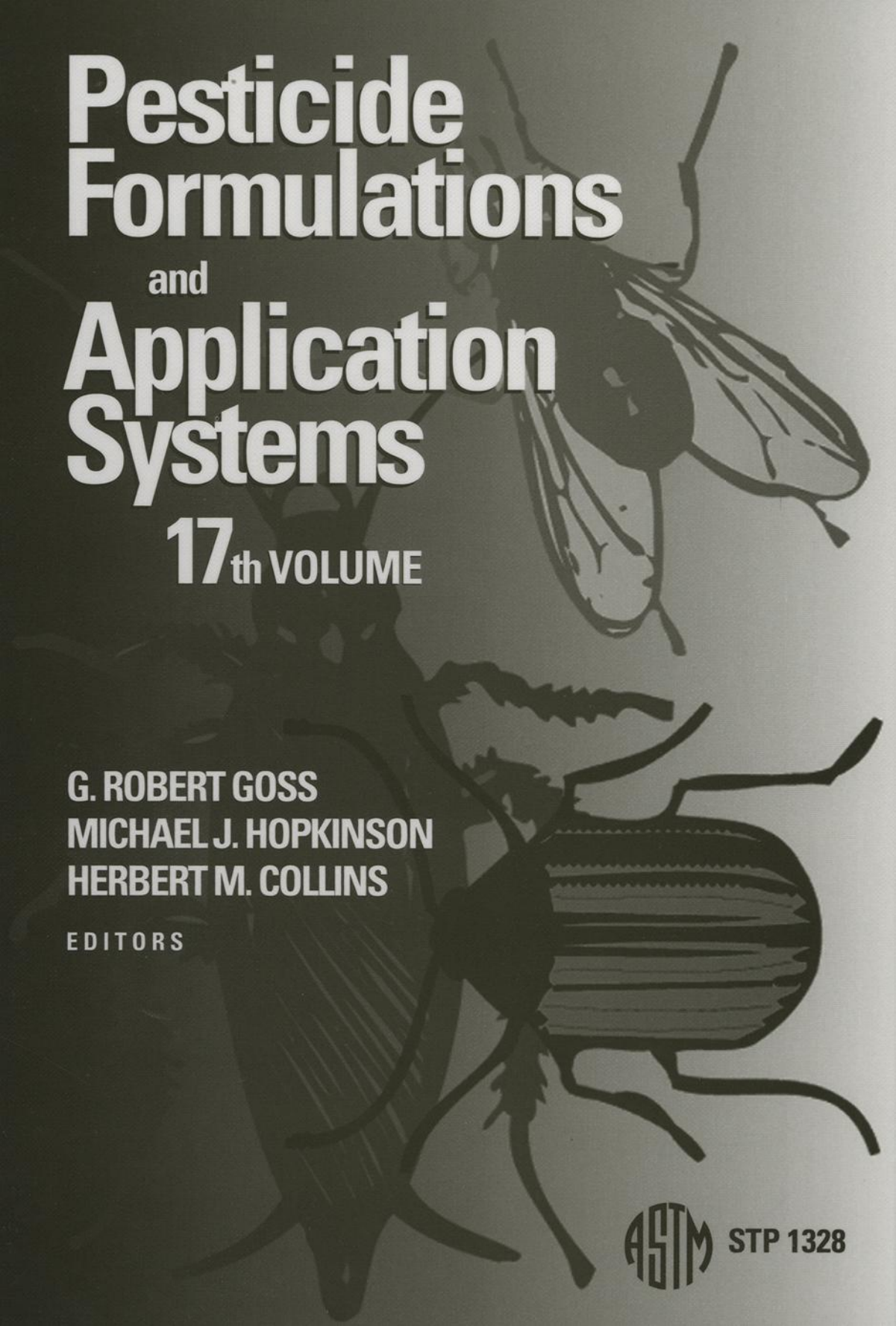
Pesticide Formulations and Application Systems

17th VOLUME



G. ROBERT GOSS
MICHAEL J. HOPKINSON
HERBERT M. COLLINS

EDITORS

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G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Editors

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The quality of the papers in this publication reflects not only the obvious efforts of the authors and the technical editor(s), but also the work of these peer reviewers. The ASTM Committee on Publications acknowledges with appreciation their dedication and contribution of time and effort on behalf of ASTM.

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Foreword

This publication, *Pesticide Formulations and Application Systems: 17th Volume,* contains papers presented at the symposium on Pesticide Formulations and Application Systems: The Changing Face of Agricultural Delivery Systems, held on 29–30 October 1996 in New Orleans, Louisiana. The symposium was sponsored by ASTM Committee E-35 on Pesticides. G. Robert Goss of Oil-Dri Corporation in Vernon Hills, Illinois; Michael J. Hopkinson of Ciba-Geigy Corporation in Greensboro, North Carolina; and John D. Nalewaja of North Dakota State University in Fargo, North Dakota presided as symposium co-chairmen. G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins of Stepan Company in Winder, Georgia are editors of the resulting publication.

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Basic Chemistry

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Overview

This book is the seventeenth in a continuing forum on one aspect of pesticide science. Specifically, this forum addresses pesticide formulations and application systems. Formulation and application of active ingredients is closely intertwined.

In this area of pesticide science, active ingredients are already of proven efficacy. The formulator is concerned about preparing the active ingredient for application. The applicator is concerned about how to deliver a formulated product to the target. Often, between formulation and application, other ingredients are added to aid efficacy. These are loosely called adjuvants. Pesticide regulations, of course, play an integral part in all these areas.

This book addresses nearly all the areas of pesticide formulations and applications. The first chapter, titled Formulation Technology gives examples of state-of-the-art development in preparing actual formulations. There are sub-sections for formulation preparation, chemical formulations, and biological formulations.

The Formulation Preparation section contains papers on the regulation of inert ingredients (Leifer), experimental design of formulation experiments (Butler), and the use of certain inert ingredients (Frisch).

Actual formulations can come in many forms. The Chemical Formulations section addresses several. There are papers on granules (Ross et al.), solid powders (Oelmüller and Müller), and even aerosols (Narayanan et al.).

While today, chemicals are the primary weapons against pests, biological agents are being increasingly used. The last section in this first chapter is on biological formulations. Levy et al. discusses the controlled release of biologically derived agents (Bacillus spp). Both Wacek and Jaronski describe the delivery of live organisms to the target.

Application Technology is the second chapter. Two papers (Downer et al. and Ozkan et al.) address spray deposition from hydraulic nozzles. While not exactly application technology, the paper by Keeney et al. describes the movement of particles through soil once applied.

One of the functions of this forum is education. Formulations and applications scientists come from across the United States, and to some extent the world, to share this knowledge. The third chapter is a review section. Papers review aspects of surface active agents, both their use (Tann) and property measurement (Pallas).

Finally, the last chapter, Surface Active Agents/Adjuvants describes surfactant basic properties and their effects on active ingredient efficacy. Hydrolytic stability (Anderson et al.), foam control (Policello and Koczo), and the mechanism of efficient organosilicane spreading (Hill and Burow) is discussed in the first section.

As described in the second paragraph of this forward, adjuvants can play an important role in the efficacy of a formulation. These are usually added as tank mixes immediately prior to application.

Recent advances in the use of solid adjuvant formulations are introduced by Roberts et al. and Narayanan and Tallon.

The balance of the papers introduce a wealth of factual information about adjuvant effects on several basic chemicals by several different methods. Three papers by Manthey et al. ("Lipophilic. . ."), Nalewaja and Matysiak, and Nalewaja et al. deal with herbicide efficacy. Contained are some of the first scanning electron micrographs of the effects of surfactants on droplet deposition. The paper by Mathey et al. ("Measuring. . .") gives a novel laboratory

method for predicting adjuvant efficacy. Fader and Bukovac confirm that even small differences in surfactant chemistry can alter formulation properties.

This volume advances our knowledge of many facets of pesticide formulation and application science. It is only one of several and hopefully one of more to come. As long as we live, chemistry will affect our lives. This forum, in its own small way, helps provide a safer environment for humanity, by delivering pesticides in a more efficient and safe manner.

G. Robert Goss
Symposium Co-Chairman.

Michael J. Hopkinson
Symposium Co-Chairman.

Herbert M. Collins
Symposium Co-Chairman.

FORMULATION TECHNOLOGY

Formulation Preparation

Kerry B. Leifer[1]

NEW DEVELOPMENTS IN THE REGULATION OF PESTICIDE INERT INGREDIENTS IN THE UNITED STATES

REFERENCE: Leifer, K. B., **"New Developments in the Regulation of Pesticide Inert Ingredients in the United States,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: The Environmental Protection Agency (EPA) has the authority to regulate inert ingredients present in pesticide products registered for use in the United States. Until recently, most of EPA's regulatory activities focused on the pesticide active ingredient, rather than the other components of the pesticide formulation known as inert ingredients. Although EPA has considered inert-specific data when reviewing some of the inert ingredients currently accepted for use in pesticides applied to food, it wasn't until the publication of EPA's Inert Strategy that a comprehensive policy regarding the information needed to determine the acceptability of a pesticide product inert ingredient was established. The implementation of this policy has led to changes in the types of substances proposed for use as inert ingredients as well as advancements in the methods for reviewing new inert ingredients. Emphasis on the long-term health and environmental effects of inert ingredients now requires pesticide registrants and others to consider these effects when developing new formulations. In order to encourage the use of less toxic inert ingredients, EPA has streamlined its review process, resulting in quicker decisions and more timely market introductions.

KEYWORDS: pesticide, inert ingredient, regulation, formulation, toxicity

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[1]Kerry B. Leifer, Registration Division, Office of Pesticide Programs, United States Environmental Protection Agency, Washington, DC 20460.

BACKGROUND

Pesticide products sold or distributed in commerce are required to be registered by the Environmental Protection Agency (EPA) in accordance with the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C.). Prior to EPA's inception in 1972, this function was within the jurisdiction of the United States Department of Agriculture (USDA). Although section 3 of FIFRA gives EPA broad authority in determining the data required to support a pesticide registration, EPA's and USDA's traditional regulatory focus has solely been on the active ingredients in pesticide products.

In addition to the authority delegated to EPA under FIFRA, the residues of pesticide chemicals in food are regulated under section 408 of the Federal, Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C.). This authority was transferred from the Food and Drug Administration (FDA) to EPA following the establishment of EPA. In 1961, FDA published a notice in the *Federal Register* stating that USDA had determined that each component of a registered pesticide product, including the inert ingredients, were pesticide chemicals and thus subject to the prescription of tolerances or the granting of tolerance exemptions under section 408 of FFDCA (U.S. FDA 1961).

With the exception of those pesticide products which consist solely of the technical grade active ingredient sold for the purposes of formulation into end use products, pesticide products are generally comprised of one or more active ingredients and various inert ingredients. These inert ingredients are formulants that typically function in such a manner as to help ensure the delivery of the active ingredient to the targeted pest or site and maintain the integrity of the formulation. Examples of inert ingredients include carriers, diluents, surfactants, buffering agents, and preservatives. The Environmental Protection Agency's Office of Pesticide Programs has identified over 2,000 substances that have been used as inert ingredients in registered pesticide product formulations.

Although FDA established a policy in 1969 regarding data requirements and review procedures for pesticide inert ingredients used on food (U.S. FDA 1969), it was not until 1987, when EPA announced its Inert Strategy (U.S. EPA 1987) that a comprehensive approach to the regulation of pesticide product inert ingredients was established.

The basic tenet of the Inert Strategy is to attempt to reduce the potential of adverse effects from substances used as inert ingredients and to ensure that the use of all inert ingredients is supported by a scientifically valid data base. Each of the existing inert ingredients was classified as belonging in one of four toxicity categories. The first category, referred to as List 1, was "Inerts of Toxicological Concern," and consisted of chemicals that had been found to produce cancer, adverse reproductive or

developmental effects, or other adverse chronic health or environmental effects. The substances on List 2 were "Inerts with a High Priority for Testing" and were primarily related by structure or class to compounds on List 1. List 3 included the "Inerts of Unknown Toxicity," which consisted of chemicals that could not be classified as belonging to one of the three other categories. List 4 constituted the "Minimal Risk" inerts and essentially was comprised of substances generally regarded as safe. In 1989, List 4 was subdivided into Lists 4A and 4B (U.S. EPA 1989). List 4A was comprised of substances judged to be of minimal risk based on their inherent nature, such as food substances like corn cobs and cookie crumbs. List 4B was reserved for chemicals for which the Agency had sufficient hazard and exposure data to make a minimal risk determination.

In addition to establishing a classification scheme for the existing inert ingredients, the Inert Strategy also identified a set of data requirements, referred to as the "base set," which would need to be addressed for any inert ingredient not previously accepted for use in a pesticide. These data would be evaluated by EPA to determine whether an inert ingredient would be considered to be safe and therefore acceptable for use in a pesticide formulation. The strategy also requires the labeling of pesticide products containing a List 1 inert ingredient.

The Inert Strategy has proven to have been very successful at meeting its objectives. Pesticide manufacturers either reformulated or discontinued products containing List 1 inert ingredients while exploring alternatives to many List 2 inert ingredients. Of the 1330 products initially containing a List 1 inert ingredient, less than 70 now continue to do so. These remaining products are being evaluated by EPA to determine if the presence of a List 1 inert ingredient in the formulation poses any unreasonable risks to human health or the environment.

CURRENT ACTIVITIES

With the virtual elimination of List 1 inert ingredients in pesticides, EPA's focus is now on attempting to obtain additional health and safety information on the substances on Lists 2 and 3. This effort has had two primary components, the first being the identification and evaluation of any data that could be used to reclassify these substances to either List 1 or List 4, and the second being a closer examination of the potential toxicity and actual uses of the substances, in order to determine which may be of greatest concern.

It is in this latter area that EPA has been the most innovative. Due to the sheer numbers of chemicals on Lists 2 and 3, it became readily apparent to EPA that a regulatory approach that strictly required the submission of test data on each chemical would prove to be extraordinarily time consuming and resource intensive, while also not being particularly protective of public health. Instead, EPA opted to develop a screening mechanism that would order this large group of chemicals based on their likelihood of causing potential harm. Since little actual test data were available for

most of the substances on these lists, EPA utilized structure activity relationships to predict toxicity and estimated potential exposures based on the use patterns of products containing these inert ingredients.

Although this prioritization effort is not yet complete, tangible results have already been realized. In 1995, 220 substances were reclassified from List 3 to List 4B utilizing these techniques; another 300-400 List 2 and List 3 chemicals are slated for reclassification or removal in 1997.

The Agency has also begun to work in partnership with chemical manufacturers to better determine the extent of use of certain inert ingredients, in order to assure that the focus of the Inert Strategy remains on those substances that are most commonly used as pesticide inert ingredients.

With the costs of developing new pesticide active ingredients continually rising, many pesticide manufacturers are now recognizing the benefits that can be realized from the development of novel formulations. Formulations that are less toxic and more effective result in a more desirable product, which can often translate into increased sales and profits. Advancements in formulation design can take many forms. The increased use of water soluble packaging has resulted in products that are safer for use by mixers, loaders and applicators. Microencapsulation technologies can result in a lower active ingredient burden, as well as mitigating the transport of pesticides into ground water.

The EPA also has a role in new product formulation development. Streamlining the review and approval of new and safer inert ingredients provides incentives to industry to continue to develop more desirable formulations which often results in a natural evolution away from more traditional, and perhaps more hazardous, inert ingredients. In addition to toxicological concerns, EPA, principally under the provisions of the Clean Air Act, is more actively considering the effects of substances used as inert ingredients on the troposphere and stratosphere. Substances that deplete the ozone layer are being phased out of production and use. Volatile organic compound (VOC) content in consumer products is also being regulated to help reduce high levels of air pollution found in certain areas of the country.

The increasing usage of polymeric materials as pesticide inert ingredients has been facilitated by OPP's adoption of the OPPT Polymer Exemption Rule (U.S. EPA 1984). Polymers meeting the criteria outlined in the rule have been determined to be of such a low order of toxicity as to allow the Agency to waive the submission of the base set data and render approvals in a more expeditious fashion then was previously possible. A recent revision to this rule has expanded the types of polymers that are now eligible for exemption.

FUTURE TRENDS

The recently enacted Food Quality Protection Act will impact the use of inert ingredients in pesticide formulations applied to food (P.L. 104-70, 1996). The new standards of safety included in the Act, as well as its requirements to consider common mechanisms of toxicity; aggregate dietary, drinking water, and residential exposures; and increased emphasis on determining the potential for chemicals to exhibit endocrine disrupting effects will need to be addressed by both the EPA and industry as new inert ingredients are proposed for use in pesticide products applied to growing crops and other food commodities. Presently, EPA scientists and others are in the process of promulgating regulations and developing additional guidance that will help ensure conformance to this new statute.

The result of EPA's consideration of the inert ingredients currently in use is likely to be reflected in a reduction in the number of older substances that are considered to be acceptable for use. An inadequate base of toxicological data will mean that many substances cannot be toxicologically supported and would be deemed unacceptable. However, a high degree of interchangeability exists between many inert ingredients, particularly within the surfactant and emulsifier classes of inert ingredients that account for a large portion of the total number of inert ingredients, rendering the likelihood of significant losses in truly functional formulation components remote. It is expected that the newly-approved inert ingredients will ensure that flexibility in pesticide product formulation design is maintained.

There will be many challenges to those who attempt to develop the next generation of pesticide formulations, but with these challenges come opportunities to effect tremendous improvements. In addition to considering the more traditional issues such as phytotoxicity, compatibility with active ingredients, formulation integrity and ease of delivery, the designers of new pesticide formulations must also be able to succeed at developing products that are not only less toxic, but use lower concentrations of active ingredients, can be applied at lower use rates, and are of less concern to applicators, nontarget species, and the environment.

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Brett J. Butler[1]

DEVELOPMENT OF EMULSIFIABLE CONCENTRATE FORMULATIONS USING EXPERIMENTAL DESIGN SOFTWARE

REFERENCE: Butler, B. J., **"Development of Emulsifiable Concentrate Formulations Using Experimental Design Software,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: This paper examines two experimentation processes for developing an emulsifier system for an EC herbicide formulation. The first process is the traditional method of single factor experimentation, and the second process uses DOE (Design of Experiments) computer software. A four component emulsifier system is optimized using both processes. The advantages and disadvantages of both processes are discussed.

The use of DOE techniques for mixtures gives the researcher a greater level of understanding of the interactions of the variables in the experimental system, compared to single factor experimentation. The software used in this study did create predictive models for the formulation developed, thus allowing the selection of the optimum blend of the components after evaluation of seventeen blends. The property selected for evaluation was emulsion separation as a function of water hardness.

KEYWORDS: EC, emulsifiable concentrate, experimental design, Design-Expert, emulsifier, surfactant, D-optimal

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INTRODUCTION:

The development of any pesticide formulation involves a good amount of "trial and error". First, the formulator screens those components that are both approved for use, and

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[1] Agricultural formulations chemist, Stepan Company, 951 Bankhead Hwy, Winder, GA 30680

are likely to work based on the formulator's past experience. After the screening process for the final components is complete, the task of closing in on the "best" formulation begins.

Traditional formulation development technique usually involves preparing a test blend, measuring the desired properties, adjusting the blend and re-measuring to determine how the changes in the blend have affected the desired properties. Hopefully, a trend becomes apparent and the experimenter can begin to understand how a given system of components interact, when levels of components are changed. Many blends may be tested before one emerges as superior, or even, acceptable. This type of work can be frustrating and tedious, even for the experienced formulator.

The main challenge of formulation development work, or for that matter, any experimental investigation, is to gain an understanding of the interaction between the variables. Part of elucidating this interaction involves identifying those variables in the system that are most important.(Snee and Marquardt 1976) Once this is achieved, one can derive the blend of those ingredients that will create the "best" formulation.

Statistical Design of Experiments (DOE)

Statistical experimental design techniques are a powerful tool that can be used on mixtures to help the formulator identify which components are important, and what the optimum ratios of the components should be.

Statistical experimental designs for mixtures have been used since the mid 1960's. The calculations involved can be time-consuming if done by hand, thus making statistical DOE an ideal task for computers.

The statistical interpretation of mixture experiments is based on the ideas of response-surface methodology developed by Box and Wilson. (1951) The procedure has four steps:

- The data are generated using a pre-planned experimental design.
- A mathematical model is fit to the data by statistical curve fitting techniques. The model is usually a polynomial.
- A plot of the model is studied for regions where the best values of the responses are likely to be obtained. If the model is a quadratic, the plotted contours can resemble those of a topographical map.
- Additional blends are made in the selected region to verify the predictions of the model.

The models for mixture experiments are different from those of conventional response surface work due to the fact that the variables (components of a blend) are not independent. In a three component blend, once levels have been selected for two of the components, the third component's level is fixed.

This constraint on mixture designs changes the shape of the factor space, compared to standard factorial response surface designs. For example, in a factorial design for three variables, the factor space is a cube. However; for a three component mixture, the factor space is a equilateral triangle.

Scheffe' (1958) suggested several canonical forms for mixture models, for linear, quadratic, special cubic, and full cubic systems. For a three component system, the linear and quadratic models are as follows:

Linear

$$E(y) = B_1x_1 + B_2x_2 + B_3x_3 \quad (1)$$

Quadratic

$$E(y) = B_1x_1 + B_2x_2 + B_3x_3 + B_{12}x_1x_2 + B_{13}x_1x_3 + B_{23}x_2x_3 \quad (2)$$

Evaluation of the size of the coefficients (B's) shows which variables (x) show strong effects, i.e. which variables are "important". Thus, one can determine whether or not a given component contributes significantly to the desired properties. Snee (1979) states that the linear and quadratic models are the most useful, with the special cubic being used occasionally.

DOE software packages are sold with promises of helping the engineer or experimenter, achieve goals quicker and with fewer experiments. The software programs use statistical formulae and algorithms to choose experimental points that allow one to model a system mathematically with the fewest number of trials. (Hanrahan and Baltus 1992) Some of the programs available are ECHIP, RS/Discover, and XStat. For this paper, we used DESIGN-EXPERT[2] mixture design software from Stat-Ease Inc.

The question being asked for this paper is, will the use of DOE software in developing EC formulations result in significant differences in composition or development efficiency, compared to standard "trial and error" methods?

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[2] DESIGN-EXPERT is a registered trademark of Stat-Ease, Inc., Minneapolis, MN

EXPERIMENTAL:

The system chosen for investigation was a 6 lb/Gal. 2,4D ester EC formulation. The objective was to optimize an emulsifier system for the product using four emulsifier components. The components are referred to as A, B, C, and D in this paper, since the experimental process is what we are studying. The total emulsifier level was fixed at 4% by weight. The emulsions were prepared by dispensing 5 mL of each trial blend into 95 mL of test water in 100 mL mixing cylinders. Spontaneity was recorded, and then the emulsions were inverted 10 times. Separation in mL was recorded at 1 hour. All emulsions were tested in 50 and 1000 ppm synthetic waters at 25°C. The test waters were prepared according to the procedure outlined in ASTM E 1116-86.

