BIOMETRICS OF PYRIDYLIUM PHOTOSYNTHATE INHIBITORS WITH *PHASEOLUS VULGARIS* FOLIAR TISSUES AND *CHLORELLA PYRENOIDOSA*

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INTRODUCTION

Pyridyliums are recently developed herbicides which are being used for the control of terrestial and aquatic weeds and for preharvest dessicants and defoliants. These chemicals give rapid dessication and top kill to a wide range of plant species infesting cropland and are effective herbicides to certain aquatic weeds. As the use of pyridyliums increases the problem of soil residues and their detoxification is receiving added attention.

Pyridyliums are rather unique herbicide compounds with cationic chemical activity. These compounds are tightly adsorbed within the soil cation exchange complex. Residues remaining in the soil are extremely difficult to determine and satisfactory procedures have not yet been devised.

The initial work of this study was concerned with the microbial breakdown of Paraquat by common soil fungi. The difficulty of determining this pyridylium in residual media by use of prescribed chemical procedures led to the initiation of studies for bioassay methods for active residue determinations. The purpose of these bioassay studies was to determine the concentrations of Paraquat that could be accurately estimated under carefully controlled conditions.

LITERATURE REVIEW

The herbicidal properties of the pyridyliums, Diquat, 6, 7-dihydrodipyrido (1,2-a:2',1'-c)-pyrazidiinium salt and Paraquat, 1,1' dimethyl-4,4' bipyridinium salt were first described by Brian et al. (7)[1] in 1955. It was found that several quaternary ammonia compounds were rapid acting dessicants which would kill the aerial part of most plants and then be rendered inactive as soon as contact was made with the soil.

The physiological factors involved in the action of pyridylium quaternary salts shows that they are not biologically active and only become active on the reduction to free radicals (12, 17, 20). This reduction involves the addition to the molecule of one electron and the loss of one positive charge. Brian (6) has shown that Paraquat is activated by metabolism of the plant and postulated that herbicide activity was a result of the addition of one electron per molecule. Homer et al. (17) in studying the redox properties and the mode of action of bipyridyl quaternary salts concluded that phytotoxicity and reduction were related. They hypothesized that herbicidal activity depends on the ability of the active compounds to form free radicals by the uptake of one electron. Commoner et al. (11) reported that free radicals are most likely to occur in biological systems from

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[1] Figures in parenthesis refer to Literature Cited.

oxidation reactions and from irradiation. They have also stated that the concentration of free radicals produced by radiation increased with increasing doses and that high unpaired electron content exists in many biological systems.

The reduction processes in plants are associated with photosynthesis and respiration. These reactions can furnish the electron needed to make the Paraquat molecule a free radical (1, 8). To produce the rapid effect of Paraquat in the field, Boon (4) has shown that light and chlorophyll are necessary indicating that the action is closely related to photosynthesis. Wilkinson (29) found a decrease in growth of common duckweed that had been treated with Paraquat as light intensity was increased. Blackburn and Weldon (3) in studying the effects of light quality on herbicidal activity of Paraquat found that the greatest activity was under red light. Compared to light used by plants in photosynthesis, the orange-red light produces the highest rate of photosynthesis and the greatest herbicide activity. Pierce et al. (22) in testing Paraquat found that highest herbicidal activity could be expected if the herbicide was applied during periods of darkness to allow translocation. Merkle (21) found that light and O_2 were essential in obtaining a bleaching effect on broadleaf bean extract that was treated with Paraquat and the herbicide action was much more rapid than the slower hormone type of herbicides.

It is now generally believed that the function of illuminated chlorophyll is to transform light energy into chemical energy by affecting electron transfer from water leading to the formation of reduced NAD and ATP. Therefore when chlorophyll is brought into contact with light its lost electron could be the one that reduces

Paraquat to its toxic free radical (4).

Paraquat is used in the field as a contact herbicide because it is inactivated upon contact with the soil. It is being used in fallow farming as a substitute for cultivation in controlling weeds. Hood et al. (16) used Paraquat to control weeds on wheat fields in place of cultivation and found that the yield was comparable to a cultivated field. Evans (13) found that in establishing wheatgrass Paraquat could be used very effectively in controlling competition. Ross and Cocks (23) reported that Paraquat was most effective in controlling grasses and Diquat gave better results on broadleafs. Shear (25) in using Paraquat with Atrazine in a no-tillage study in corn found that it was most effective for increasing the rate of kill without presenting any residue problems.

Scifres and Santelman (24) reported that Paraquat gave good weed control when used as a direct spray in sorghum and cotton fields. They found that tall cotton plants and sorghum plants over 6 inches were less susceptible to Paraquat injury than smaller plants.

The more recent work with pyridyliums has been in the area of aquatic weed control. White (28) in testing pyridylium compounds has shown that members of this family of compounds exhibit spectacular herbicidal activity under certain aquatic conditions. These compounds are rapidly adsorbed into the plant producing a rapid phytocidal action of aerial parts of the plants. MacKenzie (19) in field studies on aquatic weed control over the past five years has shown that pyridyliums make very good aquatic herbicides because of their physical and chemical characteristics. Their high solubility in water and their ability to effect a rapid knockdown and kill plant life at relatively

low concentrations has made them valuable as aquatic herbicides. Lawerence et al. (18) have reported that Paraquat will control certain aquatic plants for periods as long as several months with concentrations of .2 to .5 pound cation.

The inactivation of pyridyliums occurs immediately when they come into contact with the soil because of their strong cationic nature. Weber et al. (27) in studying the adsorption of Paraquat found that it is completely adsorbed by kaolonite and montmorillonite clays at the cation exchange sites. This strong adsorption to clay minerals present in soil or aquatic environments is a critical factor in determining fate and activity of this herbicide. Coats et al. (9) reported that Paraquat and Diquat were tightly adsorbed to bentonite and was unavailable to wheat at high rates.

Funderburk and Lawerence (14) have devised a bioassay procedure for determining residues of Paraquat applied for control of aquatic weeds. They found that Duckweed (Lemma minor) was extremely sensitive to very low concentrations. Blackburn and Weldon (3) in studying Duckweed and Azolla sensitivity to various rates of Paraquat and Diquat found increasing activity from .005 to 1.0 ppm cation. Taylor and Amling (26) studied the persistence of Paraquat in a sandy loam soil where applications of four pounds per acre were made at 2, 4 and 6 week intervals. Bioassay was preformed using wheat and it was found that after 12 weeks at the higher rates of Paraquat, 28 pounds per acre, strong inhibition of plant growth was still evident.

