SOIL BIOLOGICAL FACTORS GOVERNING PERSISTENCE

OF SODIUM OMADINE, A PYRIDYL FUNGICIDE

By

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CHAPTER I

INTRODUCTION

Highly effective biocides have recently been developed with pyridine compounds. These are used as pesticides and have value as broadleaf and grass herbicides, insecticides, nitrification inhibitors, and more recently as fungicides. These pyridines may eventually have considerable agronomic value. However, soil characteristics and microbial activities greatly influence persistence, effectiveness, and their eventual fate in soils.

The pyridine base compounds are of particular interest to the biologist because of the essential nature of the pyridines in all living cell metabolism. An outstanding characteristic of the pyridine ring is its chemical inertness and resistance to degradation, although substitutions on ring loci and condensation reactions occur readily. The principal common alkaloids are complex pyridines and are almost all poisonous.

The purpose of this study was to determine the effect of time and biological transformations on activity of 1-hydroxypyridine-2-thione (sodium omadine) in soil and to establish rates and magnitudes of degradation. Included in this study were experiments designed to determine the effects of sodium omadine on selected common soil fungi isolates and to determine chemical additives which might offset fungitoxicosis.

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CHAPTER II

LITERATURE REVIEW

In recent years substituted pyridine compounds have been utilized
for a wide array of biocide uses. Initial research and development was
directed primarily towards the formulation and testing of herbicides
and insecticides. Specific examples of such compounds would include
picloram which has proven to be one of the more effective systemic or
auxin type herbicides. A new group of insecticides, the pyridyl phos-
phates, appear to be promising compounds for the broad spectra control
of many insect pests (14)[1]. More recently a substituted pyridine com-
pound, 1-hydroxypyridine-2-thione (Omadine)[2] and its salts have been
reported to have pronounced antibacterial and antifungal activity (10).
Omadine is a derivative of a group of compounds classed as cyclic thio-
hydroxamic acids. It contains two functional groups which react read-
ily, the N-oxide of the pyridine moiety and the thione (or mercapto
groups) (2).

Horsefall (6) stated that most nitrogen heterocycles are inherent-
ly toxic, but most do not display activity as fungicides. The pyridine
nucleus is essentially inactive, but can be made quite active if it is
substituted in such a fashion as to make it permeable to the cell. He

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[1] Figures in parenthesis refer to Literature Cited.

[2] Names in parenthesis refer to the trade name of the chemical com-
pound and for simplicity will be used throughout this paper.

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found that by adding a styryl group in the 2-position or a carboxy ester in the 3-position, pyridine could be made an effective spore killer.

One of the most striking substitutions involving the pyridine compound was the formation of the metal salt of 3-pyridine thiol as reported by Soo-Hoo and Gruenberg (12). They proposed that the metal salt increased the lipoid soluble properties of the compound, thus allowing it to penetrate the fungal cell. Once inside the metal is removed by dissociation and the pyridine thiol is available to disrupt the cell.

Treatment of soil with chemicals for control of plant pathogenic soil fungi has received considerable attention in recent years. Likewise development of fungicides for application as foliar sprays is of vast importance, especially for the fruit and vegetable industry (3).

Omadine salt derivatives have been tested quite extensively as both foliage sprays and as soil drenches. Kenaga and Kiesling (7) found that sodium and copper omadine gave highly significant control of anthracnose of bean and black-leg of cabbage when applied as greenhouse foliar sprays. Couch and Cole (4) obtained similar results when using the disulfide derivative of omadine for the control of melting out disease of Kentucky bluegrass. Hamilton and Szkolnik (5) reported that iron omadine consistently provided good control of apple scab, through protection at active concentrations below that of the standard fungicides like sulfur, Ferbam, Captan and Glyodin. They also showed the eradicative action of iron omadine in greenhouse evaluations against apple scab was superior to Captan and approached that of Phygon. Szkolnik and Hamilton (13) in using iron omadine as a foliar spray on peaches found that it compared favorably with liquid lime sulfur, the

dinitros and Ferbam in the control of peach leaf curl. The zinc salt of omadine was reported in the same series of studies to give very effective control of brown rot on peaches when applied as a pre-harvest spray.

Barnes and Zerkel (3) conducted a controlled environment soil test and discovered that certain derivatives of omadine alone and in combination with pentachloronitrobenzene (PCNB) provided good control of species of Rhizoctonia, Pythium and Fusarium. They found that some stunting of the cotton and cucumber indicator plants did occur, but it was of a temporary nature after which the plants eventually recovered. These investigators noted that both cupric omadine and zinc omadine in combination with PCNB have been found to be effective in field tests conducted at various cooperating agricultural experiment stations.

It has been shown that some of the omadine derivatives have, at certain concentrations, exhibited a stimulatory effect on rooting of specific plant species. Other researchers report an inhibitory effect on normal rooting. Peterson, et al. (11) found that manganese omadine was a most effective fungicide for control of Fusarium stem rot of carnations. They also noted that it significantly stimulated the rooting of cuttings derived from the treated mother blocks. This stimulation of rooting was over and above that usually provided by conventional rooting hormones. Apparently certain concentrations of the compound are required for this to occur.

Norman (10) showed that sodium omadine and certain other derivatives at low soil concentrations caused a reduction in root elongation and in dry matter produced by cucumber and barley seedlings. However, applications of similar concentrations to the tops of these species did

not result in discernible responses in top growth and development. Seed from treated plants was found to produce normal seedlings, indicating no carry over. These findings agree with those of Kenaga and Kiesling (7) which showed root inhibition and plant stunting on Chinese cabbage at low soil concentrations. Morgan (9), working with tobacco, found that the disulfide, zinc and manganese salts suppressed normal root development.

The effect of fumigants and fungicides on the saprophytic soil population as well as the pathogens they are designed to control is always a major concern of the researcher. Alexander (1) noted that the degree of inhibition and duration of this effect varies with the chemical, the soil, and environmental conditions. Ultimately, the soil population will become re-established, but this return to normalcy may require a few days or, occasionally more than a year. Population counts of the saprophytic bacterial and fungal flora were utlized by Morgan (9) to determine the effect of zinc omadine on the over-all activity of soil microorganisms. One week after application he found the bacteria counts to be slightly higher and the fungus population counts to be significantly lower than the untreated controls. However, after five weeks the soil microflora increased significantly.

The fate of fungicides in soils has not been established. According to Martin and Pratt (8) when fumigants or fungicides are applied to the soil, a portion, if volatile, will be dissipated into the air. The remainder will be adsorbed by the soil particles. The compound then decomposes by chemical or microbial action.

Adsorption of the omadine derivatives to soil colloids accounts for a significant loss in toxicity. Norman (10) has shown that the

inhibitory effect on barley and cucumber rooting was greatly reduced when soil was used instead of vermiculite or quartz sand. Approximately the same amount of growth repression or dwarfing was obtained by the addition of 10 mg. of omadine to quartz sand, 40 mg. to vermiculite and 100 mg. to soil indicating significant adsorption by soil colloids.

Leaching as well as photodecomposition appear to be other important avenues of dissipation of the omadine salts (9).

CHAPTER III

MATERIALS AND METHODS

The sodium omadine used in these studies was a formulation of the
Olin Mathieson Chemical Corporation which consisted of a 90 percent
wettable powder.