The four emulsifier components were given to a formulator, along with technical 2,4D ester, and solvent. The formulator was instructed to use her usual standard procedures, with the stipulation that every blend prepared, would be documented, along with number of hours required to complete the project. When the product displayed the maximum achievable combination of emulsification spontaneity, and minimum amount of separation cream or oil at 1 hour, the project would be complete. "Spontaneity" refers to the rapid formation of an emulsion as the product is added to the water with only the force of gravity. "Cream" is an opaque layer of concentrated emulsion, visible at the bottom or top of the cylinder, depending on the density of the formulation. "Oil" is a layer of nonemulsified liquid.

Another formulator would also document each blend prepared, and would rely on Design-Expert software to pre-plan the experiment and then analyze the data.

Development using traditional single factor methods:

The approach for developing the system using the traditional method proceeded along two courses. First, blends were prepared volumetrically and tested one at a time, adjusting the mixture in subsequent blends to try and improve performance. Eleven such blends were produced, and the composition of each blend can be found in Table 1. Each blend was made from stock solutions containing 4% by weight of a single emulsifier component. The initial ratios of ingredients were chosen based on the formulator's past experience working with the components.

The formulator performing this section of the work concluded that component C was not improving performance significantly, and therefore, could probably be left out of this formulation.

In Table 1, it is clear that the formulator held two component levels constant, and changed the level of two variables to examine the relationship of those variables to each other. Through this process, the formulator narrowed the levels of each component to be:

A from 60-65%
B from 15-25%
C from 0 to 10%
D from 10 to 20%.

Blend	A	B	C	D	50 ppm	1000 ppm
A	50	20	20	10	7	5
B	60	30	10	0	10	6
C	60	30	0	10	7	0
D	70	20	0	10	0	6
E	65	25	0	10	1	5
F	65	35	0	0	5	6
G	65	20	0	15	4	0
H	60	25	0	15	3	0
I	65	15	0	20	5	0.25
J	60	20	10	10	2.5	0
K	65	15	5	15	0	5.5

Table 1: Compositions (% volume) and separation data (mL) of test blends for the single factor experiment. The shaded data are 1 hour separation, unshaded are after 2 hours.

Blends G-J were identified as being close to optimum performance, showing excellent spontaneity and stability in hard water, but only good spontaneity and significant creaming in the soft water.

The formulator then began the second course of experimentation which was to try to improve the soft water performance of blends G-J with the addition of more of component A. Thus, the ratios of components B, C, and D would be constant relative to each other, and component A would be increased. Fifteen additional blends were prepared, by adding more component A (in 5% increments) to G, H, I, and J. The blends prepared were made on a weight by weight basis, instead of volumetric blending.

The final formulation chosen by the formulator was based on blend J, and contained 63.2% A, 18.4% B, 9.2% C, and 9.2% D. This formulation gave 0 separation at 1 hour in 1000 ppm water, and 2 mL of cream in 50 ppm at 1 hour.

Development With DOE

A D-optimal design was selected, testing the following ranges for the four components:

A from 55% to 65%
B from 15 to 25%
C from 0 to 20%
D from 5 to 15%

D-optimal designs are particularly useful for situations where there are upper and lower bounds on ingredients. The ranges chosen for the experiment were based on data from screening work done to determine whether these ingredients were appropriate for this formulation.

A total of 17 blends were planned in the design, including 4 blends used to determine lack of fit of the models, and 3 blends used to estimate pure error. The design with the 1 hour separation data is in Table 2

Sample No.	A	B	C	D	50 ppm Separation (mL)	1000 ppm Separation (mL)
1	55	25	15	5	**3**	**0**
2	65	25	5	5	**0**	**5**
3	55	25	10	10	**3.5**	**1**
4	65	15	15	5	**0**	**5**
5	65	15	15	5	**0**	**5**
6	55	20	10	15	**4**	**2**
7	60	20	10	10	**3**	**0**
8	60	20	10	10	**3**	**0**
9	65	20	0	15	**4.5**	**3**
10	55	20	20	5	**3**	**0**
11	55	25	5	15	**4.5**	**3**
12	55	15	15	15	**4**	**2.5**
13	55	15	20	10	**3.5**	**1**
14	65	25	5	5	**0**	**5**
15	60	15	20	5	**0.5**	**0**
16	65	15	5	15	**4.5**	**2**
17	60	15	20	5	**1**	**0**

Table 2: The % by weight of each component stock solution in each blend of the computer generated design. Samples are randomized. Separation data is after 1 hour.

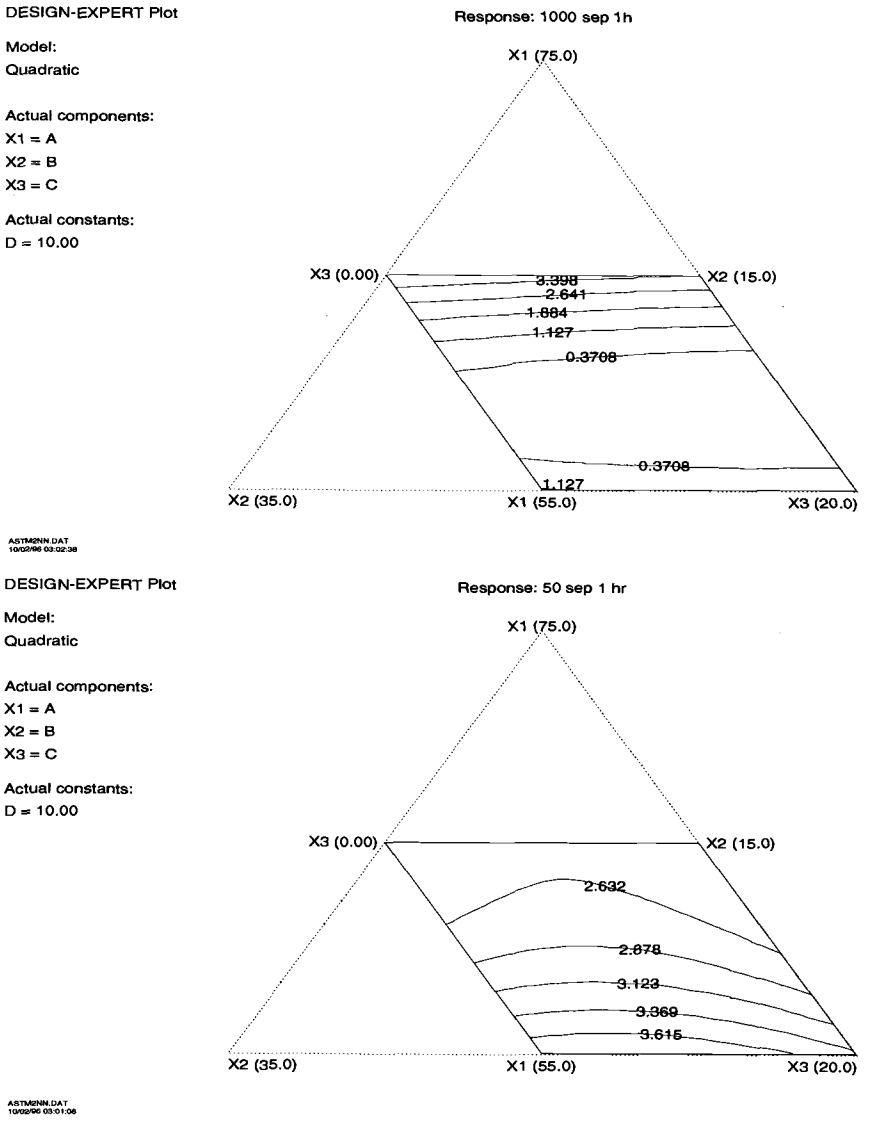
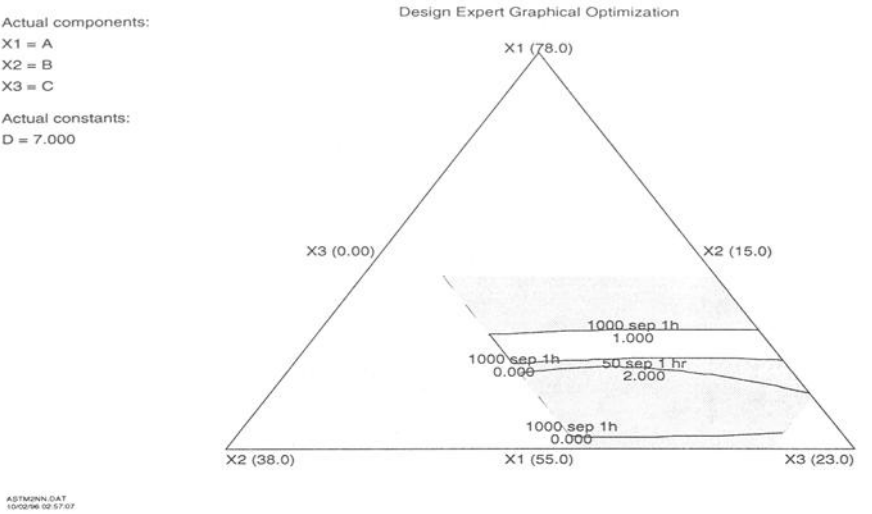


Figure 1: The plots of separation as a function of water hardness. In both cases, the plots were made holding component D constant at 10%.



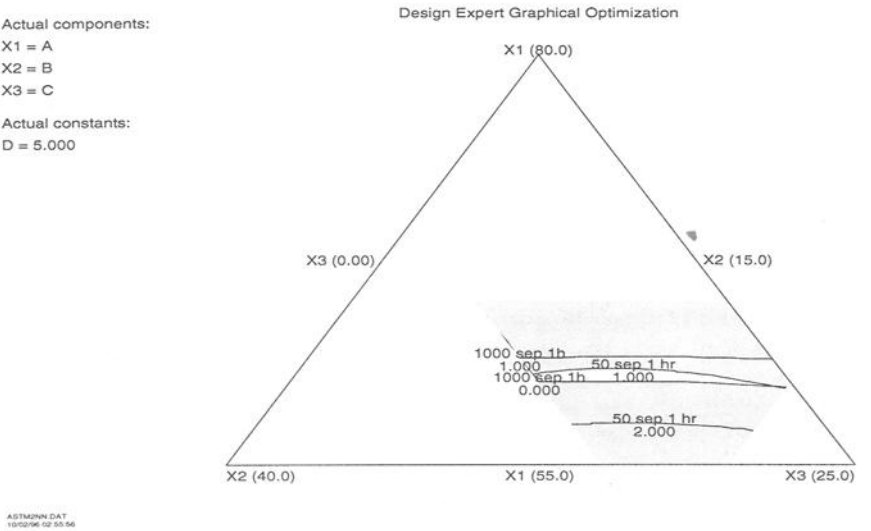


Figure 2: Graphical optimization plots for 50 ppm and 1000 ppm waters. Both the 50 ppm and 1000 ppm functions are on a single plot. Views are for D held constant at 5% and 7% by weight.

Ten grams of each blend were prepared by combining the necessary amount of stock solutions containing 4% emulsifier component, 6.2% solvent and 89.8% 2,4D ester technical. Once all 17 blends were prepared, 5 mL of each blend was dispensed into 95 mL of 50 ppm and 1000 ppm standard waters. Spontaneity was noted, then the cylinders were inverted 10 times, and allowed to stand for 1 hour at 25°C. At 1 hour, the amount of separation was recorded.

RESULTS AND DISCUSSION:

The data for separation were input into the Design Expert program and effects were analyzed. A separate analysis of variance (ANOVA) was conducted for each test water. This means that a model was generated for 50 ppm separation and a different model was generated for 1000 ppm. Both responses were modeled using quadratic functions.

In Figure 1, it is clear that minimizing separation in 50 ppm is in a region where separation is maximized for 1000 ppm (at D=10%). Therefore, optimal performance is not possible for D=10%, no matter what the levels of the other three components.

In the plots shown in Figure 2, we asked the program to show where 1 hr separation in 50 ppm water was less than 2 mL and separation in 1000 ppm was less than 1 mL. The plot for component D held constant at 5% shows that optimal performance is achieved, but the region of interest is relatively narrow. For D=7% the region of interest grows dramatically, showing this level to be a better choice because then small variations in the other levels of ingredients are less likely to result in unacceptable levels of separation.

The computer program also has a numerical optimization feature which is an iterative process of arriving at the optimal formulation. Using the numerical optimization feature with the same performance demands as those used in the graphical optimization section, the computer suggested sample A (see Table 3) as the best formulation based on the data available. The experimenter also added one more blend, B to test a slightly higher level of A and a 7% level of D. Blend B was devised based on the graphical optimization results, and the spontaneity observed in 50 ppm water. Sample B was chosen as the final blend on the basis of separation and spontaneity.

In this study, the computer program showed no advantage in terms of time (see Table 4). The formulator doing the single component studies was very efficient at arriving at an acceptable formulation.

However; the computer program does appear to give an advantage in predicting the optimal formulation. The use of DOE did mean preparing fewer blends, however more time was used due to computer analysis. The blends devised are remarkably similar with respect to components A, B and D (See Table 5).

Component	Sample A	Sample B
A	60.5	62
B	15	15
C	19.5	16
D	5	7
Separation at 1 hour:	1 mL in 50 ppm 0 mL in 1000 ppm	1 mL in 50 ppm 0 mL in 1000 ppm

Table 3: Separation data and composition of the final two formulations prepared using the DOE software program.

	Traditional Method	Computer DOE
Number of Blends	26	19
Development Time	8 hours	12 hours
Final blend performance (separation at 1 hour)	2 mL in 50 ppm water 0 mL in 1000 ppm water	1 mL in 50 ppm water 0 mL in 1000 ppm water

Table 4: Comparison of final results and relative efficiency of the development methods.

Components	Traditional	Computer DOE
A	63.2	62.0
B	18.4	15
C	9.2	16
D	9.2	7

Table 5 : Final composition of emulsifier blend for 2,4D Ester formulation.

The program allowed us to determine two important trends, that were missed in the traditional approach. First, levels of component D at 10% were deleterious to performance in soft water. Secondly, component C was contributing to performance and should not be eliminated.

One important piece of data that we were not able to use in the program was the subjective property of emulsion spontaneity. Although both formulators noted spontaneity on a relative scale (excellent, good, fair, poor), this rating could not be used in developing models for separation. The formulator performing the traditional portion of the study was able to use this information as a guide in adjusting the level of component A.

CONCLUSIONS:

The models developed using the computer program did predict optimal formulations. The use of mixture design software allows the researcher to explore and understand the interactions between variables. The use of such software requires a significant amount of time in terms of computer analysis, but we believe the time is well spent, given the level of understanding one gains about the interactions between variables in the formulation.

The use of computer software such as Design Expert does not replace single factor experimentation. Indeed, single factor experiments were run in order to identify the four emulsifier components used in this study, and the ranges of those ingredients that were explored.

Computer programs for formulation development should be viewed as helpful tools, not as replacements for experience, or background knowledge. The levels of ingredients that are explored with such a program need to be selected with great care. One must be fairly confident that the optimum formulation will be found within the ranges of ingredients chosen. Too narrow a range may exclude a desired "peak" of performance. If the range is too broad, or is selected in the wrong place, there may be poor performance in each blend.

Of course, using the computer evaluation technique will not eliminate the need for additional testing such as storage stability, or checking the effect of various lots of technical, solvents, and emulsifiers on the properties of the formulation. Indeed, gathering additional data will enable the formulator to determine how robust a formulation is.

If the development objective is to develop a formulation that is acceptable as soon as possible, then using statistical software could be viewed as overkill. If the objective is to develop a formulation where the best possible performance can be achieved consistently, DOE programs can help the formulator meet this objective.

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[3] Senior Formulation Chemist, Stepan Company

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P. Douglas Frisch[1]

OXO-ALCOHOL ACETATES: A NEW FAMILY OF INERTS FOR AGRICULTURAL CHEMICAL USE

REFERENCE: Frisch, P. D., **''Oxo-Alcohol Acetates: A New Family of Inerts for Agricultural Chemical Use,''** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: Acetate esters of oxo alcohols have been available to AgChem formulators outside the U.S. for several years and are being used as solvents and cosolvents in a variety of commercial product formulations. Only recently, as a result of EPA action to grant this family of products exemption from the requirement of a maximum residue limit, have these materials become available for use in the U.S. The product family consists of a homologous series of acetate esters derived from oxo-alcohols, ranging from carbon number C6 through C13.

This paper describes the composition and physical properties of these solvents. Important performance properties; such as solvency, low temperature capabilities and low/controlled volatility, which are most useful to the formulator will be highlighted and compared to those of other inerts commonly used by the industry. Since the entire family has received a tolerance exemption, the oxo-alcohol acetate group of solvents offers a high degree of flexibility to the formulator of agricultural chemical products.

KEYWORDS: Acetate, acetate ester, inert, oxo-alcohol acetate

INTRODUCTION

Oxo-alcohol acetate esters were introduced by Exxon Chemical Company in the early 1980's and are sold worldwide under the Exxate trade name. Their primary application is in paints and coatings. They have been

[1]Senior Staff Chemist, Basic Chemicals and Intermediates Technology, Exxon Chemical Company, 5200 Bayway Drive, Baytown, Texas 77520

highly successful in this market finding primary use in high solids coatings, maintenance and marine coatings. Since that time other application areas have been developed for this family of solvents. These include metalworking fluids, industrial cleaners/degreasers and pigment dispersing aids. Included in these other applications is agricultural chemicals where these products have been available to formulators for several years and are being used as solvents and cosolvents in a variety of product formulations.

However, since these products did not have a tolerance exemption from the EPA, the AgChem opportunities were limited to those which existed outside the U.S. Based on industry pressures to bring these formulations to the U.S., a registration data package was submitted to the USEPA and, as a result of recent action, the entire family of acetate esters ranging from oxo-alcohols of six through thirteen carbon atoms have been granted an exemption from the requirement of a maximum residue limit and may now be used as inert ingredients in agrochemical products applied to growing crops under requirements cited in 40 CFR 180.1001(d). This EPA action considerably expands the formulation possibilities for these products.

This paper describes how this new family of inerts is manufactured and gives some of their key physical and chemical properties. It focuses on where they will be most useful to the product formulator and provides some guidelines on how to formulate using them.

A FAMILY OF INERTS

Manufacture

The manufacture of this family of new inerts begins with propylene. Propylene is oligomerized over an acid catalyst (supported phosphoric acid) to produce a series of highly branched primary olefins (Eqn. 1). It is the high degree of branching in the hydrocarbon backbone which imparts the unique properties to the products. This primary olefin stream is separated on the basis of boiling point into specific carbon number fractions and then converted into a mixture of primary alcohols via the oxonation reaction using a cobalt catalyst (Eqn. 2). Each alcohol* is reacted with acetic acid to generate the corresponding acetate ester (Eqn.3). The family consists of six acetate esters ranging in carbon number from C6 to C13. Detailed compositional analysis of each of these acetate esters shows a high degree of branchiness. For example, the primary isomers in oxo-decyl acetate are trimethyl heptyl and dimethyloctyl acetates.

*These alcohols are marketed by Exxon Chemical Company under the tradename Exxal (TM).

$$C_3H_6 \rightarrow C_nH_{2n} \qquad \text{(Equation 1)}$$

$$C_nH_{2n} + CO + 2H_2 \rightarrow C_{n+1}H_{2n+3}(OH) \qquad \text{(Equation 2)}$$

$C_{n+1}H_{2n+3}(OH) + CH_3COOH \rightarrow C_{n+1}H_{2n+3}OOCCH_3 + H_2O$ (Equation 3)

Physical Properties

Typical physical properties of these oxo-alcohol acetates is given in Table I. Chemically they are all acetate esters of primary alcohols. Properties critical to the formulator of agricultural products include solvency, volatility, low temperature characteristics, water miscibility and safety (flash points, phytotoxicity). One thing which should be emphasized is the breadth of physical properties which this family of inerts allows. The fact that the entire product line is acceptable for AgChem use gives the formulator a high degree of formulation flexibility. This will be pointed out as we discuss some key properties below.

Solvency power can be determined in a variety of ways but the ones of most value to the AgChem formulator are solubility parameters (Frisch 1996) and actual solubility data on common active ingredients. In the case of solubility parameters, Table II compares the Hildebrand and Hansen solubility parameters (Barton 1991) of the oxo-alcohol acetates to those of other solvents used in the industry.

The acetate esters have solvency intermediate between that of saturated hydrocarbons like normal and isoparaffins and that of strong solvents like ketones and amides. Based on solubility parameters, their solvency is expected to be similar to the aromatic hydrocarbons although they derive their strength from different types of interactive forces. The esters have higher polarity and hydrogen bonding capabilities while the aromatic hydrocarbons have stronger dispersive forces of interaction. This means that the esters and aromatics have generally similar solvency but will display different affinities for active ingredients of different chemical types.

Table III compares the solubility of some pesticides in selected fluids. The oxo-alcohol acetates show very similar solubility profiles to the aromatic hydrocarbons with only minor differences. This is seen in the case of deltamethrin and cyfluthrin, two very similar insecticides. Cyfluthrin displays higher solubility in the esters while deltamethrin shows higher solubility in the aromatic solvents. Both are poor solvents for propoxur and carbaryl but excellent solvents for pendamethalin. As predicted by the solubility parameters, the esters and aromatics are both weaker than the ketone cyclohexanone.

Volatility is an important parameter because it controls the residence time an active ingredient remains on the surface of the plant or insect and because it is an indicator of flammability (and consequently safety) as measured by flash point. In the case of a family of inerts with systematically increasing boiling ranges, the rate of evaporation and the flash point of the formulation can be controlled by the choice of carrier fluid. Boiling points and flash points are presented in Table I while Table IV summarizes the vapor pressure and relative evaporation

TABLE I. TYPICAL PHYSICAL PROPERTIES OF OXO-ALCOHOL ACETATES

PROPERTY	C6	C7	C8	C9	C10	C13
Distillation Range, °C ASTM D86	162-176	176-200	186-215	205-235	220-250	240-285
Flash Point, °C ASTM D56	57	66	77	90	100	127
Specific Gravity, 20/20 °C ASTM D1298	0.87	0.87	0.87	0.87	0.87	0.87
Viscosity, mPa @ 25°C ASTM D445	1.0	1.2	1.7	2.2	2.6	4.6
Acidity, wt.% as Acetic ASTM D1613	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Purity, wt.% as Ester	>99.0	>99.0	>99.0	>99.0	>99.0	>99.0
Water Solubility, 25°C						
wt. % in water	0.02	0.01	0.02	0.02	<0.01	<0.01
wt. % water in	0.66	0.58	0.35	0.29	0.26	0.18

TABLE II. SOLUBILITY PARAMETERS* OF SELECTED INERTS

FLUID	HILDEBRAND	HANSEN		
		DISPERSION	POLARITY	HYDROGEN BONDING
Oxo-Heptyl Acetate	8.3	7.8	1.3	2.6
Oxo-Decyl Acetate	8.2	7.9	0.8	2.0
C10-11 Alkyl Benzene	8.5	8.3	0.5	1.5
C10-12 Alkyl Naphthalene	8.6	8.4	0.3	1.5
Cyclohexanone	9.7	8.7	3.1	2.5
N-Methyl Pyrrolidone	11.3	8.8	6.0	3.5
C12-15 Isoparaffin	7.2	7.2	0.1	0.1
C15 Normal paraffin	7.1	7.1	0.2	0

*Units are $cal^{½}\ cm^{-3/2}$. To convert to $MPa^{½}$ multiply by 2.05.