Bozarth et al. (5) conducted studies to determine if Paraquat could be degraded by soil microorganisms. They found that it could be degraded by an unidentified bacteria species and isolated two

degradation products. Funderburk and Lawerence (15) in studying degradation of labled Paraquat to CO_2 found no breakdown occurred in alligatorweed and broadleaf beans.

MATERIALS AND METHODS

The Paraquat used in all of these studies was a commercial formulation of the California Chemical Company containing two pounds of Paraquat cation per gallon.

Use of Chlorella Pyrenoidosa in Determination of Paraquat Activity:

The culture used in these studies was Chlorella pyrenoidosa, Chick, 1AC 251 obtained from the Culture Collection of Algae at Indiana Univ.

Cultures of Chlorella were grown autotrophically on an inorganic-distilled water medium under continuous light of 500 footcandles supplied by Gro-lux florescent lamps. The cultures were maintained at 30°C in 2.8 liter flasks and a constant flow of saturated compressed air was bubbled through the cell cultures to allow aeration and prevent settling. The inorganic salt medium was composed of 3 grams each: NH_4NO_3, K_2SO_4, and Phillips-Hart salt mix per liter of distilled water. P.-H. salt mixture in percent was: K_2HPO_4 32.2, $CaCO_3$ 30.0, NaCl 16.7, $MgSO_4$ 10.2, $CaHPO_4$ 7.5, Ferric Citrate 2.75, $MnSO_4$ .51, KI .08, $CuSO_4$ .03, $ZnCl_2$ .025, and Cobalt Chloride .005.

Stock cultures were developed to near the estimated peak log growth phase and cells were centrifuged from the culture medium and resuspended in distilled water. Equal volume of cell concentrations from the resuspended cultures were allotted to smaller flasks for differential Paraquat treatments.

For the described experiments algae cells suspended in distilled water were incubated in 125 ml Erlenmeyer flasks with Paraquat added to give a total volume of 25 mls. Following the incubation period, cells were separated by centrifugation and were frozen and ruptured, then washed with acetone to remove extractable chlorophyll. Percent transmission of the extractable chlorophyll was read on a Beckman spectrophotometer at 660 mμ. Dry cell weights were obtained by transferring cell material after chlorophyll extraction to tared weighing pans. Pans were oven dried overnight at 90°C and weights determined to nearest tenth a milligram.

Residual media remaining from Chlorella studies was quantitated for Paraquat by using prescribed sodium dithionite procedure (2). Two ml sodium dithionite solution (.2% in .3N NaOH) was added to 10 ml residual media and read as percent transmission on Beckman spectrophotometer at 394 mμ.

Initial work with Chlorella was conducted to determine at what concentrations, length of incubation and culture conditions were required to give a measure of Paraquat activity.

To determine the effect of length of incubation on Paraquat activity experimental cultures of Chlorella were treated with Paraquat to make 0, .5, 1, 1.5 and 2 ppm solutions. Treated cultures were incubated under continuous light of 500 footcandles at 30°C. Incubation times were 48, 72 and 96 hours and determinations were made of extractable chlorophyll, cell weights and Paraquat in residual media. Treatments were made in triplicate for each incubation period and cultures were shaken by hand three times a day.

The effect of agitation on Paraquat activity was studied using the same procedure as previous study except that Paraquat concentrations were 0.0, 0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 ppm. Cultures were incubated under the same light intensity and temperature but for only 72 hours. After stock cultures were centrifuged and resuspended in distilled water, the cell suspension was adjusted to an optical density of 0.75. Measurements were made of extractable chlorophyll, cell weights, optical density read at 600 mμ and Paraquat in residual media. One series of treated cultures was incubated quiescent and another series gently agitated on a Lab-Line water-bath shaker.

Paraquat Determinations Using Foliar Tissue:

Phaselous vulgaris L., variety Indian bean, leaf tissue was obtained from plants grown under greenhouse conditions in 4 inch square plastic pots in a loamy sand soil. All leaf tissue used was from seedling plants 10 to 20 days old.

Disks 6 mm in diameter were taken from the primary leaves with a paper hole puncher and placed five each, dorsal side down, in 60x20 mm disposable petri dishes. The dishes contained Paraquat solutions of 0, .01, .025, .05, .075, .1, .25, .5, .75 and 1 ppm and were incubated at 30°C for 36 hours with three replications of each treatment. Continuous light of 500 footcandles was supplied by Gro-lux lamps.

Following incubation, leaf disks were taken from petri dishes and extractable chlorophyll was removed with 10 ml of acetone after mastication in a tissue homogenizer. Percent transmission of extractable chlorophyll was read on a Beckman spectrophotometer at 660 mμ to give an estimation of Paraquat activity.

After establishing at what concentrations Paraquat could be detected with the leaf disk method, the influences of light intensity, temperature and length of incubation were measured.

The effect of light intensity was determined by preparing leaf disk in similar manner as described in the previous study. Concentrations of Paraquat were 0, .025, .05, .1, .5 and 1 ppm with light intensities of 0, 200, 500 and 750 footcandles. Leaf disks were incubated for 24 hours and measurement of activity was determined using the extractable chlorophyll.

The effect of temperature was measured by incubating leaf disks at 1, 15, 25, 35 and 45°C for 24 hours in Paraquat concentrations of 0, .025, .05, .1, .5 and 1 ppm. The light intensity was 750 footcandles and activity was measured by chlorophyll extraction.

The length of incubation on Paraquat activity was determined by incubating leaf disks in Paraquat solutions of 0, .025, .5 and 1 ppm under light intensity of 750 footcandles. After twelve hours a full series of treated leaf disks were removed and subsequent treated disks were removed on 2 hour intervals for an entire incubation of 24 hours. Chlorophyll was extracted to give a measurement of Paraquat activity.

Degradation Studies Using Soil Microorganisms:

The fungi, Trichoderma viride, Aspergillus niger and Aspergillus flavus were selected for this study. These are common soil inhabiting organisms and have demonstrated an ability to degrade many resistant organic compounds. The pure cultures were obtained from a collection of the U. S. Army Q. M. Natick Labs, Natick, Massachusetts.

