatment were used and incubation was 24 hrs in the dark. The plants were allowed to dry in the growth chamber for one half hour following the spray application. After exposing the plants to a range of light periods they were encapsulated and placed in the dark. All control plants evolved less than 20 ppb ethylene. The results (Table 6) show that the highest ethylene concentration is produced by palnts encapsulated and placed in the dark after one half hour of preincubation time, which is also the period required for drying. For cucumbers the optimum light period is approximately 1 hr after spraying.

An alternative test was done to determine if exposure to light for different periods after encapsulation had any effect on the rate of ethylene evolution. All plants were encapsulated one half hour after spraying in order to dry. Thereafter one series was placed immediately in the dark for 24 hr and the other exposed to light in the growth chamber for 2 hr and placed in the dark for 22 hr thereafter.

The results (Table 7) show that cucumber plants as well as the beans not treated with a toxicant (control) produce more ethylene if 2 hr of light is given before the dark incubation. A temperature increase of 11°C in the container during the light period probably causes enough stress to account for the ethylene increase. This interference alone would make it less advisable to apply light after encapsulation.

Beans and cucumbers sprayed with Phytar also showed an increase in ethylene when exposed to 2 hr of light before dark incubation (Table 7). When sprayed with sodium fluoride, however, there appears to be a reduction in both cases. We believe that this provides adequate information to decide that dark incubation for 24 hr immediately after encapsulation is most desirable.

5. Determination of Incubation Period. To establish some definite period during which the treated plants were allowed to evolve ethylene during the dark incubation, beans were treated with Endothall and cucumbers with Phytar. Rates of evolution were recorded for 24 hr or longer. Both plants had sigmoid rate curves (Figure 19). The bean plants had plateaued or were regressing at 24 hr. The cucumbers reached a plateau between 30 and 46 hr and 70% of the total having been evolved in 24 hours. Because the beans had plateaued and 70% of maximum ethylene had been produced by the cucumbers, 24 hr was selected as a fixed period for sampling. This time is also very convenient logistically.

6. Formulation of toxic substances prior to application to plants. Preparation of suitable dilutions of test compounds which have limited water solubility can present problems. Oil soluble materials can often be dissolved in acetone and/or a non-toxic oil, such as olive oil, an emulsifier added, and a stable oil-in-water emulsion prepared. Odorless kerosene can also serve as a primary solvent. These emulsions can often be diluted ad lib to obtain suitable concentrations. Less soluble compounds can often be dissolved in acetone which is then dispersed in water with violent agitation. Compounds with adequate water solubility but which dissolve very slowly can be suspended in a cloth bag in a tank. This allows the dense solution which forms at the solid-liquid surface to sink thus renewing the dilute solution and aids in rapidity of solution. Water solutions require a non-toxic wetting agent to obtain uniform leaf coverage. We have used X-77* at 0.625 ml/l.

These procedures were used for formulation of the compounds tested as follows:

1. Paraquat - water solution + X-77

*Colloidal Products Corp.
P.O. Box 666
Sausalito, CA 94965

2. Phytar - water solution + X-77
3. Endothall - water solution "
4. Sodium Fluoride - water solution "
5. Sodium Chlorate - water solution "
6. Orthene - water solution "
7. Diazinon - Dissolve in acetone-dispersed in water + X-77

7. Effects of Toxic Substances, Evaluation of Data. Tingey et al. (1976) found that stress ethylene produced by plants when exposed to ozone increased proportionately with the ozone concentration and that up to a limit the increase of the stress ethylene production can be modeled using the following equation: Log_e (ethylene conc.) = Log_e A + B (concentration of the toxicant). In this equation A is an estimate of the ethylene production of nontreated plants and B is the slope parameter which is a measure of the increase in stress-induced ethylene production in relation to the stress concentration. Our studies showed that this slope parameter can be used to express the relative toxicity of aqueous solutions or suspensions of toxicants on vegetation. All plots and slope parameters are based upon the values on the linear portion of the curve plus the control values. During the later phases of our work we found that the Log_e ethylene response is sigmoid, so in the future the control and possibly some of the very low levels should be excluded. This also implies the need for a number of concentrations larger than the 5 we used. The general procedure was first to determine a range of concentrations below the point where the Log_e ethylene response ceases to be linear. Four concentrations of the toxicant over a wide range using 8 plants per concentration, repeated 2 or 3 times was usually sufficient for an approximation. After this range finding test 5 concentrations within this range were applied using 8 plants per concentration and the slope of the linear regression of the Log_e ethylene response determined. The compounds used to establish this methodology were: Endothall, Phytar, sodium fluoride, sodium chlorate, Orthene and Diazinon.

8. Determination of reproducibility of method. In order to determine the reproducibility of the method, 2 tests were carried out with Endothall. The slopes were respectively 143.6 and 136.6 and the correlation coefficients .91 and .96 (Figures 5-6). Analysis of covariance (Snedecor and Cochran 1978; Bennettand Franklin 1963) showed that there is no significant difference between these slopes and that the 95% confidence interval of the mean is 140 ± 8.0. This interval was determined for n=40, namely 5 concentrations at 8 plants each. For n=30 the interval would be 140 ± 10 and for n=20 140 ± 12. These results suggest that a smaller number of plants per concentration would not affect the results dramatically.

9. Determination of relative toxicity of test compounds.

A. Endothall

Endothall caused visible injury to plants at low levels so a narrow range was tested. Kidney beans (Run #103) and cucumbers (Run #35) were used. The concentrations applied ranged from 0.01 to 0.04 grams/liter. these concentrations are much lower than would be used for weed control. Kideny beans proved to be very sensitive to Endothall and produced large amounts of ethylene as shown in Figure 5. This increase in stress ethylene was apparent at a dosage of 0.01 grams/liter, but visible injury was not observed until a dosage of 0.02 grams/liter and higher were applied. Cucumbers showed only a slight increase in ethylene even when visible damage occurred (Figure 7).

B. Phytar 560

Phytar 560, which is also an herbicide, produced high levels of stress ethylene in both kidney beans and cucumbers. In both species there was a high correlation between dosage of Phytar 560 and ethylene production. Run 102 tested kidney beans (Figure 8) treated with 0.3 to 0.6 g/l. Ethylene increased at each concentration as did injury.

Cucumbers in Run #21 (Figure 9) showed visible damage at lower concentrations than beans. A range of 0.09 to 0.37 g/l caused substantial ethylene increases. We recommended that Phytar 560 be used at the rate of 0.30 g/l as a positive control compound for both test species.

C. Paraquat

Paraquat was different from other compounds tested because it needs light to cause injury. As previously stated light is usually accompanied by increased temperatures, therefore, we preferred to use dark incubation. Beans and cucumbers which were incubated in the dark produced very low levels of ethylene and the plants failed to show visible damage until they were placed in the light. However, even with light incubation, Paraquat produced very low levels of ethylene compared with Endothall and Phytar 560. Both kidney beans and cucumbers were tested with a range of 0.3 to 2.4 g/l of Paraquat. Kidney beans in Run #77 (Figure 10) showed a slight increase in ethylene at each dosage with some visible damage at all dosages. Cucumbers in Run #78 (Figure 11) exposed to the same range showed only a slight increase in ethylene but 100% damage on all test plants.

D. Sodium Fluoride

Sodium fluoride was chosen to evaluate the effect of an inorganic compound. In Run #42 (Figure 12) kidney beans were treated with a range of 5.25 to 42.0 g/l of sodium fluoride and showed increasing levels of ethylene and visible injury. Cucumbers proved to be more sensitive as illustrated in Run #94 (Figure 13). A smaller range of 2.6 g/l to 21 g/l was used, but ethylene levels peaked at 10.5 g/l although visible injury continued to increase.

The major difference found in the behavior of plants treated with NaF and those treated with organic compounds involved incubation temperature. With higher temperatures the NaF treated plants produced less ethylene while the organic treated plants produce more (Table 7).

E. Sodium chlorate

Limited testing was performed with $NaClO_4$ because of the low correlation between dosage and ethylene production. In Run #55 (Figure 14) kidney beans were treated with a range of 6.65 to 26.61 g/l. High levels of ethylene were produced but visible injury was high at all dosages. Cucumbers in Run #56 (Figure 15) produced very low levels of ethylene when exposed to a range of 1.66 to 13.30 g/l, but showed considerable injury. When higher dosages were tested inury increased but ethylene remained low.

F. Orthene

Orthene is a widely used, broad spectrum insecticide. It is not considered to be toxic to plants and indeed none of our test plants showed any visible injury. We used a range of 3.0 to 24.0 g/l on both

kidney beans and cucumbers. These concentrations are much higher than would be used for insect control. Kidney beans in Run #45 (Figure 16) showed a very slight increase in ethylene at 12.0 g/l and higher. The cucumbers treated in Run #44 (Figure 17) showed no increase in ethylene.

G. Diazinon

A second broad spectrum insecticide was found to be more toxic to cucumbers than Orthene. This compound was not water soluble at the concentrations studied. It was dissolved in 50% acetone. Cucumbers were sprayed with 1.32 to 10.56 g/l Diazinon in Run #43 (Figure 18). An increase in ethylene was observed in the 5.28 and 10.56 g/l groups. Slight visible injury occurred but only in the 10.56 g/l group.

C. Discussion

The purpose of this study was the development of a rapid, simple, reproducible, bioassay procedure for determining the relative effects of "Toxic Substances in the Environment" on vegetation and provide a protocol whereby investigators could evaluate the many and diverse substances to be considered. Accordingly, the major effort has been to evaluate the relative effects of cultural, environmental and manipulative factors on the primary response, the evolution of stress ethylene. Because rapidly growing plants are so sensitive to practically all of these factors it was necessary to evaluate, define and standardize each of the operations, if reproducible responses were to be obtained. Some of the operations had to be fixed arbitrarily so that the other factors could be studied.

Selection of the two plant species for use from the eight tested, pink kidney beans and cucumbers, was made because they grew rapidly, gave uniform sized plants, had large flat leaves which could be sprayed uniformly and evolved large amounts of stress ethylene. To grow plants of a uniform size it was extremely important that the seed all germinate promptly when planted. This necessitated a soil mix, hence "Jiffy Mix," which gave proper moisture, aeration and mineral nutrients. Also, the watering, temperature and light regimen must be rigidly controlled if the same kind of plants be produced from run to run. This will of necessity require that plants be grown in a growth chamber.

The spray application with the pendulum sprayer gave very uniform dosages, proceeded rapidly and was essentially trouble free. By enclosing the apparatus in a plastic curtain and exhausting vapors contamination of the laboratory was avoided. The studies of light periods and temperature regimen were cursory but sufficed to produce plants which evolved sufficient stress ethylene for easy measurement by the gas chromatograph. The gas chromatograph, once calibrated, was essentially trouble free.

Evaluation of the seven test compounds shows that correlation coefficients on some earlier runs showed little significance. However, as the above-named factors were recognized and controlled much better results were obtained and reproducibility from run to run was good, (see runs #103 and 104, Figures 5 and 6). The sigmoid nature of the response curve could interfere with the suggested Log_e evaluation of the data unless several (7-9) concentrations of toxicant are applied, the shape of the curve determined and those falling on both ends, where less linearity occurs, could then be discarded.