TABLE III. SOLUBILITY OF PESTICIDES IN SELECTED INERTS

FLUID	DELTAMETHRIN	CYFLUTHRIN	PROPOXUR	CARBARYL	PENDIMETHALIN
Oxo-Hexyl Acetate	13/8	34/27	8/5	2/<0.5	55/46
Oxo-Heptyl Acetate	-	-	-	2/<0.5	55/53
Oxo-Decyl Acetate	6/2	22/10	4/2	-	-
C10-11 Alkyl Benzene	11/4	12	2	1/<0.5	47/47
C10-12 Alkyl Naphthalene	18/13	16	6	1/<0.5	53/53
Cyclohexanone	45/38	54/46	36/20	-	-

Figures are on a wt/wt% basis where two values are at 23/0°C, single values are at 23°C.

rates of several acetate esters and compares them with other solvents commonly used in the industry. Critical flammability break points occur at flashpoints of 142° F. and 200° F. These flashpoints define flammable, combustible and non-regulated DOT categories for shipping products over land within the U.S.

Vapor pressure is important in the consumer products and home and garden markets. State of California consumer products regulations set 0.1 mm Hg at 20°C as a vapor pressure cutoff point between a reportable and a non-reportable Volatile Organic Compound (VOC). This means that in California the two heaviest members of the family are non-reportable VOC and exempt from reporting considerations.

Low temperature performance is particularly important in the cooler climates of the Northern U.S. and Canada. Key properties here are pour point and freeze point. These properties are compared in Table V where one can see that oxo-heptyl and oxo-decyl acetates have the lowest pour and freeze points of common solvents used as carriers. Comparison of the branched acetates with equivalent molecular weight methyl esters of linear acids shows the effect of a linear versus a branched structure on the low temperature properties. These data indicate that there needs to be no solvent related concern for low temperature storage of any member of the oxo-acetate family.

Water miscibility is detrimental to the stablity of many active ingredients. Many active ingredients hydrolyze slowly over time in the presence of water and consequently cannot be stored in the presence of water or solvents which have an affinity for water. In addition, one of the primary applications for solvents in agricultural formulations is in emulsifiable concentrates where low/no water solubility is important to the storage stability of the formulated product and the stability of the emulsion on dilution. As shown in Table I, the saturation levels of water in the oxo-alkyl acetates is very low, typically between 0.7 and 0.2 wt. percent.

Phytotoxicity

One of the most critical properties of an inert is that it displays no biological activity. It must have no/minimal phytotoxic effects on the targeted crop plants. In an extensive greenhouse study (Krenek and King 1985) of the phytotoxic effect of the 20 solvents and oils on four major agricultural crops (corn, soybeans, wheat and cotton), it was shown (Table VI) that the oxo-alcohol acetate esters possess approximately the same level of phytotoxicity as common industry standards like xylene range aromatic solvents. This study was conducted with neat solvents at concentrations much higher than normally used in the field ("over the top" in a one time application at rate of 32.7 L/Ha (3.5 gal./acre) to two week old plants).

In a subsequent follow up study (Sandler et al. 1995) which looked at emulsufiable concentrates (EC) fully formulated in aromatic solvents and applied at concentrations closer to actual pesticide treatment rates,

TABLE IV. VOLATILITY COMPARISON OF SELECTED INERTS

FLUIDS	BOILING RANGE ASTM D86, °C	VAPOR PRESSURE ASTM 2879, mmHg			RVOC*	EVAP. RATE+ ASTM D3539 (mod.)
		20°C	50°C	100°C		
Oxo-Heptyl Acetate	176-200	1.3	6.5	55	yes	8
Oxo-Decyl Acetate	220-250	<0.1	0.7	8	no	<1
Xylene	139-141	14	53	270	yes	66
C10-11 Alkyl Benzene	184-204	0.5	2.1	30	yes	5.3
C10-12 Alkyl Naphthalene	231-276	<0.1	0.6	7	no	<1
C12-15 Isoparaffin	223-254	<0.1	0.7	8	no	<1
C15 Normal Paraffin	255-279	<0.1	0.2	3	no	<1

* Reportable as VOC under CARB regulations governing consumer pesticides
+ Relative evaporation rate based on n-butylacetate = 100.

TABLE V. COMPARISON OF LOW TEMPERATURE PROPERTIES OF SELECTED INERTS

FLUID	VISCOSITY mPa.s, 25°C	FREEZE POINT °C (°F)	POUR POINT °C (°F)
METHOD	ASTM D445	ASTM D1015	ASTM D97
Oxo-Heptyl Acetate	1.2	<-60(<-76)	<-57(<-70)
Oxo-Decyl Acetate	2.6	<-60(<-76)	<-57(<-70)
C10-11 Alkyl Benzene	1.2	-43(-45)	-32(-26)
C10-12 Alkyl Naphthalene	2.6	-8(18)	-26(-15)
Cyclohexanone	2.1	-32(-26)	-
N-Methyl Pyrrolidone	1.7	-24(-11)	-
Methyl Caprylate (C8)	1.6	-40(-40)	-
Methyl Caprate (C10)	2.3	-18(0)	-

TABLE VI. RELATIVE PHYTOTOXICITY* OF SELECTED INERTS

FLUID	CROP			
	CORN	WHEAT	COTTON	SOYBEAN
C12-15 Isoparaffin	A	A	A	A
N-C15 Paraffin	A	A	A	A
C11-15 Mixed Aliphatic (aromatics <0.5)	A	A	A	A
Xylene Range Aromatic	C	D	C	C
C10-11 Alkyl Benzene	C	D	C	C
C10-12 Alkyl Naphthalene	D	D	C	C
Oxo-C6 Acetate	C	D	C	C
Oxo-C7 Acetate	C	D	D	C

* Scale: A = No observed effect
B = Slight effect
C = Moderate effect
D = Maximum observed effect

the heavy aromatic solvents were shown to be non phytotoxic to a broad range of sensitive crops (tomatoes, curcurbits,cotton and soybeans) under two different sets of climatic conditions. Based on this study which showed that aromatic solvents pose minimal phytotoxic risk, it is assumed that if the oxo-alcohol acetate esters were similarly formulated into EC's and sprayed at similar levels, they also would pose no phytotoxic risk.

Formulation Guidelines and Use

The primary use of the oxo-alcohol acetates will be as solvents or co-solvents for liquid based formulations. Their solvency, low water miscibility and low temperature properties makes them candidates for carriers in emulsifiable concentrate formulations. A screening of the patent literature (Wicke 1988, 1992, 1993; Narayanan et al. 1992; Smith et al. 1994; Martin et al. 1995) reveals some new formulations based on these materials as preferred solvents.

In liquid pesticide concentrates, the addition of these esters have a predictable effect on the viscosity of formulations. In the case of ultralow volume (ULV) spray oils like soybean or mineral crop spray oils, viscosity can be lowered and controlled (because of the low volatility) by the addition of an oxo-alcohol acetate ester. Figure 1 presents viscosity reduction curves for soybean oil and several blends of soybean oil with the oxo-C7 alcohol acetate. By the proper choice of acetate ester and blend ratio, one can obtain a more optimal viscosity for spray application.

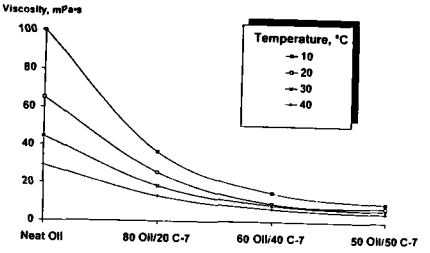

Figure 1. Viscosity reduction curves, wt. basis

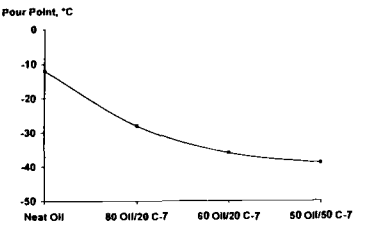

Figure 2. Pour point depression curve, wt. basis

Due to the strong solvency and low pour point of the acetate cosolvent, added benefits of higher active ingredient concentrations and better low temperature properties are achieved. Figure 2 shows the almost linear decrease in pour point of soybean oil with addition of C7 alcohol acetate. Furthermore, these benefits will accrue whether the formulation is emulsifiable or non-emulsifiable since the only difference is the addition of small amounts of emulsifiers (up to 8% in EC's).

Since the majority of applications will involve emulsified formulations, a consideration of the hydrophilic/lipophilic balance (HLB) requirements of these new esters compared to those of commonly used solvents may be useful. Since the oxo-alcohol acetate esters are a homologous series, it is expected that the HLB values will vary systematically. In the case of microemulsions formed using 1/1 mixtures of nonionic and anionic surfactants, Graff and co-workers (1988) have shown that the HLB values of the acetate esters (Figure 3) decreased linearly with molar volume (molecular weight divided by density).

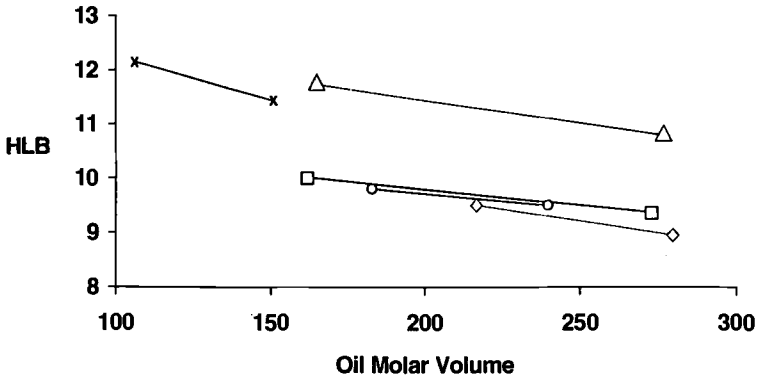

Figure 3. HLB correlation with molar volume (cc/mole): X, alkylbenzenes; *, oxo alcohol acetates; □, isoparaffins; o, mixed aliphatic; ◊, normal paraffins

This correlation follows very closely that of the aromatic solvents, with the aromatic series being lower in molar volume and consequently requiring a higher HLB emulsifier package. Comparison with other series of solvents like isoparaffins, normal paraffins and mixed aliphatics, the acetate esters are considerably more hydrophilic. Although the branchiness of hydrocarbons does effect the H/L balance, there is little difference expected between the HLB requirements of branched and linear esters; e.g., oxo-heptyl acetate vs. methyl caprylate. There is, however, a considerable difference in the stability of emulsions of these esters with the branched esters showing preferred higher stability (Graff et al. 1988; McKay 1996). These guidelines will vary somewhat with the inclusion of an active ingredient but they should provide useful starting points in replacing solvents.

SUMMARY

It is not often that a new inert receives tolerance exemption from the USEPA and it is highly unusual that an entire family of new inerts receives approval. It is felt that the registration of the oxo-alcohol acetate family of esters will increase the flexibility of the formulator of agricultural chemical pesticides because of the broad range of physical characteristics which a family of products offers. In this

paper, those properties which are of greatest importance to the AgChem formulator have been discussed. Solvency for selected a.i's., low/controlled volatility which relates to safety (flammability), excellent low temperature performance, low water miscibility and low phytotoxicity were highlighted and some formulation guidelines were given. It is envisioned that the major use of these new oxo-alcohol acetate ester, will be in liquid formulations either of the low volume, ultralow volume (ULV) or emulsifiable concentrate and concentrated emulsion types. These products compliment the extensive line of hydrocarbon inerts already on the market.

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Chemical Formulations

Kolazi S. Narayanan[1], Milla Kaminsky,[2] Domingo Jon[3] and Robert M. Ianniello[4]

WATER IN OIL MICROEMULSION AEROSOL SYSTEMS FOR INSECTICIDAL COMPOSITIONS

REFERENCE: Narayanan, K. S., Kaminsky, M., Jon, D., and Ianniello, R. M., **''Water in Oil Microemulsion Aerosol Systems for Insecticidal Compositions,''** Pesticide Formulations and Applications Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: Conventional aerosol as a delivery system for hydrophobic insecticides, formulated with hydrocarbon or freon type propellants [(A46) or Freon 11/12] are derived from matrices based on non-aqueous organic solvents, i.e., either hydrocarbons or halogenated hydrocarbons. Such systems pose potential environmental hazards like high flammability (hydrocarbon emission) and depletion of the ozone layer from fluorinated hydrocarbons, and emission of chlorinated hydrocarbons as cancer suspect agents. Totally aqueous systems are not easy to formulate in a single phase system as are aerosols. While O/W microemulsions are described in the literature, their use as trigger spray or aerosol systems produced low knockdown rates (speed of 'kill'). A W/O microemulsion which will accommodate high levels ($\geq$ 35%) of conventional hydrocarbon propellant (A46) would be safer and will improve the knockdown rate.

This paper describes our effort in successfully formulating such W/O microemulsion systems. A systematic approach to stabilize W/O microemulsions that can accommodate high level of water (25-40%) as well as high level of hydrocarbon oil and hydrocarbon propellant (40-50%) based on partial phase diagrams produced several prototype formulations. These formulations matrices essentially consist of: nonylphenol ethoxylates as primary emulsifiers and long chain (C_8) alkyl pyrrolidone/pentanol/ glycerol as cosurfactant/cosolvents, C_{12} hydrocarbon and water. Mixed pyrethroids and propellants can be loaded at appropriate levels.

Examples of prototype formulations, stability data, and biological efficacy are provided. A working model that would explain the high biological performance is also provided.

KEYWORDS Water-in-oil microemulsions, aerosols, insecticides, pyrethroids, N-alkyl pyrrolidones, nonylphenol ethoxylates, hydrocarbon propellants, water-hydrocarbon matrix, cosolvents, glycerol, pentanol, anionic emulsifiers, optimization, partial phase diagrams, stability

[1]Research Fellow, [2]Chemist, and [3]Research Chemist, Agricultural Products, and [4]Director, Pharmacuetical, Agricultural and Beverage Technologies, International Specialty Products, 1361 Alps Road, Wayne, NJ 07470.

INTRODUCTION

Microemulsions are thermodynamically stable oil and water dispersions stabilized by the choice of specific surfactants, producing isotropic, transparent or low turbidity systems(Zana and Lang, 1987). Microemulsions are formed when the surfactant and cosurfactant, in just the right ratio, produce a mixed adsorbed film that reduces the O/W interfacial tension transiently below zero(Rosano et. al, 1987). Use of N-alkylpyrrolidones along with nonylphenol ethoxylates and EO/PO blocks for the formation of O/W microemulsions and the corresponding concentrates for several pyrethroids is described in the literature(Narayanan et. al., 1993). It is desirable for insecticide aerosol systems to be formulated with hydrocarbon propellants in the form of W/O microemulsions for speed of insect knockdown. O/W microemulsions formulated as above without modification were not compatible with hydrocarbon propellants(Narayanan et. al., 1993). This paper offers a systematic approach for optimization of surfactants/cosurfactants or cosolvents to generate W/O microemulsions matrices (single-phase-compositions) capable of high loading ($\geq$ 25%)of hydrocarbon propellant and high water content (~ 50%).

APPROACH

Figure 1 is a flow chart outlining the steps for generating clear one-phase compositions capable of accommodating high levels of water/oil/propellant. The following components were used in the

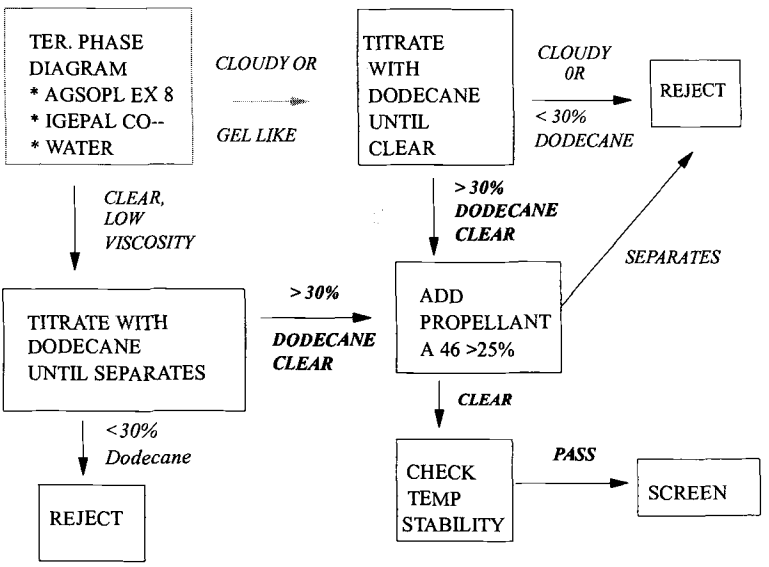

FIG. 1 -- Approach for Aerosol Microemulsion Concentrate Formulation

first phase of development: N-octyl pyrrolidone[1], nonylphenol ethoxylates surfactants[2], water, dodecane, and A46 propellant[3]. Effect of cosolvents (pentanol, glycerol) and anionic surfactants was investigated in order to increase the water content in the composition and decrease total surfactant levels.

EXPERIMENTAL SECTION

Matrix Preparation

All materials used are commercially available. Partial phase diagrams were constructed to identify broad clear, single phase regions for three component systems. An experimental design with three components: N-octyl pyrrolidone, nonylphenol (9) ethoxylates[4], and water was established varying the concentration of each component from 0-100% with an increment of 5% to cover the entire triangular space. Appropriate mixed compositions (10g)were prepared by weighing the individual components on an analytical balance in a 2 oz bottle. The contents were mixed in an automatic orbital shaker for a period of 15 minutes. The clear phases were further centrifuged for 30 minutes at 3000 rpm to ensure absence of phase separation. The compositions with different phase changes were plotted in triangular diagrams.

The procedure was repeated with other nonylphenol ethoxylates surfactants in order to investigate the effect of hydrophilic/ lipophilic character of the system. the following nonionic sufactant series was used: nonylphenol (5)ethoxylates[5], nonylphenol (10)ethoxylates[6], and nonylphenol (15)ethoxylates[7]. Figure 2 summarizes the partial phase diagrams for the above cases.

Hydrocarbon Compatibility

The compositions yielding clear-single-phase regions were titrated with n-dodecane in order to establish an upper limit of hydrocarbon solubility in the system. Compositions capable of accommodating 30% dodecane and still yielding clear systems were further evaluated. These compositions were centrifuged in 50 ml

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[1]Agsol Ex™ 8 a trademark of International Specialty Products

[2]Igepal CO™ series surfactants are trademarks of Rhone Poulenc Corp.

[3]An aerosol grade propellant containing 20% propane and 80% isobutane obtained from AGL, Clifton NJ.

[4]Igepal CO 630

[5]Igepal CO 530

[6]Igepal CO 660

[7]Igepal CO 730

centrifuge tubes at 3000 rpm for 30 minutes. If no separation was detected after centrifugation then the samples qualified for the next experimental stage.

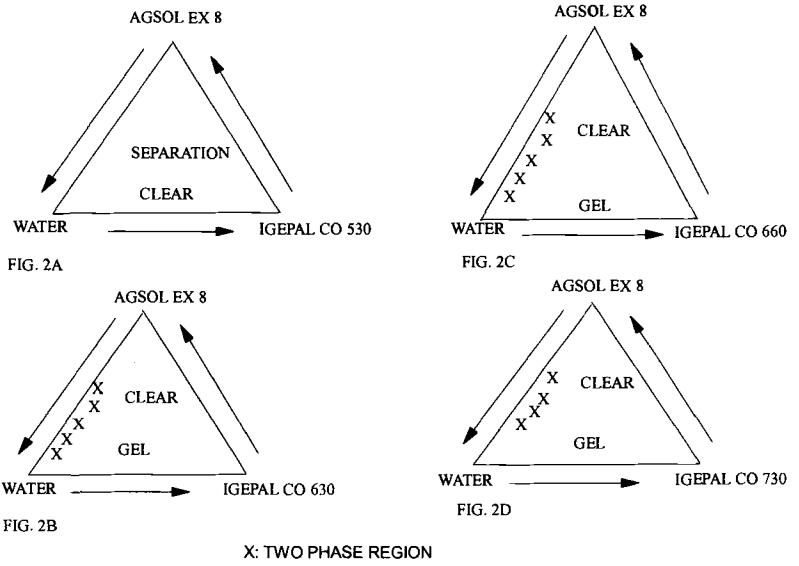

FIG. 2 -- Three component phase diagram including Agsol EX8, Water, and Igepal a)CO 530, b)CO 630, c)CO 660, and d)CO 730

Solubility of Active Ingredients

Solubilities of several pyrethroids at room temperature were evaluated in matrices qualified by the above tests. The pyrethroids were added to the matrices either as solids or, preferably, in liquid form. The actives were found to dissolve high levels (> 5%) of active ingredients.

Propellant Substitution

The successful matrix formulations from above, were reformulated by substituting the 25wt% dodecane with 25wt% A46 propellant. The resulting formulation would contain up to 10 wt% dodecane and 25 wt% A46 propellant. All other components were kept constant. Propellant was filled into clear 100 ml bottles with the total formulation weight not exceeding 50 grams. If formulations remained clear after propellant filling, the matrices were subjected to a series of stability tests.

Stability Testing and System Characterization

Samples were kept in glass jars at room temperature, 5°C, and 40°C for 24 hours in order to test their physical stability and phase

separation. Particle size analyses by a Leeds Northrup Microtrac (Northwales, PA) were carried out, and Brookfield viscosity measurements (from Brookfield Engineering Laboratories, Inc., Stoughton, WA) were taken at different dilution levels in water and n-dodecane.

Particle Size Measurements

Emulsion droplets (5-50 microns) were analyzed via an optical microscope, model Nikon S-Kt (Garden City, NY) at 250 X magnification. Particle size distribution (1-100 microns) for aqueous dispersions and emulsions were measured using a Microtrac particle size analyzer. Microemulsion range particle size distribution (0.01-0.1 microns) were measured using Leeds Northrup, Microtrac ultra fine particle analyzer, containing software package for data analysis (Narayanan, et. al., 1993).