The cultures were developed on a liquid media containing 0, .5, 1, 2, 5, 10, 20 and 100 ppm of Paraquat cation. The basic medium in grams per liter consisted of sucrose 40, citric acid 2, potassium sulfate 1, ammonium nitrate 3 and inorganic salt mixture (Phillip-Hart) 3. Composition of salt mixture is recorded in the section containing the constituents of the Chlorella medium.

Forty-five ml of medium was used in 500 ml cotton stoppered Erlenmeyer flasks with added Paraquat to give a total volume of fifty ml. Treatments were replicated three times in each study. Autoclave sterilization was used at 15 psi for 20 minutes and cultures were inoculated with spore suspensions after cooling. Cultures were incubated at 32°C for 96 hours.

Following incubation the weights of the fungi mycelial pads were obtained by placing the pads in tared weighing pans and drying them overnight at 100°C.

Residual media remaining after fungi growth were kept and the bioassay procedure using leaf disks were employed to obtain an estimate of Paraquat activity. Paraquat was also quantitated using the recommended sodium dithionite method.

Reduction of Paraquat activity by media alone was determined using the leaf disk method of bioassay with uninoculated treated media having the same concentrations as in the fungi growth studies. A series of like treatments in distilled water were used as a comparison of activity reduction.

Statistical significance of treatment F values and coefficient of variation were determined using conventional analysis of variance procedures for all experimental results in these studies.

RESULTS AND DISCUSSION

The unicellular photolithotroph, Chlorella pyrenoidosa 251, was highly sensitive to length of incubation, agitation and Paraquat concentration. Chlorella cultures not treated with Paraquat were used as a base of zero percent reduction for evaluation of treated cultures. There was always some color development in the chlorophyll extracting procedure even when cultures appeared to be completely chlorotic. These chlorotic cultures were based as 100 percent reduction even though a slight amount of color was measured. Data from these studies are presented in three dimensional graphs, when possible, to show relative reduction in chlorophyll as a result of culture conditions and Paraquat concentration. Data otherwise will be shown in tables.

Results of length of incubation and Paraquat levels for Chlorella are shown in Figure 1. Extractable chlorophyll decreased with increased Paraquat additions and incubation time. Dry weights obtained are shown in Table I. No consistent decrease or increase could be noted in weights and due to this lack of consistency no attempt was made to express this data in percent reduction. An increase in weight was noted after 48 hour period but no correlation could be made with Paraquat treatment. Results of chemical recovery of Paraquat using the sodium dithionite method are shown in Table II. A series of uninoculated treated media was incubated along with algae to find effects of media on Paraquat. Paraquat recovery was higher from residual algae

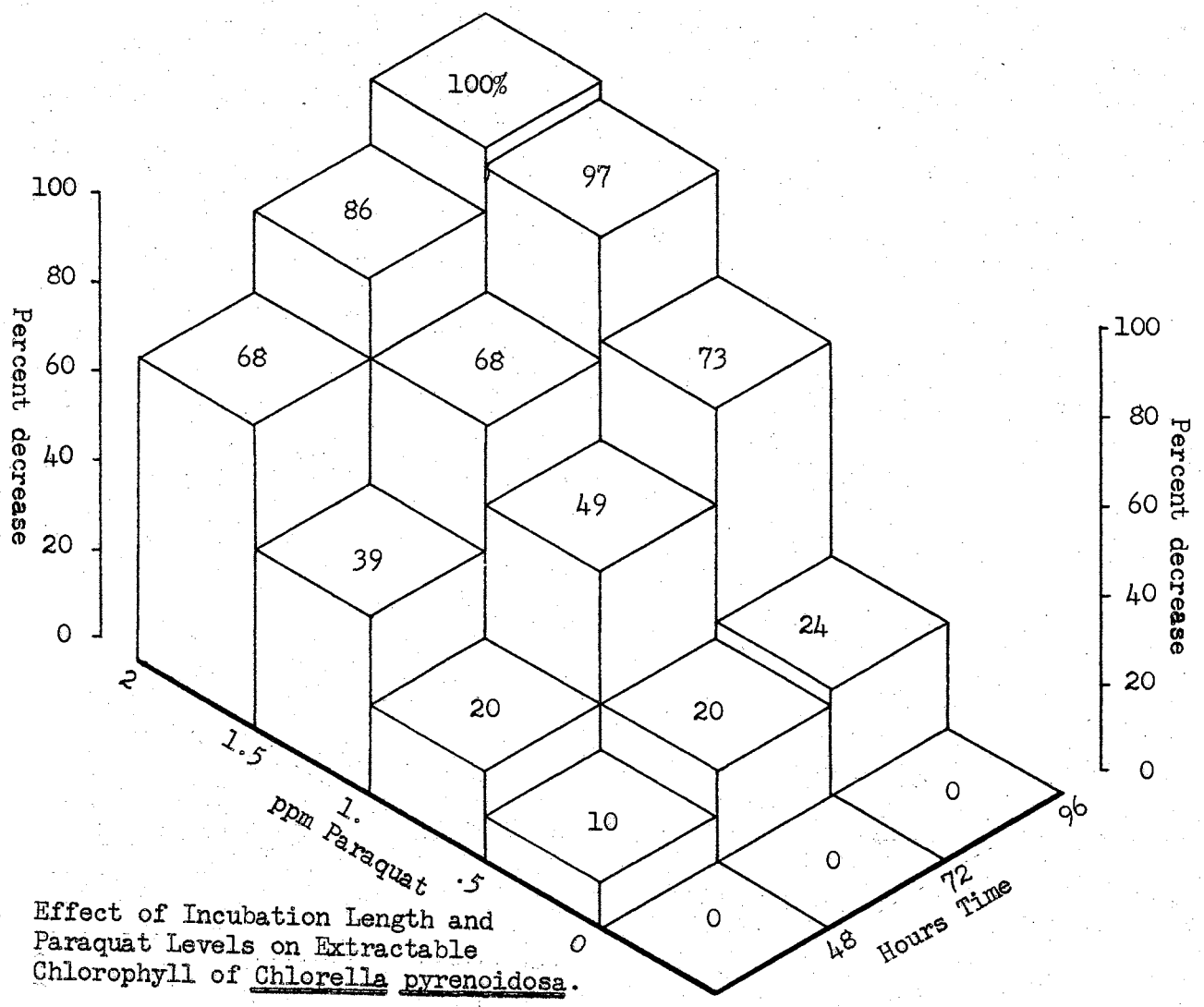

Figure 1. Effect of Incubation Length and Paraquat Levels on Extractable Chlorophyll of Chlorella pyrenoidosa.