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Table 1

Reproducibility of Sprayer Delivery

Date	8/22/79	1/24/79	11/29/78	12/27/78
Nozzle #	4001	4001	6501	6501
Distance from Sprayer Tip (cm)	30	30	45	45
Delivery (Mg/cm^2)	2.06	2.63	1.18	1.77
	2.07	2.67	1.26	1.77
	2.14	2.70	1.31	1.79
	2.09	2.66	1.26	1.78
		2.79	1.31	1.76
			1.28	1.77
			1.31	1.79
			1.22	1.68
			1.33	1.75
			1.31	1.77
Mean	2.09	2.69	1.28	1.76
Standard Deviation	.04	.06	.05	.03

Table 2

Ethylene[1] produced by five different plant species after spraying with PHYTAR or ENDOTHALL

g/l	Banana squash	Corn	Cucumber	Bush beans	Kidney beans
			PHYTAR		
0.0	48	10	63	37	45
0.095		2.8	81		
0.19		5.3	144		
0.38	58	11	443		232
0.76	86	11	1722	146	517
1.82	146		2673		744
3.04				669	
Age, days	21	80	14	9	9
Leaf area, cm^2	190	--	67	130	165
			ENDOTHALL		
0.0	47	0.5	60	16	24
0.01	74		84		
0.02				810	938
0.03			151		
0.04	161			1223	2835
0.08	667				
0.125					
0.250		1.2			
0.375					
0.500		1.6			
Age, days	17	8	14	9	9
Leaf area, cm^2	115	—	72	130	165

[1]Parts per billion.

[2]6-8 plants per concentration

Table 3

ETHYLENE ACCUMULATED DURING 24 HRS AFTER SPRAYING WITH PHYTAR AT .37 g/l USING TWO DIFFERENT SOIL MEDIA

Plant No.	Jiffy Mix	Jiffy Mix/Sponge Rock (1:2)
1	428 ppb	68 ppb
2	465	186
3	577	279
4	291	50
5	471	415
6	291	68
7	223	12
8	310	25
Average	382	138

Table 4. Ethylene Evolution of Kidney Beans and Cucumbers Grown with 2 Photoperiods

Run 3	Light hrs	Control ppb	.020 g/l ppb	.040 g/l ppb
		Endothall (Beans)		
98	12	19	604	3195
98	16	11	196	774
97	12	6	609	1833
97	16	5	133	77
99	12	16	1363	3229
99	16	17	326	1437
		Phytar (Beans)		
		0	.30 g/l	.60 g/l
101	12	12	171	318
101	16	10	16	256
		NaF (Cucumbers)		
			2.62 g/l	5.25 g/l
100	12	19	62	139
100	16	33	51	296

Table 5

EFFECT OF LIGHT PERIOD BEFORE SPRAY APPLICATION
ON ETHYLENE EVOLUTION (ppb)

Light Period (hrs)	Beans			Cucumbers		
	Endothal .02 g/l			Sodium Fluoride 5.25 g/l		
	Run 58	Run 69	Run 70	Run 51	Run 63	Run 72
0	44.97 (6.4)				0.42 (0)	80.14 (19.7)
0.5		374.54 (5.6)	786.63 (8.6)			77.22 (9.6)
1					20.20 (.6)	
1.5	/272.26/ (4.4)	509.81 (27.8)	/814.96/ (1.1)	/158.40/ (20.2)		68.43 (12.1)
2					/86.45/ (0)	
2.5		/609.71/ (10.7)	523.70 (0)			/93.19/ (12.8)
3					27.69 (1.7)	
3.5	242.46 (2.4)	489.99 (14.5)	419.53 (0)	21.00 (8.4)		55.51 (4.8)
5.5	155.16 (0)			21.58 (1.2)		
7.5	15.73 (0)			3.63 (0)		

/ / = maximum response

( ) = nontreated controls

TABLE 6

Effect of Preincubation [1/]
Period of Ethylene Evolution

Preincubation period (hrs)	BEANS			CUCUMBERS		
	Endothall Run 74	Phytar Run 84	NaF Run 85	NaF Run 82	Phytar Run 83	Endothall Run 87
0.5	179	478	479	66.6	168	62.6
1.0	53.1	382	257	166	291	70
1.5	13.1	427	237	125	219	46
2.0	0.88			40	200	
2.5	0.88					

[1/] Preincubation is the period after spraying and before encapsulation. Plants were placed in lighted growth chamber immediately after spraying.

Table 7. Ethylene Response of Plants Incubated Under Two Different Conditions

KIDNEY BEANS

Incubation Conditions	Run 84		Run 85	
	Control	.37 g/l Phytar	Control	10.5g/l NaF
24 hrs in dark[1/]	18.6	478.1	10.6	479
2 hrs in light[2/] 22 hrs in dark	61.2	575.8	43.4	375
PPB Increase	42.6	97.6	32.7	-104
% Increase over non-preincubated	229%	20%	307%	-21%

CUCUMBERS

	Run 83		Run 82		Run 87	
	Control	.37 g/l Phytar	Control	5.25g/l NaF	Control	.04 g/l Endothall
24 hrs in dark[1/]	11.8	291.3	7.7	166.9	11.0	70.6
2 hrs in light[2/] 22 hrs in dark	44.1	603.2	26.8	66.6	37.8	98.8
PPB Increase	32.2	311.8	19.0	-100.3	26.7	28.1
% Increase over non-preincubated	273%	107%	249%	-60%	242%	39%

1/ Plants were allowed to dry in lighted growth chamber before encapsulation.

2/ Plants were encapsulated in jars and therefore temperatures rose at least 11°C during that period due to radiation. Chamber temperature 24°C.

PARTS DESCRIPTION FOR FIGURE 1

1. Pillow blocks with 1/2" bearing.
2. 1/2" shaft.
3. Teflon washers.
4. Flange with set screw (Lab frame foot).
5. Aluminum cam. 2" x 4 1/2: attached to flange to activate microswitch (6). Cam is vertically adjustable through slotted holes.
6. Microswitch.
7. 1/4" galvanized TEE bored out to accommodate 1/2: shaft. A hole is drilled and tapped for a setscrew to fix TEE to the shaft.
8. 1/4" nipple.
9. Galvanized reducer 1/2: x 1/4:.
10. 1/2" nipple.
11. Galvanized TEE 1/2" x 1/2: x 1/4". This allows wiring and pressure tube (15 and 17) inside the pendulum.
12. 1/2" galvanized pipe.
13. Conduit fitting 1/2" (type C).
14. Main switch (toggle type).
15. Electrical wiring for the microswitch, solenoid (20), main switch circuit.
16. 3-way valve. One position allows the reservoir to be filled with the spray formulation and the other the air to pressurize the system.
17. 1/4" polyethylene tubing for compressed air supply.
18. Stainless steel ball joints (18 mm) modified to fit the valve and solenoid.
19. Glass reservoir; 40 mm diameter, 220 mm long (ball joints included).
    Reservoir is normally exposed to 30 lbs pressure.
    Ball joint size is 18 mm and the clamps (not in sketch) are the screw lock type.
20. Solenoid valve with stainless steel valve body.
    110-115 volt; 100-psi; 1/8" orifice; 10 watt;
    valve no V52 DA 2100 Code no VC7.
    Skinner Electric Valve Div.
    New Britain, Conn. USA
21. L/4 TT Tee-jet stainless steel spray nozzle assembly with interchangeable tip. The tip used in this application is Tee-Jet flat spray tip no 4001.

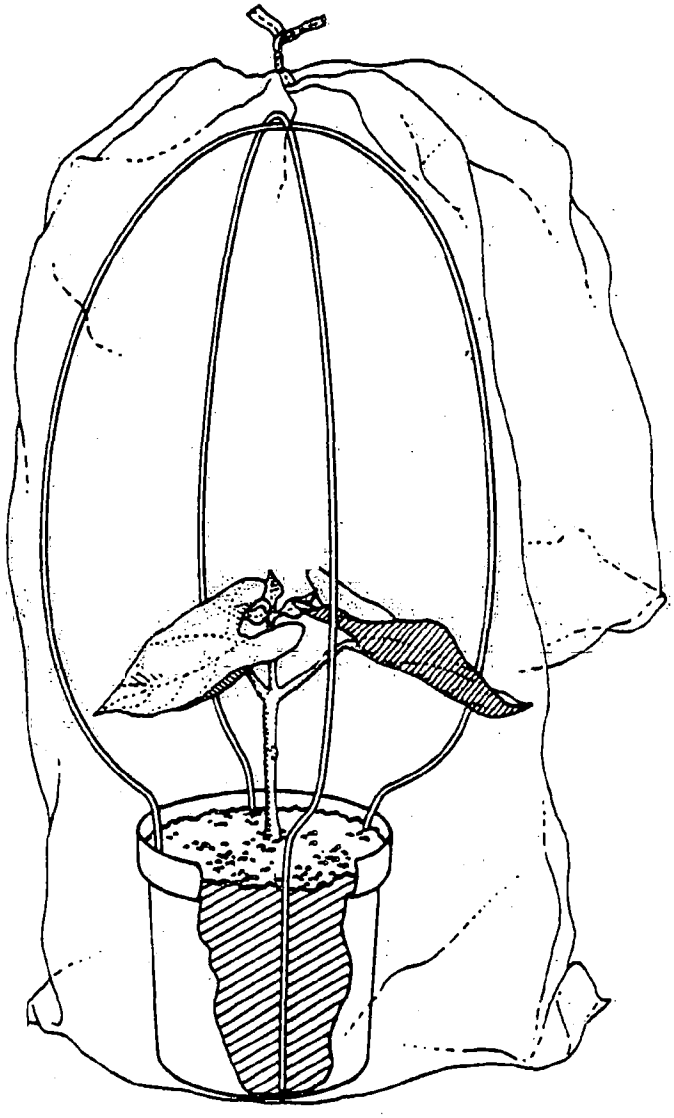

Figure 2. Cut-away, side view of bag and fram encapsulation system.

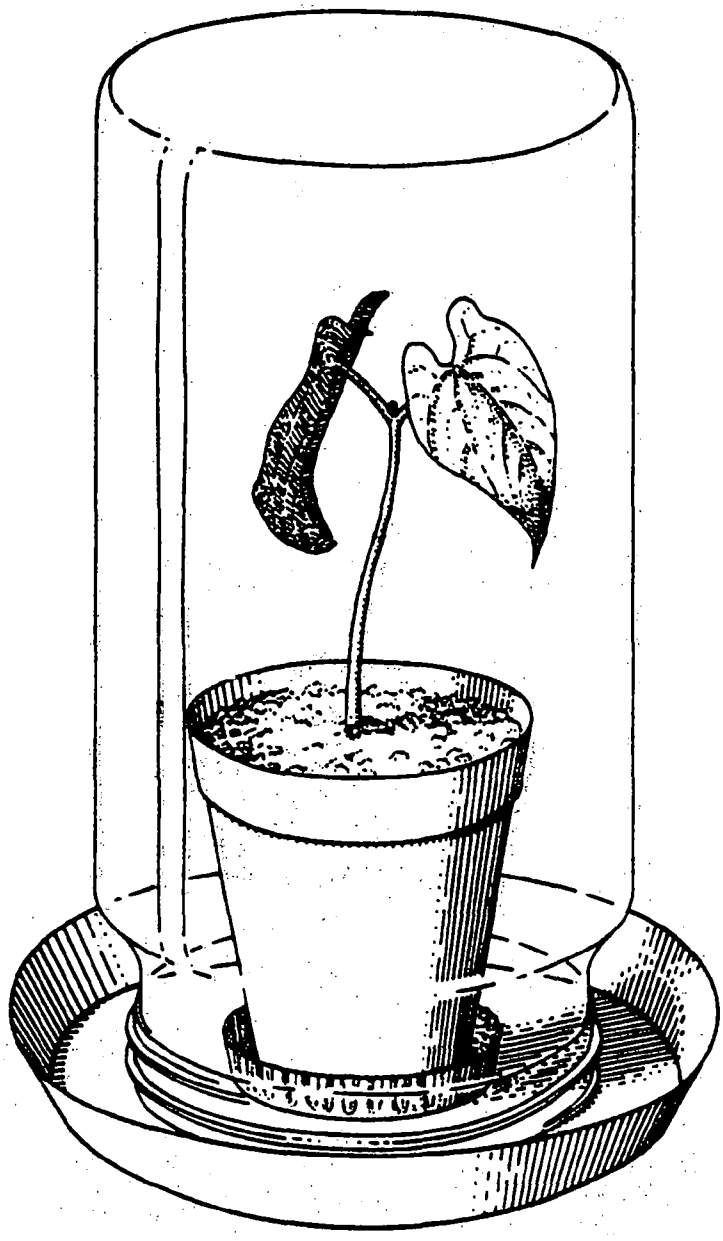

Figure 3. Side view of glass jar encapsulation system.