Viscosity Measurements

Viscosity of the appropriate compositions was measured by weighing the required quantity of the formulation to produce 250 g of final sample at the required dilution. The samples were stirred by a magnetic stirrer for one hour, and the viscosities were measured as a function of dilution with water. The viscosities were measured with a Brookfield digital viscometer DV-II Model # RVT DV-II using a RV spindle #1.

RESULTS AND DISCUSSIONS

Mutual solubilities of water and hydrocarbons in water/alcohol/N-alkyl pyrrolidone[8] were recently published(Adamy 1995). A large proportion of N-alkyl pyrrolidone is required to solubilize the hydrocarbons in water. Efforts to solubilize the hydrocarbons in water by optimizing the surfactant compositions are described in the literature (Rosano et al., 1979; Shinoda and Friberg 1983; and Sagitini and Friberg 1980). The general effects of emulsifier compositions on thermal stability of w/o and o/w emulsion systems have been studied(Chen and Ruckenstein 1991; Davies et al., 1987, and Rosen 1978). Based on the literature and our past experience a combination of N-alkyl pyrrolidone and nonylphenol ethoxylates series was chosen as an efficient emulsifier system to solubilize o/w compositions. A systematic approach was undertaken to identify clear phase regions for three components not including the oil.

Figure 2 shows the transparent and homogeneous phase regions for three component compositions comprising of N-octyl pyrrolidone, water and different members of the nonylphenol ethoxylates series of surfactants. The phase diagram was very similar for the ethoxylated series having 9 EO, 10 EO and 15 EO. Three distinct regions were identified: a clear region, a gel region, and a multiphase region. The area of clear region was greater with higher HLB surfactants. The phase diagram was different with 5 EO, a clear region and an area of separation were observed.

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[8]Agsol EX™ is a trademark of International Specialty Products

Accommodation of C_{12} hydrocarbon was evaluated by following the phase behavior starting from clear compositions in Figure 2 and titrating with C_{12} hydrocarbon. This procedure was used to construct the fourth dimension of the phase diagram as summarized in Figure 3. The procedure is similar to the one described by Friberg (Shinoda and Friberg, 1983). Table 1 shows a few typical

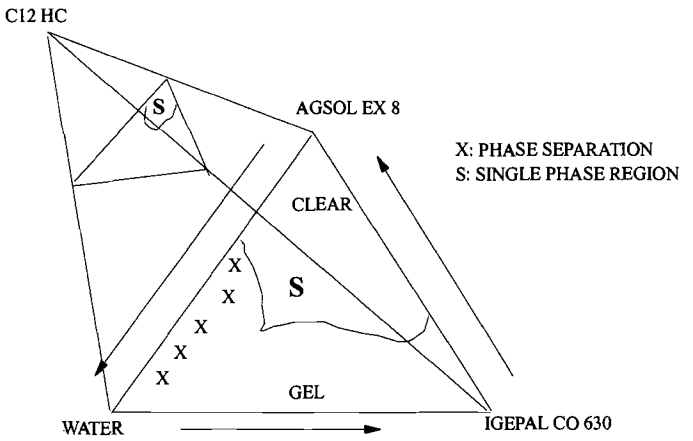

FIG. 3 -- PARTIAL PHASE DIAGRAM FOR A FOUR COMPONENT SYSTEM

clear, single phase compositions containing C_{12} hydrocarbon generated using the clear regions from Figure 2B and Figure 2C. Compositions containing nonylphenol with 12 EO[9] and 15 EO did not hold n-dodecane. Nonylphenol compositions containing 5 EO produced cloudy emulsions on adding n-dodecane.

Single phase aerosols were prepared by introducing 25% A46 propellant, replacing part of the n-dodecane from clear single phase compositions containing $\geq$ 30-35% n-dodecane. Alternately, 25% A46 propellant was introduced leaving behind 5-10% n-dodecane from clear compositions shown in Figure 3. In Figure 3, S refers to the single, clear phase region in the three component system. As hydrocarbon is added to the system, the single phase region is seen to project closer toward n-dodecane. Both the aerosol compositions and their non-aerosol matrices remained stable when monitored for 8 weeks, both, at room temperature and at 40°C. Figure 4 shows the particle size distribution of a typical matrix composition (Table 1, A). The particle size distribution was found to be small, within the range of 0.05 micron.

Figure 5 shows viscosity changes when a typical clear matrix

[9]Igepal 720

composition (Table 1A) was diluted with water. A low viscosity region at low water concentration occurs followed by increased viscosity as a function of dilution. The viscosity reads a

TABLE 1 -- Clear Matrix Compositions (wt%)With C_{12} Hydrocarbon

Ingredients/ Composition No	A	B	C	D
C_{12} (Hydrocarbon)	23.1	37.5	15.2	12.4
Water	15.4	12.5	35.6	22.6
N-octyl pyrrolidone	23.1	37.5	33.4	38.7
Nonylphenol 9 EO	38.4	12.5	0	0
Nonylphenol 10 EO	0	0	15.8	26.3
Starting phase diagram	2A	2A	2B	2B

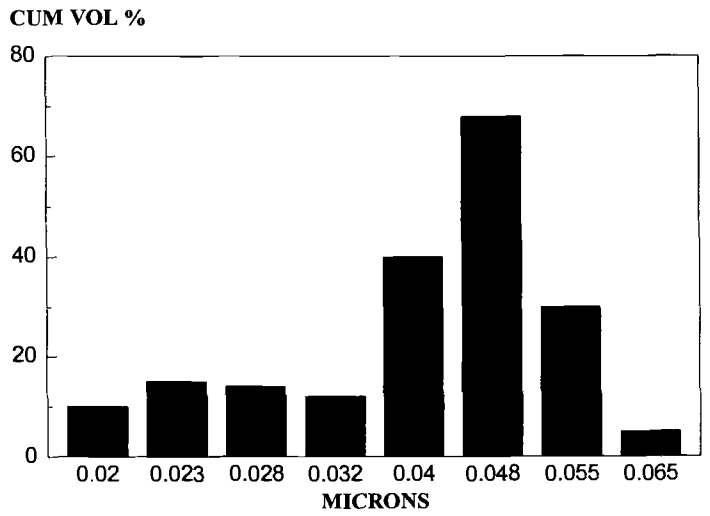

FIG. 4 -- Particle Size Distribution of Matrix 1A

maxima, and is accompanied by a decrease in viscosity with subsequent dilution. The value approaching that of water at high dilution is characteristic of phase inversion(Rosano 1987). Viscosity maxima would be consistent with a lamellar structure.

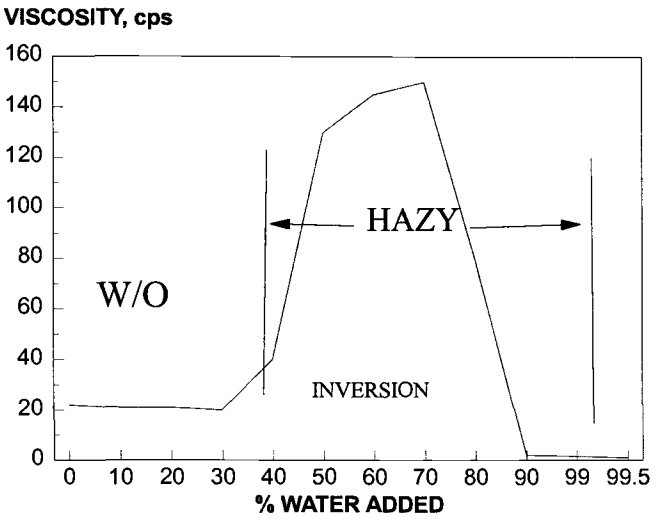

FIG. 5 -- Viscosity on Dilution of Matrix 1A

Fine Tuning

A new objective for fine tuning was to reduce the total surfactants in the above systems. Water and n-dodecane were kept as constant components. The surfactant system was modified by using combinations of N-octyl pyrrolidone with mixed nonylphenol ethoxylate surfactants to obtain the optimum HLB, similar to the combination of N-octyl pyrrolidone and a single nonylphenol ethoxylate of the typical clear systems. Effective HLB of mixed surfactant systems were calculated from Eq (1)

$$\text{EFFECTIVE HLB} = \Sigma\ f_i(\text{HLB})_i \quad \text{---------- (1)}$$

where f_i, and $(\text{HLB})_i$ represent the weight fraction and HLB of the i^{th} surfactant in the system summed for all surface active components. Use of mixed nonylphenol ethoxylates produced the following general observations. Systems containing low HLB ($\leq$ 7.0) accommodated high n-dodecane but showed poor heat stability. High HLB systems ($\geq$ 11) produced good heat stability but showed poor n-dodecane loading. This result was constant with repeated observations(Chen and Ruckenstein, 1991).

Alternately, low HLB, 5 EO Nonylphenol, coupled with N-octyl pyrrolidone and small quantities of anionic surfactants nonyl phenol ethoxylated phosphate ester[10] produced promising results. Use of cosolvents like pentanol or glycerol could replace part of

[10]Rhodofac RE™ 610

N-octyl pyrrolidone in the matrices. Typical single phase matrices capable of loading $\geq$ 25% A46 propellant containing reduced levels of surfactants are summarized in Table 2 along with stability data.

TABLE 2 -- Typical Clear Matrix Compositions (wt%) With Reduced N-octyl pyrrolidone/ Surfactants and Stability

Composition/properties	D	E	F
n-dodecane	14	15	12
Water	36	50	35
Others[1]	50	25	53
Stability			
Room temp.	Clear	Clear	Clear
40^{0}C	Cloudy	Cloudy	Clear
Appearance with Propellant	Clear	Clear	Clear
Max A 46 propellant	>25%	~ 25%	>35%

[1]includes optimized N-octyl pyrrolidone/surfactants and cosolvents

CONCLUSION

The function of co-solvents is not clearly understood. However, accommodation of the co-solvent molecules sandwiched between pairs of surfactants could compel bending of the emulsifier facilitating a spherical micelle formation which is necessary in order to achieve a microemulsion (Shinoda and Friberg 1983). Water-in-Oil aerosol microemulsions were developed by optimizing A46 propellant along with Igepal CO series surfactants and N-octyl pyrrolidone by maximizing water in the presence of C_{12} hydrocarbon. Use of cosolvents like pentanol/ glycerol was beneficial in reducing the total surfactant levels. Optimization was accomplished via partial phase diagrams. The resulting single phase matrix and aerosol systems showed acceptable stability, high water content, high a.i. loading and small droplet size distribution.

Biological evaluations of the insecticide formulations using the above matrices are in progress.

ACKNOWLEDGMENT

Support of this work and permission to publish by International Specialty Products is gratefully acknowledged.

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Harvey Ross[1] , Barry Omilinsky[2], A. D. Lindsay[3], D. Creech[4], J. Glatzhofer[5], B. Tickes[6]

Trifluralin 10% Granular Formulation Prepared On Biodac® And Clay For The Control Of Annual Ryegrass, Giant Foxtail and Carpet Weed.

REFERENCE: Ross, H., Omilinsky, B.A., Lindsay, A.D., Tickes, B., Creech, D., Glatzhofer, J. **"Trifluralin 10% Granular Formulation Prepared On Biodac® And Clay For The Control Of Annual Ryegrass, Giant Foxtail and Carpet Weed,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT:

The development of a new cellulosic granular carrier (Biodac®) with characteristics somewhat different from the clay and botanical carriers presently used in the agricultural industry required further evaluation especially in regard to efficacy due to the extremely uniform distribution of the particles within the targetted range (see Figs.1&2). Tests conducted in 1995 in Arizona and in Pennsylvania, showed that 10% Trifluralin formulations prepared on clay and Biodac® performed as follows: Trifluralin on clay ≥ Biodac® 30/50 ≥ Biodac® 20/40 > Biodac® 16/30 >Biodac® 12/20. These data clearly show that the locus of activity for each unit area will be greatly decreased if a 16/30 or 12/20 Biodac® material is applied rather than a 20/40 or 30/50 material.

KEYWORDS: granular, clay, cellulosic, particle size, trifluralin, particle size.

The objective of these tests was to evaluate the calibration and number of granules per ft^2 with a 30/60 clay granule (Treflan TR10), 30/50 Biodac® and 16/30 Biodac®. All materials contained 10% Trifluralin and were applied at a rate of 10 lbs/A.

The relative metering rate of the three granules was determined by setting application equipment at a uniform setting and measuring the output. A Valmar PT 1220 Airflo pneumatic herbicide applicator with ground drive metering was set to apply 10 lbs/A of the 30/60 clay granule. Cotton calibration bags were placed over all outlets and the weight of the granules applied from ten revolutions of the metering roller was determined. This procedure was repeated four times for

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[1]Manager of Sales, Grantek, Inc.
[2] Vice President of Development, Formulogics, Inc.
[3] Vice President of Research, Formulogics, Inc.
[4] Plant Chemist, Gowan Co.
[5] Formulation Chemist, Gowan Co.
[6] Cooperative Extention, U of Arizona, Yuma Arizona

each granule type tested. 6" x 8" Sentry "sticky boards" were used to determine the number of granules per ft^2 applied. The Valmar PT 1220 applicator was set to apply 10 lbs per acre and driven over the sticky boards placed five feet apart and parallel to the 16.5 ft. applicator boom. This procedure was replicated three times for each formulation. This test was designed to compare the weed control activity of the three granules when applied by air and by ground. These tests were conducted at the University of Arizona Maricopa Agriculture Center, approximately 20 miles south of Phoenix, Arizona on a four

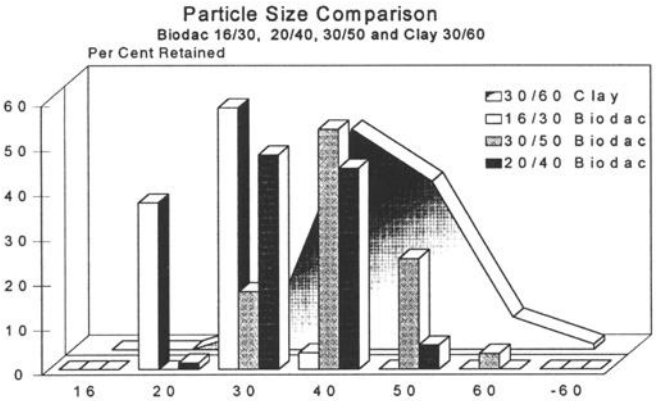

Fig. 1

year old stand of CUF 101 alfalfa. Soil type was a silt loam. Annual ryegrass (*lolium multiflorum)* was planted into the alfalfa as an indicator crop to measure weed control activity. The treatments were 1.0 lb a.i. per acre of the three 10% trifluralin granules applied by ground and by air and an untreated check. The ground applications were made with a Valmar -PT1220 applicator with a 16.5 ft. boom and the aerial applications were made with a fixed wing Ayers Turbothrust airplane flown 50 ft. above the ground. Plot size was 16.5 ft. by 550 ft. for the ground applications and 165 by 550 ft. for the aerial applications. Treatments were replicated three times in a randomized complete block. The tests were established on January 23, 1996 and evaluated 20 days after treatment on February 13, 1996. Weed control was measured by counting annual ryegrass seedlings in a one ft^2 grid dropped randomly in ten locations in each plot for the aerial applied test and six locations in each plot for the ground applied tests.

Table 1. Application Rate and granules/ft^2 of the 10% Trifluralin Products Tested

Granule Source	Granule Size	Calibration - % Difference When Set to Apply 10 lbs. of 30/60 Clay Formulation	Granules/ft^2 (10 lb/A Rate)
Clay	30/60	-	873-1420
Biodac ™	30/50	0	858-1391
Biodac ™	16/30	+15-19	437-710

According to Table 1, no significant differences were measured between the metering rate of the 30/60 clay and the 30/50 Biodac granules when run through the Valmar applicator set to apply 10 lbs/acre. Significantly more of the 16/30 Biodac granules (15-19%) is applied at this same setting.

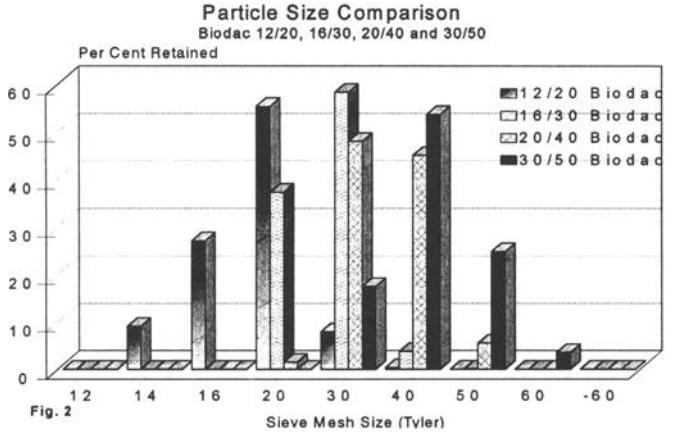

Fig. 2

The number of granules per square foot between the 30/60 clay and the 30/50 Biodac™ granules were essentially equivalent. The numbers for the 30/60 clay granule ranged from 873 to 1420 per ft^2 at the 10 lb/acre rate. The 30/50 Biodac granule ranged from 858 to 1391 particles per ft^2. The 16/30 deposited approximately 50% fewer granules than the 30/50 0r 30/60 products.

Table 2 - Weed Control for the Granules Tested

Granule Source	Granule Size	Aerial Application Ryegrass (1) seedlings/ft^2	Ground Application Ryegrass (1) seedlings/ft^2
Attapulgite Clay	30/60	4.8 (a)	2.9(a)
Biodac ™	30/50	17.7(a)	4.1(a)
Biodac ™	16/30	60.1(b)	32.8(b)
Untreated	-	92.1(c)	78.8(c)
		LSD (0.05)=23.3	LSD (0.05) = 15.0
		LSD (0.10) =18.5	LSD (0.05) = 11.9

(1) Avg. of 3 replications. (a-c) indicates that the arithmetic means within a column with no common subscript are significantly different.

The number of ryegrass seedlings were significantly lower (see table 2) with both the 30/60 clay and the 30/50 Biodac granules than with the 16/30 Biodac and the untreated control in both the aerial and ground applied tests. The 16/30 Biodac® granule seedling

counts were significantly lower than the untreated check in the aerial and applied tests. No significant differences in the seedling counts was detected between the 30/60 clay and the 30/50 Biodac™ treatments using the least significant difference (LSD) analysis of variance of both the 0.05 and 0.10 levels of significance.

In 1995, trifluralin 10% formulations were prepared on 20/40, 16/30, and 12/20 Biodac. The materials were pre-plant incorporated and compared to a standard Treflan 10G on clay and tested at .05 and 0.75 lb A.I. for non-crop control of giant foxtail, *Setaria faberi*, and carpetweed, *Mollugo verticillata.* To emphasize residual effects of the treatments, no crop was planted. The data are presented in table 3 at the 0.75 lb A.I./A rate at 15 days after treatment and 54 days after treatment.

These data show that the 20/40 Biodac mimicked the standard clay treatment at both rates (only the 0.75 lb A.I. rate is shown) with insignificant differences in control of both weed species.

Only the 0.75 lb a.i./A rates of 20/40 Biodac® and the Treflan clay standard provided greater than 80% control at 54 days. The performance for the treatments after 54 days (Table 3) may be categorized as:

Treflan clay standard ≥ 20/40 Biodac® > 16/30 Biodac® > 12/20 Biodac®.

Table 3 - 10% Trifluralin @ 0.75 lbs A.I./A for Control of Giant Foxtail and Carpetweed

Treatment	Granule Size	Giant Foxtail % Control 15DAT*	Carpetweed % Control 15DAT*	Giant Foxtail % Control 54DAT*	Carpetweed % Control 54DAT*
Biodac®	12/20	68.3(c)	93.3(ab)	40(def)	70.0(bc)
Biodac®	16/30	75.0(abc)	96.7(a)	60.0(a-d)	76.7(abc)
Biodac®	20/40	88.3(ab)	98.3(a)	81.7(ab)	88.39(ab)
Clay Standard	30/60	93.3(a)	98.3(a)	86.7(a)	95.0(a)
Untreated		0.0(d)	0.0(c)	0.0(g)	0.0(d)
		LSD (0.05)= 19.3	LSD (0.05)= 8.5	LSD (0.05)= 26.3	LSD (0.05)= 18.4

*DAT - Days after treatment

(a-d) indicates that the arithmetic means within a column with no common subscript are significantly different.

These data as well as the previous data clearly show that the choice of a highly uniform granule material such as Biodac competes effectively with clay materials which have a wider distribution of particles, for the active ingredient used, under the conditions described.

Rolf Oelmüller[1], and Astrid Müller[2]

STABLE FORMULATION OF EASY HYDROLIZING ACTIVES BASED ON SPECIALTY SILICAS SHOWN ON MALATHION AS A MODELLING SUBSTANCE.

REFERENCE: Oelmüller, R. and Müller, A., **"Stable Formulation of Easy Hydrolizing Actives Based on Specialty Silicas Shown on Malathion as a Modelling Substance,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: There are some active ingredients (a.i.) such as phosphoresters which can be hydrolyzed by even the small amount of water available from the surface of natural carriers or synthetic silica carriers. Dry formulations, i.e. wettable powders (WP) and dispersible granules (WG), cannot be formulated with these compounds (Ferch et al. 1990). It has been shown, that the controlled hydrophobicity of the surface of a new type of carrier silica can improve the stability of such a.i., thus, enabling the formulator to produce and apply stable WP and WG.

KEYWORDS: Formulation, wettable powder, dispersible granule, hydrolysis, natural carrier, silica carrier, hydrophilic, hydrophobic

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[1] Manager Applied Technology Silicas,
[2] Research Scientist Applied Technology Silicas, both at Degussa AG, Inorganic Chemical Division, P.O.Box 1345, D-63403 Hanau, Germany

EXPERIMENTAL METHODS

Test methods

To determine hydrophobicity, the „methanol wettability" test method was used. This is an internal Degussa method not published. A publicly known method which also may be used is the determination of the carbon content (Oelsen et al. 1951, 1952, Abresch and Büchel, 1962).

The DBP absorption value was obtained according to DIN 53 601 / ASTM D 2414.

To further characterize the silica, a particle size analysis by Coulter Counter (100 µm aperture, water/methanol, ultrasonic) was performed.

For Malathion content and suspensibility WHO/SIF/10.R5, CIPAC MT 15/1 and CIPAC 12/3/(M)/1 were used (WHO Specifications, 1985), (CIPAC Handbook 1A and F, 1970 and 1995).

To determine the surface area the BET method has been used Brunauer et al., 1938), however, in the context of the paper only the relative value corresponding to a low and high amount of OH-groups is important.