TABLE I

EFFECT OF LENGTH OF INCUBATION AND PARAQUAT LEVELS ON DRY WEIGHT OF *CHLORELLA PYRENOIDOSA*[1]

Time Hours	Treatment ppm Paraquat	Dry weight grams*
48	0	.0045
	.5	.0042
	1	.0054
	1.5	.0061
	2	.0052
72	0	.0084
	.5	.0102
	1	.0090
	1.5	.0071
	2	.0067
96	0	.0122
	.5	.0079
	1	.0076
	1.5	.0081
	2	.0101

* Average of three determinations
48 hour F=30.28** C.V.= 4.57%
72 hour F= 6.02 C.V.=11.96%
96 hour F= 3.40 C.V.=20.00%

** Significant at .01 level

[1] Incubated under 500 footcandles of light at 30°C.

TABLE II

SODIUM DITHIONITE DETERMINATIONS OF PARAQUAT

FROM CHLORELLA RESIDUAL AND UNINOCULATED MEDIA[1]

Time Hours	Algae residual Treatment ppm Paraquat	Mean*	Time Hours	Uninoculated media Treatment ppm Paraquat	Mean*
48	0	86	48	0	100
	.5	75		.5	87
	1	65		1	75
	1.5	55		1.5	67
	2	48		2	57
72	0	85	72	0	97
	.5	74		.5	86
	1	64		1	82
	1.5	55		1.5	72
	2	48		2	65
96	0	85	96	0	95
	.5	75		.5	83
	1	64		1	67
	1.5	52		1.5	56
	2	43		2	48

* Average of three determinations read as percent transmission.

[1] Indubated under 500 footcandles of light at 30°C.

cultures than from the uninoculated medium and both could be correlated with concentration. Opaque interferences were encountered in both Chlorella and uninoculated media and little value was placed on these readings.

Results from the study comparing agitation during incubation versus complete quiescence are shown in Tables III, IV and V. Optical density readings (Table III) show that gently shaking the cultures caused an increase in cell growth. Readings of untreated check and of lower concentrations are all higher than initial optical density readings although the Paraquat treated cultures are not as high as the untreated check. In the higher concentrations optical density readings decreased as Paraquat levels increased. In the quiescent incubation, readings remained somewhat the same in both treated and untreated cultures and all readings were lower than the initial reading.

Cell weights from the cultures that were agitated showed that as Paraquat concentration increased weights decreased (Table IV). An initial weight was not taken but it was assumed that with the increasing of the optical density readings cell weights would increase. Average weights from treated cultures were not as high as the average weights of the untreated check. In the quiescent cultures, both treated and untreated check, the weights remained the same.

Extractable chlorophyll readings from the cultures that were agitated showed an increase as Paraquat increased (Table V). Overall readings were somewhat erratic but the average of the three replications showed a constant decline. In the quiescent cultures, readings were all erratic and no trend could be detected due to treatment.

TABLE III

EFFECT OF AGITATION AND PARAQUAT LEVELS

ON OPTICAL DENSITY OF CHLORELLA PYRENOIDOSA[1]

Treatment ppm Paraquat	Agitated Reading*	Percent decrease	Quiescent Reading*
0	1.02	0	.67
.05	.84	30	.65
.1	.85	32	.68
.5	.59	72	.66
1	.57	75	.67
2	.53	81	.63
5	.42	100	.69
	F=66.02**	C.V.=6.8%	C.V.=4.5%

* Average of three determinations read as optical density
** Significant at .01 level
[1] Incubated under 500 footcandles of light at 30°C for 72 hours.

TABLE IV

EFFECT OF AGITATION AND PARAQUAT LEVELS

ON DRY WEIGHT OF CHLORELLA PYRENOIDOSA[1]

Treatment ppm Paraquat	Agitated Weight*	Percent decrease	Quiescent Weight*
0	.0110	0	.0079
.05	.0095	28	.0073
.1	.0098	23	.0077
.5	.0072	73	.0075
1	.0063	90	.0071
2	.0065	87	.0074
5	.0059	100	.0073
	F=18.78**	C.V.=10.9%	C.V.=7.1%

* Average of three determinations in grams
** Significant at .01 level
[1] Incubated under 500 footcandles of light at 30°C for 72 hours.

TABLE V

EFFECT OF AGITATION AND PARAQUAT LEVELS ON

EXTRACTABLE CHLOROPHYLL FROM CHLORELLA PYRENOIDOSA[1]

Treatment ppm Paraquat	Agitated Reading*	Optical Density	Percent decrease	Quiescent Reading*	Optical Density
0	42	.377	0	44	.357
.05	40	.398	0	39	.409
.1	48	.319	11	41	.387
.5	64	.194	41	45	.347
1	87	.061	66	42	.377
2	94	.027	100	49	.310
5	95	.022	100	40	.398
	F=24.21**		C.V.=2.98%		C.V.=12.19%

* Average of three determinations read as percent transmission
** Significant at .01 level

[1] Incubated under 500 footcandles of light at 30°C for 72 hours.

TABLE VI

SODIUM DITHIONITE DETERMINATION OF

PARAQUAT FROM RESIDUAL MEDIA[1]

Treatment ppm Paraquat	Agitated Reading*	Quiescent Reading*
0	100	100
.05	100	100
.1	100	100
.5	85	86
1	78	78
2	65	63
5	35	35

* Average of three determinations read as percent transmission

[1] Incubated under 500 footcandles of light at 30°C for 72 hours.

Chemical determination of Paraquat remaining in residual media read the same in both agitated and quiescent cultures (Table VI). Readings could be correlated with Paraquat additions.

The results of the first leaf disk study (Table VII) indicated that Paraquat could be detected at concentrations as low as .025 and possibly as low as .01 ppm. The extractable chlorophyll readings were somewhat erratic within treatments but were generally correlated with Paraquat concentration.