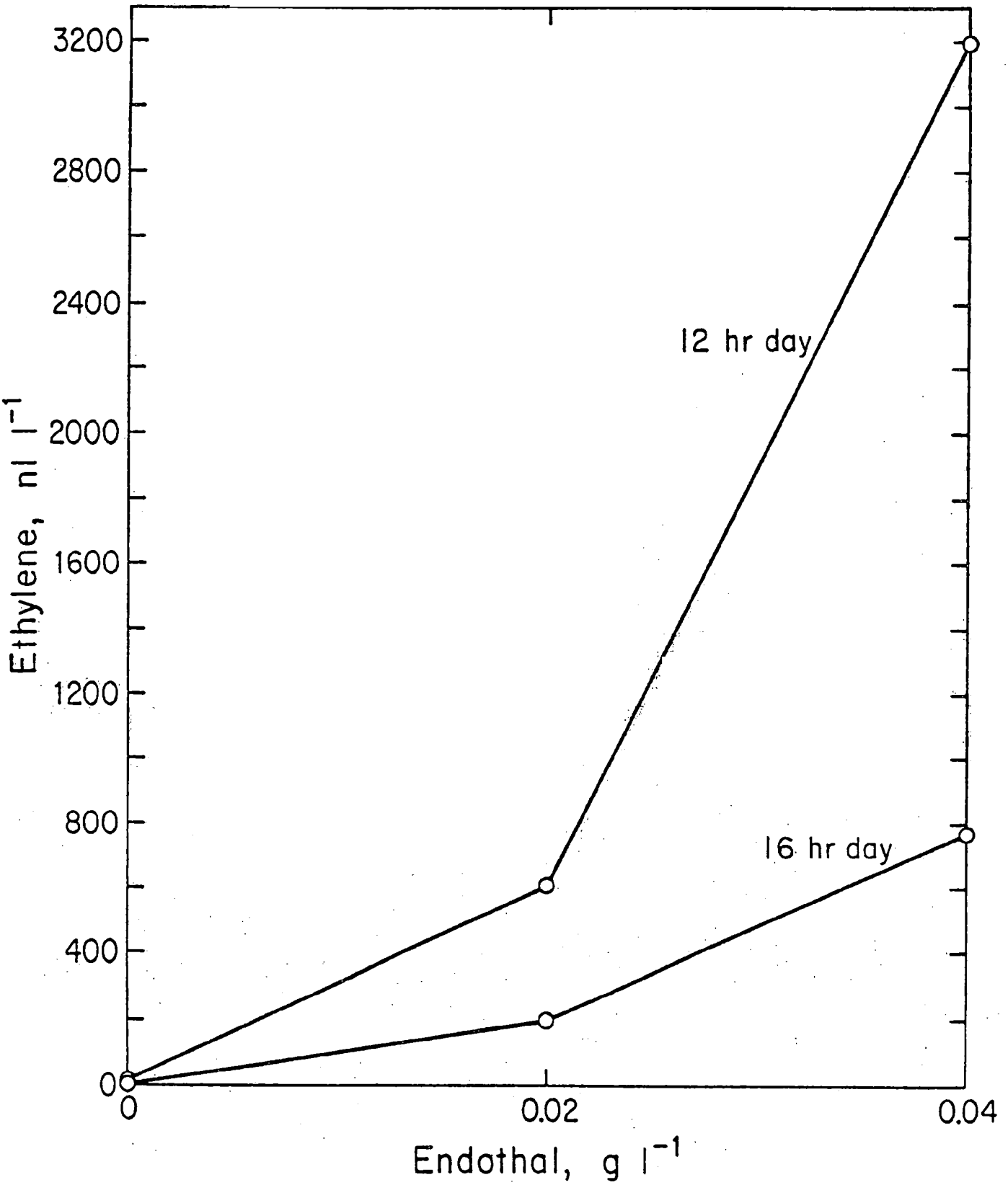
3200
2800
2400
2000
1600
1200
800
400
0
Ethylene, nl l^{-1}
12 hr day
16 hr day
0
0.02
0.04
Endothal, g l^{-1}

Figure 4

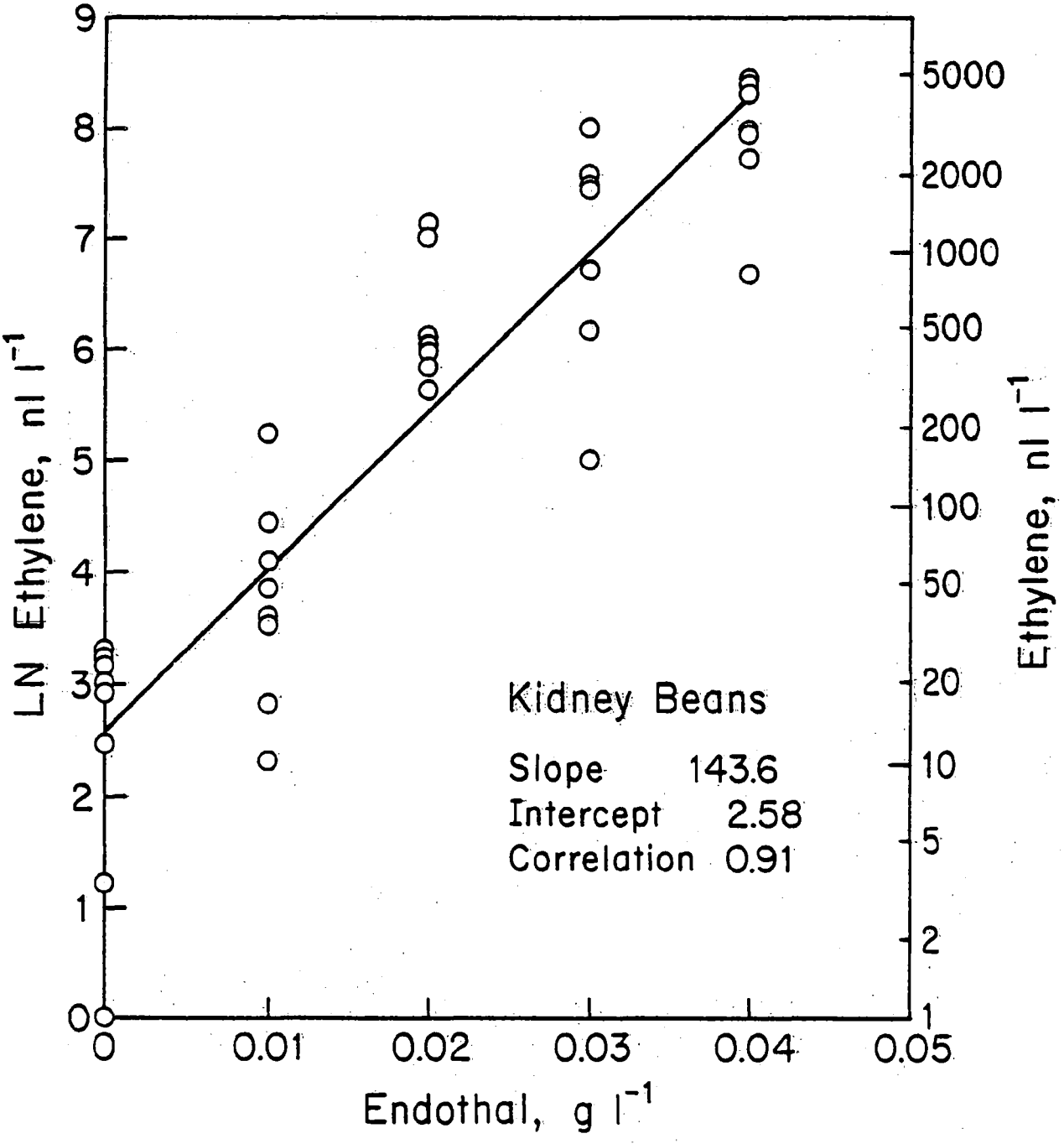
LN Ethylene, nl l^{-1}
Ethylene, nl l^{-1}
Endothal, g l^{-1}
Kidney Beans
Slope 143.6
Intercept 2.58
Correlation 0.91
0
1
2
3
4
5
6
7
8
9
0
0.01
0.02
0.03
0.04
0.05
1
2
5
10
20
50
100
200
500
1000
2000
5000

