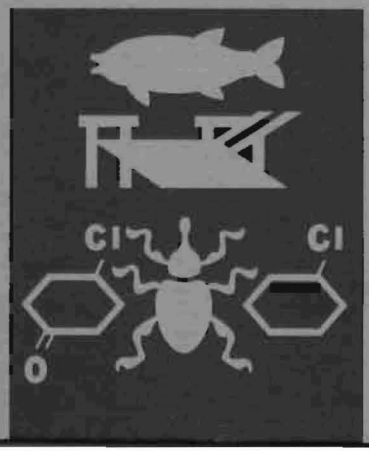

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COMPATIBILITY OF A *CERSCOSPORA RODMANII* FORMULATION WITH SELECTED HERBICIDE SPRAY ADDITIVES

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Cercospora rodmanii Conway, a fungal pathogen, offers promise as a biocontrol of the aquatic plant waterhyacinth (*Eichhornia crassipes* (Mart.) Solms.). A powdered formulation of the pathogen has been developed by Abbott Laboratories, Chicago, Ill. To facilitate the spray application of this formulation, a chemical additive was needed that would not be inhibitory to the fungus. Nine additives commonly used in application of herbicides for control of aquatic plants were tested: Agridex, Big Wet, Cide-Kick "SA-77," Lo-Drift,

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Nalco-Trol, Ortho X-77 Spreader, Peg, Sterox NJ, and Surfel. Each additive was tested at the lowest and highest concentrations recommended by its label. Tests were performed in laboratory cultures by comparing the number of colonies of *C. rodmanii* produced in the presence of each test agent with the number produced in untreated controls. All additives except Ortho X-77 Spreader exhibited significantly lower counts than controls, indicating that they inhibited the growth of the *C. rodmanii* formulation. Ortho X-77 Spreader consistently produced more colonies than did controls at the lowest recommended concentration signifying enhancement rather than inhibition.

Results of this study affirm that it would be inadvisable to use eight of these additives with the *C. rodmanii* formulation at the concentrations recommended by their labels. Ortho X-77 Spreader is recommended for use at the lowest concentration listed on its label.

PREFACE

Funding for this study was provided by the Office, Chief of Engineers, under appropriation number 96X3122, Construction General, to the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss., Aquatic Plant Control Research Program (APCRP).

This report describes laboratory tests of the compatibility of spray additives with a formulation of the fungus *Cercospora rodmanii* Conway, which offers promise as a biological control agent of waterhyacinth. The tests were performed to determine the viability of the fungus in the presence of nine additives commonly used in the application of herbicides for the control of this species. The additives possess properties that would make them very useful in the application of the formulation, provided that the additives do not inhibit the germination or growth of the fungus.

The tests were conducted by Mrs. Judith C. Pennington and Mr. Edwin A. Theriot of the Wetland and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), WES. Abbott Laboratories, Inc., Chicago, Ill., provided the *C. rodmanii* formulation and various manufacturers of spray additives supplied samples of their products.

The study was conducted under the technical guidance of Dr. Dana R. Sanders, Sr., WTHG; under the direct supervision of Dr. Hanley K. Smith, WTHG; and under the general supervision of Dr. Conrad J. Kirby, Jr., Chief, ERD. Dr. John Harrison was Chief, EL. Manager of the APCRP was Mr. J. Lewis Decell.

During preparation of the report, COL Nelson P. Conover, CE, and COL Tilford C. Creel, CE, were Commanders and Directors of the WES; Mr. F. R. Brown was Technical Director.