Effects of Paraquat on leaf disks as a function of various light intensities is illustrated in Figure 2 as percent decrease in extractable chlorophyll. As light intensity and concentration of Paraquat increased the amount of reduction increased to where greatest reduction was obtained at high light intensities and high concentrations. Treated disks not exposed to light showed no loss of extractable chlorophyll.

Figure 3 shows the results of the study in which leaf disks were incubated at temperatures ranging from 1° to 45°C. The measurable effect of temperature was similar to that of light in that as temperature and concentration increased, rates of loss of extractable chlorophyll increased. At the lowest temperature (1°C) no decrease was obtained from any treatments.

Results from the time-course study are shown in Figure 4 as percent decrease in extractable chlorophyll. The loss of chlorophyll shows a steady decline as length of incubation period and Paraquat concentration increased. At the low concentration no reduction could be detected for 18 hours, however, at the end of 24 hours a measurable

TABLE VII

EFFECT OF PARAQUAT ON THE EXTRACTABLE CHLOROPHYLL FROM LEAF DISKS[1]

Treatment ppm Paraquat	Reading*	Optical Density	Percent decrease
0	30	.523	0
.01	34	.469	6
.025	56	.252	37
.05	58	.237	40
.075	75	.125	64
.1	76	.119	66
.25	97	.013	100
.5	96	.018	100
.75	97	.013	100
1	98	.009	100

F=291.7** C.V.=7.29%

* Average of three determinations read as percent transmission
** Significant at .01 level

[1] Incubated under 500 footcandles of light at 30°C for 36 hours.

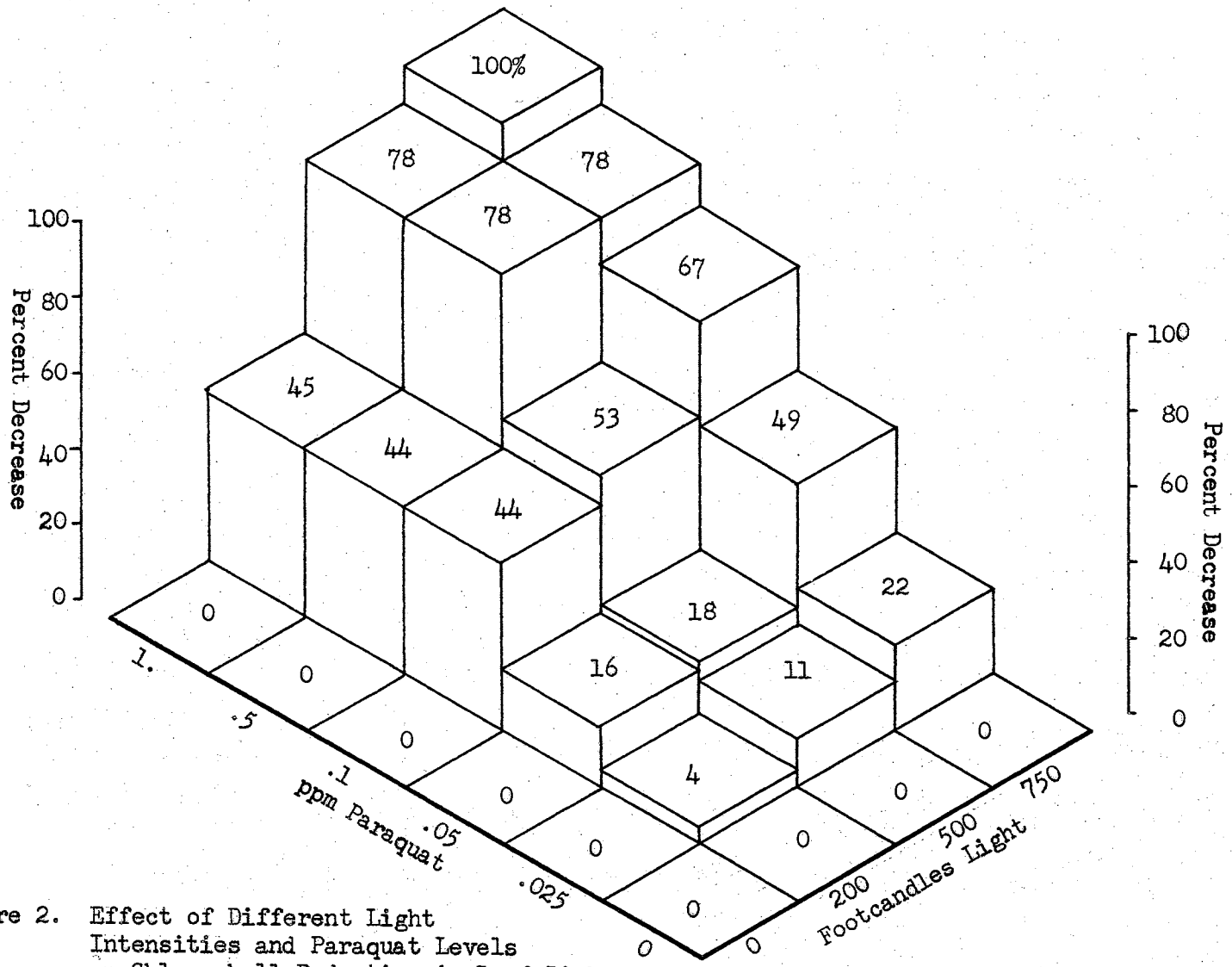

Figure 2. Effect of Different Light Intensities and Paraquat Levels on Chlorophyll Reduction in Leaf Disks.