Experiment I. Effect of Sodium Omadine on Five Fungi Isolates

Five common soil inhabiting fungi; Curvularia lunta, Aspergillus
flavus, A. niger, A. tamarii and A. oryzae were cultured on a liquid
broth media containing 0, 1, 5, and 10 ppm levels of sodium omadine.
The basic media in grams per liter consisted of: sucrose 100, am-
monium nitrate 1.5, potassium sulfate 1.5, inorganic salt mixture
(Phillips-Hart) 1.5, and citric acid 1. Composition of the salt mix-
ture in percent was: dipotassium phosphate 32.2, calcium carbonate
30.0, sodium chloride 16.7, magnesium sulfate 10.2, monocalcium phos-
phate 7.5, ferric citrate 2.75, maganese sulfate .51, potassium iodide
.08, copper sulfate .03, zinc chloride .025, and cobalt chloride .005.

Fifty milliliters of media were used in 500 milliliter cotton
stoppered Erlenmeyer flasks for each culture. Each treatment was rep-
licated three times. These flasks were autoclave sterilized at 15 psi
for 20 minutes and, upon cooling, all cultures were inoculated with
spore suspensions of the respective organisms. Cultures were incubated
at 30° C. for 5 days. Following incubation the mycelial pads were re-
moved from the flasks, washed, oven dried at 100 degrees C. for 24

hours, and weighed.

Experiment II. Effect of Chemical Additives on Sodium Omadine
Fungitoxicity

Two soil inhabiting fungi; A. niger and A. tamarii were utilized
in a series of studies to find if there were compounds that would re-
duce the toxicity of sodium omadine to these two Aspergilli. The base
medium used in this study was the same as that described in Experiment
I. In addition to the base medium, eight growth factor additives were
used at varying levels. These included: yeast extract, peptone,
casein, pyridine chloride, pyridoxine base, ribose nucleic acid, ribo-
flavin and picolinic acid. Sodium omadine levels of 0, 1, 2.5, 5 and
10 ppm were added to media before sterilization. The bioassay pro-
cedures were the same as used in Experiment I.

Experiment III. Sodium Omadine Interaction With Selected Chemical
Additives

Aspergillus niger was found to be the organism most consistently
sensitive to sodium omadine toxicity. This selection was cultured on
the same base medium and under the same conditions as those described
in Experiment I. However, the sodium omadine levels were 0, 5, 10, 20
and 40 ppm. The amendments used in the three growth response studies
included picolinic acid, riboflavin and 4-amino-3, 5, 6-trichloropi-
colinic acid-K salt (picloram). The additives were applied at levels
of 0, 100, 200, 400 and 800 ppm per culture. The bioassay procedures
were the same as used in Experiment I.

PLANT BIOASSAY

In order to evaluate the rate of degradation with time six common plant species including oats (Avena sativa, L. var. Cimarron), grain sorghum (Sorghum vulgare, Pers. var. Ok 612), sunflower (Helianthus annus, L.), wheat (Triticum aestivum, L.), soybeans (Glycine max, L.), and cucumber (Cucumis sativus, L. var. Long green), were screened for susceptibility to sodium omadine toxicity.

The soil used in this investigation was a Psammentic Paleustalf, Eufaula sand. The chemical and physical epipedon properties of this soil are shown in Table I.

Air dried soil that had previously passed through an 8-mesh sieve was placed in $2\frac{1}{2}$ inch round styro-cups. Two hundred gram soil cultures were used in each experiment. All treatments were replicated three times.

These preliminary screening studies indicated Cimarron oats, and Ok 612 grain sorghum were more sensitive than the other four species tested. These species were used as indicator plants for the following study.

Experiment IV. Effect of Soil Sterilization on Sodium Omadine Toxicity

Sterilized and unsterilized Eufaula sand were used in this experiment. The soil was steam sterilized for twelve hours at 15 psi. Half of the pots were planted with approximately 20 Cimarron oat seeds. The remaining pots were planted with 20 grain sorghum seeds per pot. These pots were later thinned to uniform stands of 15 seedlings per pot. Sodium omadine levels of 0, 5, 10, 20, 40, 80, 160, 320, 640 and 1280 ppm were applied as solutions to both the sterilized and unsterilized soil

TABLE I

CHEMICAL AND PHYSICAL PROPERTIES OF EUFAULA
SOIL AS DETERMINED BY LABORATORY ANALYSES

Determination	Eufaula 0-6"
Mechanical Analysis	
% Sand	90.0
% Silt	7.0
% Clay	3.0
Textural Class	Sand
pH	5.7
% Organic Matter	0.5
% Total Nitrogen	0.04
Cation Exchange Capacity*	2.7
Exchangeable Cations*	
Calcium	1.1
Magnesium	0.9
Potassium	0.3
Available Phosphorus**	6.8

*m.e. per 100 grams soil (1N Ammonium acetate)
**pounds per acre (0.25N HCl)

at the time of planting. The seedlings were grown under continuous light of 500 foot candles furnished by fluorescent "Grolux" lamps and maintained at room temperature. At the end of 18 days, the above-ground plant parts were harvested and green and dry weights taken. The soils were air dried, mixed and replanted. The same growing and harvesting procedures were followed in the second and third harvests as in the previous one. The second planting was harvested at the end of 19 days and the third crop was also grown for 19 days. Following each harvest a small soil sample was taken from each pot, oven dried and saved for later flask bioassay studies.

Experiment V. Extent of Sodium Omadine Degradation with Time

Aspergillus niger was cultured on the base media described in Experiment I. One gram oven dried soil samples from Experiment IV were added to the media and steam sterilized. The fungicide levels in the flasks after incorporation of the soil were 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, and 25.6 ppm. Equivalent concentrations of sodium omadine were added to check flasks for comparison purposes. The bioassay procedures were the same as used in Experiment I.

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

CHAPTER IV

RESULTS AND DISCUSSION

The discussion of results from these studies is presented chrono-
logically by experiment numbers as indicated in Chapter III, Methods
and Materials. Data from these studies are presented both in data
tables and in three dimensional graphs showing relative growth as a
function of sodium omadine levels in conjunction with selected treat-
ment additives.

Experiment I. Effect of Sodium Omadine on Five Fungi Isolates

Sodium omadine levels up to 10 ppm severely inhibited the growth
of the following soil inhabiting fungi: Aspergilli: niger, tamarii,
oryzae and Curvularia lunta. Threshold levels for these microorganisms
were apparent between 1 and 5 ppm. As illustrated in Table II and Fig-
ure 1, only a slight depression in mycelial growth was shown by Asper-
gillus flavus. In a similar study with sodium omadine levels, A.
flavus growth was reduced approximately 50 percent at 1000 ppm. As
shown in Figure 1, A. niger and A. tamarii were the two organisms most
adversely affected by this compound.

The thermal stability of sodium omadine was confirmed in this
study. Additions of the fungicide to the basic medium were made both
prior to and after steam sterilization at 15 psi for 20 minutes. No
apparent decomposition occurred as measured by fungi bioassay. Thus,
in subsequent flask culture studies, the fungicide was added before

TABLE II

EFFECT OF FOUR SODIUM OMADINE LEVELS
ON FIVE SELECTED SOIL FUNGI ISOLATES

Fungus	ppm Sodium Omadine					
	0	1	5	10	C.V.	F
	Fungus Growth					
Aspergillus flavus	2.59	2.48	2.44	2.41	5.69	.94 (n.s.)
Curvularia lunta	2.28	1.87	.82	.00	18.21	62.69
Aspergillus oryzae	1.70	1.11	.55	.00	34.99	18.49
Aspergillus tamarii	2.15	1.37	.09	.00	8.39	570.91
Aspergillus niger	3.26	2.76	.00	.00	13.06	206.96