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CONTENTS

COMPATIBILITY OF A *CERCOSPORA RODMANII* FORMULATION WITH SELECTED HERBICIDE SPRAY ADDITIVES

PART I: INTRODUCTION

Background

1. A powdered formulation of mycelial cells of the fungus *Cercospora rodmanii* Conway has been developed as a potential biological agent for control of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms.). *Cercospora rodmanii* was first isolated in December 1973 from declining waterhyacinths in Rodman Reservoir, Florida, by Dr. K. E. Conway of the University of Florida (Conway, Freeman, and Charudattan 1974). After establishing the pathogenicity and host-specificity of the fungus (Conway and Freeman 1977), the University was granted a patent for its use for the control of waterhyacinth. They subsequently granted Abbott Laboratories, Inc., Chicago, Ill., the right to develop it as a marketable product. As a part of the U. S. Army Engineer Waterways Experiment Station's (WES) Large-Scale Operations Management Test with Insects and Plant Pathogens, field tests of this formulation were conducted by the WES in Louisiana (Sanders et al. 1979).

Rationale

2. Most herbicides commonly used for the control of waterhyacinth are mixed with chemical agents that facilitate the application process by controlling drift and/or by enhancing the adherence of the herbicide to plant surfaces. Because the *C. rodmanii* formulation is a wettable powder containing large, heavy particles that suspend poorly in the tank mix, additives that stabilize the suspension and increase adherence of the particles to leaves would be useful. The possibility existed, however, that these agents might be toxic or inhibitory to the *C. rodmanii* formulation. For this reason, a laboratory study was designed to determine the viability of the formulation in the presence of commonly used

additives at the lowest and highest concentrations recommended by their labels for general field application.

Purpose and Objectives

3. The purpose of this study was to determine the effects of nine commercially available spray additives on the viability of the Abbott Laboratories formulation of *C. rodmanii*. The specific objectives were as follows:

a. To determine the compatibility of the formulation with each additive at its minimum recommended concentration.

b. To determine the compatibility of the formulation with each additive at its maximum recommended concentration.

c. To contrast the effects of the various additives.

PART II: MATERIALS AND METHODS

Selection of Test Additives

4. Waterhyacinth control programs are conducted primarily by State and local agencies and Corps of Engineers District Offices. For this reason, a telephone poll of such agencies was conducted to identify spray additives presently in use. Results of the poll indicated that the following additives are commonly used:

Additive	Producer
Agridex	Helena Chemical Co.
Big Wet	JLB International Chemical, Inc.
Cide-Kick "SA-77"	JLB International Chemical, Inc.
Lo-Drift	AmChem Products, Inc.
Nalco-Trol	Nalco Chemical Co.
Ortho X-77 Spreader	Chevron Chemical Co.
Peg	Helena Chemical Co.
Sterox NJ	Monsanto Co.
Surfel	AmChem Products, Inc.

5. Invert-emulsifying agents were not tested because they require the addition of xylene, diesel, or similar oils strongly suspected of being toxic to the fungus. Seeking nontoxic components to produce these emulsions was considered to be beyond the scope of the present study.

Considerations for Culturing the *C. rodmanii* Formulation

6. By experimenting with various media, Freeman et al. (1981) found potato dextrose agar (PDA) with yeast extract to be most suitable for culturing *C. rodmanii*. Since it was necessary to quantify colonies rather than to produce luxurious growth, the yeast extract was considered unnecessary and PDA alone was used. When grown on PDA, *C. rodmanii* produces light- to dark-gray, fluffy colonies (Figure 1). A pigment