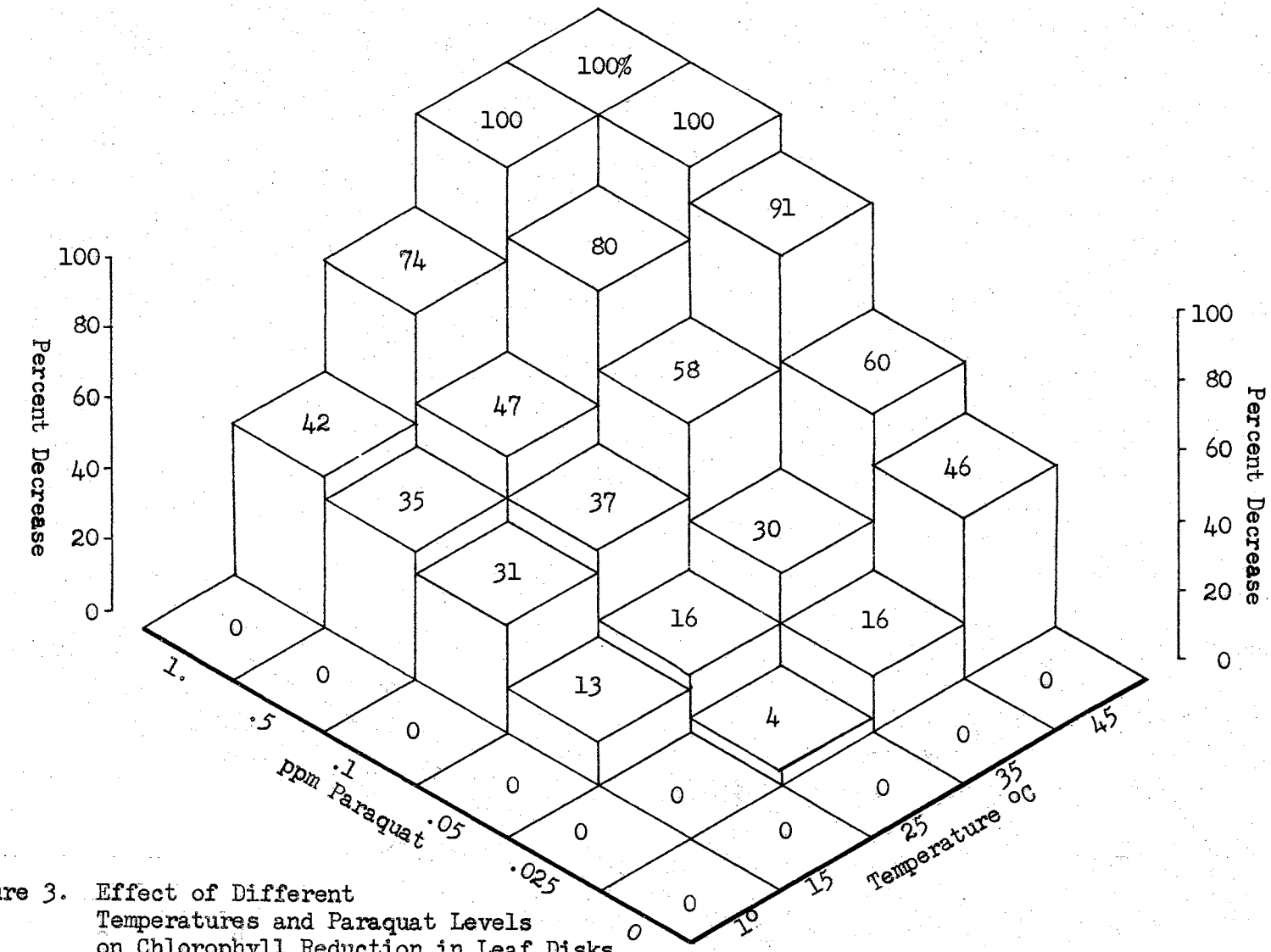

Figure 3. Effect of Different Temperatures and Paraquat Levels on Chlorophyll Reduction in Leaf Disks.