Figure 5

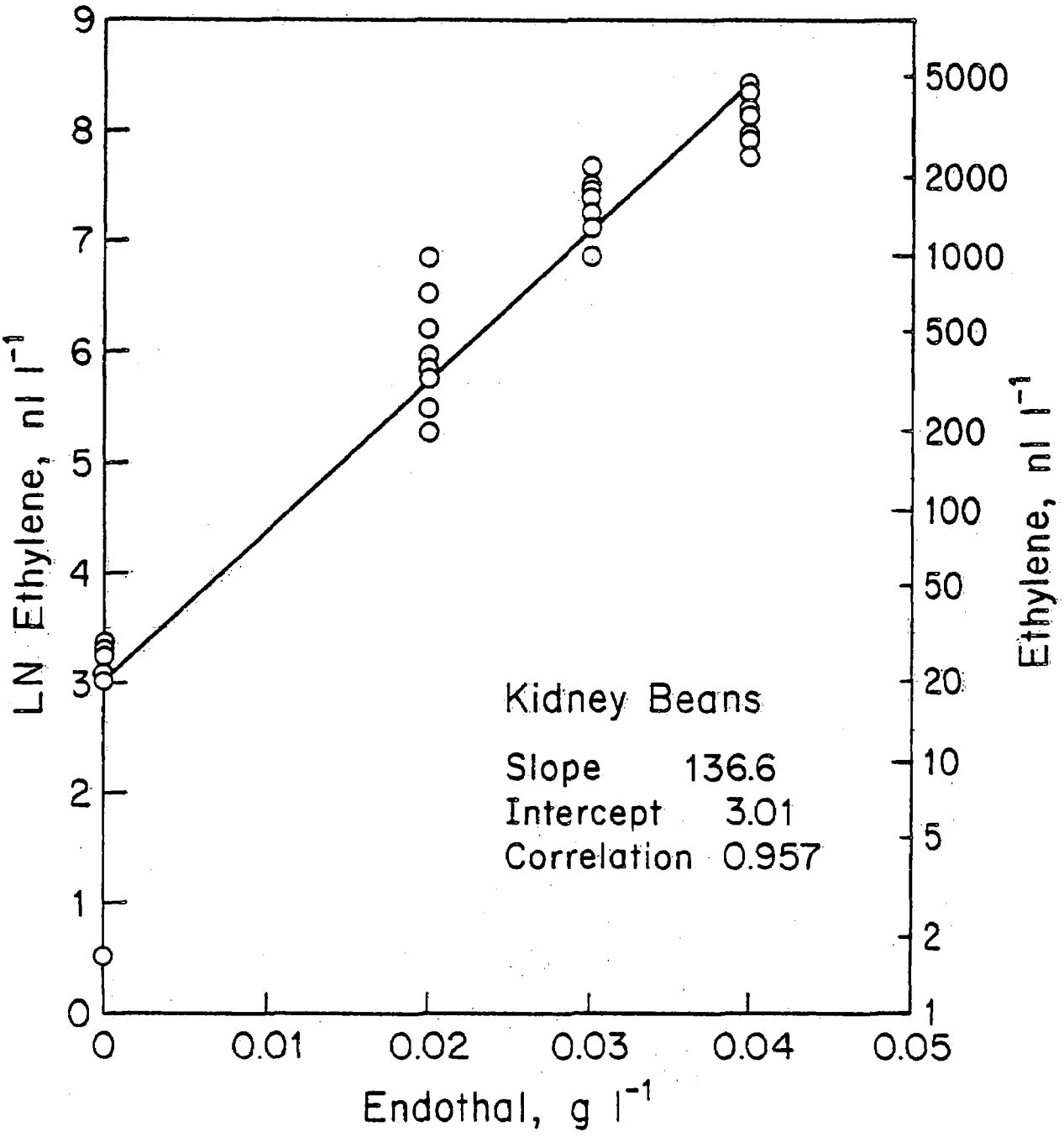
LN Ethylene, nl l^{-1}
Ethylene, nl l^{-1}
Endothal, g l^{-1}
Kidney Beans
Slope 136.6
Intercept 3.01
Correlation 0.957
0
1
2
3
4
5
6
7
8
9
0
0.01
0.02
0.03
0.04
0.05
1
2
5
10
20
50
100
200
500
1000
2000
5000

Figure 6

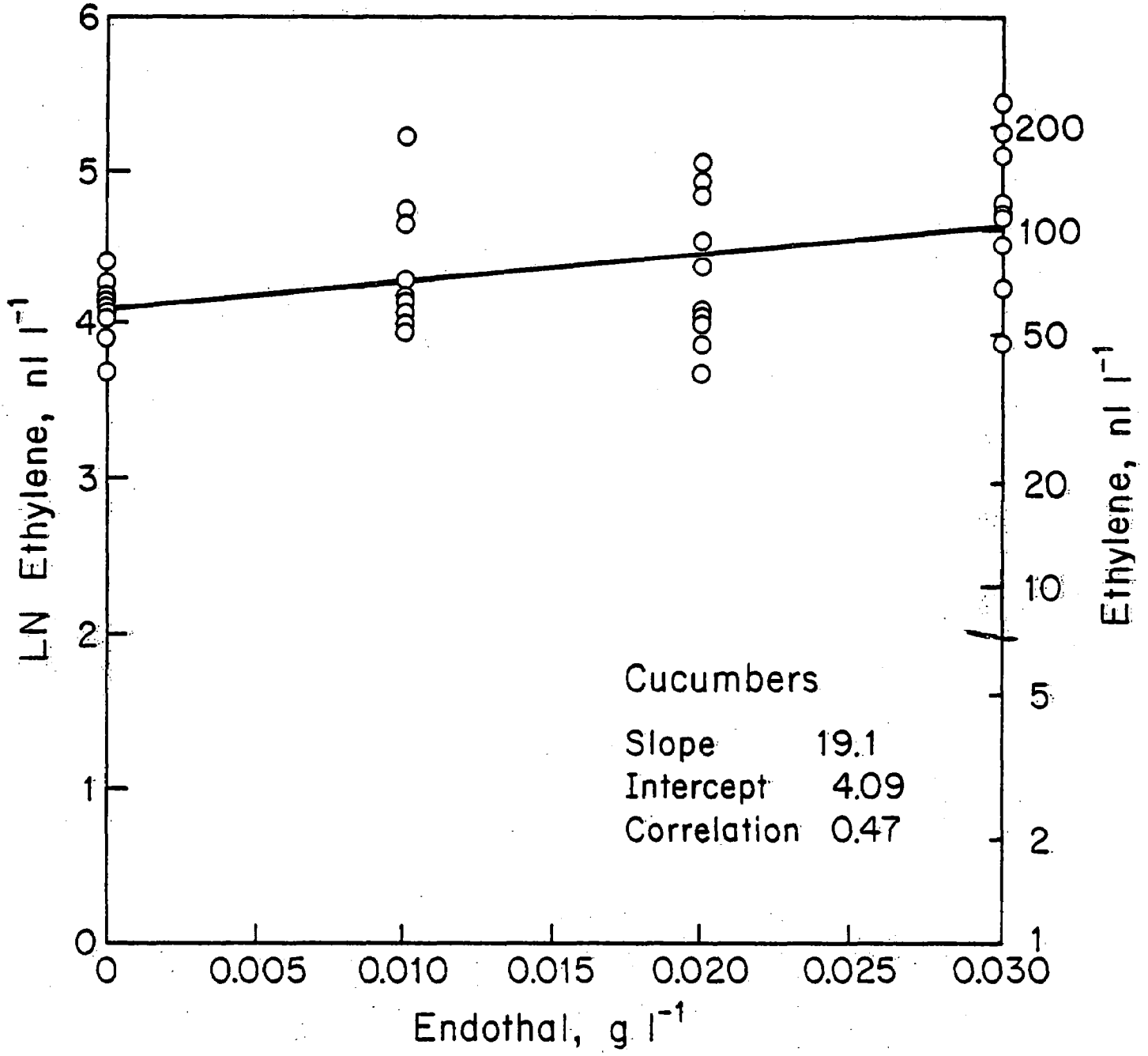
LN Ethylene, nl l⁻¹
Ethylene, nl l⁻¹
Endothal, g l⁻¹
Cucumbers
Slope 19.1
Intercept 4.09
Correlation 0.47
0
1
2
3
4
5
6
0
0.005
0.010
0.015
0.020
0.025
0.030
1
2
5
10
20
50
100
200

Figure 7

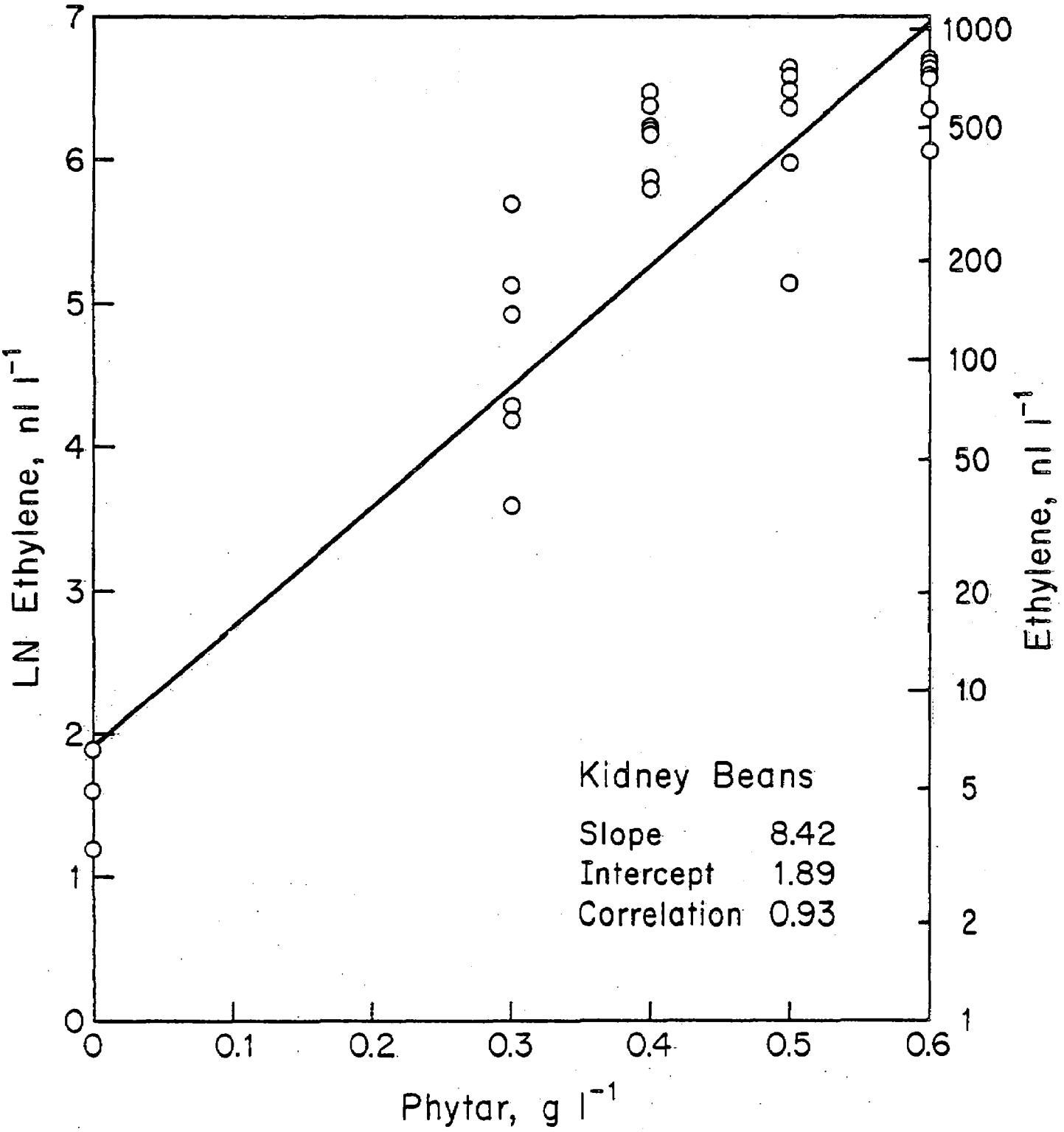
LN Ethylene, nl l^{-1}
Ethylene, nl l^{-1}
Phytar, g l^{-1}
Kidney Beans
Slope 8.42
Intercept 1.89
Correlation 0.93
0
1
2
3
4
5
6
7
0
0.1
0.2
0.3
0.4
0.5
0.6
1
2
5
10
20
50
100
200
500
1000