Abbreviations used within figures

NO	low surface of silica
MO	medium surface of silica
HO	high surface of silica
OH	without hydrophobicity
NH	with low hydrophobicity
HH	with high hydrophobicity

Formulation and modelling substance

On the base of hydrophilic synthetic precipitated silicas new products with varying hydrophobicity have been produced and tested. Malathion was used as a modelling substance in our silica laboratory keeping in mind its relatively low toxicity. Proprietary customer work has shown that other actives are giving similar positive results.

TABLE 1--Test formulation for 50WP Malathion.

premix I	3 g	wetting agent
	3 g	hydrophilic, spray dried, ground, precipitated silica
premix II	28 g	specialty silica (treated)
	52.1 g	Malathion
add	3 g	dispersing agent
add	10.9 g	diluent (chaulk)

A testing formulation of 50WP Malathion, prepared by simple mixing without any grinding, which is used in our laboratories for quality control indicated the trend towards enhanced stability of the active, however, the influence of surfactants, dispersing agents and other additives did not allow a clear picture (see FIG. 1). It also became clear, that the WHO method (marked GC in the graph) showed advantages over the CIPAC method (marked UV in the graph).

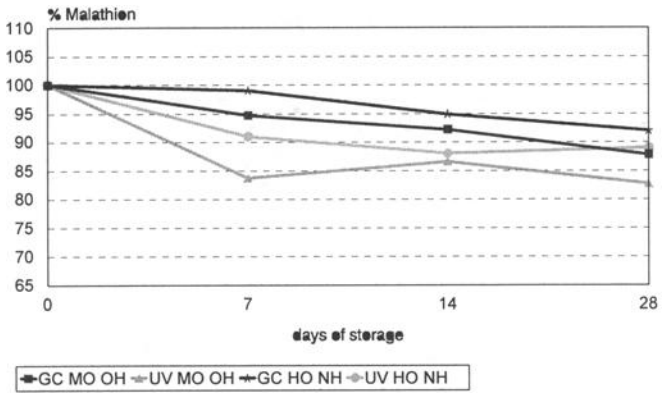

FIG. 1--Loss on active after acc. storage - 50WP Malathion (relative).

As a result we switched to 1:1 blends of silica carrier and Malathion to replace the WP and stopped using the CIPAC method to determine the Malathion content.

RESULTS

FIG. 2 shows the decline of stability when a high amount of free water is available from hydrophilic silicas with higher BET surface.

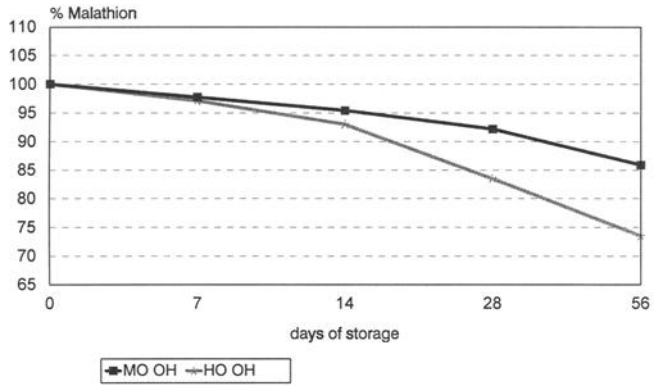

FIG. 2--Loss on active after acc. storage - hydrophilic carrier silica - 1:1 blend.

As can be seen from FIG. 3 the stability of the a.i. has been increased when hydrophobic silicas are used as a carrier. All lines representing blends based on hydrophobic carrier silicas are above the ones from FIG. 2.

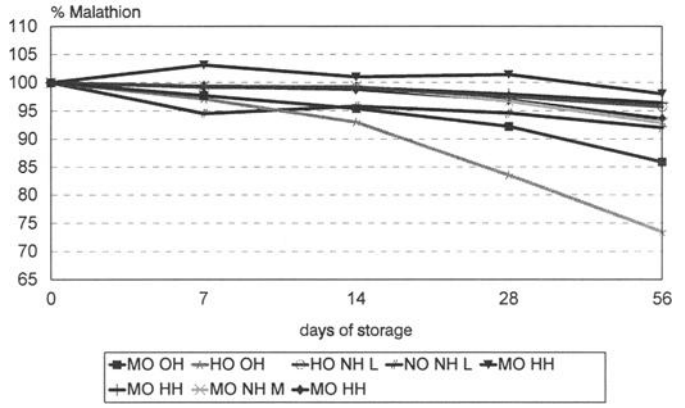

FIG. 3--Loss on active after acc. storage - hydrophobic carrier silica - 1:1 blends.

The 1:1 blends of hydrophobic precipitated silicas and Malathion showed improved stability when silicas with the following characteristics have been used: methanol wettability = 30 +/- 20%, absorption >= 250 g DBP/100g, and particle size = 5-7 µm.

To our surprise the a.i. was not only available when a modified WP formulation based on the new type of silica was applied on house flies, but it

outperformed a regular WP (FIG. 4). This result on availability still needs to be confirmed, however, it is very encouraging and currently work is being done to confirm this result.

Malathion conc.	control *	code 616	code 620	code 622	code 623
g/m²	%	%	%	%	%
0,25	0	100	100	100	100
0,05	0	10	40	40	80
0,01	0	0	0	0	0
0,002	0	0	0	0	0

* control is a commercial WP

FIG. 4--Mortality results in house fly test.

Concept of practical use

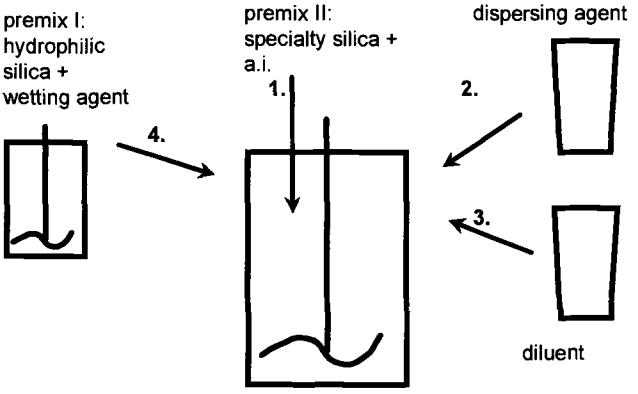

FIG. 5--Concept of practical use.

Wettable powders can be formulated by blending two premixes. The first premix contains a liquid surfactant carried on hydrophilic silica carrier, the second one contains the active carried on the new class of silicas. It is essential that no grinding is done after completion of the formulation, because grinding means shear and shear would press the liquid off the pores. Thus, all ingredients have to be preground before mixing. Dispersible granules (WG) can be formulated accordingly with a granulation process included.

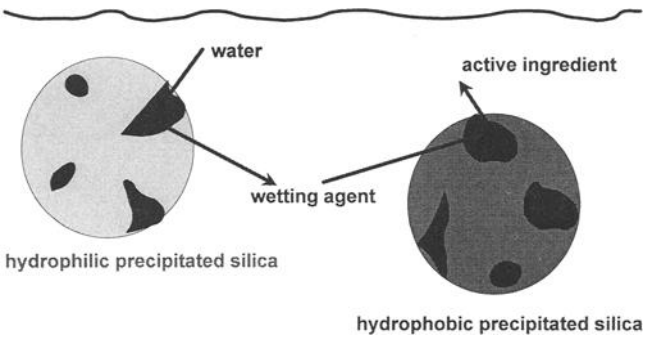

FIG. 6--Schematic function of the release of the a.i.

As schematicly shown in FIG. 5 the a.i. is protected in the pores of the silica. When the farmer applies the WP or the WG the surfactant is released from the hydrophilic carrier silica, thereby wetting out the hydrophobic carrier for the a.i. As a result, the a.i. becomes available.

Patents A patent for the new type of silica and the principle of its use has been applied for (Oelmüller et al., 1997). The goal of the patent is to hinder others from applying to patent the concept. This will assure that every interested formulator can make use of the principle.

CONCLUSION

Our work has shown a strong possibility to formulate stable WP and WG of easy hydrolizing actives when precipitated, spray dried, and finely ground silicas of defined hydrophobicity are being used as a carrier.

ACKNOWLEDGMENT

I wish to acknowledge with gratitude the help I have received from Anita Wengel et al of Cheminova, Lemvig, DK who performed analytical testing and did the availability studies. I also thank my co-author, Astrid Müller, who did most of the practical laboratory work.

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Biological Formulations

Richard Levy[1], Michael A. Nichols[1], and W.R. Opp[1]

TARGETED DELIVERY OF PESTICIDES FROM MATRICAP™[2] COMPOSITIONS

REFERENCE: Levy, R., Nichols, M.A., and Opp, W.R., **"Targeted Delivery of Pesticides from Matricap™ Compositions,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: A novel encapsulation system was developed for controlled delivery of bioactive agents from solid matrices. The efficacy of several encapsulation techniques and polymer or nonpolymer coating/coating-complex formulations in regulating the controlled-delivery duration and profile of the biopesticides *Bacillus thuringiensis* var. *israelensis* or *Bacillus sphaericus* and the insect growth regulators methoprene or pyriproxyfen from solid carrier matrices such as Biodac or corn cob granules was evaluated against larvae of the mosquitoes *Aedes taeniorhynchus*, *Anopheles albimanus,* and *Culex quinquefasciatus* or nymphs of the German cockroach *Blattella germanica.* Results of a series of bioassays against mosquito larvae in a variety of water qualities suggested that the solid controlled-delivery compositions could be used to direct the biopesticides or growth regulators to specific surface and/or subsurface areas of a water column to target the feeding zones and/or orientation patterns of each type of mosquito for prolonged periods. Cockroach bioassays indicated that a bait-growth regulator formulation could be encapsulated within several types of polymer-base compositions and slow-released for extended periods.

KEYWORDS: coatings, coating complex, encapsulation, controlled delivery, biopesticides, growth regulators, mosquito larvicides, German cockroaches

A variety of solid and liquid controlled-delivery compositions of bioactive agents for control of aquatic and terrestrial pests (e.g., insects and weeds) have been reviewed by Kydonieus (1980), Baker (1987), Duncan and Seymour (1989), and

[1]Research coordinator, research assistant, and director, respectively, Lee County Mosquito Control District, P.O. Box 60005, Ft. Myers, FL 33906.
[2]Matricap is a trademark of the Lee County Mosquito Control District, Ft. Myers, FL.

Wilkins (1990). Although the efficacy of several controlled-delivery compositions of biopesticides such as *Bacillus thuringiensis* var. *israelensis (B.t.i.)* or *Bacillus sphaericus* and insect growth regulators (IGRs) such as methoprene, diflubenzuron, or pyriproxyfen for control of mosquito larvae has been demonstrated (Wilkins 1990, Levy et al. 1992; 1993a; 1993b; 1993c; 1995a; 1996b) only a few products are commercially available at this time.

New delivery systems are needed to improve the controlled-release profiles, controlled-release duration, and the range of effectiveness of the larvicides in a variety of water qualities while targeting one or more pest species in desired portions of the water column. Controlled-delivery bait-insecticide compositions are also needed to improve prolonged targeting of adult and/or immature stages of urban pests such as cockroaches in various interior and exterior environmental niches that are subjected to a variety of temperature and humidity conditions (Levy et al. 1995b).

The objective of this research was to evaluate the potential applications of the Matricap™ coating-carrier formulation system (Lee County Mosquito Control District, Ft. Myers, FL) for prolonged controlled delivery of one or more biopesticides or growth regulators for control of mosquito larvae or cockroaches (Levy et al. 1996a; 1996b). U.S. and overseas patents are pending on this novel encapsulation system for controlled delivery of bioactive agents in aquatic and terrestrial environments.

EXPERIMENTAL METHOD

Solid Matricap™ controlled-delivery compositions were formulated in a simple formulation blending system that matched a variety of coating/encapsulating agents and solid carriers/matrices to specific bioactive agents in a manner to achieve short or long-term targeted delivery of one or more bioactive agents from solid compositions in aquatic or terrestrial environments. Active ingredients incorporated in the compositions included solid and/or liquid bioactive agents utilized for pest management. Inert ingredients constituted one or more polymer or non-polymer carriers/matrices in the form of powders, granules, pellets, extrusions, composites, briquets, etc., and one or more solid or liquid polymer and/or non-polymer coating encapsulating agents, with or without optional polymer or non-polymer formulating agents. Coating/encapsulating agents were utilized to control the rate and duration of delivery of the bioactive agent(s) from the composition and to help protect the bioactive agent(s) from environmental degradation. Low and high speed mixing techniques were used to encapsulate a granular carrier with formulations of coatings and bioactive agents to produce dry flowable granules.

Our current research on encapsulated controlled delivery systems was aimed at evaluating the efficacy of several encapsulation techniques and coating complexes in regulating the release rate of *B.t.i.*, *B. sphaericus,* methoprene, or pyriproxyfen from a variety of solid matrices that could be used to carry and deliver these biorational insecticides in aquatic or terrestrial habitats. Biodegradable 12/20 mesh cellulose complex granules called Biodac® (Edward Lowe Industries, Inc., Cassopolis, MI), and 10/14 mesh corn cob granules (Mt. Pulaski Products, Inc., Mt. Pulaski, IL) were selected as matrices for use in this study. Several proprietary nontoxic and biodegradable coating complexes consisting of a blend of two or more polymer and/or

nonpolymer coatings were formulated with a granular carrier and a *B.t.i.* formulation labeled Vectobac® Technical Powder (5000 ITU/mg; Abbott Laboratories, North Chicago, IL) or Bactimos® Primary Powder (7000 ITU/mg; Abbott Laboratories, North Chicago, IL), a *B. sphaericus* formulation labeled Vectolex® Technical Powder (600-700 ITU/mg; Abbott Laboratories, North Chicago, IL), a methoprene formulation labeled Dianex® Emulsifiable Concentrate (32.8% S-methoprene; Sandoz Agro, Inc., Dallas, TX), or a pyriproxyfen formulation labeled Nylar® 10% Emulsifiable Concentrate (10% pyriproxyfen; McLaughlin Gormley King Company, Minneapolis, MN). Active and inert components were combined into solid controlled-delivery compositions in a sequential series of admixing and curing procedures that were dependent on the type and concentration of ingredients utilized in a formulation. Small quantities of granules were prepared with a KitchenAid® KSM 90 (KitchenAid Portable Appliances, St. Joseph, MI) while large quantities of granules were prepared with an Arimex® MG 80 Mixing Machine (Am-Mac Incorporated, West Caldwell, NJ). All percentage compositions are given in weight/weight in this study.

The biopesticide and growth regulator compositions were formulated for prolonged surface and/or subsurface delivery to target *Anopheles albimanus, Aedes taeniorchynchus,* and *Culex quinquefasciatus* larvae in fresh water (i.e., well water purified by reverse osmosis filtration) or brackish water (i.e., 10% or 50% artificial sea water - Instant Ocean®; Aquarium Systems, Mentor, OH), or 100% seawater (Instant Ocean). The pretreatment potential of the controlled-release granules was also evaluated against larvae of *Ae. taeniorhynchus.* Encapsulated polymer-base films, extrusions, or coatings containing a synthetic vegetable gum-base bait and an insect growth regulator were evaluated against nymphs (7 to 10 mm) of the German cockroach *Blattella germanica.*

Mosquito Biopesticide Bioassays

A series of stress-test granule-transfer bioassays were designed to simulate pretreatment of flooded semipermanent brackish water habitats that initially have no larval breeding and direct treatment of multiple broods of mosquito larvae in permanent fresh or brackish water and seawater, or in semipermanent brackish water habitats that periodically flood and dry. The bioassay protocol consisted of challenging the *Aedes, Anopheles* or *Culex* larvae with matrices comprised of Biodac or corn cobs, a coating complex, and a microbial larvicide or IGR formulation for ca. 90 to 100 days. The controlled-delivery granules were applied at rates of 5.6 kg/ha (5 lb/acre), 7.8 kg/ha (7 lb/acre) or 11.2 kg/ha (10 lb/acre).

Bioassays were conducted in 1.9 liter plastic cups containing 1 liter of fresh water, brackish water, or seawater and 10 1st to 3rd instar *Aedes, Anopheles,* or *Culex* larvae, or in 18.9 liter plastic buckets containing 17.0 liters of brackish water and a mixed population of 10 *Anopheles* and 10 *Aedes* larvae. Larvae in plastic cups were treated with 4 corn cob-base or 6 Biodac-base biopesticide or IGR granules/cup which was equivalent to an application rate of 5.6 kg/ha. Larvae in buckets were treated with 31 and 42 Biodac-base biolarvicide granules/bucket or 7.8 and 11.2 kg/ha, respectively.

Larvae were fed ground rabbit chow throughout a test series. Tests were

conducted in a room maintained at ca. 27°C. Bioassays with each controlled-delivery granular composition were replicated 3 times.

Bioassays with *Aedes, Anopheles,* and *Culex* larvae were conducted according to the following protocol. Controlled-delivery granules were introduced into a semi-permanent habitat containing brackish water and no larvae to simulate a pretreatment habitat for 9 days. Granules were then removed from the water, washed, and dried for 2 days. Dry granules were reintroduced into new semipermanent brackish water habitats containing larvae. In simulated direct treatments, granules were introduced into semipermanent brackish water, or permanent fresh water, brackish water, or seawater habitats containing larvae. Percentage larval mortality was recorded at 24 hr posttreatment intervals. A test was terminated if average larval control was less than 100% or if average control mortality exceeded 10%.

Tests were continued if the termination parameters were not observed. In semi-permanent brackish water tests, granules remained in the water with the dead larvae and rabbit chow for an additional 9 days. Granules were then removed from the water, washed and air dried for 2 days. Dry granules were transferred to new semi-permanent brackish water habitats containing larvae. In permanent fresh water, brackish water or seawater tests, granules remained in the water with dead larvae and rabbit chow for 10 to 22 days after reaching 100% mortality. Granules were then washed and transferred to new fresh or brackish water habitats containing larvae. Sequential transfer of granules to challenge larvae in new semipermanent or permanent water habitats were continued according to the aforementioned protocol for ca. 90 to 100 days or until control was ineffective.

Cockroach IGR Bioassays

Liquid formulations composed of a polymer, Nylar 10% EC, and a bait complex were extruded into cubical chambers, poured into thin film sheets, or lightly coated on the interior of dark green glass 10 mL screw cap vials. These bait station compositions were evaluated against 25 German cockroach nymphs (Navy 3 strain) in 41 by 23 by 17 cm plastic trays. Trays contained one cubical chamber or film sheet that was placed in a 35 by 10 mm plastic petri dish that was positioned in a corner. A coated vial was laid directly on the floor in the corner of each tray. A 35 x 10 mm petri dish containing 6 g of rabbit chow pellets was positioned in the opposite corner of each bait station as an alternate food source. A cotton-plugged vial of water (35 mL) was placed in a 100 x 15 mm square petri dish and positioned in the center of each tray. Control trays contained 25 German cockroaches, each type of IGR-free polymer-base bait station composition, rabbit chow pellets, and a cotton-plugged vial of water. Tests with each bait station composition were replicated 3 times. Cockroach bioassays were shielded from direct light in a room maintained at ca. 26 to 28°C and 50 to 78% RH.

Adult cockroaches exposed to the 3 types of Nylar-bait compositions as 7 to 10 mm nymphs exhibited twisted wing/dark pigmentation abnormalities as indicators of IGR-induced sterility. The average percent abnormalities observed were used as the main criterion to evaluate the efficacy of the controlled-delivery IGR compositions.

Twisted wing data was recorded at 24 hr posttreatment intervals throughout a test

series. Twisted wing or highly pigmented adults were removed from each tray on a daily basis to prevent errors in data recording. When exposure of the polymer-base IGR composition to the cockroaches in each tray resulted in twisted wing/dark pigmentation abnormalities in 100% of the cockroach population in a test series and when control mortality did not exceed 10%, the cubical chambers, film sheets, or coated vials were allowed to remain in the trays for an additional 2 days before being transferred to new trays containing new cockroach nymphs, rabbit chow pellets, and water. Sequential transfer of each type of controlled-delivery IGR-bait composition was continued according to this procedure until one or more normal adults with straight wings were observed in a test series or until average cockroach mortality in controls exceeded 10%.

RESULTS

Mosquito Biopesticide Bioassays: *B.t.i.*

Comparative pretreatment and direct treatment bioassays were conducted against subsurface-feeding one day old larvae of *Ae. taeniorhynchus* and *Cx.quinquefasciatus* at 5.6 kg/ha in shallow semipermanent or permanent water habitats (ca. 7 cm deep) with controlled-delivery granules composed of 92.3% Biodac matrix, 3.8% coating complex A/B, and 3.9% Vectobac TP. Results indicated that *B.t.i.* can be slow-released from submerged Biodac matrices (specific gravity > 1) in fresh or brackish water for at least 3 months and effectively control multiple broods of 2nd or 3rd instar *Aedes* or *Culex* larvae, even though the granules were subjected to a number of repetitive flooding and/or drying cycles during the granule transfer challenges (Fig. 1). This granular composition was also evaluated against surface-feeding 1st or 2nd instar *An. albimanus* larvae in shallow permanent water habitats (ca. 7 cm deep) in 10, 50, and 100% seawater (Fig. 2); however, the age of the granules were 121 days old when the tests were initiated.

Figure 1 indicated that the coating complex-regulated controlled-delivery profiles followed first-order or square-root-of-time kinetics. Mortality recorded at 24 hr posttreatment intervals suggested that a "burst-effect" or the initial release of high concentrations of *B.t.i.* from the granules occurred within the first 2 weeks after treatment, followed by a general decrease in the rate of kill over time. This trend was characteristic in both water qualities. Controlled-delivery profiles of *B.t.i.* matrices over the 98 to 105 day test periods were correlated to the rates of biodegradation and hydrolysis of the coating complex as well as to the gradual decomposition of the Biodac granules.

The data from Figure 2 suggested that submerged coating-regulated Biodac granules can maintain controlled delivery of *B.t.i.* to the surface of the permanent shallow water habitats in sufficient concentrations to achieve sustained 100% control of surface-feeding *Anopheles* larvae at an application rate of 5.6 kg/ha for 63 to 91 days. The effect of water quality on the release of *B.t.i.* from the granules was indicated by the number of broods controlled. Six broods of larvae were controlled in 10 and 50% seawater, while only 5 broods were controlled in 100% seawater; however, the rate of kill of each of the 5 broods was faster than in the other water qualities.