Figures shown are sums of three replications as dry weight in
grams
All F values significant at .005 level except for A. flavus
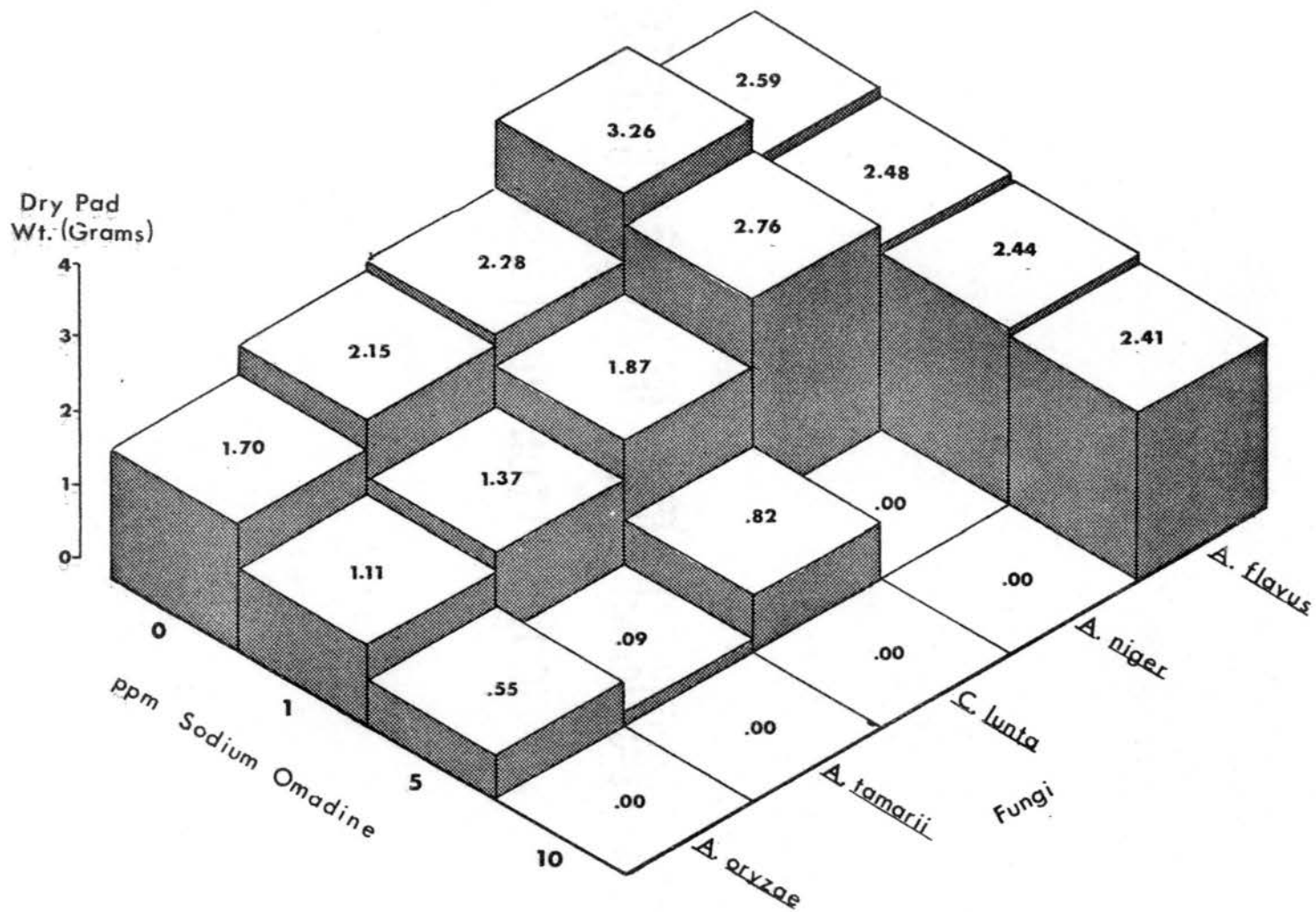
Figure 1. Effect of Sodium Omadine Levels on Five Fungi Isolates.

sterilization to minimize the possibility of contamination.

Experiment II.   Effect of Chemical Additives on Sodium Omadine
Fungitoxicity

The growth factor additives utilized in this experiment showed a
wide and varied effect both with A. niger and A. tamarii proliferation
as shown in Tables III and IV, respectively.   Three pyridine compounds,
two organic nitrogenous materials, a nucleic acid and a vitamin were
incorporated as variables in this study.

Pyridine chloride, pyridoxine base, and casein included in culture
media at 400 and 800 ppm failed to offset sodium omadine toxicity as
indicated with A. niger proliferation.   As shown in Table III, a re-
duction in growth at the 10 ppm fungicide concentration resulted with
all three of these compounds.   The 400 ppm levels of these compounds
were apparently more effective than the 800 ppm levels.   Similar re-
sults were obtained with ribose nucleic acid at 200 and 800 ppm and
peptone at 200 ppm.   However, yeast extract, riboflavin, and picolinic
acid stimulated growth of this fungus when applied separately at 200
and 400 ppm in combination with 1, 2.5, 5, and 10 ppm sodium omadine.
Very similar trends were observed when these compounds were added to
A. tamarii cultures in combination with the same concentrations of
sodium omadine as shown in Table IV.

The organic nitrogenous materials used in these cultures may be
termed as components containing presynthesized growth factors.   Yeast
extract provides amino acids, vitamins, nucleic acids, and other es-
sential growth factors in a somewhat heterogeneous combination.   The
growth response obtained with this material indicated promise for seek-
ing single growth factors that have similar effects on culture yields

TABLE III

EFFECT OF CHEMICAL ADDITIVES ON
SODIUM OMADINE FUNGITOXICITY

Additive	ppm	0	1	2.5	5	10	Σ
				Aspergillus niger			
				Grams Dry Mycelium			
Check	0	3.78	3.63	2.53	.00	.00	9.94
Pyridine Chloride	400	3.67	3.66	3.58	2.97	.33	14.21
" "	800	3.90	3.71	1.25	.79	.00	9.65
Pyridoxine Base	400	3.50	3.68	3.38	3.33	.00	13.89
" "	800	3.78	3.63	2.53	.00	.00	9.94
Casein	400	4.28	3.50	3.24	.93	.00	11.95
"	800	3.29	3.51	3.44	.00	.00	10.24
Ribose Nucleic Acid	200	3.78	3.67	2.45	.00	.00	9.90
" " "	800	4.01	4.11	3.30	.26	.00	11.68
Riboflavin	200	3.43	3.10	3.08	2.32	.11	12.06
"	400	3.64	3.67	2.61	2.41	2.14	14.47
Yeast Extract	200	3.45	3.43	3.31	.83	.00	11.02
" "	400	3.85	3.80	3.56	1.59	.00	12.80
Picolinic Acid	400	4.14	3.99	4.17	3.83	3.72	19.85
Peptone	200	2.79	2.97	2.93	1.93	.00	10.62

Yield figures are sums of three replicate cultures per treatment

TABLE IV

EFFECT OF CHEMICAL ADDITIVES ON
SODIUM OMADINE FUNGITOXICITY

| | | | | Sodium Omadine ppm | | | |
Additive	ppm	0	1	2.5	5	10	Σ
				Aspergillus tamarii			
				Grams Dry Mycelium			
Check	0	2.18	1.53	1.20	.36	.00	5.27
Pyridine Chloride	400	1.81	2.14	1.69	1.62	.40	7.66
" "	800	2.32	2.29	2.57	1.61	.58	9.37
Pyridoxine Base	400	2.55	2.24	2.22	2.17	2.03	11.21
" "	800	2.66	2.45	1.85	2.02	1.68	10.66
Casein	400	2.59	2.65	2.25	2.18	1.64	11.31
"	800	2.49	2.41	2.61	1.87	.96	10.34
Ribose Nucleic Acid	200	1.83	2.16	1.78	1.25	.66	7.68
" " "	800	1.81	2.58	2.61	1.42	.00	8.42
Riboflavin	200	1.64	1.86	1.87	1.79	1.06	8.22
"	400	2.00	2.55	2.12	1.69	1.08	9.44
Yeast Extract	200	2.17	2.27	2.19	1.77	1.34	9.74
" "	400	2.08	2.20	2.01	1.19	1.12	8.60
Picolinic Acid	400	2.17	2.45	2.30	2.62	2.18	11.72
Peptone	200	2.21	2.07	2.21	1.91	1.12	9.52

Yield figures are sums of three replicate cultures per treatment

with A. niger and A. tamarii at various sodium omadine levels. Of the
separate materials used, riboflavin (vitamin B-2) was most promising in
these studies. Both A. niger and A. tamarii yields were slightly great-
er at 400 ppm riboflavin than this same level with yeast extract. This
indicated favorable effects from combinations of some pre-synthesized
factors within the yeast that may offset sodium omadine toxicity to the
fungus.