Figure 8

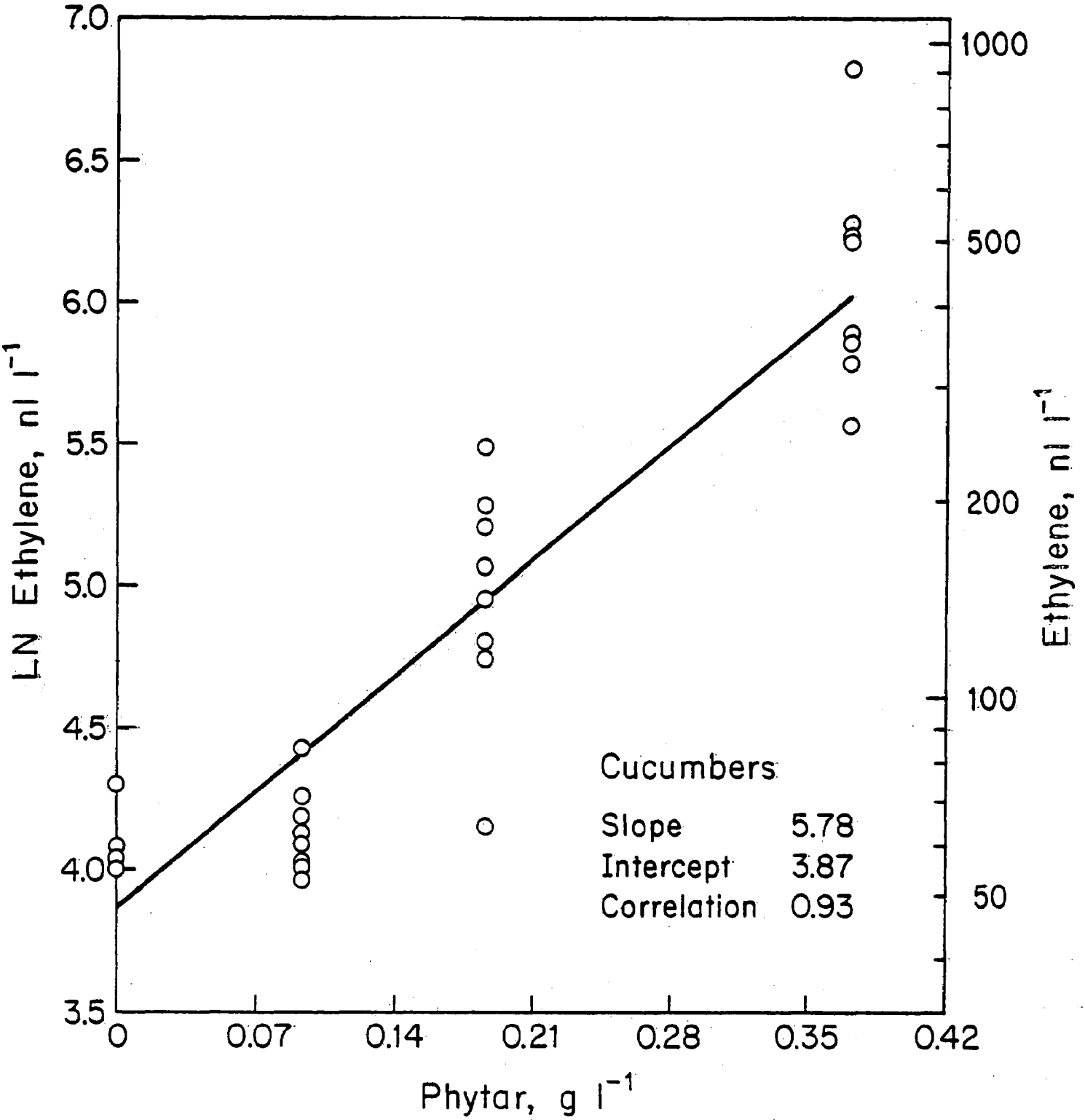
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
LN Ethylene, nl l⁻¹
1000
500
200
100
50
Ethylene, nl l⁻¹
Cucumbers
Slope 5.78
Intercept 3.87
Correlation 0.93
0
0.07
0.14
0.21
0.28
0.35
0.42
Phytar, g l⁻¹

Figure 9

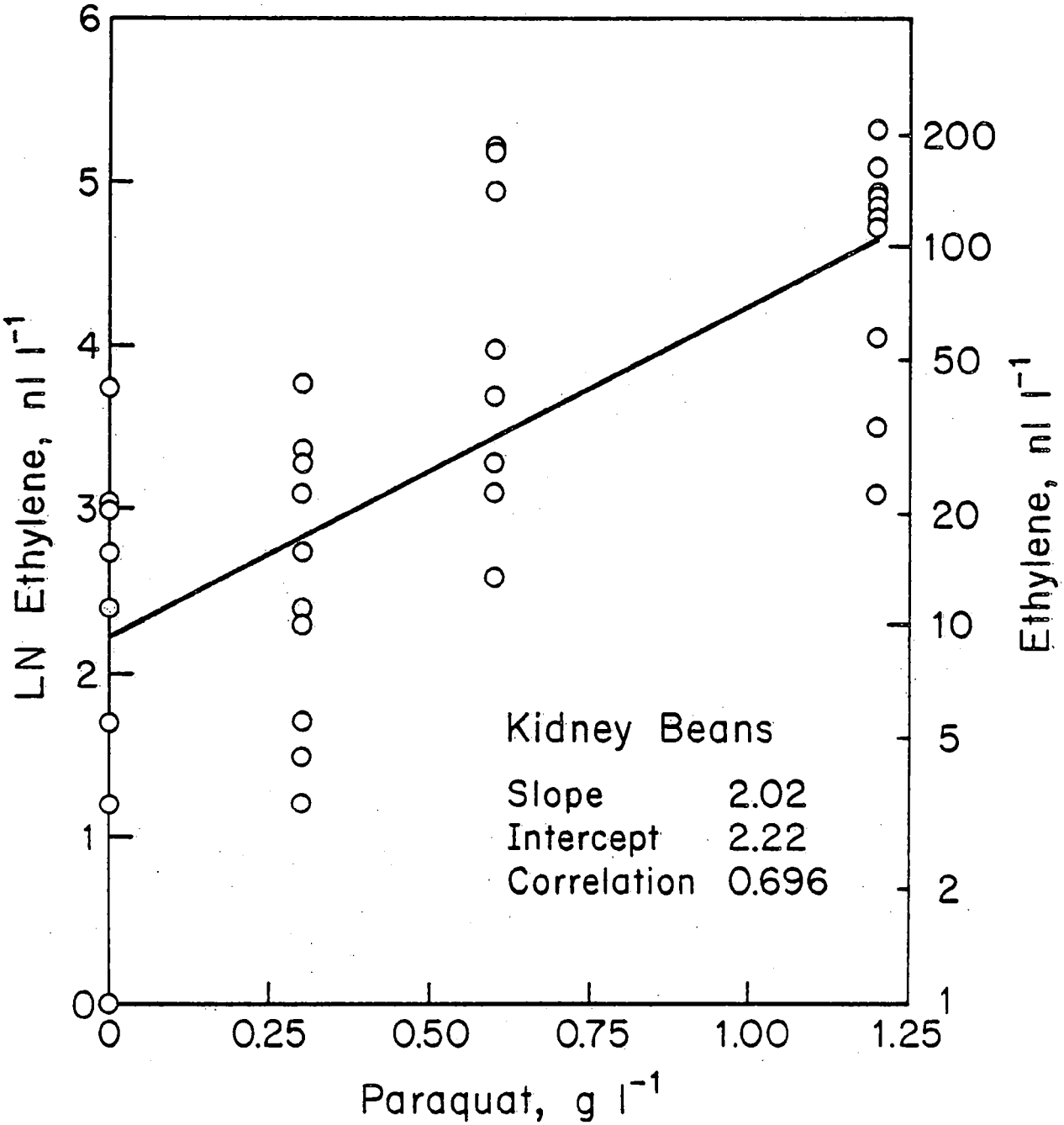
LN Ethylene, nl l^{-1}
Ethylene, nl l^{-1}
Paraquat, g l^{-1}
Kidney Beans
Slope 2.02
Intercept 2.22
Correlation 0.696
0
1
2
3
4
5
6
0
0.25
0.50
0.75
1.00
1.25
1
2
5
10
20
50
100
200

Figure 10

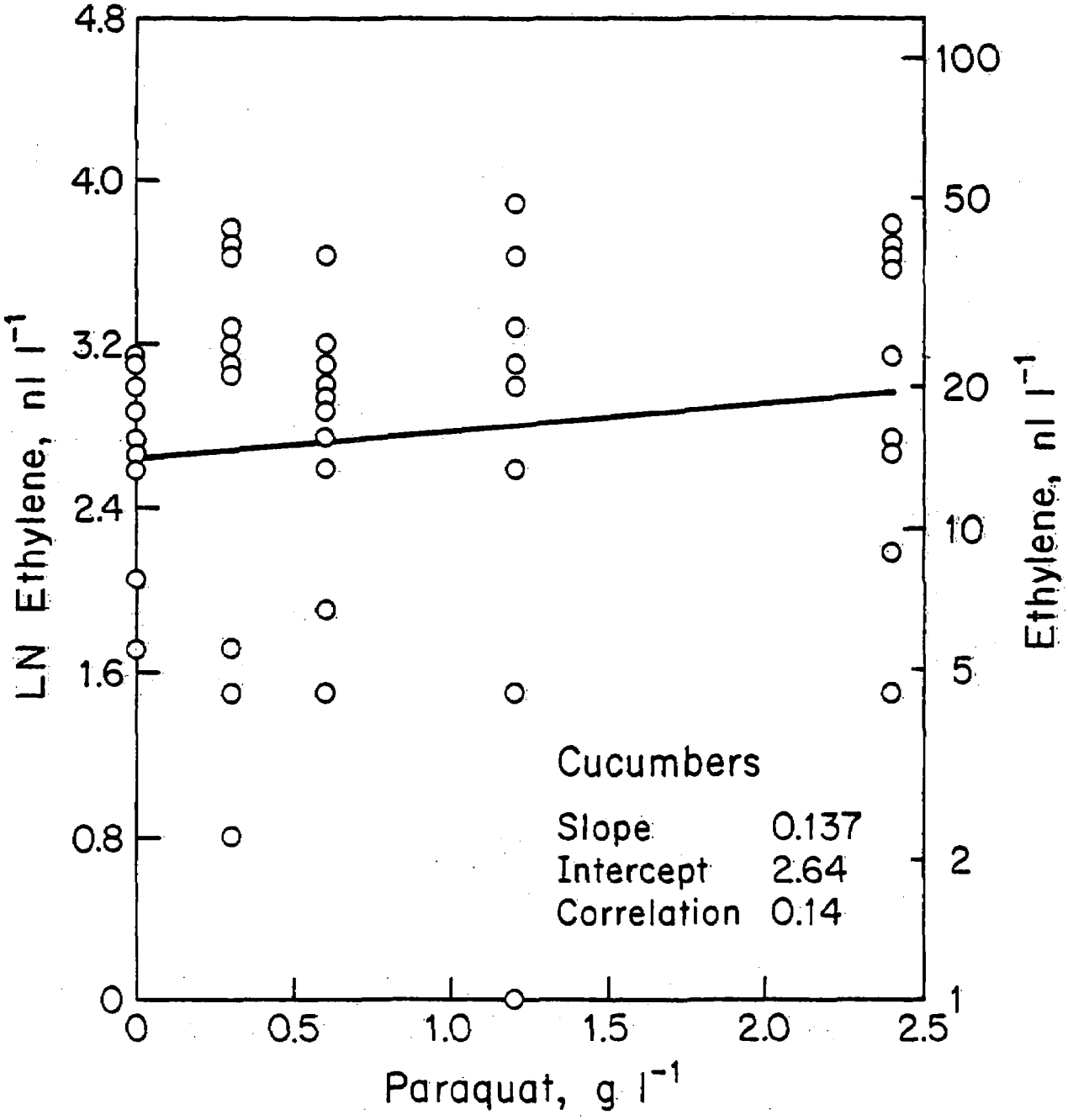
4.8
4.0
3.2
2.4
1.6
0.8
0
LN Ethylene, nl l^{-1}
100
50
20
10
5
2
1
Ethylene, nl l^{-1}
0
0.5
1.0
1.5
2.0
2.5
Paraquat, g l^{-1}
Cucumbers
Slope 0.137
Intercept 2.64
Correlation 0.14