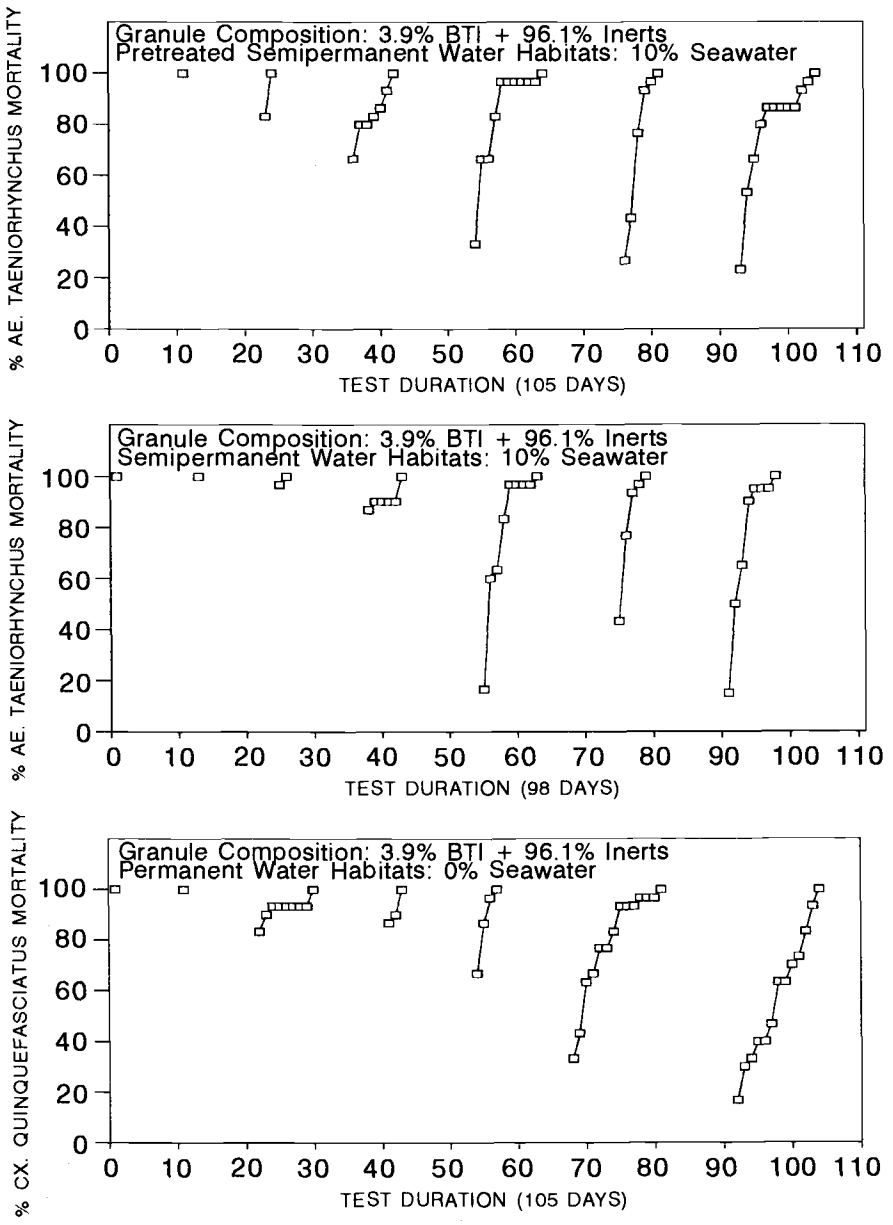


FIG. 1--Controlled delivery of Vectobac TP from Biodac granules encapsulated with coating complex A/B.

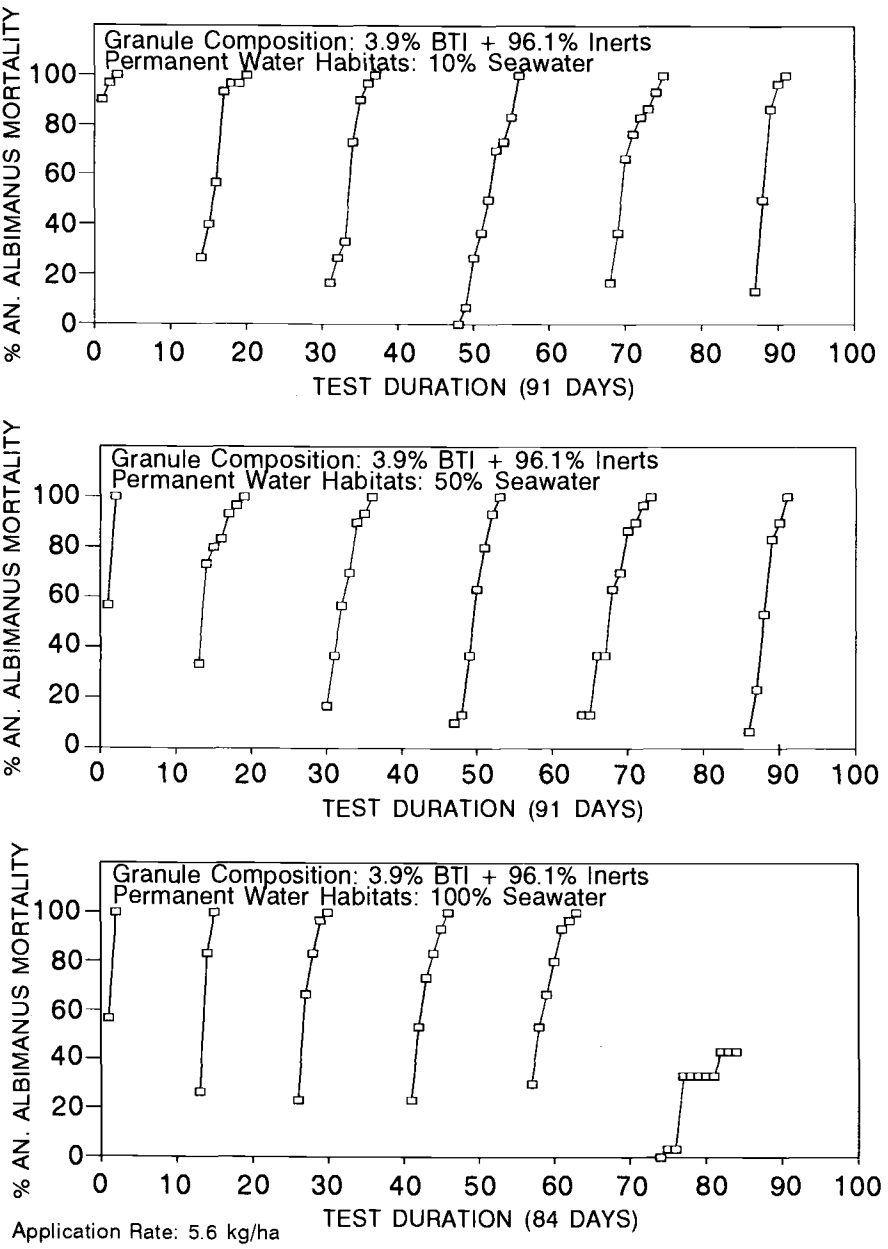


FIG. 2--Controlled delivery of Vectobac TP from Biodac granules encapsulated with coating complex A/B.

Controlled release of *B.t.i.* from the Biodac granules in *Anopheles* tests showed first-order or square-root-of-time kinetics; however, the "burst effect" duration and pattern of release were significantly less pronounced when compared to the *Culex* and *Aedes* bioassays (Fig. 1). In general, differences in the delivery profiles of *B.t.i.* from the granules and mosquito-controlling efficacy between the *Anopheles* and *Culex* or *Aedes* bioassays were related to the subsurface orientation of the granules in relation to the subsurface or surface filter feeding habits of the target mosquito species, the type of permanent or semipermanent habitat, biodegradation and hydrolysis of the coating complex, and to slow decomposition of the Biodac granules.

Anopheles larvae were also challenged with other coating complex granular formulations of *B.t.i.* Tests against 2nd or 3rd instar *An. albimanus* larvae in shallow permanent fresh and brackish water (ca. 7 cm deep) with 5.6 kg/ha of 156 day old granules consisting of 90.5% Biodac, 4.7% Vectobac TP, and 4.8% coating complex C/E (Fig. 3) or 93.2% Biodac, 3.4% Vectobac TP, and 3.4% coating complex B/C (Fig. 4) resulted in 100% control of 6 broods of larvae over the 84 or 101 day test periods with either type of coating complex. Larvicidal action was significantly faster in 10% seawater than in 0% seawater habitats (Fig. 3), and was attributed to the effects of water quality on the coating complex C/E-*B.t.i.* formulation. Variations in the rate of delivery of *B.t.i.* to the surface of the water from both types of submerged Biodac granules, and the subsequent larvicidal action, were presumed to be due to differences in *B.t.i.* concentration, differences in biodegradation and hydrolysis of the 2 coating complexes, and to the gradual decomposition of the matrices in the 2 water qualities. The release profiles and duration of larval control were comparable for both types of granules in 10% seawater. The *B.t.i.* release profiles of granules encapsulated with coating complex C/E or B/C also followed first-order or square-root-of-time kinetics and exhibited an initial "burst effect."

Bioassays were also conducted against mixed populations of surface and subsurface feeding 1st or 2nd instar larvae of *Anopheles* and *Aedes* species in 31.8 cm deep permanent water habitats containing 10 and 50% seawater with 121 day old Biodac granules composed of 3.9% Vectobac TP and 3.8% coating complex A/B (Fig. 5 and 6) or 3.3% Vectobac TP and 3.3% coating complex A/F (Fig. 7 and 8) at application rates of 7.8 and 11.2 kg/ha, since initial bucket tests with the 2 types of Biodac controlled-delivery compositions in these water qualities at 5.6 kg/ha did not produce sustained 100% control of *Aedes* and *Anopheles* larval populations for greater than 40 days. The test objective was to determine if the coating complexes utilized in the formulations could effectively distribute the high molecular weight *B.t.i.* particles throughout the water column of relatively deep water habitats at levels that would maintain persistent long-term larval control for at least 90 days. Comparative bioassays were conducted against the *Anopheles* and *Aedes* species under the same test conditions with standard commercial *B.t.i.* granules from Abbott Laboratories (Vectobac CG granules; Abbott Laboratories, North Chicago, IL).

Controlled-release profiles exhibited first-order or square-root-of-time kinetics and a defined "burst effect" in both water qualities and application rates (Fig. 5 to 8). Granular application rates of 11.2 kg/ha resulted in faster larvicidal action than rates of 7.8 kg/ha with both coating complex formulations; however, it is interesting to note that granular compositions with coating complex A/F contained less *B.t.i.* than

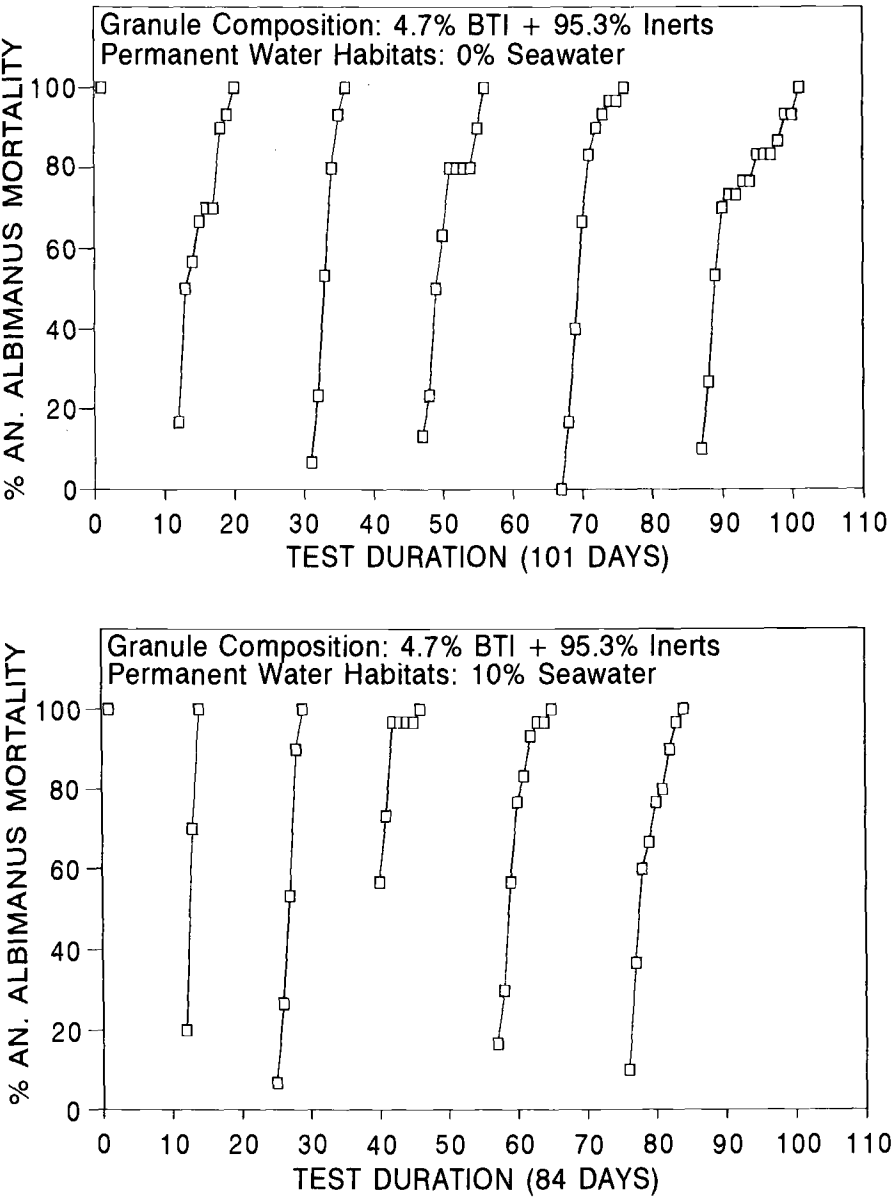
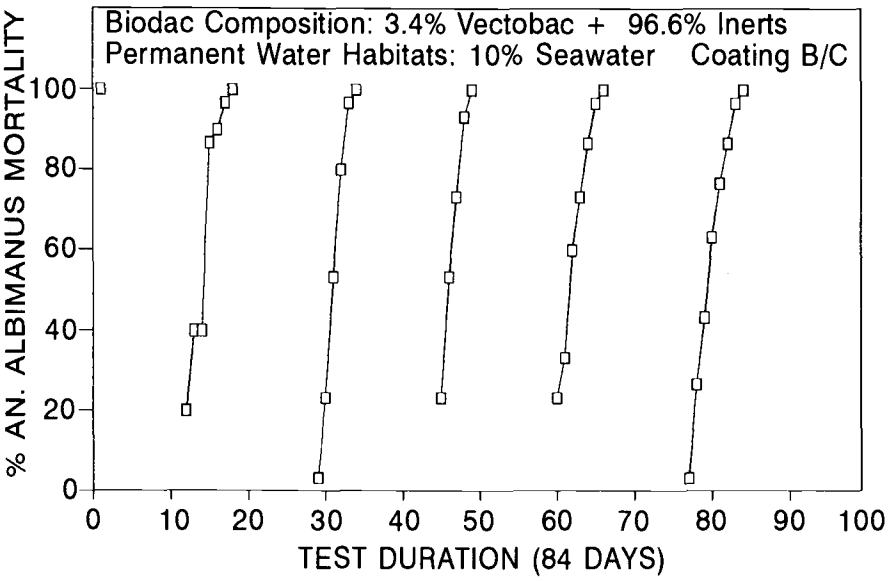
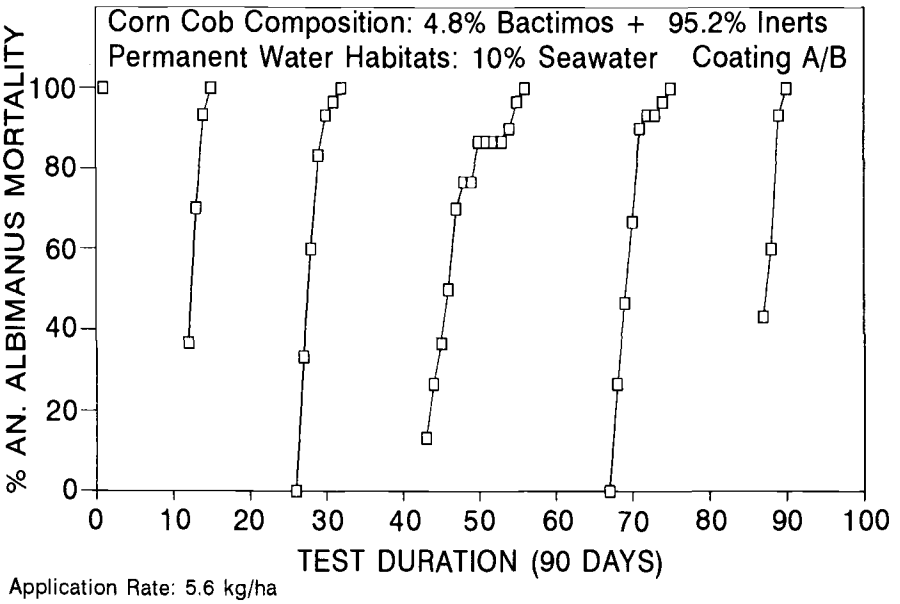


FIG. 3--Controlled delivery of Vectobac TP from Biodac granules encapsulated with coating complex C/E.





Application Rate: 5.6 kg/ha

FIG. 4--Controlled delivery of Vectobac TP or Bactimos PP from Biodac or corn cob granules encapsulated with coating complex B/C or A/B.

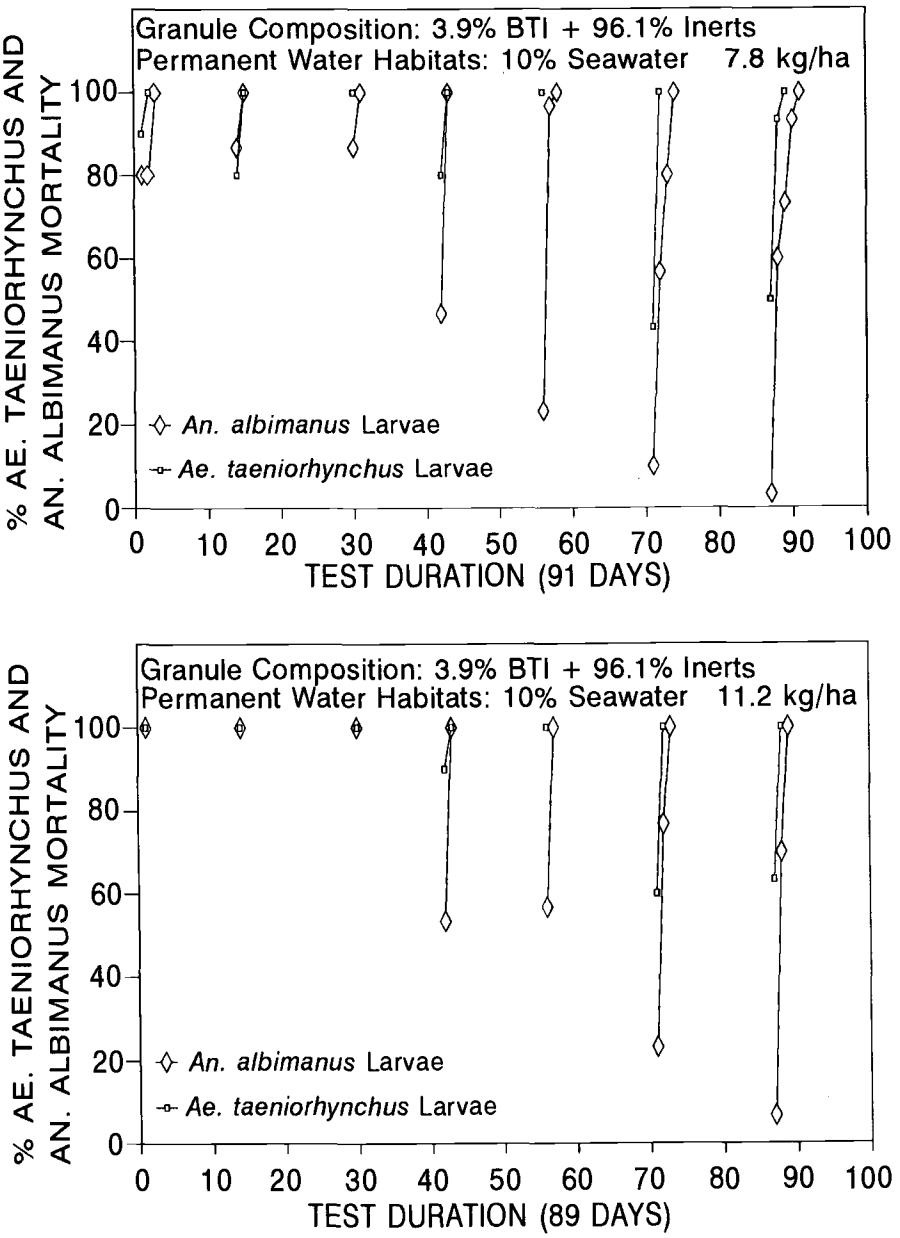


FIG. 5--Controlled delivery of Vectobac TP from Biodac granules encapsulated with coating complex A/B.