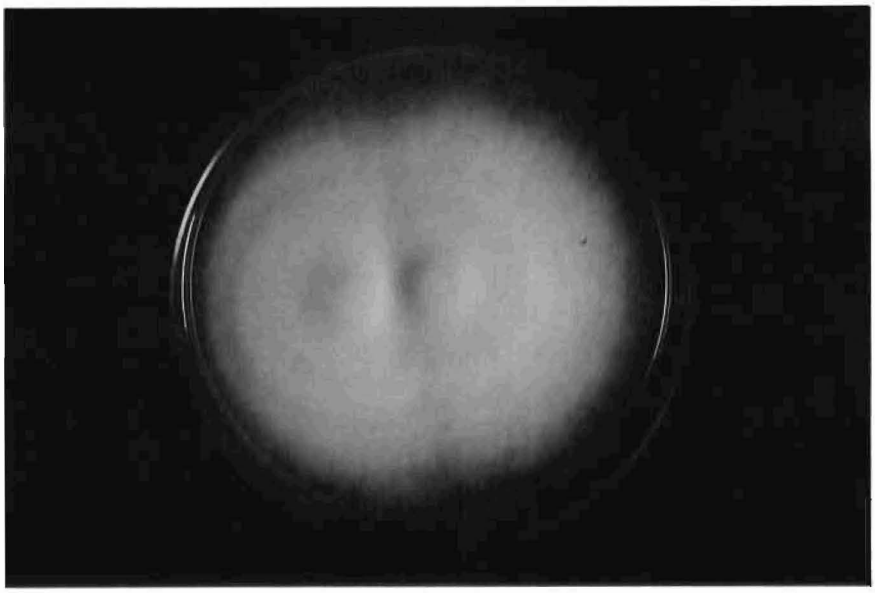

Figure 1. Surface characteristics of two colonies of *C. rodmanii* grown from the Abbott Laboratories formulation (PDA-LA)

identified as the phytotoxin, cercosporin (Freeman et al. 1981), diffuses into the media beneath the colonies, coloring it various shades of pink, red, or brown (Figure 2).

7. Bacterial contaminants, a recurring problem in fungal cultures in Petri dishes, are frequently so numerous that they completely overcome the slow-growing *C. rodmanii*. These bacteria were inhibited by adding 1 ml of 25 percent (volume/volume) lactic acid to each 100 ml of PDA (PDA-LA).* Contaminating fungal colonies are usually present in smaller numbers and can be ignored.

8. The exact nature of the particles within the formulation of *C. rodmanii* is proprietary information; however, they are described by researchers at Abbott Laboratories as mycelial segments, some of which may exist as single viable propagules and some as several such propagules growing together or adhering to each other. This lack of uniformity in distribution of colony forming units (CFU) introduces a great deal of

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* Personal Communication, Susan Woodhead, July 1980, Abbott Laboratories, Inc., Chicago, Ill.

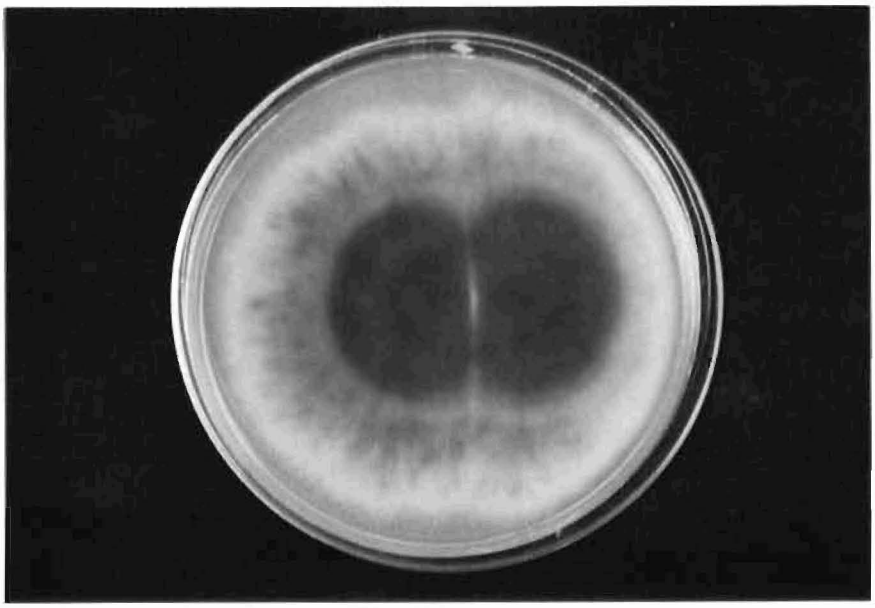

Figure 2. Subsurface characteristics of the same two colonies of *C. rodmanii* shown in Figure 1

variability when sampling the formulation. To reduce this variability, the formulation was passed through a 100 mesh sieve prior to weighing samples for individual tests.