Figure 11

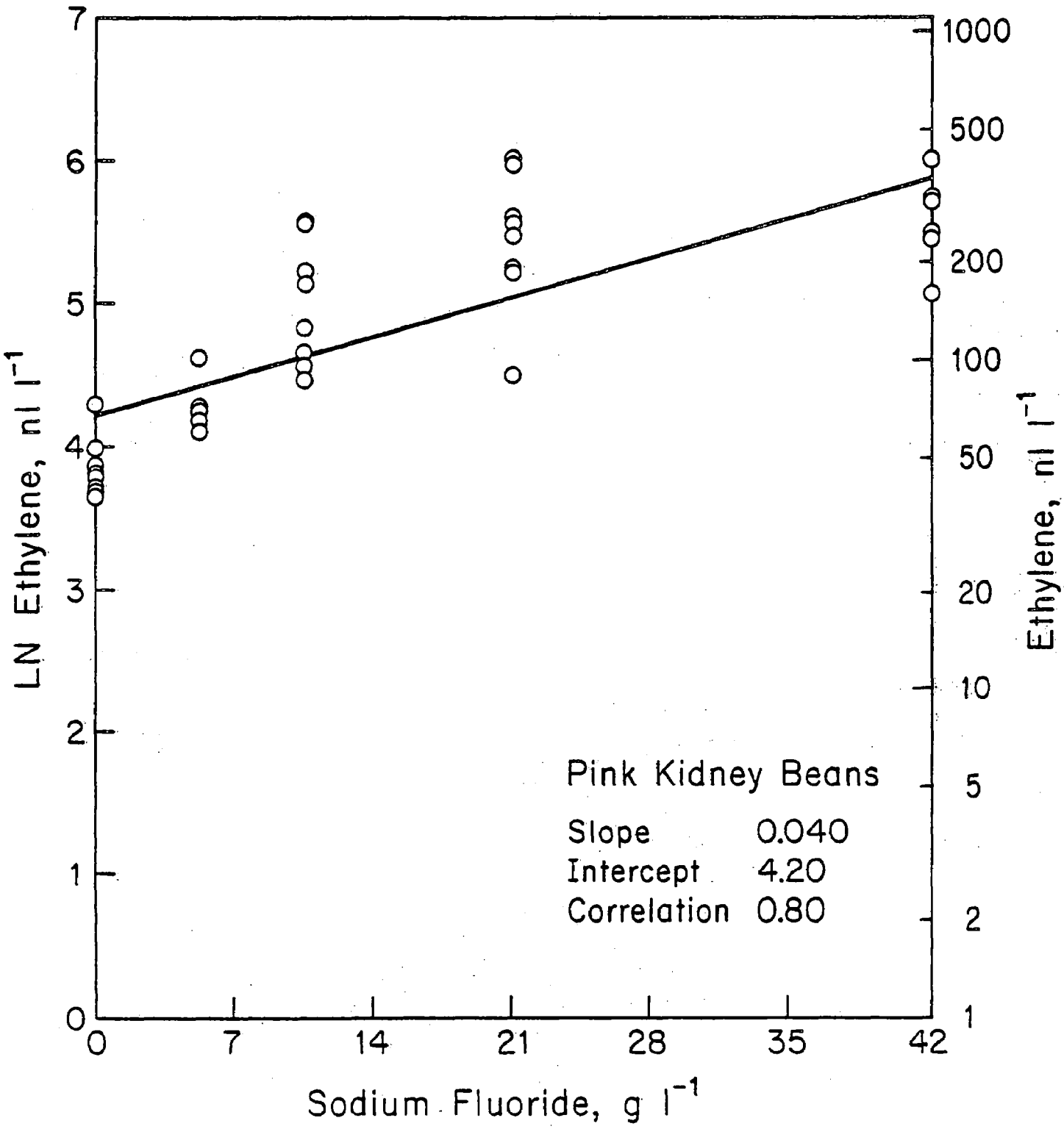
LN Ethylene, nl l⁻¹
Ethylene, nl l⁻¹
Sodium Fluoride, g l⁻¹
Pink Kidney Beans
Slope 0.040
Intercept 4.20
Correlation 0.80
0
1
2
3
4
5
6
7
1
2
5
10
20
50
100
200
500
1000
0
7
14
21
28
35
42

Figure 12

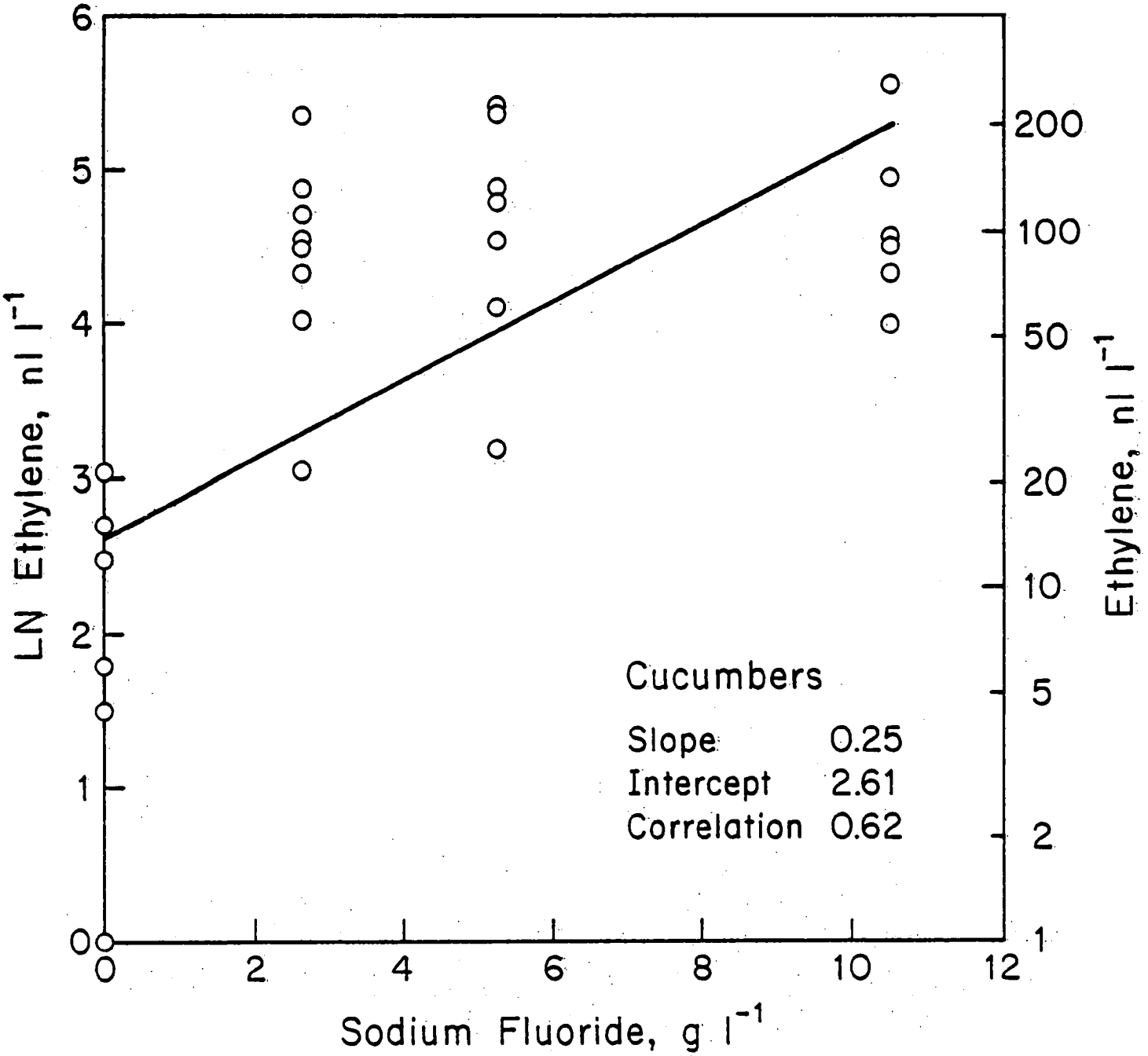
LN Ethylene, nl l-1
Ethylene, nl l-1
Sodium Fluoride, g l-1
Cucumbers
Slope 0.25
Intercept 2.61
Correlation 0.62
0
1
2
3
4
5
6
0
2
4
6
8
10
12
1
2
5
10
20
50
100
200

Figure 13

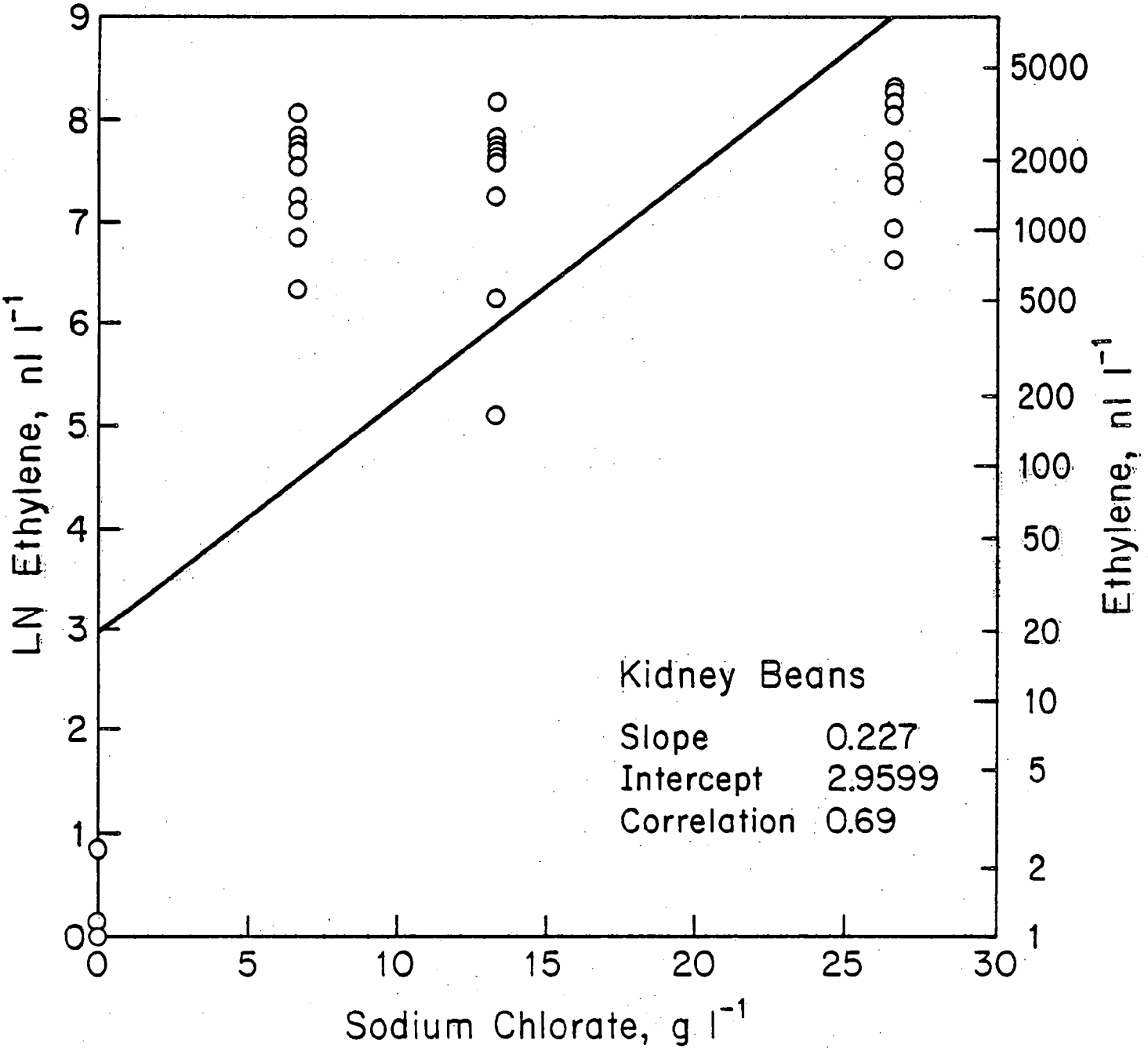
LN Ethylene, nl l^{-1}
Ethylene, nl l^{-1}
Sodium Chlorate, g l^{-1}
Kidney Beans
Slope 0.227
Intercept 2.9599
Correlation 0.69
0
1
2
3
4
5
6
7
8
9
5000
2000
1000
500
200
100
50
20
10
5
2
1
0
5
10
15
20
25
30