Of all of the treatments used picolinic acid, which is a pyridine
monocarboxylic acid and an analog of nicotinic acid, provided the most
promising results. Some component within this pyridine molecule ap-
parently blocks the toxic effect of sodium omadine on these selected
microorganisms.

Experiment III. Sodium Omadine Interactions with Selected Chemical
Additives

Aspergillus niger, being the more sensitive of the microorganisms
tested in Experiment II, was utilized in these growth response studies
with increased sodium omadine rates of 5, 10, 20, and 40 ppm.

Picolinic acid, applied at levels of 100, 200, 400, and 800 ppm
was found to be an effective additive for reducing sodium omadine
toxicity (Table V). As shown in Figure 2, this compound applied at
800 ppm offset the effect of sodium omadine levels up to 40 ppm.

Picloram, a picolinic acid derivative, supplied at equivalent
rates in A. niger cultures was found to offset the effects of sodium
omadine levels up to 10 ppm (Table VI). Apparently some free picolinic
acid was present in the solution in order for this to occur. However,
picloram is a potassium salt of picolinic acid and some hydrolysis may
occur or the K salt form may be utilized as such without modification.

TABLE V

PICOLINIC ACID INTERACTION WITH SODIUM OMADINE
ON <u>ASPERGILLUS</u> <u>NIGER</u> GROWTH

Picolinic Acid ppm	Sodium Omadine Level ppm					
	0	5	10	20	40	Σ
	Grams Dry Mycelium					
0	3.39	.00	.00	.00	.00	3.39
100	3.53	3.35	.00	.00	.00	6.88
200	3.52	3.39	2.45	.00	.00	9.36
400	3.31	3.51	3.21	.05	.00	10.08
800	3.32	3.61	3.21	2.12	.20	12.46
Σ	17.07	13.86	8.87	2.17	.20	42.17

Yield figures are sums of three replicate cultures per treatment
All F values were statistically significant at the .005 level
C.V. = 16.31%, F: Treatment = 109.81, Sodium Omadine = 419.46,
Picolinic Acid = 94.29, Sodium Omadine X Picolinic Acid = 36.27
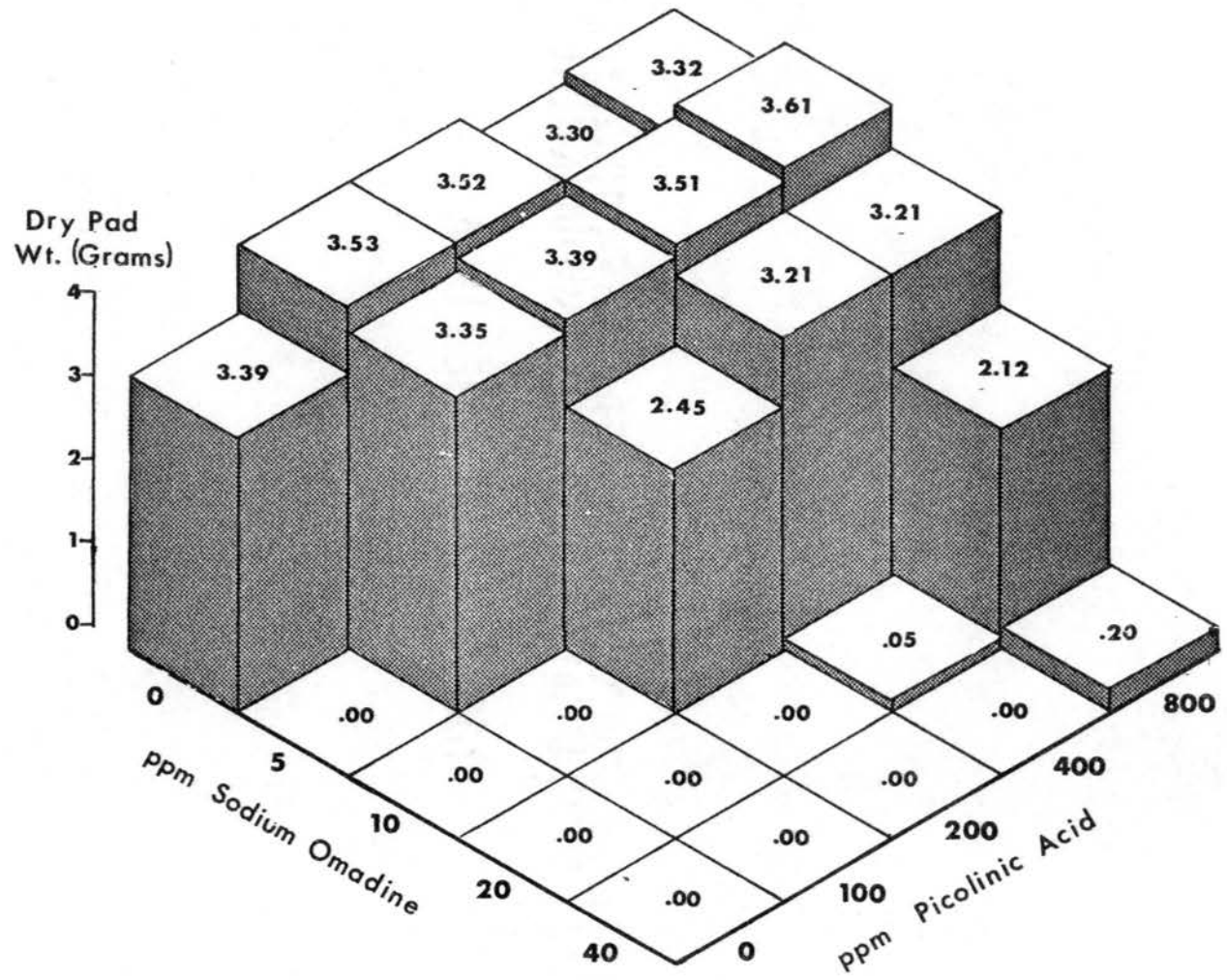
Figure 2. Interactions of Sodium Omadine and Picolinic Acid Levels on
Aspergillus niger Growth.

TABLE VI

PICLORAM INTERACTION WITH SODIUM OMADINE
ON ASPERGILLUS NIGER GROWTH

Picloram Level ppm	Sodium Omadine Level ppm					
	0	5	10	20	40	Σ
	Grams Dry Mycelium					
0	4.38	.00	.00	.00	.00	4.38
100	3.45	.00	.00	.00	.00	3.45
200	2.78	.06	.00	.00	.00	2.84
400	2.70	1.70	.00	.00	.00	4.40
800	2.41	2.20	.80	.00	.00	5.41
Σ	15.72	3.96	.80	.00	.00	20.48

Yield figures are sums of three replicate cultures per treatment
All F values were statistically significant at the .005 level
C. V. = 15.39%, F: Treatment = 341.58, Sodium Omadine Level =
1690.71, Picloram Level = 36.72, Sodium Omadine X Picloram = 80.51

As shown in Table VII and Figure 3, riboflavin applied at 100,
200, 400, and 800 ppm apparently offset sodium omadine toxicity up to
the 20 ppm level.