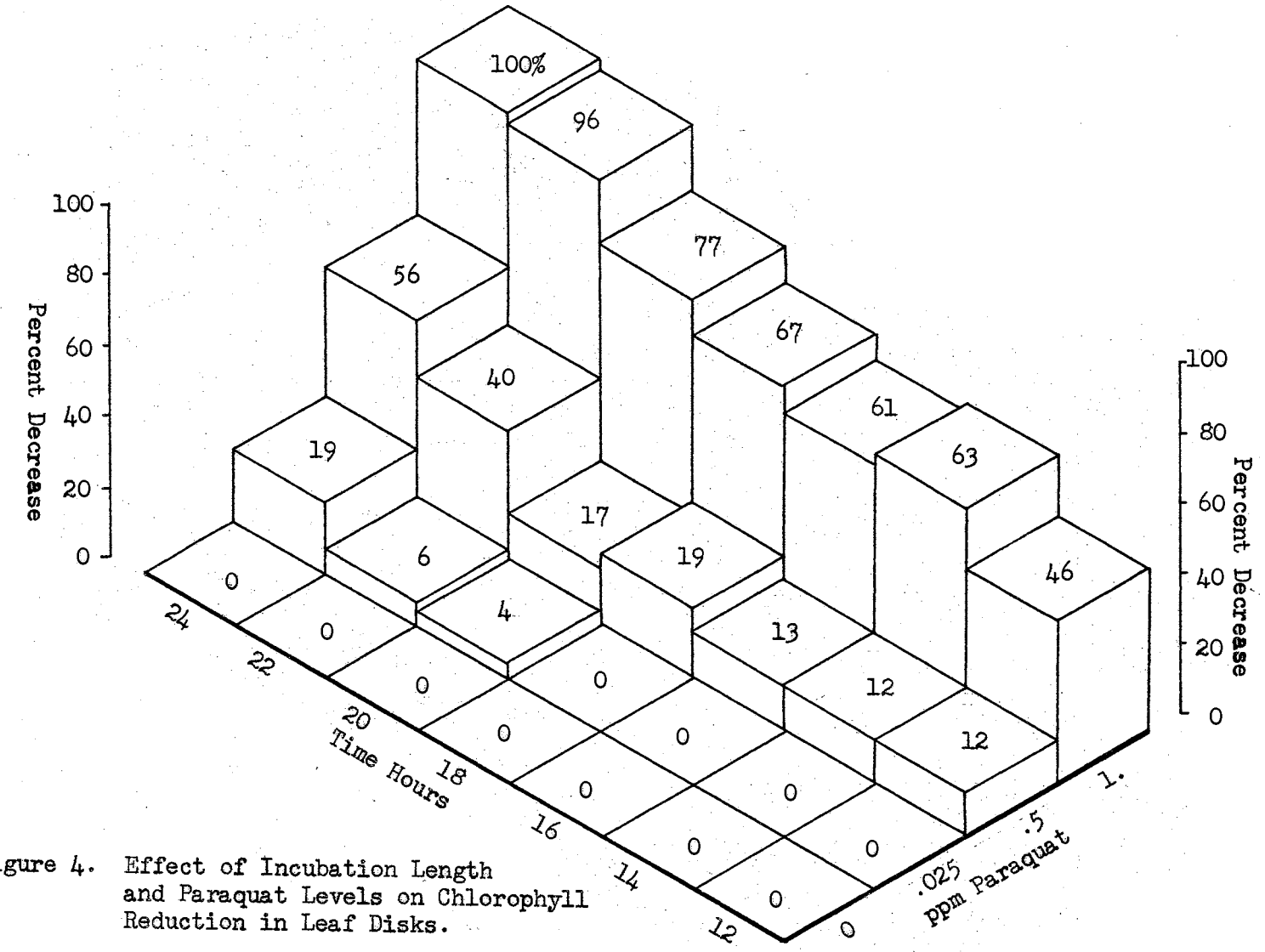

Figure 4. Effect of Incubation Length and Paraquat Levels on Chlorophyll Reduction in Leaf Disks.

reduction could be obtained. The higher concentrations showed reduction after 12 hours incubation and this reduction increased with increasing time.

Pad weights of fungi tested are given in Table VIII and results show no reduction in growth due to Paraquat additions.

Table IX illustrates the effects of media alone on Paraquat activity as compared to the same concentrations in distilled water. These concentrations are the same used in the fungus studies and are all high enough to give 100 percent reduction in extractable chlorophyll as indicated by previous studies. Dilutions of 1:10, 1:5 and 1:1 of treated media were made with distilled water to give concentrations at which treatment differences could be detected. These results indicate that the media alone will cause a measurable amount of reduction in Paraquat activity.

Bioassay of fungus media was done using the same dilution procedure. Results in Table X show that Paraquat activity in the residual media from the three organisms is reduced as compared to the results obtained from the uninoculated check in Table VIII. It is possible however that the Paraquat cation may have become attached to the fungal mycelium and become inactivated.

Results of sodium dithionite determinations for Paraquat in residual media are shown in Table XI. Readings correspond to the dilutions and it was apparent that leaf disks gave a more precise measure of remaining active Paraquat in the media.

TABLE VIII

EFFECT OF PARAQUAT ON WEIGHT OF FUNGUS PADS

Treatment ppm Paraquat	Dry weight in grams*		
	A. niger	A. flavus	T. viride
0	.530	.544	.662
.5	.533	.534	.612
1	.530	.524	.683
2	.531	.522	.724
5	.521	.527	.667
10	.522	.512	.692
20	.534	.510	.683
100	.546	.531	.654

* Average weight of three replications
A. niger C. V. = 4.88%
A. flavus C. V. = 3.32%
T. viride C. V. = 9.74%

TABLE IX

EFFECT OF MEDIA ON PARAQUAT ACTIVITY

AT THREE DIFFERENT DILUTIONS USING LEAF DISKS[1]

Treatment ppm Paraquat	Media 1:10 Reading*	Optical Density	Percent decrease	Water 1:10 Reading*	Optical Density	Percent decrease
0	41	.387	0	38	.420	0
.5	40	.398	0	45	.347	12
1	47	.328	11	49	.229	34
2	51	.292	18	85	.071	79
5	63	.201	39	92	.036	93
10	96	.018	96	96	.018	100
20	99	.004	100	95	.022	100
100	98	.009	100	97	.013	100
	F=24.61** C.V.=14%			F=64.34** C.V.=22.0%		
	Media 1:5			Water 1:5		
0	42	.377	0	41	.387	0
.5	41	.387	0	65	.187	47
1	52	.284	19	86	.066	82
2	60	.222	33	93	.032	95
5	81	.092	72	96	.018	100
10	96	.018	100	97	.013	100
20	95	.022	100	96	.018	100
100	96	.018	100	96	.018	100
	F=75.74** C.V.=21.78%			F=42.73** C.V.=20.43%		
	Media 1:1			Water 1:1		
0	45	.347	0	42	.377	0
.5	65	.187	38	84	.076	78
1	82	.086	71	95	.022	100
2	98	.009	100	96	.018	100
5	95	.022	100	95	.022	100
10	96	.018	100	97	.013	100
20	96	.018	100	96	.018	100
100	97	.013	100	96	.018	100
	F=27.23** C.V.=4.73%			F=216.9** C.V.=7.99%		

* Average of three determinations read as percent transmission

** Significant at .01 level

[1] Incubated under 750 footcandles of light at 30°C for 24 hours.

TABLE X

BIOASSAY OF RESIDUAL MEDIA FROM FUNGUS GROWTH USING LEAF DISKS[1]

Treatment ppm Paraquat	*A. niger* Percent decrease*	*A. flavus* Percent decrease*	*T. viride* Percent decrease*
	1:10 Dilution		
0	0	0	0
.5	0	0	0
1	0	0	0
2	0	0	0
5	26	25	18
10	49	28	30
20	100	100	100
100	100	100	100
	1:5 Dilution		
0	0	0	0
.5	0	0	0
1	0	24	0
2	0	45	25
5	48	89	75
10	96	100	92
20	100	100	100
100	100	100	100
	1:1 Dilution		
0	0	0	0
.5	36	50	26
1	50	48	22
2	48	65	46
5	50	78	62
10	100	100	100
20	100	100	100
100	100	100	100

* Average of three determinations
1:10 F=28.72** C.V.=4.16%
1:5 F=46.60** C.V.=3.03%
1:1 F=37.86** C.V.=6.82%
** Significant at .01 level

[1] Incubated under 750 footcandles of light at 30°C for 24 hours.

TABLE XI

SODIUM DITHIONITE DETERMINATION OF

RESIDUAL PARAQUAT FROM FUNGUS STUDIES

Treatment ppm Paraquat	A. niger*	A. flavus*	T. viride*
	1:10 Dilution		
0	100	100	100
.5	100	100	100
1	100	100	100
2	100	100	100
5	92	92	92
10	83	81	85
20	64	56	57
100	17	14	17
	1:5 Dilution		
0	100	100	100
.5	100	100	100
1	100	100	100
2	95	95	93
5	90	77	81
10	79	67	68
20	47	39	52
100	13	8	13
	1:1 Dilution		
0	100	100	100
.5	100	100	100
1	100	93	98
2	95	78	88
5	74	53	71
10	58	38	49
20	24	18	24
100	4	3	4

* Average of three determinations

SUMMARY AND CONCLUSIONS

The objectives of this study were to determine factors affecting Paraquat phytotoxicity and to develop bioassay procedures for determining Paraquat residues. The unicellular photolithotrophic organism, Chlorella pyrenoidosa, Chick, and foliar tissue as leaf disks from Indian beans (Phaseolus vulgaris L.) were used in these studies. Three soil inhabiting fungi known to be degraders of organic compounds were grown on liquid medium containing Paraquat and determinations of remaining residues activity were measured.