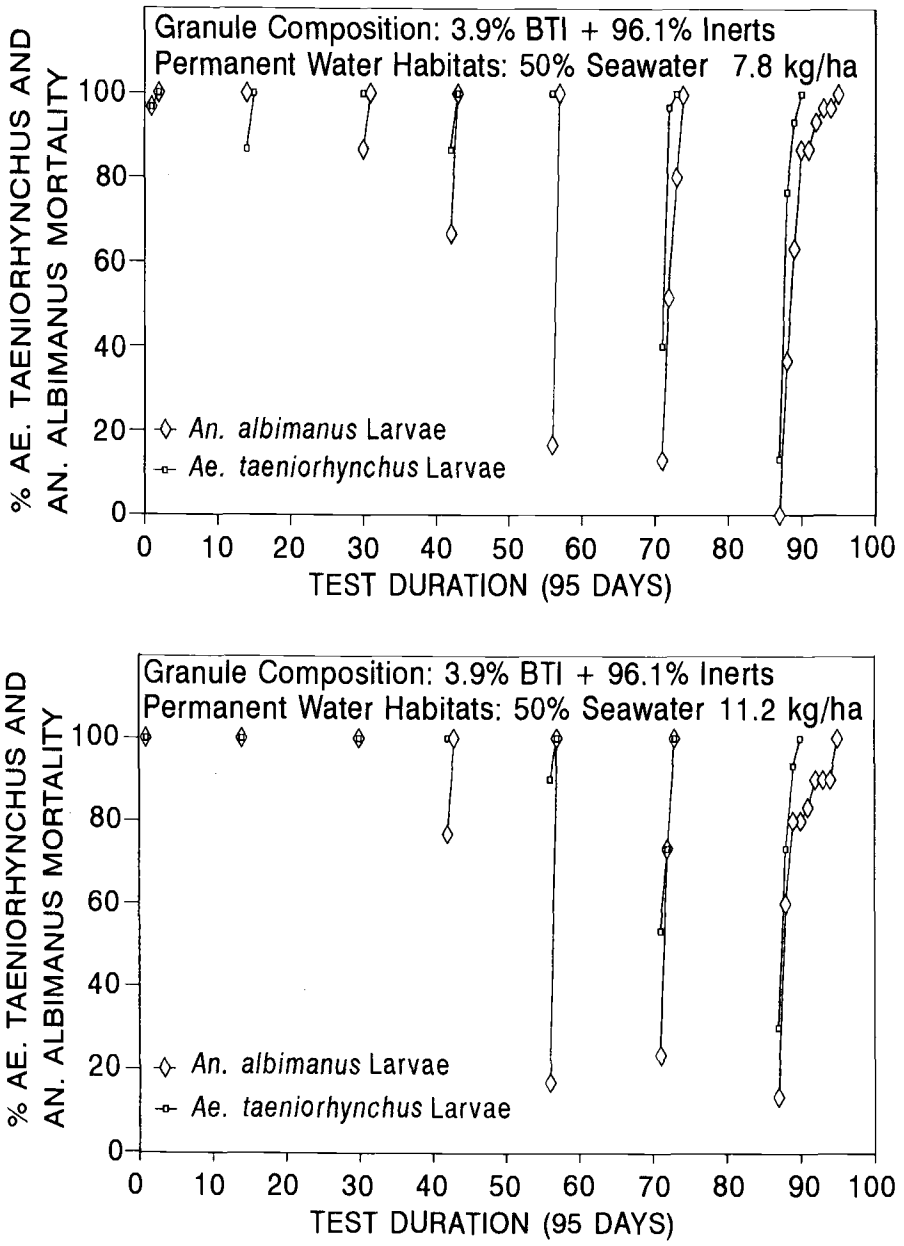


FIG. 6--Controlled delivery of Vectobac TP from Biodac granules encapsulated with coating complex A/B.

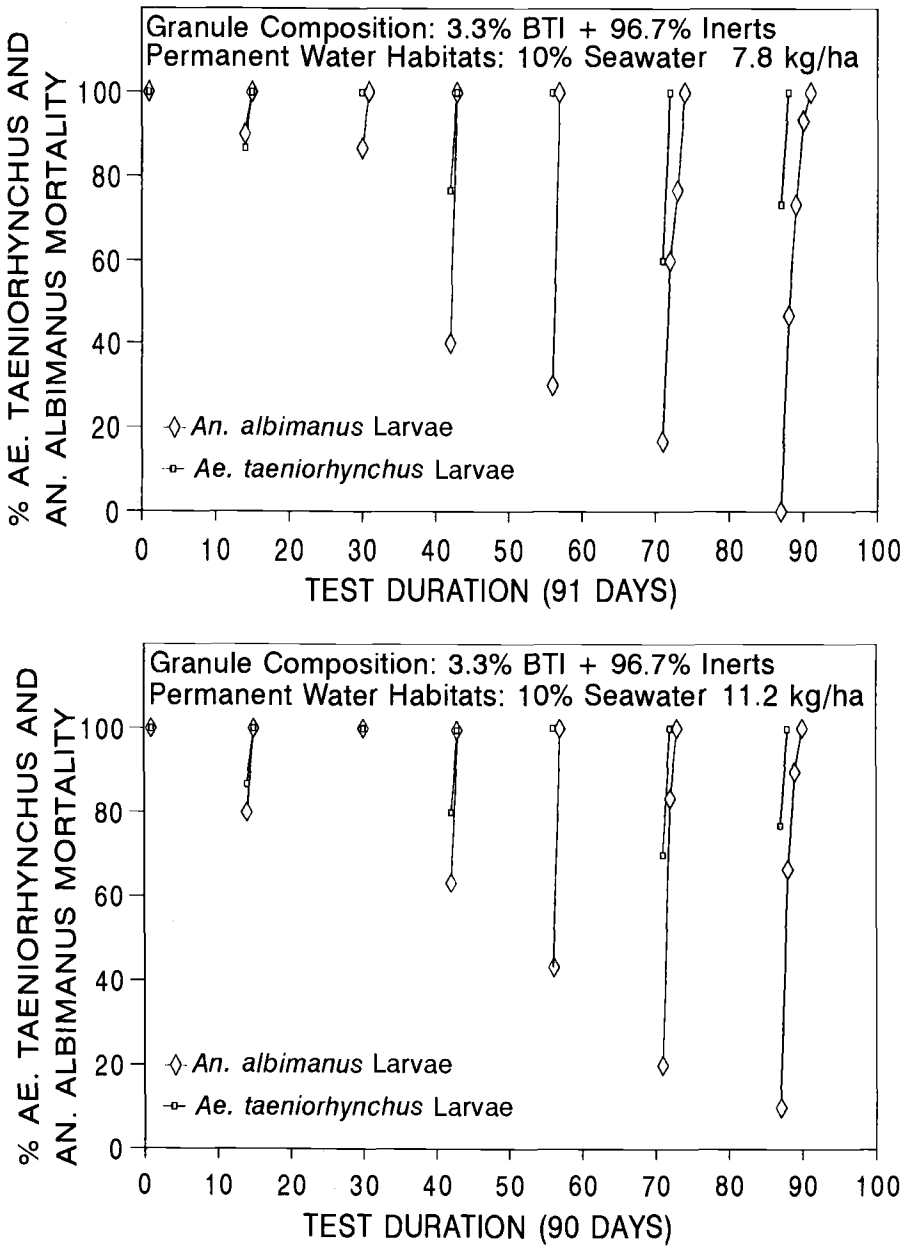


FIG. 7--Controlled delivery of Vectobac TP from Biodac granules encapsulated with coating complex A/F.

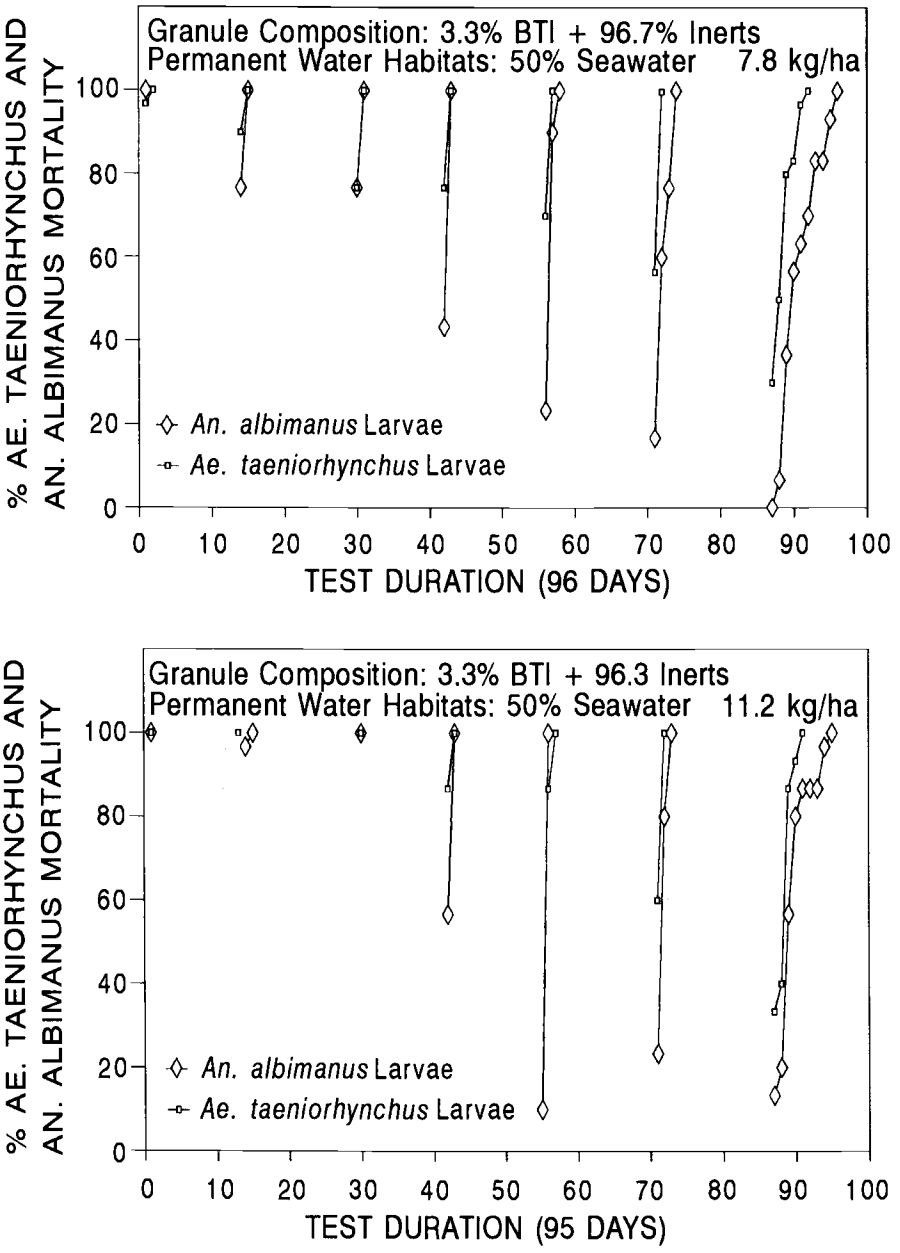
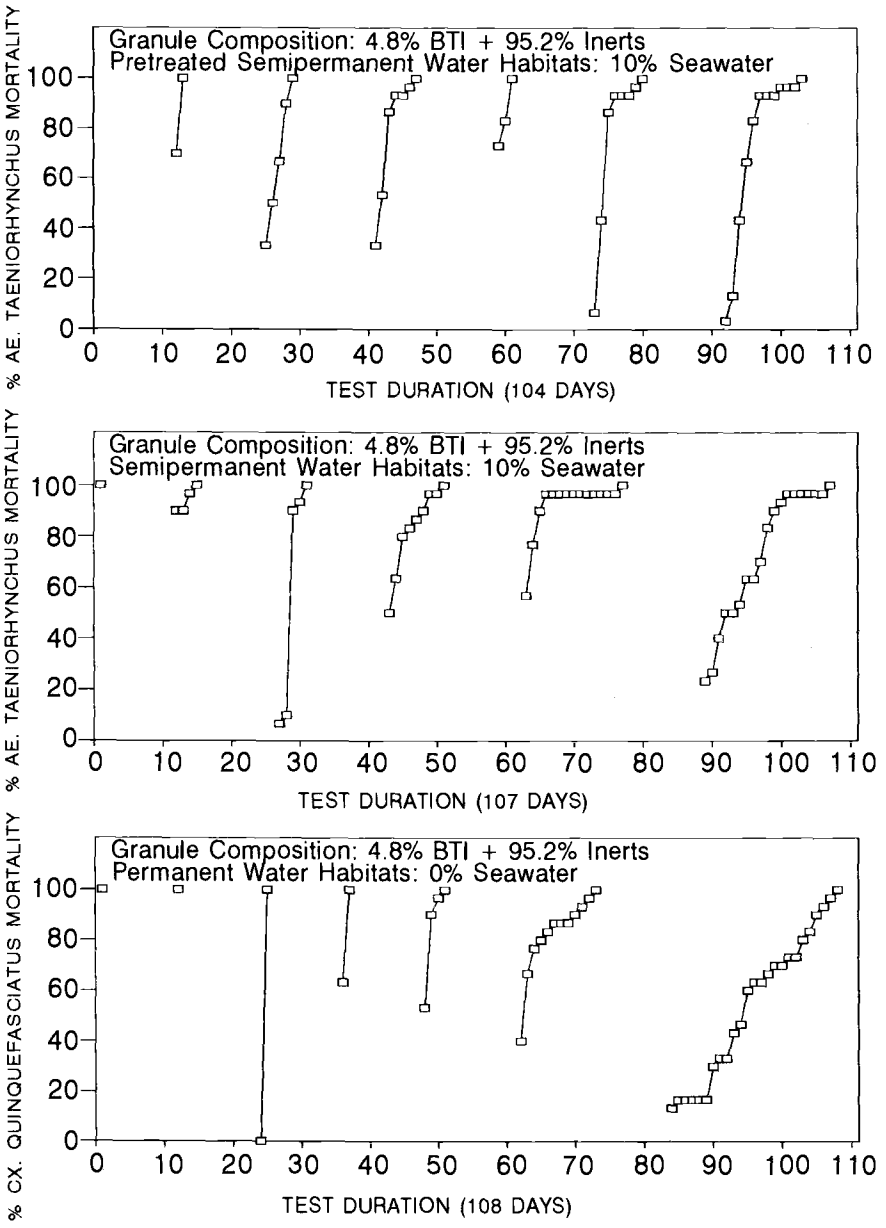


FIG. 8--Controlled delivery of Vectobac TP from Biodac granules encapsulated with coating complex A/F.

granules formulated with coating complex A/B. Nevertheless, the duration of effective control of the *Aedes* and *Anopheles* larvae (i.e., 89 to 96 days) and the number of broods controlled (i.e., 7 broods) in the 2 water qualities were comparable for both coating complex formulations at the low and high application rates. In general, the data indicated that subsurface-feeding *Aedes* larvae were easier to kill than surface-feeding *Anopheles* larvae; however, both types of coating complexes were capable of translocating sufficient quantities of *B.t.i.* from the submerged granules through the water column to the surface-water feeding zone of the *Anopheles* larvae (i.e., a distance of ca. 32 cm) to obtain effective control of both species for ca. 3 months. It should be noted that Vectobac CG granules only produced effective control of one larval brood.

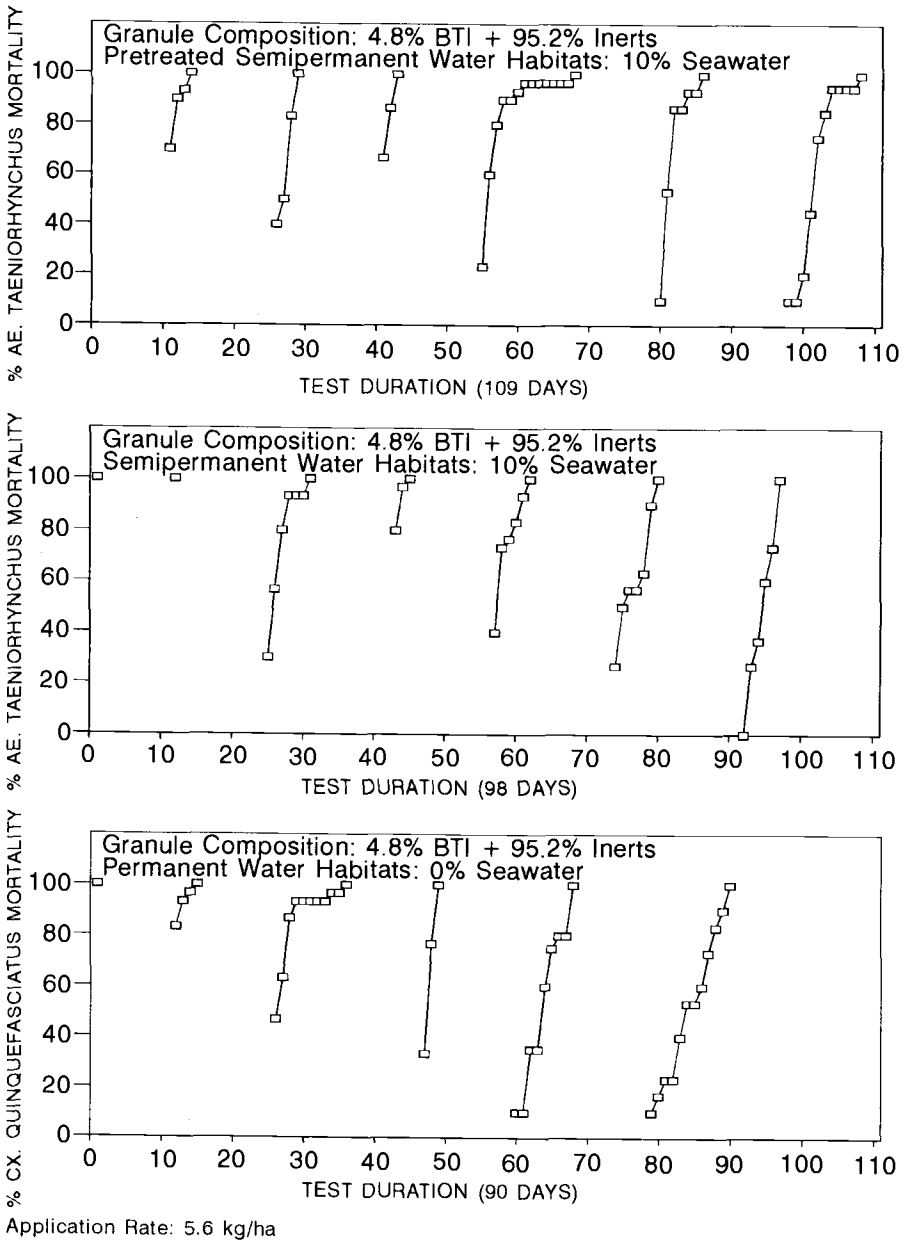
Corn cob granules were utilized as matrices for 2 commercial formulations of *B.t.i.* and 2 coating-complex formulations in another series of bioassays in shallow water habitats (ca. 7 cm deep). The first type of controlled-delivery granules consisted of 90.4% corn cob, 4.8% coating complex A/D, and 4.8% Vectobac TP. Results of a series of comparative granule-transfer bioassays against 2nd and 3rd instar larvae of *Ae.taeniorhynchus* and *Cx. quinquefasciatus* at 5.6 kg/ha in 3 types of simulated fresh and brackish water habitats indicated that encapsulated Vectobac TP can be slow-released from submerged corn cob-base matrices at levels that were effective in producing 100% control of multiple broods of *Aedes* and *Culex* larvae for at least 104 to 108 days, even though the *B.t.i.*-encapsulated corn cob granules were subjected to a variety of repetitive flooding and drying cycles (Fig. 9). The data suggested that the duration of controlled delivery of Vectobac TP from the Biodac granules formulated with coating complex A/B was comparable to the controlled-delivery duration of Vectobac TP from corn cob granules formulated with coating complex A/D; however, the rate of larvicidal action at each transfer period was observed to vary with the type of coating complex formulated with each matrix. Nevertheless, both types of granular formulations exhibited first-order or square-root-of-time kinetics and a "burst effect" during the initial release of *B.t.i.* Corn cob granules showed no signs of decomposition at the termination of the bioassays.

The second series of *B.t.i.* tests with corn cob matrices were conducted with Bactimos Primary Powder. Controlled delivery granules prepared for these evaluations consisted of 90.4% corn cob, 4.8% coating complex A/B, and 4.8% Bactimos PP (Fig. 10). Results of granule-transfer bioassays against larvae of *Ae. taeniorhynchus* and Cx. *quinquefasciatus* in shallow fresh or brackish (10% seawater) water (ca. 7 cm deep) with this granular composition were similar to the previous results with corn cob matrices formulated with coating complex A/D and Vectobac TP (Fig. 9), and indicated that encapsulated Bactimos PP can also be effectively slow-released for 90-109 days from corn cob granules that were subjected to intermittent periods of submergence in fresh or brackish water and drying during the course of bioassays in simulated permanent and semipermanent habitats. Our data suggested the controlled delivery of larvicidal levels of Bactimos PP or Vectobac TP encapsulated on corn cob granules was effective for about 3 months; however, the release profiles in the 2 water qualities were shown to vary with the type of coating complex. The controlled delivery of Bactimos PP in both water qualities followed first-order or square-root-of-time kinetics and was comparable to the release kinetics of encapsulated Vectobac TP



Application Rate: 5.6 kg/ha

FIG. 9--Controlled delivery of Vectobac TP from corn cob granules encapsulated with coating complex A/D.



Application Rate: 5.6 kg/ha

FIG. 10--Controlled delivery of Bactimos PP from corn cob granules encapsulated with coating complex A/B.

from corn cob or Biodac granules in similar habitats.

A corn cob formulation of Bactimos PP was also evaluated against 2nd or 3rd instar *An. albimanus* larvae at an application rate of 5.6 kg/ha (Fig 4). Results of bioassays in shallow permanent water habitats (ca. 7 cm deep) containing 10% seawater with 130 day old granules composed of 90.4% corn cob, 4.8% Bactimos PP, and 4.8% coating complex A/B indicated that this coating complex was also effective in delivering sufficient levels of *B.t.i.* to the surface feeding zone of the *Anopheles* larvae from the submerged granules to maintain 100% control of 6 broods for 90 days. This granular composition was also highly effective against subsurface-feeding *Aedes* and *Culex* larvae (Fig. 10). The controlled-release profiles and kinetics appeared to be similar to other bioassays against *Anopheles* larvae with Biodac granules encapsulated with Vectobac TP and coating complex A/B (Fig 2).

Coating complex A/B or A/F were also used to encapsulate corn cob granules with 6.9 or 9.3% Vectobac TP or Bactimos PP. However, this level of *B.t.i.* could not be encapsulated on Biodac granules. This was presumed to be due to differences in the surface characteristics of Biodac (i.e., smooth) and corn cob (i.e., rough) granules, and subsequent adhesion of a *B.t.i.*-coating complex formulation to the granules.

It should be noted that past studies (Levy et al. 1995a, Levy et al. Unpublished) have shown that silicon dioxide powder or wood chips can be formulated with Acrobe® Technical Powder (American Cyanamid Company, Wayne, NJ) and a coating complex into floating compositions or agglomerated into cubettes, pellets, etc., for controlled delivery of *B.t.i.* from floating or submerged compositions. Encapsulation of sand granules with coating complex-regulated controlled-delivery formulations of Acrobe TP were also shown. Results of bioassays with these compositions in fresh or brackish water indicated that the floating or submerged matrices could release *B.t.i.* at and/or below the surface of the water for ca. 60 days to control surface-feeding *An. quadrimaculatus* and *An. albimanus* larvae or subsurface-feeding *Ae. taeniorhynchus* and *Cx. quinquefasciatus* larvae in permanent or semi-permanent habitats.