Picolinic acid was the most effective of these three additives at
the levels used. Riboflavin was more effective than picloram. This
suggests different biological mechanisms involved with the materials as
two are pyridines in contrast to the basic flavin configuration of the
vitamin.

Experiment IV. Effect of Soil Sterilization on Sodium Omadine Toxicity

Results of sodium omadine phytotoxicity to three grain sorghum
plantings are shown in Tables VIII (fresh weight) and IX (dry weight).
Oat responses are illustrated in Tables X (fresh weight) and XI (dry
weight). Sodium omadine significantly reduced both oat and sorghum
growth at levels in excess of 320 ppm in both sterilized and nonsterile
soil. Yields for both crops were higher in the nonsterilized soil in-
dicating some microbial degradation that reduced the fungicide toxicity
in those cultures. However, this compound was apparently non-toxic at
lower levels associated with normal soil application rates. Visual
symptoms of toxicity accelerated with growth time on the affected
plants. All plants appeared normal the first week, then visible signs
of stunting and chlorosis became apparent. Desiccation appeared first
in the oldest tissue of the affected plants and progressed upward.
Plants growing at the lethal sodium omadine level were dead fourteen
to sixteen days after emergence. Germination of both oats and grain
sorghum at the highest fungicide application level was reduced approxi-
mately 50 percent.

Differential response to sodium omadine levels as influenced by

TABLE VII

RIBOFLAVIN INTERACTION WITH SODIUM OMADINE
ON <u>ASPERGILLUS</u> <u>NIGER</u> GROWTH

Riboflavin	Sodium Omadine Level ppm					
ppm	0	5	10	20	40	$\leq$

Grams Dry Mycelium

Riboflavin ppm	0	5	10	20	40	$\leq$
0	3.69	.00	.00	.00	.00	3.69
100	3.59	.33	.00	.00	.00	3.92
200	3.54	.43	.00	.00	.00	3.97
400	3.53	.63	.45	.00	.00	4.61
800	3.55	1.25	.52	.19	.00	5.51
$\leq$	17.90	2.64	.97	.19	.00	21.70

Yield figures are sums of three replicate cultures per treatment
All F values statistically significant at .005 level
C. V. = 21.26%, F= Treatment = 175.92, Sodium Omadine = 1029.34,
Riboflavin = 9.55, Sodium Omadine X Riboflavin = 4.16
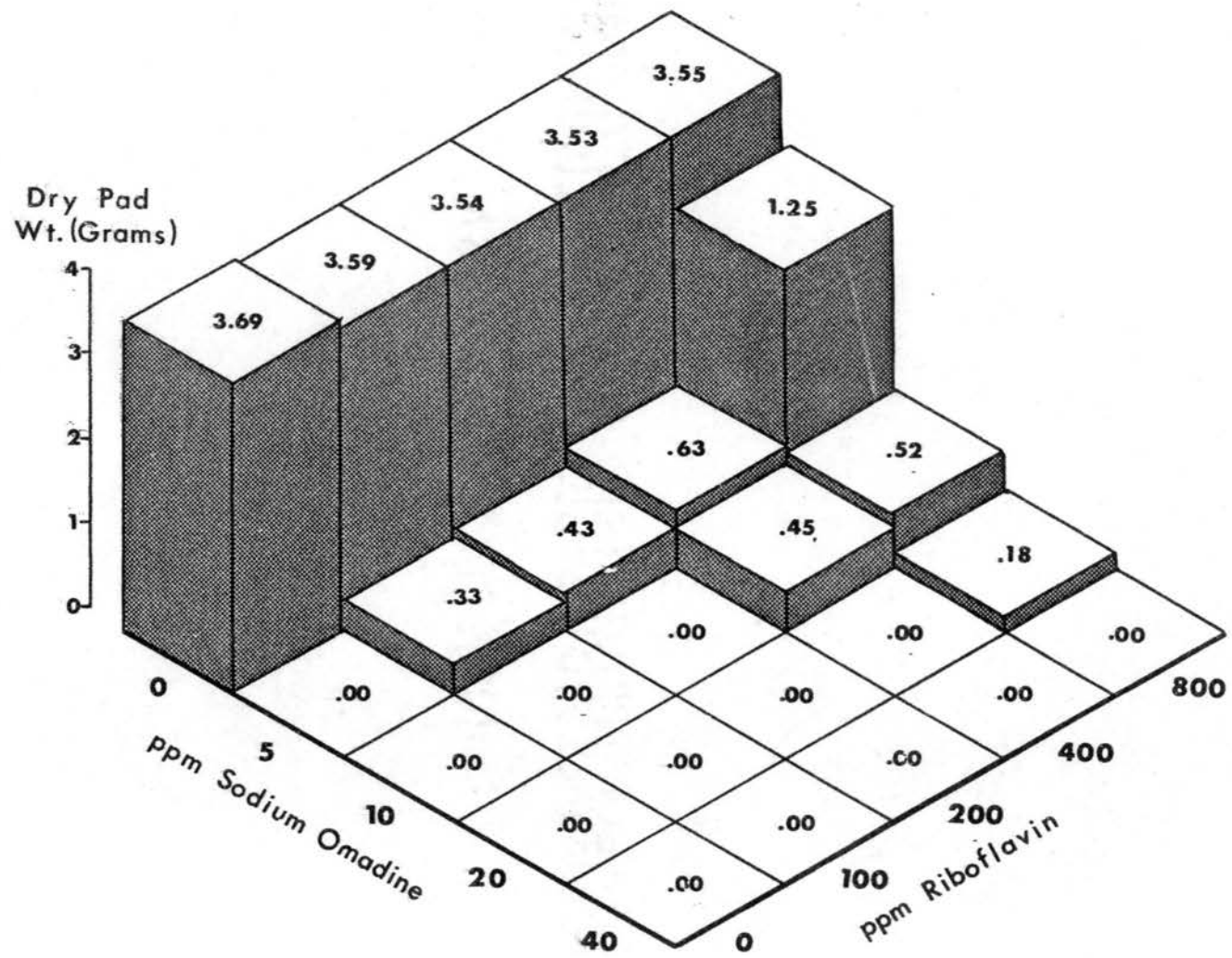
Figure 3. Interactions of Sodium Omadine and Riboflavin Levels on
Aspergillus niger Growth.