Test Procedure

9. A 0.100-g sample of the formulation having 5.0×10^4 CFU per gram was added to 100 ml of sterile distilled water in a 125-ml Erlenmeyer flask containing a magnetic stirring bar. The flask was placed on a magnetic stirrer at medium speed while 40 PDA-LA plates were inoculated by transferring 0.9 ml of the suspension to each plate. Twenty of the plates were used as controls and were treated by adding 0.1 ml of sterile distilled water; the other twenty were used as tests and were treated by adding 0.1 ml of additive diluted to 10 times its recommended concentration (Figure 3). The materials were mixed and spread over the surface of the plates with a flame-sterilized, bent glass rod. The same procedure was used for both the low and high recommended concentrations of test products (Table 1). All plates were

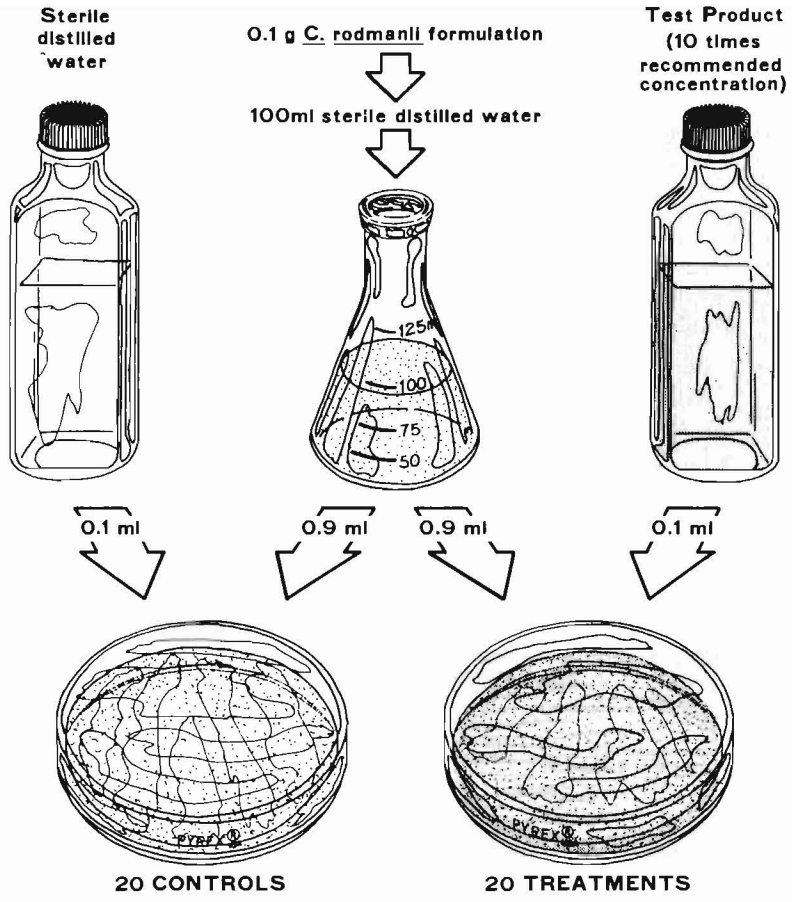

Figure 3. Flow diagram of procedure for diluting the *C. rodmanii* formulation and spray additives

incubated at 25° to 27°C (Freeman et al. 1981) for 5 days before individual colony counts were made.

Methods of Data Analysis

10. A standard t-test was used to compare means for each set of controls and treatments. Data were tabulated as colony counts and a square root transformation was employed prior to analysis (Steel and Torrie 1960). T-tests were performed on data for each of eight spray additives at the lowest recommended concentrations and for each of nine spray additives at the highest recommended concentrations. Only eight additives were tested at the low rate because no minimum concentration

for Sterox NJ was given on the label.