Figure 14

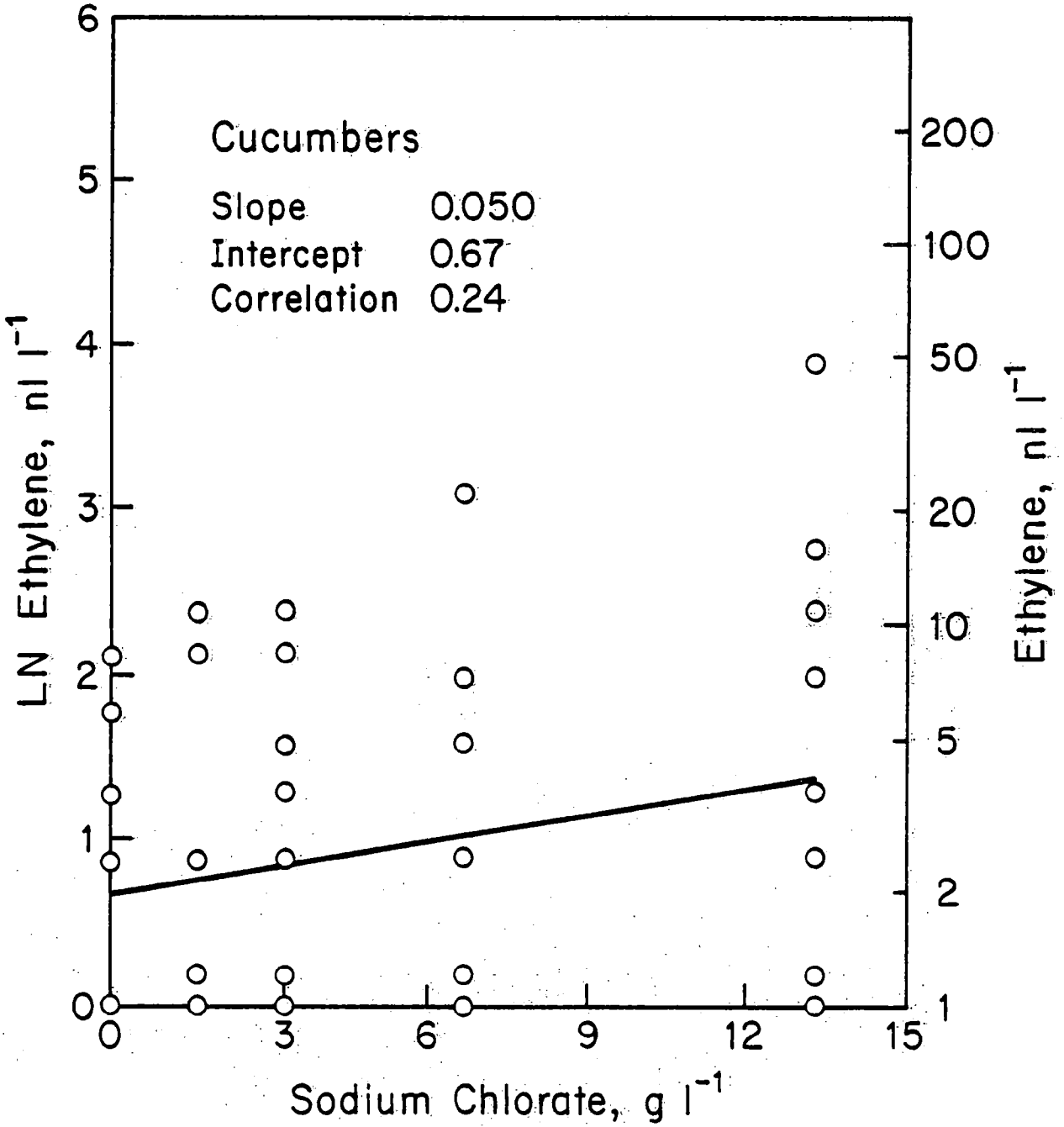
Cucumbers
Slope 0.050
Intercept 0.67
Correlation 0.24
LN Ethylene, nl l-1
Ethylene, nl l-1
Sodium Chlorate, g l-1
0
1
2
3
4
5
6
0
3
6
9
12
15
1
2
5
10
20
50
100
200

Figure 15

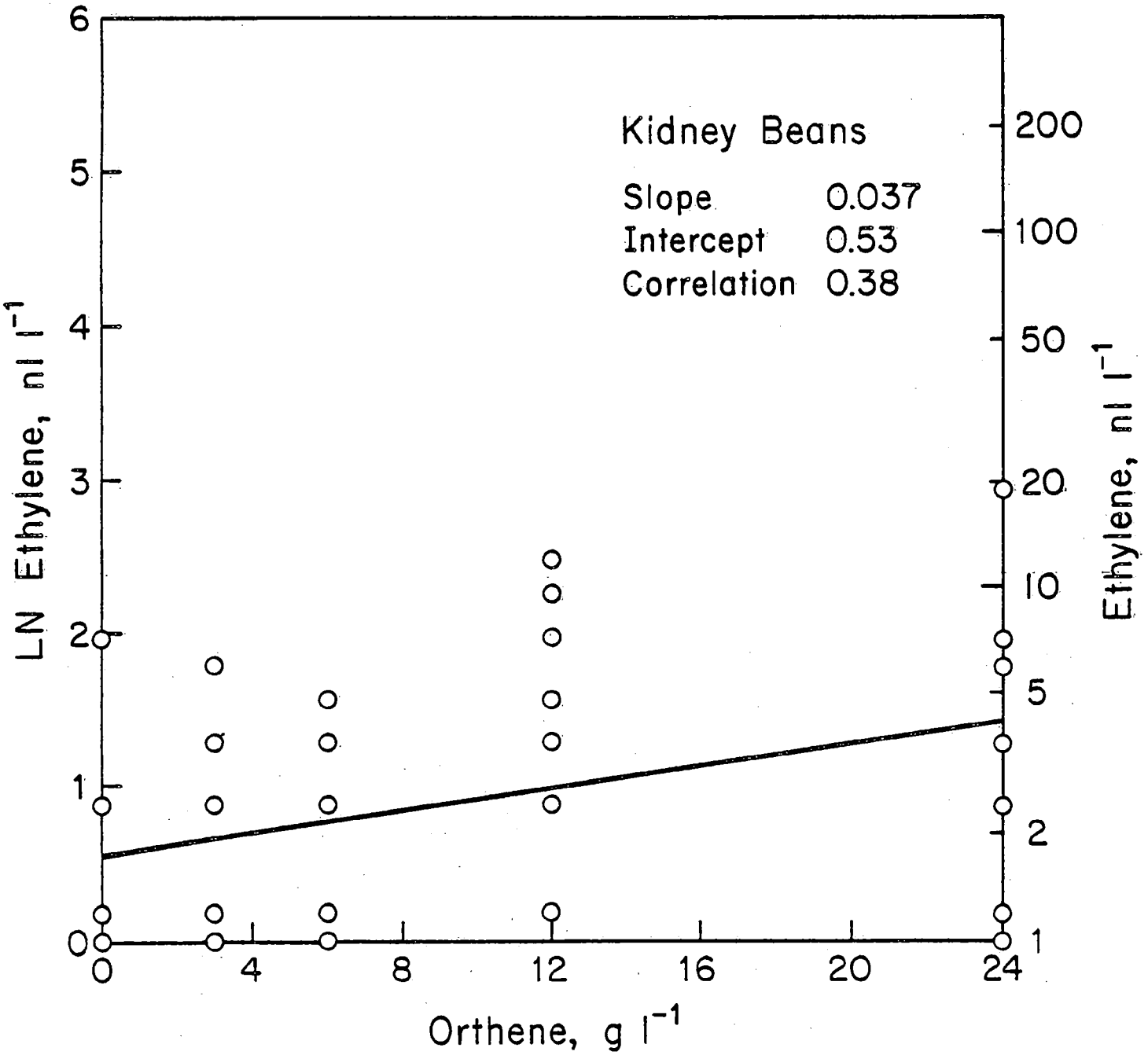
Kidney Beans
Slope 0.037
Intercept 0.53
Correlation 0.38
LN Ethylene, nl l⁻¹
Ethylene, nl l⁻¹
Orthene, g l⁻¹
0
1
2
3
4
5
6
0
4
8
12
16
20
24
1
2
5
10
20
50
100
200