TABLE VIII

EFFECT OF SODIUM OMADINE ON SORGHUM VULGARE PERS. GROWTH AS FRESH WEIGHT IN STERILIZED AND NONSTERILIZED SOIL

Treatment ppm Sodium Omadine	First	Second	Third	Total
		Sterilized Soil		
0	4.33	1.45	5.07	10.85
5	4.23	2.27	4.26	10.76
10	4.12	5.61	3.28	13.01
20	2.74	4.03	4.02	10.79
40	3.22	4.69	1.75	9.66
80	2.71	2.45	5.18	10.34
160	3.33	4.76	5.54	13.63
320	2.43	3.46	4.86	10.75
640	.76	1.95	3.12	5.83
1280	.33	1.57	1.84	3.74
Total	28.20	32.24	38.92	99.36
		Unsterilized Soil		
0	3.91	3.53	3.84	11.28
5	5.41	2.68	4.48	12.57
10	4.38	3.60	2.35	10.33
20	4.51	2.31	3.47	10.29
40	4.19	2.60	4.18	10.97
80	3.83	4.12	4.36	12.31
160	3.45	4.04	5.77	13.26
320	3.08	3.39	5.77	12.24
640	2.13	2.88	5.25	10.26
1280	.95	1.87	4.74	7.56
Total	35.84	31.02	44.21	111.07

Figures shown are sums of three replications, with fresh weight in grams
All F values statistically significant at .005 level except those des-
ignated n.s. (non significant)

	C.V.%	Treatment	Fungicide	Time	Fungicide X Time
			F Values		
Sterilized	23.46	19.57	2.88 n.s.	2.79 n.s.	1.40 n.s.
Unsterilized	20.35	7.93	4.74	23.64	7.78

TABLE IX

EFFECT OF SODIUM OMADINE ON SORGHUM VULGARE PERS. GROWTH
AS DRY WEIGHT IN STERILIZED AND NONSTERILIZED SOIL

Treatment ppm Sodium Omadine	Harvests			
	First	Second	Third	Total

Sterilized Soil

Treatment ppm Sodium Omadine	First	Second	Third	Total
0	.64	.31	.72	1.67
5	.49	.38	.59	1.46
10	.62	.82	.45	1.89
20	.48	.58	.61	1.67
40	.51	.68	.50	1.70
80	.41	.59	.92	1.92
160	.54	.75	.88	2.17
320	.29	.73	.78	1.80
640	.18	.70	.75	1.63
1280	.08	.26	.43	.77
Total	4.24	5.81	6.63	16.68

Unsterilized Soil

Treatment ppm Sodium Omadine	First	Second	Third	Total
0	.56	.56	.60	1.72
5	.71	.41	.57	1.69
10	.60	.52	.38	1.50
20	.58	.35	.45	1.38
40	.55	.43	.55	1.53
80	.76	.59	.47	1.82
160	.48	.62	.74	1.84
320	.46	.50	.75	1.71
640	.32	.38	.63	1.33
1280	.20	.35	.56	1.11
Total	5.22	4.71	5.70	15.63

Figures shown are sums of three replications with dry weight in grams
All F Values statistically significant at .005 level except those des-
ignated n.s. (non significant)

		F Values			
	C.V.%	Treatment	Fungicide	Time	Fungicide X Time
Sterilized	27.63	5.42	5.84	18.78	3.72
Unsterilized	24.72	3.21	3.26	4.51 n.s.	3.03

TABLE X

EFFECT OF SODIUM OMADINE ON AVENA SATIVA L. GROWTH AS
FRESH WEIGHT IN STERILIZED AND NONSTERILIZED SOIL

Treatment ppm	Harvests			
Sodium Omadine	First	Second	Third	Total

		Sterilized Soil		
0	2.64	2.39	1.94	6.97
5	2.64	2.67	1.98	7.29
10	2.96	2.48	2.31	7.75
20	2.93	2.67	1.92	7.52
40	2.88	2.75	2.94	8.57
80	2.57	2.71	2.70	7.98
160	2.70	2.53	2.53	7.76
320	2.34	2.42	2.70	7.46
640	2.21	2.30	2.47	6.98
1280	.93	2.12	2.23	5.28
Total	24.80	25.04	23.72	73.56
		Unsterilized Soil		
0	3.74	3.08	1.08	7.90
5	4.02	2.78	1.41	8.21
10	3.75	4.07	1.22	9.04
20	3.69	4.74	1.49	9.92
40	3.42	3.08	1.65	8.15
80	2.59	2.79	4.06	9.44
160	2.60	3.46	3.68	9.74
320	2.72	3.48	3.94	10.14
640	2.12	2.77	3.20	8.09
1280	.89	1.46	2.44	4.79
Total	29.54	31.71	24.17	85.42

Figures shown are sums of three replications with fresh weight in grams
All F values statistically significant at .005 level except those des-
ignated n.s. (non significant)

		F Values			
	C.V.%	Treatment	Fungicide	Time	Fungicide X Time
Sterilized	16.80	2.95	4.54	.88 n.s.	2.39 n.s.
Unsterilized	15.21	17.13	12.90	24.18	18.46

TABLE XI

EFFECT OF SODIUM OMADINE ON <u>AVENA</u> <u>SATIVA</u> L. GROWTH AS
DRY WEIGHTS IN STERILIZED AND NONSTERILIZED SOIL

Treatment ppm	Harvests			
Sodium Omadine	First	Second	Third	Total

<table>
<tr><td colspan="5"><u>Sterilized</u> <u>Soil</u></td></tr>
<tr><td>0</td><td>.45</td><td>.35</td><td>.31</td><td>1.11</td></tr>
<tr><td>5</td><td>.43</td><td>.43</td><td>.30</td><td>1.16</td></tr>
<tr><td>10</td><td>.44</td><td>.34</td><td>.36</td><td>1.14</td></tr>
<tr><td>20</td><td>.49</td><td>.39</td><td>.33</td><td>1.21</td></tr>
<tr><td>40</td><td>.42</td><td>.40</td><td>.39</td><td>1.21</td></tr>
<tr><td>80</td><td>.31</td><td>.41</td><td>.36</td><td>1.08</td></tr>
<tr><td>160</td><td>.30</td><td>.38</td><td>.37</td><td>1.05</td></tr>
<tr><td>320</td><td>.34</td><td>.33</td><td>.49</td><td>1.16</td></tr>
<tr><td>640</td><td>.20</td><td>.32</td><td>.49</td><td>1.01</td></tr>
<tr><td>1280</td><td>.20</td><td>.28</td><td>.37</td><td>.85</td></tr>
<tr><td>Total</td><td>3.58</td><td>3.63</td><td>3.77</td><td>10.98</td></tr>
<tr><td colspan="5"><u>Unsterilized</u> <u>Soil</u></td></tr>
<tr><td>0</td><td>.43</td><td>.44</td><td>.40</td><td>1.27</td></tr>
<tr><td>5</td><td>.51</td><td>.38</td><td>.48</td><td>1.37</td></tr>
<tr><td>10</td><td>.56</td><td>.50</td><td>.59</td><td>1.65</td></tr>
<tr><td>20</td><td>.42</td><td>.59</td><td>.58</td><td>1.59</td></tr>
<tr><td>40</td><td>.30</td><td>.37</td><td>.48</td><td>1.15</td></tr>
<tr><td>80</td><td>.36</td><td>.35</td><td>.48</td><td>1.19</td></tr>
<tr><td>160</td><td>.39</td><td>.44</td><td>.52</td><td>1.35</td></tr>
<tr><td>320</td><td>.35</td><td>.42</td><td>.49</td><td>1.26</td></tr>
<tr><td>640</td><td>.26</td><td>.29</td><td>.44</td><td>.99</td></tr>
<tr><td>1280</td><td>.20</td><td>.23</td><td>.39</td><td>.82</td></tr>
<tr><td>Total</td><td>3.78</td><td>4.01</td><td>4.85</td><td>12.64</td></tr>
</table>

Figures shown are sums of three replications with <u>dry</u> weight in grams
All F values statistically significant at .005 level except those des-
ignated n.s. (non significant)

		F Values		
	C.V.%	Treatment	Fungicide	Time	Fungicide X Time
Sterilized	21.40	2.66	1.82 n.s.	.58 n.s.	3.31
Unsterilized	17.11	6.05	12.00	17.93	1.74 n.s.

soil sterilization was most apparent with grain sorghum at the higher application levels. A similar response was obtained with the oat cultures, but it was not as well defined. The 640 and 1280 ppm levels markedly inhibited growth of the first sorghum crop. However, with the second crop phytotoxicity at these levels was greatly reduced.

The influence of sodium omadine had largely subsided after 3 plantings or 56 days. However, some stunting of both sorghum and oats at the 1280 ppm level was apparent.