11. The eight sets of controls in the low concentration test and the nine sets in the high concentration test were subjected to a one-way analysis of variance (ANOVA) to determine differences among controls.

12. In addition, an ANOVA was conducted on weighted count data, i.e., individual treatments divided by the mean of the controls for that test. Means were separated using Duncan's Multiple Range Test to show significant differences among spray additives.

PART III: RESULTS AND DISCUSSION

Compatibility of the *C. rodmanii* Formulation with Spray Additives

13. *Cercospora rodmanii* colony counts and statistics for the low and high concentration tests are given in Tables 2 and 3, respectively. A decrease in the viability of the *C. rodmanii* formulation with time was evident when control data means were examined. The control means for the low concentration test were slightly less than those for the high concentration test, which was performed 10 days earlier. Controls from both tests showed a decrease from the 5.0×10^4 CFU per gram determined just prior to the study and upon which the dilution procedure was based.

14. All but one spray additive tested produced colony counts significantly lower than the controls at both concentrations. This indicated that all but one exerted an inhibitory effect on the *C. rodmanii* formulation. The exception was Ortho X-77 Spreader at the low concentration, which produced more colonies than its controls. To rule out the possibility that these exceptional results represented an error in the experiment, an additional replicate using a new aliquot of formulation and of Ortho X-77 Spreader was performed. The mean number of colonies for the control group and for the test group were 4.2 and 13.1, respectively. These means were smaller than those of the first replicate because of a decrease in the viability of the *C. rodmanii* formulation with time. They were, however, proportional to means of the first replicate and confirmed that the formulation produced significantly more colonies in the presence of Ortho X-77 Spreader than in water alone. These results suggested an enhancement effect rather than the suppressive effect evident with all other additives at both rates. Several possible factors may account for these results. Ortho X-77 Spreader may have a threshold concentration below which it is nontoxic to *C. rodmanii*. Below that level, its modification of the physical environment surrounding the particles of formulation may actually assist germination. For example, the Ortho X-77 Spreader, which is a surfactant, may reduce the

surface tension surrounding the more minute colony forming units, thus facilitating their germination. It may also tend to break apart large clumps of propagules, thereby resulting in several colonies where a single colony would have grown in the presence of other products or in water alone. If this is the case, Ortho X-77 Spreader should be expected to exert the same effect in field applications, thereby enhancing the infectivity of the formulation. Stimulation of germination by some chemical mechanism is also possible.

Comparison of Effects of Additives with Each Other

15. Analysis of variance among controls indicated significant differences. This result is not surprising considering the nature of the formulation. Sieving prior to testing increased uniformity in the distribution of colony forming units, but variation still existed. For this reason, a weighted ANOVA was used to compare spray additives with each other. Duncan's Multiple Range Test was applied to these data and results are presented in Table 4 for the low and high concentration tests. The use of Ortho X-77 Spreader resulted in greater colony counts than all other spray additives in the low concentration test. In the high concentration test, the number of colonies resulting from the use of Cide-Kick "SA-77" was significantly lower than for all other additives, which indicated that it exerted the greatest inhibitory effect on the *C. rodmanii* formulation. The relative positions of other spray additives in the low and high concentration tests overlapped considerably and, because there was significant variation among the controls, no definite conclusions could be drawn of their relative effects on the viability of the formulation.

PART IV: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

16. All nine spray additives tested exerted significant effects on the *C. rodmanii* formulation. Conclusions that can be drawn from the results of this study are as follows:

a. The *C. rodmanii* formulation was completely incompatible with seven of the spray additives tested at their minimum recommended concentrations for field use.

b. Ortho X-77 Spreader was not only compatible with the *C. rodmanii* formulation at its lowest recommended concentration, but actually caused an increase in the number of colonies produced by the formulation as compared with controls.

c. None of the nine spray additives were compatible with the formulation when tested at their maximum recommended concentrations.