Paraquat was found to be more toxic to Chlorella as length of incubation and concentration increased. Agitating treated Chlorella cultures during incubation resulted in greater sensitivity to Paraquat additions than quiescent cultures. Optical density, cell weights and extractable chlorophyll were correlated with Paraquat treatments.

Reduction of extractable chlorophyll in leaf disks was closely related to Paraquat concentrations. Time, temperature and light intensity were all found to influence the activity of Paraquat on leaf disks. Increased reduction in extractable chlorophyll could be measured as time, light and temperature increased.

The growth of fungi species Aspergillus niger, Aspergillus flavus and Trichoderma viride was not affected by Paraquat additions. Bioassay of residual media using leaf disks indicated a decrease in Paraquat activity with all three organisms. A reduction in activity was also

measured where Paraquat was added to media alone. Reduced activity was also thought to result from adsorption of the cation by fungal mycelium.

The bioassay procedures were more precise than the sodium dithionite method for active Paraquat determinations. Active Paraquat was measured at lower concentrations with the leaf disk method than with the prescribed chemical method.

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A P P E N D I X

TABLE XII

EFFECT OF LENGTH OF INCUBATION AND PARAQUAT LEVELS ON EXTRACTABLE CHLOROPHYLL IN *CHLORELLA PYRENOIDOSA*[1]

Time Hours	Treatment ppm Paraquat	Reading*	Optical Density	Percent decrease
48	0	54	.267	0
	.5	57	.295	10
	1	62	.208	20
	1.5	70	.155	39
	2	82	.086	68
72	0	53	.276	0
	.5	62	.208	20
	1	74	.131	49
	1.5	81	.092	68
	2	89	.051	86
96	0	52	.284	0
	.5	64	.194	24
	1	83	.081	73
	1.5	93	.032	97
	2	95	.022	100

*Average of three determinations read as percent transmission
48 hour F=37.29** C.V.=1.53
72 hour F=53.40** C.V.=1.91
96 hour F=40.37** C.V.=2.09
**Significant at .01 level

[1]Incubated under 500 footcandles of light at 30°C.

TABLE XIII

EFFECT OF DIFFERENT LIGHT INTENSITIES AND PARAQUAT LEVELS ON CHLOROPHYLL DECREASE IN LEAF DISKS[1]

Treatment ppm Paraquat	Footcandles of light	Reading*	Optical Density	Percent decrease
0	0	41	.387	0
.025		40	.398	0
.05		40	.398	0
.1		41	.387	0
.5		42	.377	0
1		41	.387	0
0	200	39	.409	0
.025		41	.387	4
.05		48	.319	16
.1		63	.201	44
.5		63	.201	44
1		64	.194	45
0	500	38	.420	0
.025		44	.357	11
.05		48	.319	18
.1		67	.174	53
.5		81	.092	78
1		81	.092	78
0	750	37	.432	0
.025		49	.310	22
.05		64	.194	49
.1		74	.131	67
.5		80	.097	78
1		92	.036	100

0 F= .29 C.V.=4.08%
200 F= 8.02** C.V.=4.25%
500 F=40.45** C.V.=2.73%
750 F=32.85** C.V.=2.97%

* Average of three determinations read as percent transmission
** Significant at .01 level
[1] Incubated at 30°C for 24 hours

TABLE XIV

EFFECT OF DIFFERENT TEMPERATURES AND PARAQUAT LEVELS ON CHLOROPHYLL DECREASE IN LEAF DISKS[1]

Treatment ppm Paraquat	Temperature degrees centigrade	Reading*	Optical Density	Percent decrease
0	1	41	.387	0
.025		41	.387	0
.05		41	.387	0
.1		41	.387	0
.5		41	.387	0
1		41	.387	0
0	15	51	.292	0
.025		51	.292	0
.05		54	.268	13
.1		63	.201	31
.5		65	.187	35
1		68	.168	42
0	25	46	.337	0
.025		49	.310	4
.05		58	.237	16
.1		70	.155	37
.5		74	.131	47
1		87	.061	74
0	35	49	.310	0
.025		58	.237	16
.05		65	.187	30
.1		78	.108	58
.5		89	.051	80
1		98	.009	100
0	45	53	.276	0
.025		66	.181	46
.05		73	.137	60
.1		88	.056	91
.5		98	.009	100
1		98	.009	100

1° F= .615 C.V.=29.26%
15° F=46.02** C.V.=32.10%
25° F=23.24** C.V.=27.94%
35° F=40.74** C.V.=22.50%
45° F=18.63** C.V.=29.49%

* Average of three determinations
** Significant at .01 level

[1] Incubated under 750 footcandles of light for 24 hours.

TABLE XV

EFFECT OF INCUBATION LENGTH AND PARAQUAT LEVELS ON CHLOROPHYLL DECREASE IN LEAF DISKS[1]

Treatment ppm Paraquat	Time hours	Reading*	Optical Density	Percent decrease
0	12	39	.409	0
.025		39	.409	0
.5		45	.347	12
1		63	.201	46
0	14	39	.409	0
.025		39	.409	0
.5		45	.347	12
1		72	.143	63
0	16	41	.387	0
.025		41	.387	0
.5		48	.319	13
1		73	.137	61
0	18	42	.377	0
.025		42	.377	0
.5		52	.284	19
1		77	.114	67
0	20	43	.367	0
.025		45	.347	4
.5		54	.268	17
1		83	.081	77
0	22	42	.377	0
.025		45	.347	6
.5		63	.201	40
1		92	.036	96
0	24	42	.377	0
.025		52	.284	19
.5		71	.149	56
1		94	.027	100

* Average of three determinations
F=106.36** C.V.=14.23%
** Significant at .01 level

[1] Incubated under 750 footcandles of light at 30°C.

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of

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