Experiment V. Extent of Sodium Omadine Degradation with Time

Bioassay of soil samples for fungitoxicity showed that small residual amounts of sodium omadine were still present 56 days after soil application. After each oat harvest, a one gram soil sample from each level of treatment was taken from the oat cultures in Experiment IV. The soil was oven-dried and added to the basic A. niger flask culture medium. The initial equivalent effective fungicide concentrations within the one gram soil samples were 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, and 25.6 ppm. Results from these flask studies are shown in Table XII.

In general, sodium omadine residues present in samples from sterilized pot cultures were higher than those from the unsterilized. As shown in Figure 4, A. niger growth was noticeably reduced, especially with the first series of flask inoculations. Marked reductions in growth were evident at the three higher treatment levels and complete inhibition occurred at the 25.6 ppm level. With samples derived from soil not sterilized, A. niger growth was not affected markedly. Only a slight depression in growth was noted at the higher levels (Figure 5). At the conclusion of the three series of soil samplings the only

TABLE XII

EXTENT OF SODIUM OMADINE DEGRADATION
IN SOIL WITH TIME

Treatment ppm Sodium Omadine	Grams A. niger Dry Mycelium Harvests			
	First	Second	Third	Total

Sterilized Soil

Treatment ppm Sodium Omadine	First	Second	Third	Total
0.0	4.41	5.05	4.82	14.28
0.1	4.15	4.85	4.96	13.96
0.2	4.30	4.79	4.99	14.08
0.4	4.13	4.76	4.87	13.76
0.8	4.07	4.74	4.82	13.63
1.6	3.79	4.73	4.82	13.34
3.2	3.96	4.67	4.78	13.41
6.4	3.96	4.66	4.71	13.33
12.8	2.02	2.36	3.51	7.89
25.6	.00	.00	1.03	1.03
Total	34.79	40.61	43.31	118.71

Unsterilized Soil

Treatment ppm Sodium Omadine	First	Second	Third	Total
0.0	4.70	4.72	4.72	14.14
0.1	4.55	4.67	4.85	14.07
0.2	4.99	5.01	4.93	14.93
0.4	4.94	4.95	4.98	14.87
0.8	4.92	4.94	5.00	14.86
1.6	4.88	4.90	4.72	14.50
3.2	4.74	4.75	4.98	14.47
6.4	3.50	3.53	4.10	11.13
12.8	2.66	2.69	3.11	8.46
25.6	1.10	1.14	1.20	3.44
Total	40.98	41.30	42.59	124.87

Check

Treatment ppm Sodium Omadine	First	Second	Third	Total
0.0	3.15	3.57	3.21	9.93
0.1	3.58	3.68	3.42	10.68
0.2	3.45	3.55	3.43	10.43
0.4	3.43	3.52	3.62	10.57
0.8	2.94	3.13	3.17	9.24
1.6	2.88	2.94	2.48	8.30
3.2	.00	.00	.00	.00
6.4	.00	.00	.00	.00
12.8	.00	.00	.00	.00
25.6	.00	.00	.00	.00
Total	19.43	20.39	19.33	59.15

Figures shown are sums of three replicate cultures
F values are significant at .005 level except when designated n.s.
(non significant)

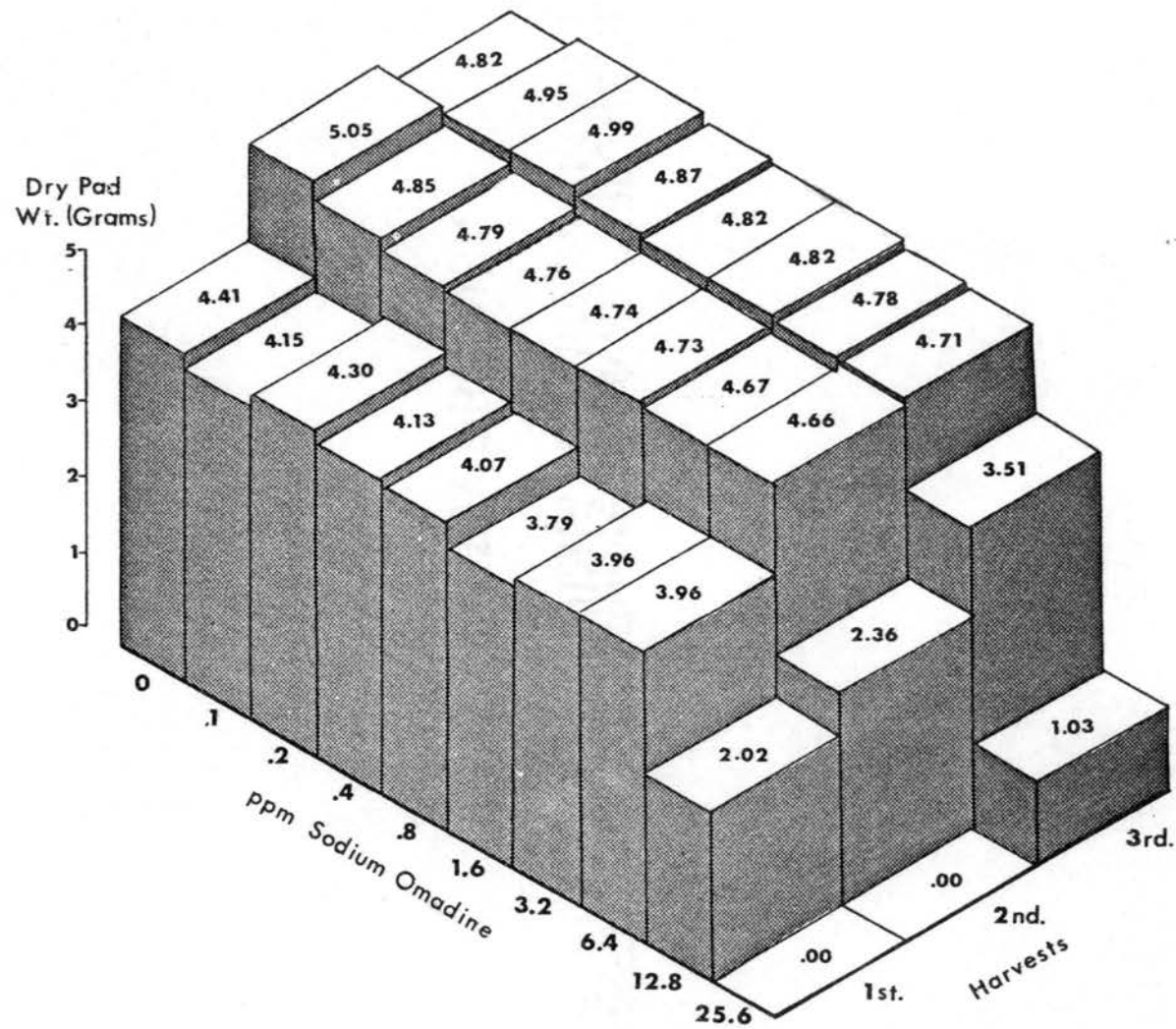|	C.V.%	Treatment	Omadine	Time	Omadine X Time
			F Values		
Check	10.65	185.86			
Sterilized	14.05	24.29	71.85	22.69	.69 n.s.
Unsterilized	10.44	24.08	76.76	1.14 n.s.	.29 n.s.