17. Duncan's Multiple Range Test showed considerable overlapping of test additives, making contrasting of effects among them difficult. However, since all but one additive significantly inhibited the *C. rodmanii* formulation, contrasting of effects was unnecessary.

Recommendations

18. Based on the results of this study, Ortho X-77 Spreader is recommended as an additive for the application of the *C. rodmanii* formulation when used at its lowest recommended concentration. None of the other spray additives tested can be recommended for use with the formulation at the concentrations given by their labels. It is possible that some of these would show effects similar to those of Ortho X-77 Spreader if they were used at concentrations lower than those tested. However, further reductions in concentration may simultaneously reduce the ability of the additives to stabilize the suspension of the formulation adequately, decrease adherence of the particles to leaf surfaces, or otherwise affect the properties for which these additives are used.

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Table 1

Minimum and Maximum Concentrations of Spray Additives as Recommended by Labels

Additive	Min (% V/V)	Max (% V/V)
Agridex	0.500	1.000
Big Wet	0.125	0.166
Cide-Kick "SA-77"	0.125	1.000
Lo-Drift	0.016	0.125
Nalco-Trol	0.156	0.250
Ortho X-77 Spreader	0.031	0.500
Peg	0.500	1.000
Sterox NJ	*	0.250
Surfel	0.125	0.500

* No minimum concentration recommended.

Table 2

Colony Counts and Statistics on *C. rodmanii* Formulation Grown in the Presence of Additives at Their Lowest Recommended Concentrations

Plate Number	Agridex		Big Wet		Cide-Kick		Lo-Drift		Nalco-Trol		Ortho		Peg		Surfel	
	C*	T**	C	T	C	T	C	T	C	T	C	T	C	T	C	T
1	7	4	12	2	8	1	12	1	11	3	4	34	2	0	8	1
2	15	4	10	3	17	1	13	1	7	3	5	34	3	0	3	0
3	20	3	7	2	16	1	12	1	5	2	3	39	10	1	17	1
4	17	7	10	5	18	2	8	4	7	11	14	29	7	1	6	3
5	22	6	8	3	24	3	7	2	12	4	5	40	5	3	6	1
6	22	3	11	3	19	2	14	3	9	4	3	35	13	1	8	2
7	24	6	15	3	11	1	12	2	12	3	11	30	8	0	5	4
8	18	8	14	4	19	7	12	5	18	12	2	35	6	5	11	0
9	14	13	16	6	17	9	18	8	11	6	11	30	6	3	11	2
10	14	4	14	8	21	6	13	7	3	7	17	28	7	3	7	1
11	13	8	9	7	17	3	3	2	3	11	6	42	7	8	38	1
12	17	6	16	11	13	3	15	5	5	3	13	27	8	3	4	2
13	31	11	10	4	18	3	14	4	14	13	14	32	0	3	5	8
14	19	8	13	2	8	2	7	7	14	5	7	37	0	7	5	3
15	23	11	14	6	21	3	20	3	10	5	15	34	7	2	12	4
16	17	15	17	5	21	8	15	7	11	9	12	36	16	0	16	1
17	31	10	15	9	27	9	9	4	17	4	13	35	11	0	7	6
18	26	8	15	12	21	2	13	9	22	7	6	35	9	2	9	5
19	23	10	13	13	19	7	19	5	17	16	15	37	5	3	10	6
20	20	10	22	10	16	12	11	16	20	8	18	41	13	4	12	3
Mean	19.6	7.8	13.0	5.9	17.6	4.2	12.4	4.8	11.4	6.8	9.7	32.6	7.2	2.4	10.0	2.7
Standard Deviation	5.9	3.4	3.6	3.5	4.8	3.3	4.2	3.6	5.5	4.0	5.2	8.2	4.2	2.3	7.6	2.2
T-Test†		7.93		6.43		10.05		6.19		3.06		-13.45		3.84		5.59

* Control.
** Test.
† T-test on transformed data ($P < 0.05$).