Figure 16

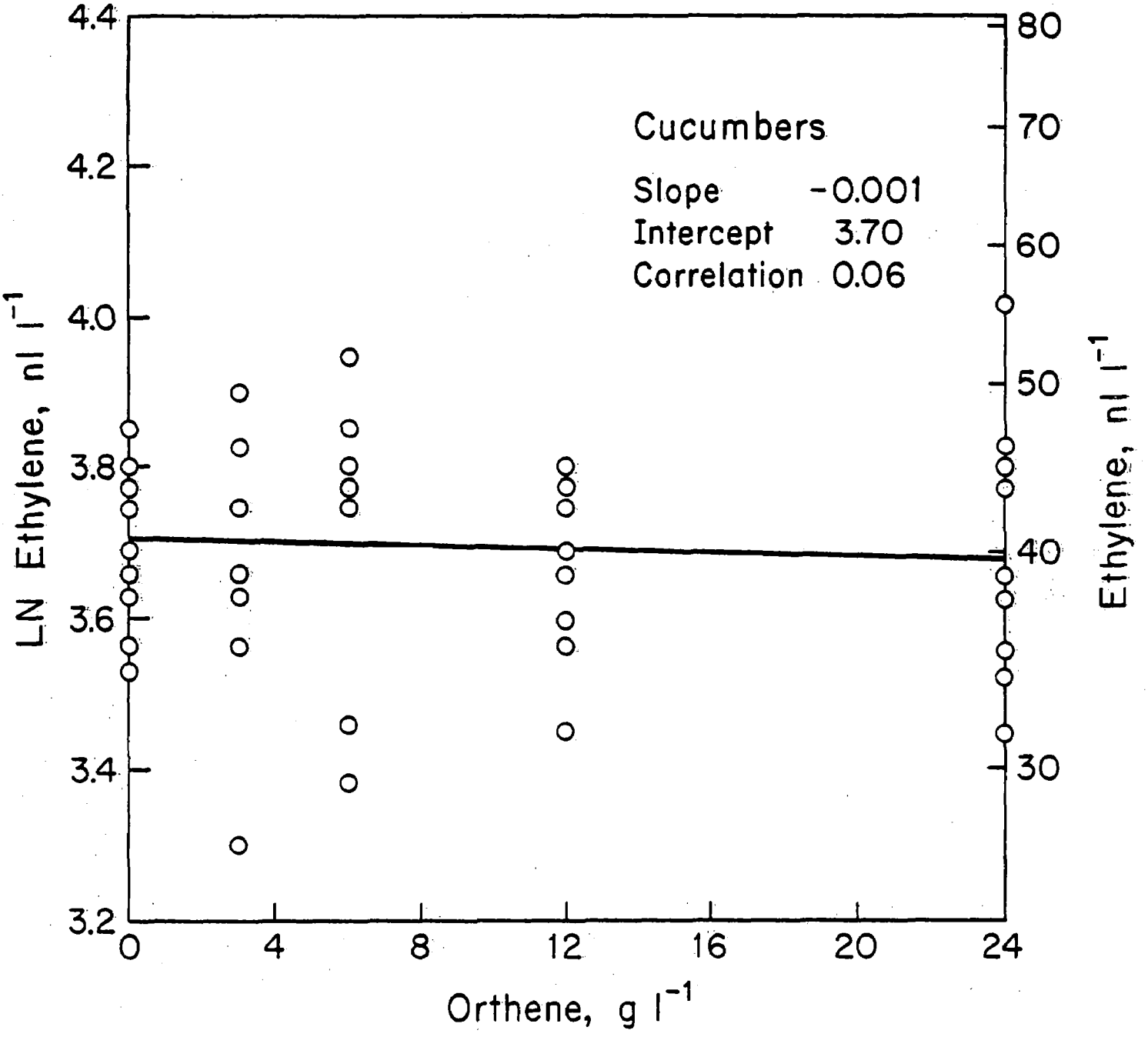
Cucumbers
Slope -0.001
Intercept 3.70
Correlation 0.06
LN Ethylene, nl l^{-1}
Ethylene, nl l^{-1}
Orthene, g l^{-1}
4.4
4.2
4.0
3.8
3.6
3.4
3.2
80
70
60
50
40
30
0
4
8
12
16
20
24

Figure 17

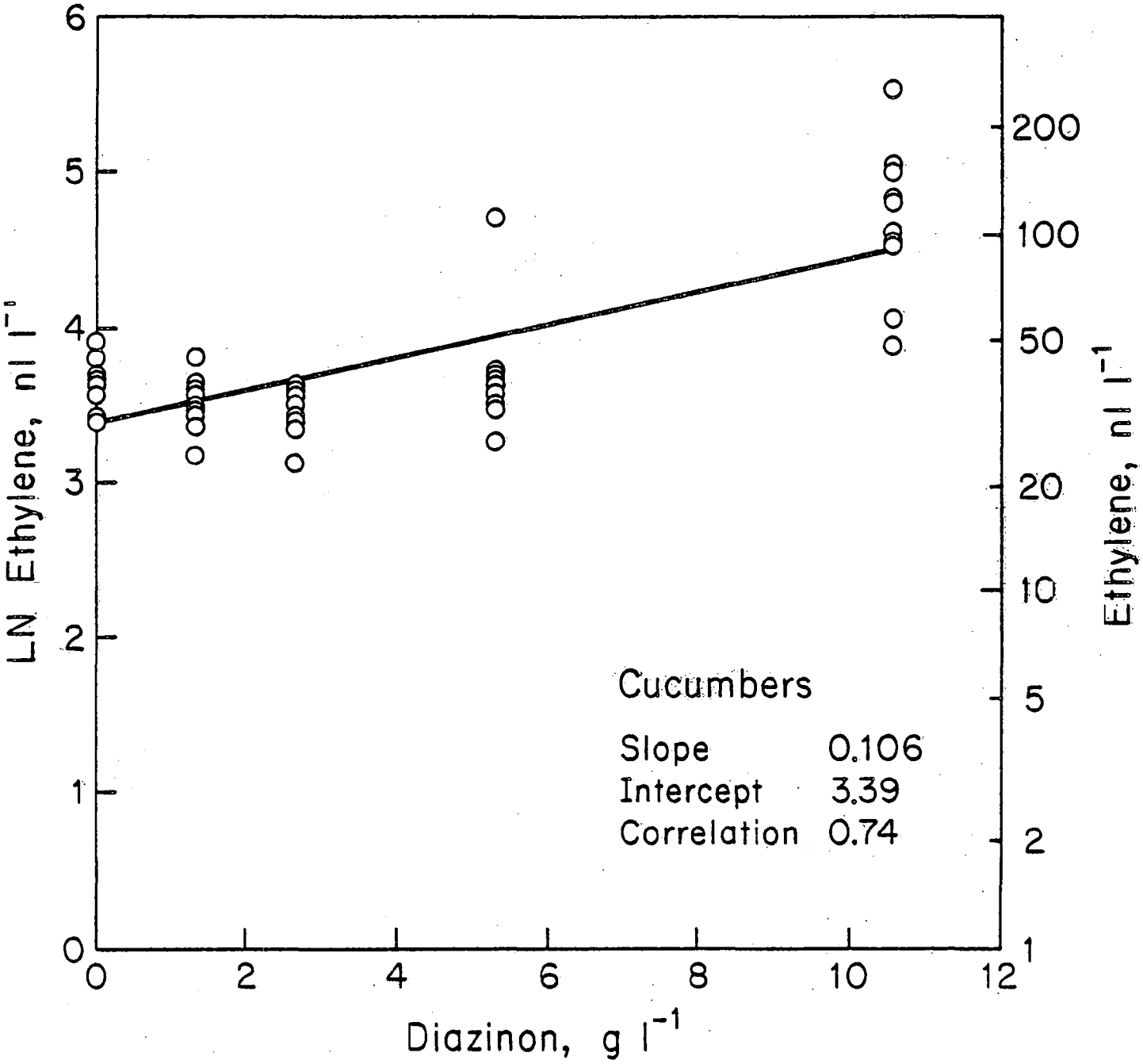
LN Ethylene, nl l⁻¹
Ethylene, nl l⁻¹
Diazinon, g l⁻¹
Cucumbers
Slope 0.106
Intercept 3.39
Correlation 0.74
0
1
2
3
4
5
6
0
2
4
6
8
10
12
1
2
5
10
20
50
100
200

Figure 18

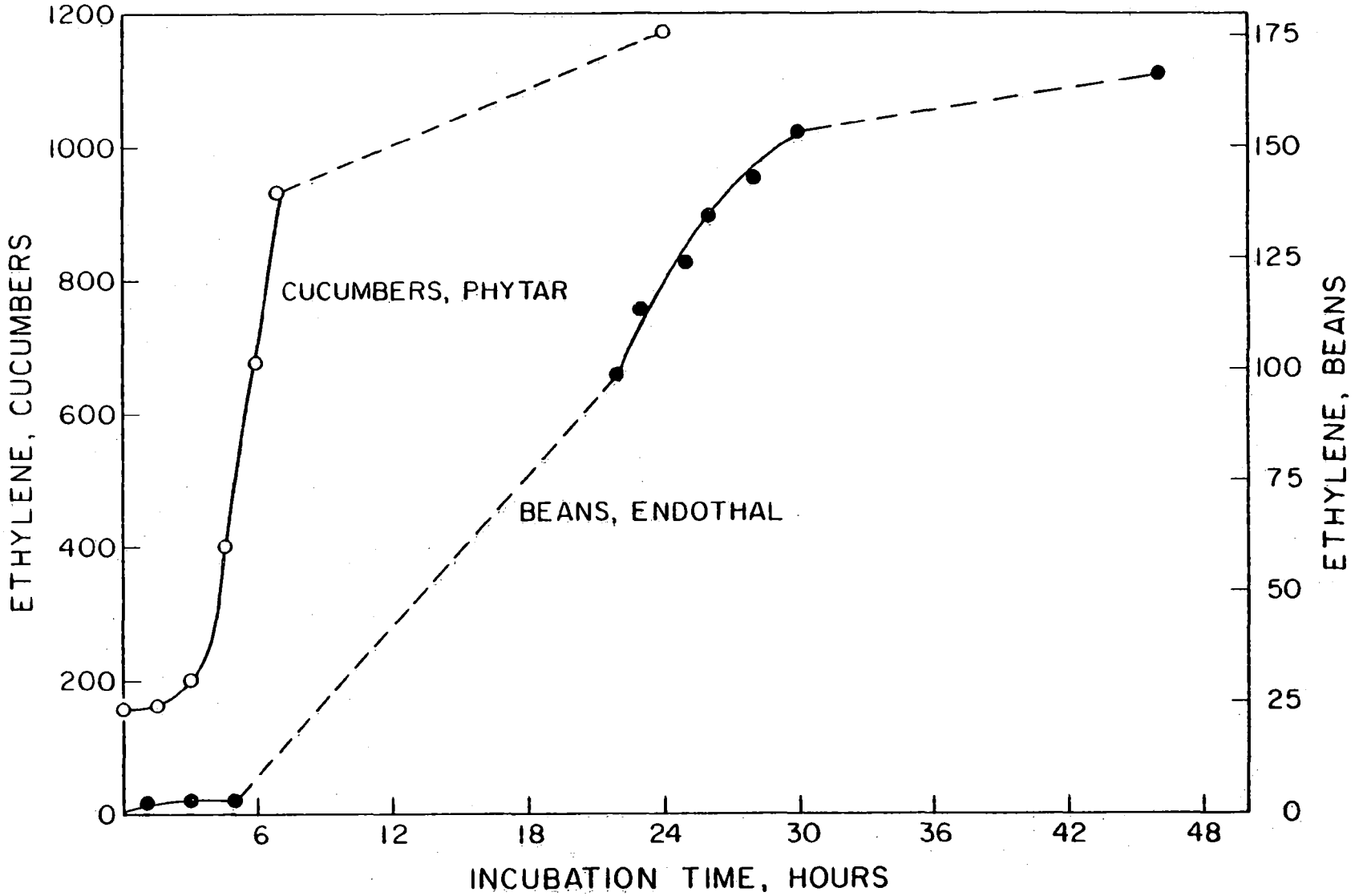

Figure 19. Ethylene evolution from cucumbers treated with Phytar and beans treated with Endothall.