Figure 4. Influence of Time on Sodium Omadine Level Toxicity to
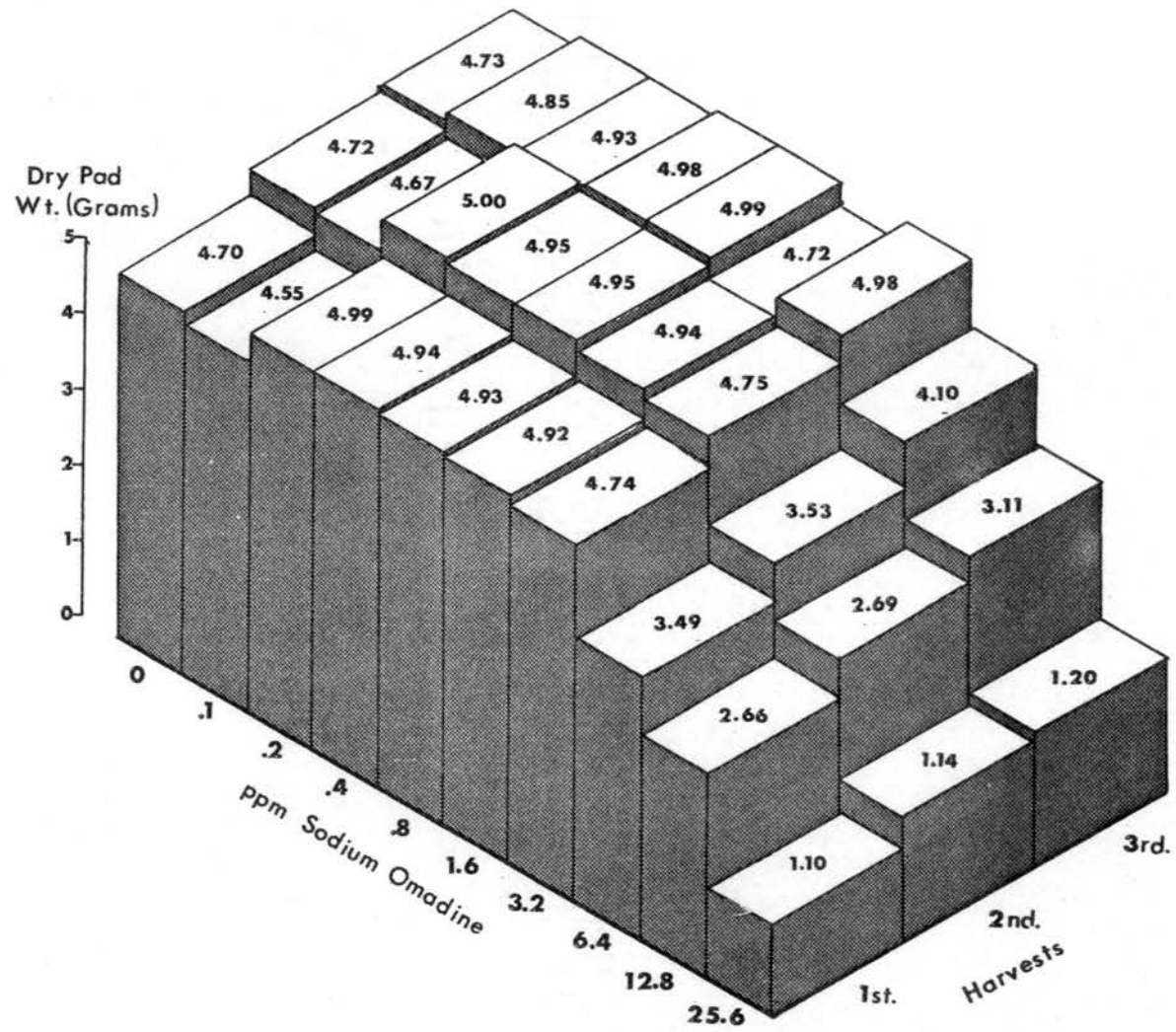Aspergillus niger with Sterilized Eufaula Sand.

Figure 5. Influence of Time on Sodium Omadine Level Toxicity to Aspergillus niger with Nonsterilized Eufaula Sand.

evidence of A. niger inhibition was at the highest level.

The differences in responses obtained from the two soil steriliza-
tion treatments could best be explained by the presence of an estab-
lished soil population in the unsterilized samples. These active micro-
floral populations could initiate degradation immediately, whereas the
sterilized samples would require a period of time to build up an adapted
soil microflora population.

As noted by Norman (10) sodium omadine is rapidly adsorbed on the
soil colloids. Thus, some inactivation may have occurred in these
studies along with some possible volatilization. However, both reac-
tions should have been near the same magnitude regardless of the soil
sterilization treatment.

CHAPTER V

SUMMARY AND CONCLUSIONS

The objectives of this study were to establish the rates of soil biological transformations on activity of sodium omadine and to determine factors that influence rates and magnitude of degradation. Oats and grain sorghum were used in bioassay studies. Five soil inhabiting fungi were screened with media containing various levels of sodium omadine to establish threshold toxicity levels. Pyridines and other compounds were used in culture amendment studies to ameliorate the toxicity of sodium omadine to Aspergillus niger and Aspergillus tamarii.

Sodium omadine was found to be non-phytotoxic to oats and grain sorghum at levels below 320 ppm. Both oats and grain sorghum growth was markedly inhibited above the 320 ppm level. Evidence of phytotoxicity was more pronounced with plants on sterilized soil than those on unsterilized soil. The influence of sodium omadine had largely subsided after 56 days. Fungus bioassay of soil samples indicated that at the highest application level, small residual amounts of sodium omadine were present.

Aspergillus flavus was the only fungi tested that was not inhibited by sodium omadine at 10 ppm. The Aspergilli: niger, tamarii, oryzae, and Curvularia lunta were inhibited at levels between 1 and 5 ppm.

Three compounds, picolinic acid, riboflavin, and picloram were

found to modify sodium omadine toxicity to <u>Aspergillus niger</u>. Of these, picolinic acid was the most effective in counteracting sodium omadine toxicity.

LITERATURE CITED

1. Alexander, Martin. 1964. Introduction to Soil Microbiology.
       John Wiley and Sons, Inc. New York, N. Y.

2. Anonymous. 1960. Zinc and Sodium Compounds of Omadine.
       Technical Bulletin No. 1. Olin Mathieson Chemical Corpora-
       tion. Rochester, New York.

3. Barnes, G. L. and R. S. Zerkel. 1961. Effectiveness of mixtures
       of pyridinethiol derivatives and PCNB (Terraclor) for control
       of a complex of soil fungi. Plant Disease Reporter 45:426-
       431.

4. Couch, H. B. and H. Cole, Jr. 1957. Chemical control of melting
       out of Kentucky bluegrass. Plant Disease Reporter 41:205-
       207.

5. Hamilton, J. M. and M. Szkolnik. 1957. Performance of Omadine,
       A. C. 5223 and other promising fungicides in the control of
       apple scab and cedarapple rust. Plant Disease Reporter 41:
       293-300.

6. Horsefall, J. G. 1956. Principles of Fungicidal Action.
       Chronica Botanica Company. Waltham, Mass.

7. Kenaga, C. B. and R. L. Kiesling. 1957. Control of three foliar
       diseases by several fungicides in greenhouse tests. Plant
       Disease Reporter 41:303-307.

8. Martin, J. P. and P. F. Pratt. 1958. Fumigants, fungicides, and
       the soil. J. of Agric. and Food Chem. 6:345-348.

9. Morgan, O. D. 1959. The use of chemical soil drenches to control
       Phytophthora parasitica var. nicotianae and their phytotoxic
       effects on tobacco and toxicity to other soil flora. Plant
       Disease Reporter 43:755-761.

10. Norman, A. G. 1957. Growth repression of higher plants by 2-
       pyridinethiol, 1-oxide. Plant Physiol. 32:16-19.

11. Peterson, L. J., R. Baker, and R. E. Skiver. 1959. Control of
       fusarium stem rot of carnations. Plant Disease Reporter
       43:1204-1208.

VITA

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