Table 3

Colony Counts and Statistics on *C. rodmanii* Formulation Grown in the Presence of Additives at Their Highest Recommended Concentrations

Plate Number	Agridex		Big Wet		Cide-Kick		Lo-Drift		Nalco-Trol		Ortho		Peg		Sterox		Surfel†	
	C*	T**	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
1	11	2	2	2	13	0	6	5	13	4	12	3	10	3	7	3	7	1
2	12	9	13	8	11	1	20	8	19	4	9	1	16	2	9	2	8	1
3	23	9	6	2	18	0	19	6	27	5	15	3	9	4	2	1	4	0
4	9	3	11	5	24	0	10	8	15	5	8	5	11	6	6	2	4	0
5	21	5	14	2	14	1	9	10	31	10	11	5	15	3	5	1	9	3
6	17	1	6	7	15	1	13	11	31	6	13	5	24	5	3	1	7	1
7	23	9	12	3	23	1	25	11	22	7	12	6	12	11	10	1	5	3
8	20	9	14	5	18	0	11	9	22	19	12	5	16	4	7	0	5	0
9	20	11	12	4	15	0	19	5	33	14	17	7	10	7	9	0	5	1
10	26	8	11	5	18	0	29	6	26	16	13	7	17	9	12	5	9	2
11	28	11	12	5	23	0	12	6	18	6	13	3	10	1	17	2	4	1
12	28	13	20	9	19	1	14	9	29	14	25	2	18	5	14	5	12	2
13	20	10	10	11	15	1	20	13	25	14	17	5	13	4	18	2	10	2
14	21	10	13	6	18	0	8	16	33	13	16	5	17	6	13	5	5	3
15	22	12	19	3	30	0	21	16	24	14	26	6	12	8	21	5	10	3
16	18	11	40	5	19	0	22	10	33	25	68	4	25	9	13	4	8	0
17	33	27	24	16	18	0	24	15	38	13	26	9	16	11	9	9	1	4
18	40	14	35	19	27	1	21	13	30	30	10	4	28	6	18	5	5	4
19	36	14	19	8	21	3	29	14	33	17	13	3	16	10	13	5	7	5
20	39	34	32	12	23	3	23	19	19	18	18	7	13	7	15	6	12	9
Mean	23.4	11.1	16.2	6.8	19.1	0.6	17.8	10.5	26.0	12.7	17.7	4.8	15.4	6.0	11.0	3.2	6.8	2.2
Standard Deviation	8.7	7.7	9.9	4.6	4.8	0.9	6.9	4.1	6.9	7.1	13.0	1.9	5.2	2.9	5.2	2.4	2.9	2.2
T-Test††		4.97		4.20		20.35		3.95		5.99		6.74		7.36		6.19		5.67

* Control.

** Test.

† This additive was tested 10 days later than all other additives in this table; therefore, its control and test means reflect a decrease in the viability of the formulation with time.

†† T-test on transformed data ($P < 0.05$).

Table 4

Duncan's Multiple Range Tests Using Transformed Data*

Additive	Means**
Low Concentration Test	
Ortho X-77 Spreader	3.556701^{a}
Nalco-Trol	0.578723^{b}
Big Wet	0.452107^{bc}
Agridex	0.394402^{bcd}
Lo-Drift	0.388664^{bcd}
Peg	0.342657^{cd}
Surfel	0.270000^{cd}
Cide-Kick "SA-77"	0.242165^{d}
High Concentration Test	
Lo-Drift	0.591549^{a}
Nalco-Trol	0.487524^{ab}
Agridex	0.475375^{ab}
Big Wet	0.421538^{bc}
Peg	0.392857^{bc}
Surfel	0.328467^{c}
Sterox NJ	0.289593^{c}
Ortho X-77 Spreader	0.268362^{c}
Cide-Kick "SA-77"	0.034031^{d}

* Each test was performed on 20 plates.

** Means followed by the same superscript within tests are not significantly different ($P < 0.05$).

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