Mosquito Biolarvicide Bioassays: *B. sphaericus*

The coating complex-regulated controlled delivery of Vectolex TP from Biodac or corn cob granules was also evaluated against *Culex* larvae in shallow permanent brackish water habitats (ca. 7 cm deep) at application rates of 5.6 kg/ha. Comparative bioassays against 2nd instar *Cx.quinquefasciatus* larvae with 18 day old granules composed of 93.2% Biodac, 3.4% Vectolex TP, and 3.4% coating complex A/B or 15 day old granules composed of 90.5% corn cob, 4.8% Vectolex TP, and 4.8% coating complex A/B indicated that *B . sphaericus* could be slow-released from both types of encapsulated matrices at levels that would effectively control 7 or 8 broods of *Culex* larvae for 106 or 99 days, respectively (Fig. 11). Results against 2nd instar *Culex* larvae in comparable habitats with 11 day old granules composed of 93.2% Biodac, 3.4% Vectolex TP, and 3.4% coating complex A/F or 12 day old granules composed of 90.2% corn cob, 4.9% Vectolex TP, and 4.9% coating complex A/F (Fig. 12) showed that effective control of 8 or 7 larval broods was obtained over an

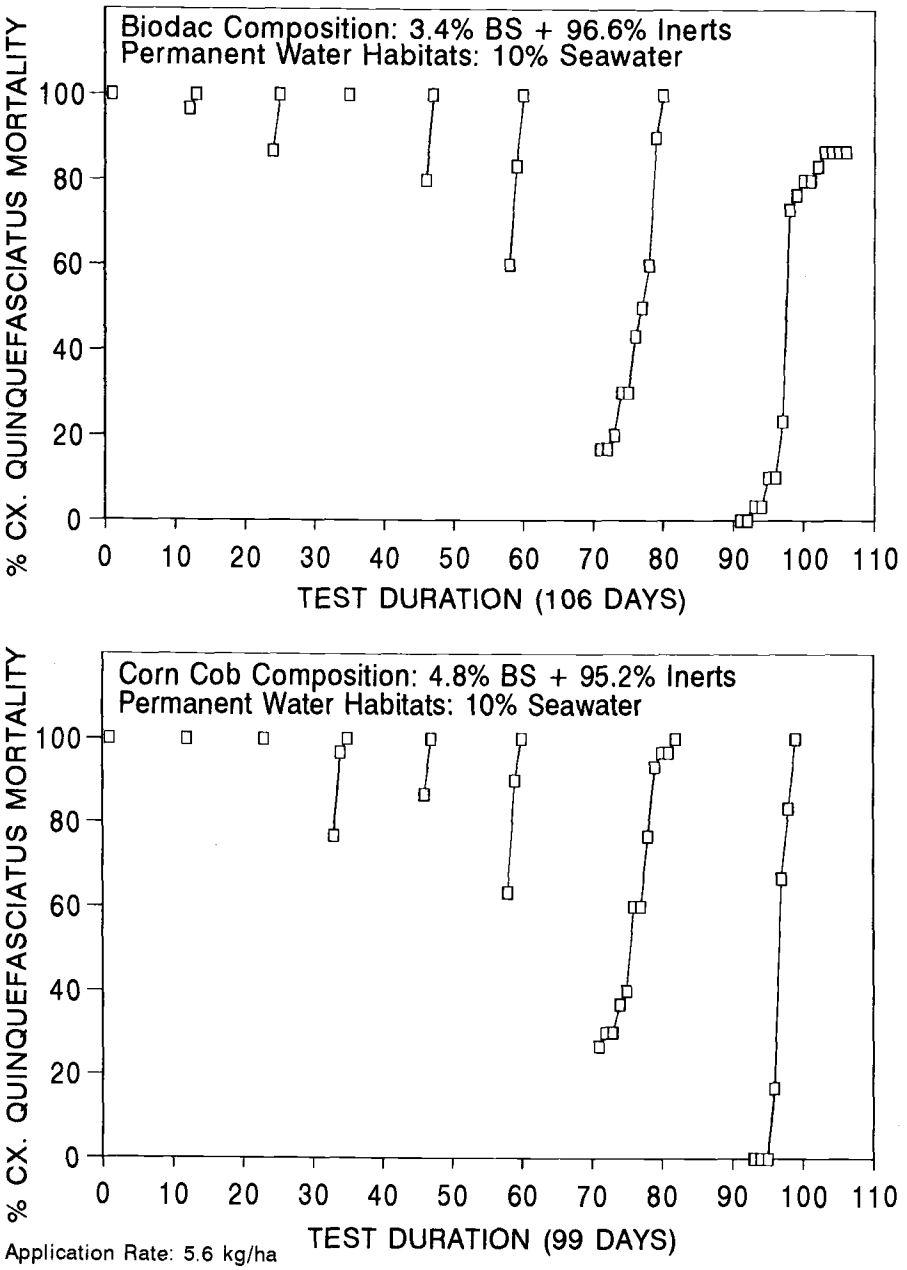
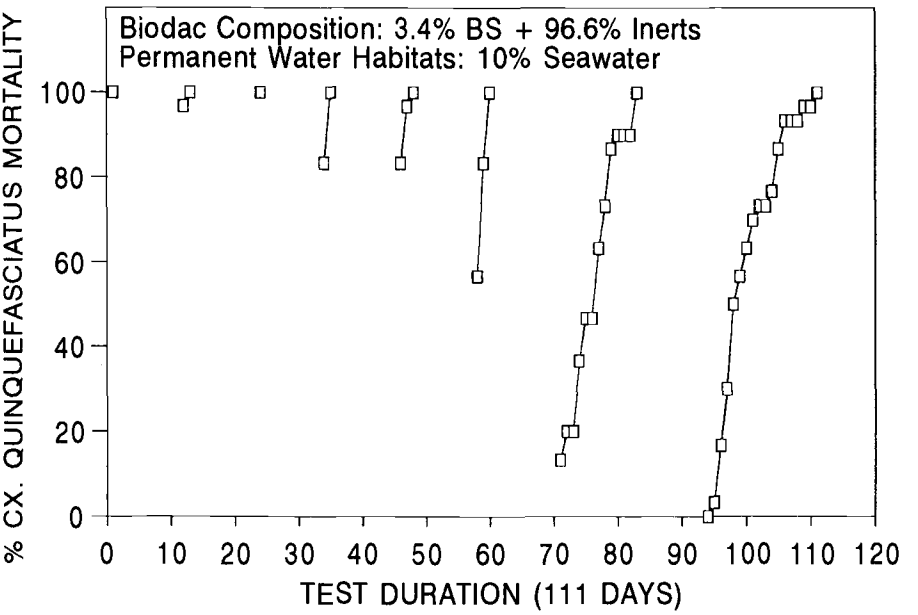
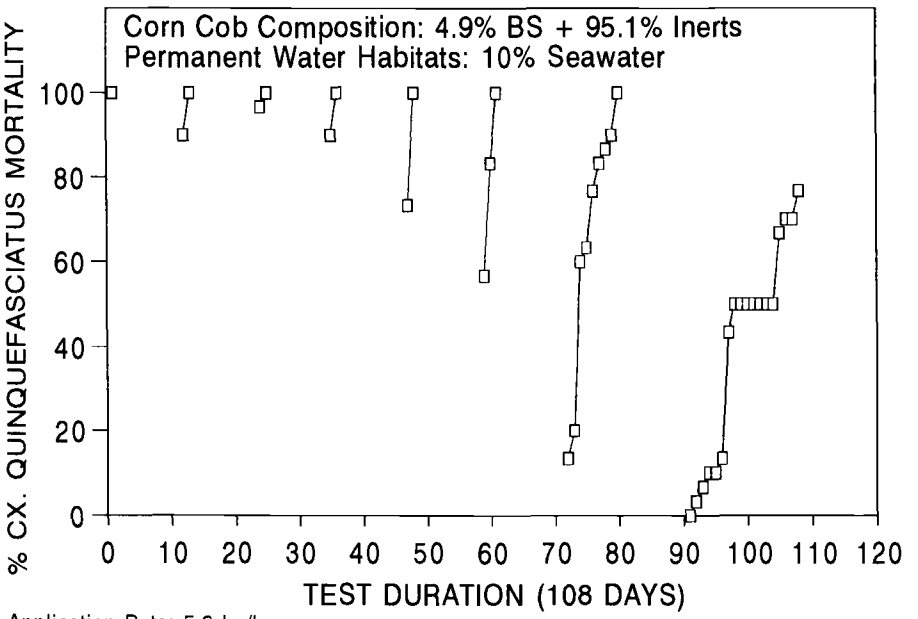


FIG. 11--Controlled delivery of Vectolex TP from Biodac and corn cob granules encapsulated with coating complex A/B.





Application Rate: 5.6 kg/ha

FIG. 12--Controlled delivery of Vectolex TP from Biodac and corn cob granules encapsulated with coating complex A/F.

111 or 108 day test period, respectively.

In general, larval mortality from each encapsulated composition followed first-order or square-root-of-time kinetics, with kill being observed in 1 to 3 days for 6 broods, 9 to 13 days for brood 7, and 7 to 18 days for brood 8. Biodac granules contained less Vectolex TP and coating complex than the corn cob granules, nevertheless, the larvicidal efficacy of one corn cob (Fig. 11) and one Biodac® (Fig. 12) composition was generally comparable. It is also interesting to note that the potency differences between *B.t.i.* (5000 ITU/mg) and *B. sphaericus* (600-700 ITU)/mg) utilized in the controlled-delivery granules in the bioassays indicated that *Culex* larvae were significantly more susceptible to *B. sphaericus* than to *B.t.i.*

Mosquito IGR Bioassays: Methoprene

Two formulations of Dianex EC were encapsulated on Biodac matrices to determine the controlled-delivery potential of methoprene in simulated shallow water habitats (ca. 7 cm deep) containing *Aedes, Anopheles* or *Culex* larvae. Granules were composed of 95.3% Biodac, 1.2% methoprene, and 3.5% coating complex A/B (Fig. 13 and 14) or 95.2% Biodac, 1.2% methoprene, and 3.6% coating complex C/E (Fig. 15) as a pretreatment and/or direct treatment to semipermanent or permanent water habitats against 2nd or 3rd instar *Aedes, Anopheles* or *Culex* larvae at application rates of 5.6 kg/ha.

Results of these comparative tests indicated that the controlled-delivery profiles and subsequent mosquito-controlling efficacy varied with the type of coating complex utilized in the granular compositions. Release rates were functions of the water quality, biodegradation and hydrolysis of the coating complex, and degradation of the carrier matrix over time. The larvicidal efficacy of granules containing coating complex A/B and C/E were comparable against *Aedes* larvae in pretreatment evaluations (i.e., 100% control of 3 broods for 90 to 92 days); however, coating complex C/E seemed to provide better long-term efficacy against *Aedes* larvae in semipermanent brackish water habitats or against *Culex* larvae in permanent fresh water habitats (i.e., 100% control of 4 broods for 94 to 97 days) when compared to similar tests with coating complex A/B (i.e., 100% control of 3 broods for 78 to 79 days). Previous bioassays against the *Aedes, Anopheles* or *Culex* species with a standard Altosand® (Sandoz Agro, Inc.) sand granule methoprene formulation resulted in control of only one larval brood.

Methoprene granules encapsulated with coating complex A/B (141 days old) produced significantly better mosquito-controlling efficacy against surface-orienting *Anopheles* larvae (Fig. 13) than against subsurface-orienting *Aedes* or *Culex* larvae (Fig. 14) that were challenged with 8 to 9 day old granules that were also encapsulated with coating complex A/B. The increase in the rate of mortality, number of broods controlled, and the duration of *Anopheles* control were attributed to the low specific gravity of Dianex EC (<1) in conjunction with the coating complex that was encapsulated on the Biodac granules.

Mortality data in these tests suggested that the coating complex-regulated controlled-delivery profiles resembled "pseudo" zero-order release kinetics against *Aedes* and *Culex* larvae. Based on the number of days to reach 100% control of a larval

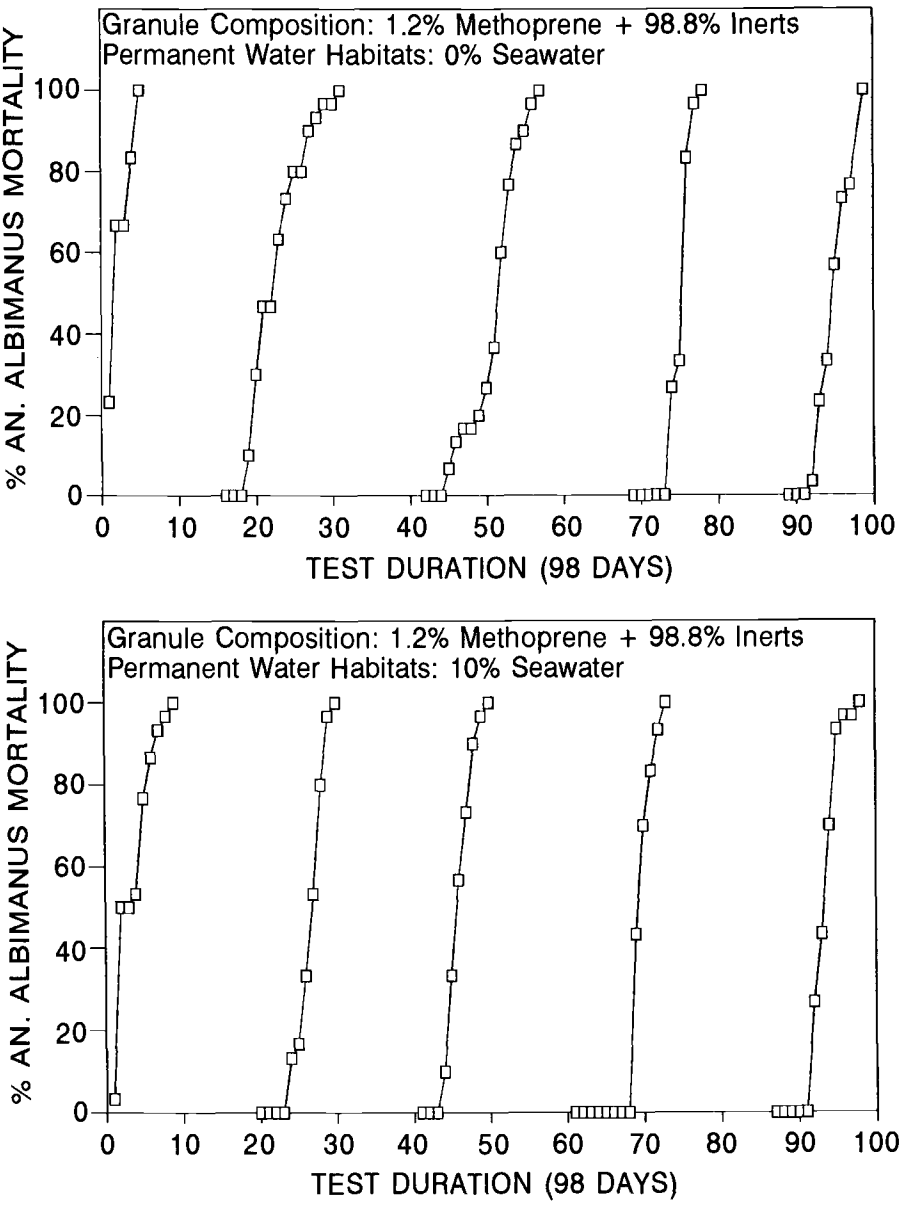


FIG. 13--Controlled delivery of Dianex EC form Biodac granules encapsulated with coating complex A/B.

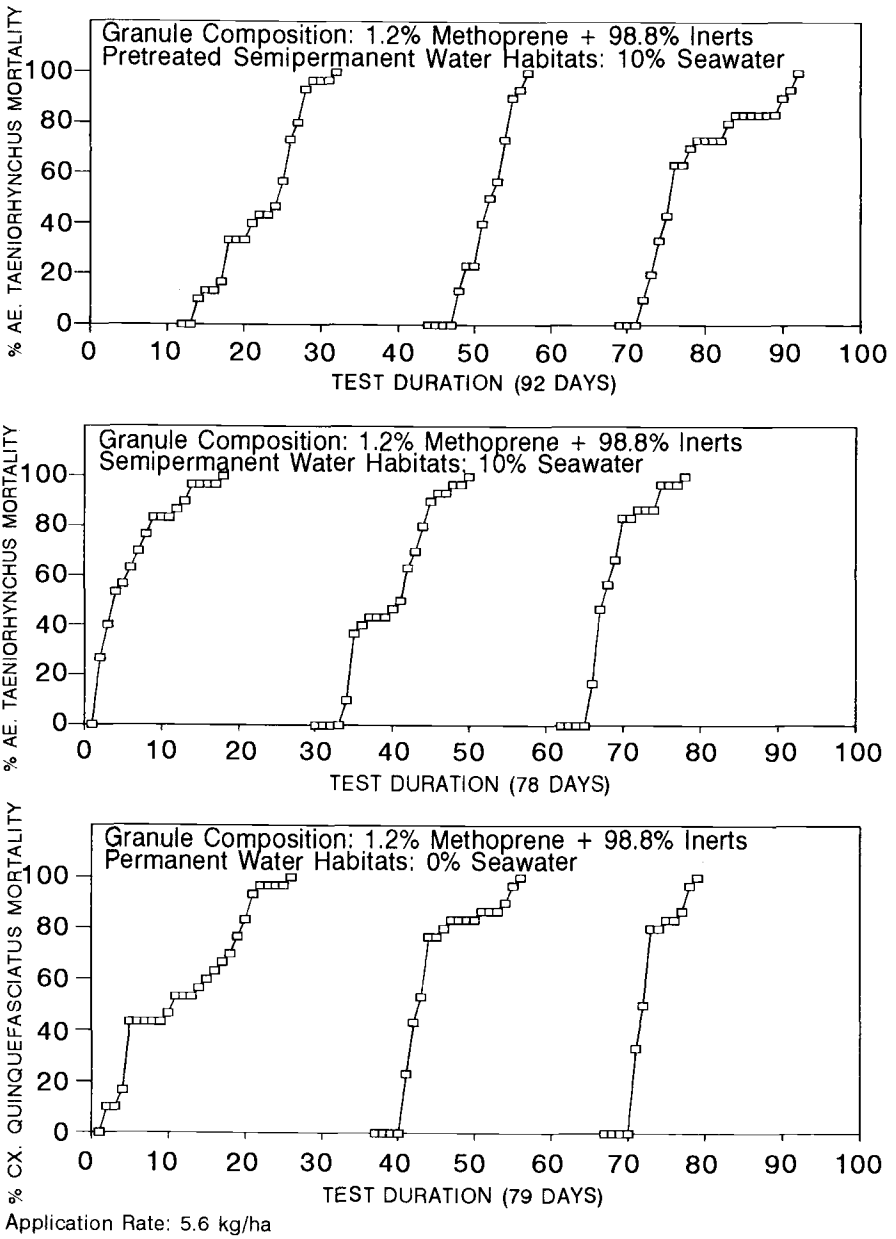


FIG. 14--Controlled delivery of Dianex EC from Biodac granules encapsulated with coating complex A/B.

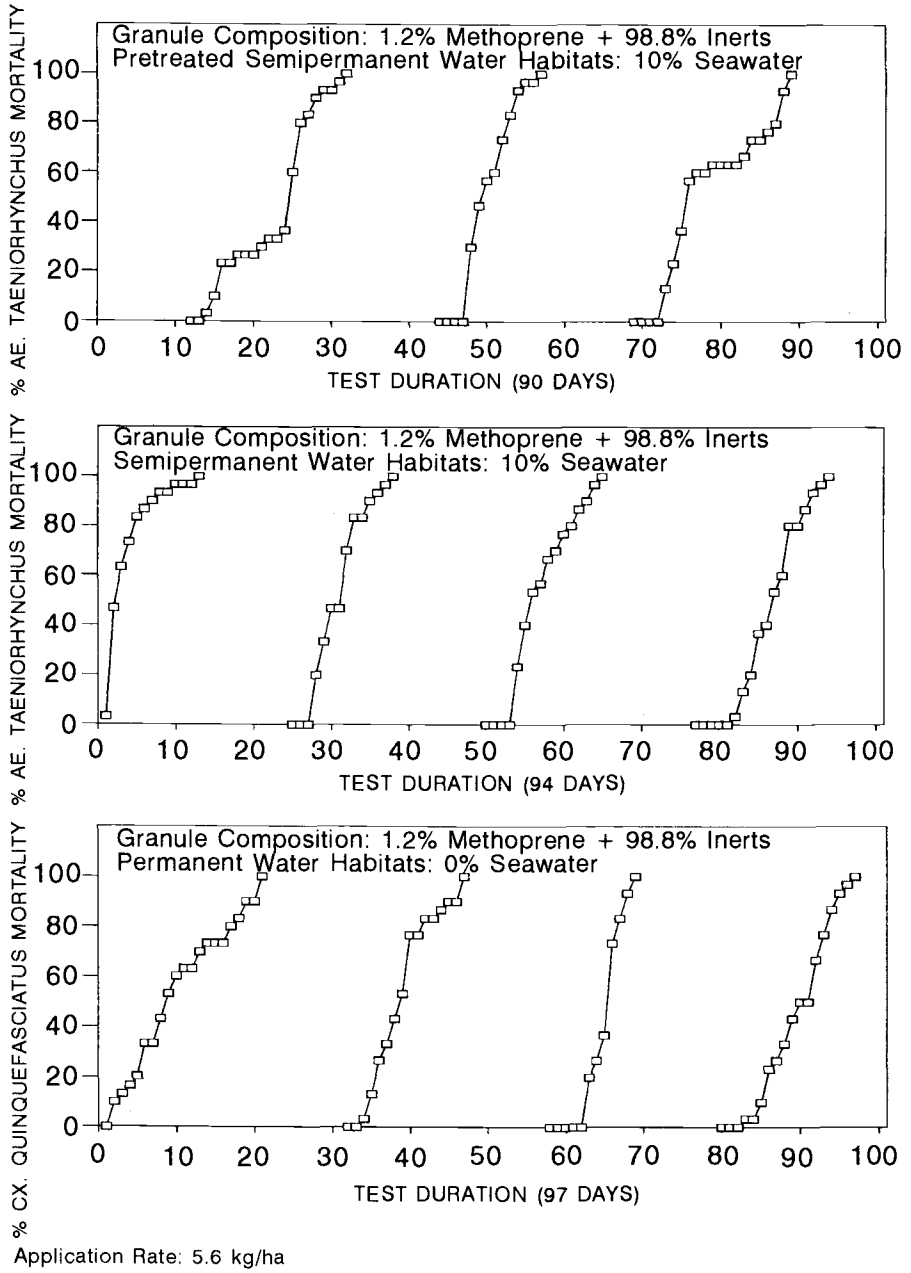


FIG. 15--Controlled delivery of Dianex EC from Biodac granules encapsulated with coating complex C/E.

brood at each transfer period, it appeared that the coating complexes A/B and C/E maintained relatively constant methoprene release rates throughout a test series. No pronounced "burst effect" was noted in the initial test periods. However, a modified "burst effect" was noted against the 1st brood of *Anopheles* larvae, particularly in the habitat containing 0% seawater (i.e., general "pseudo" zero-order kinetics).

Mosquito IGR Bioassays: Pyriproxyfen

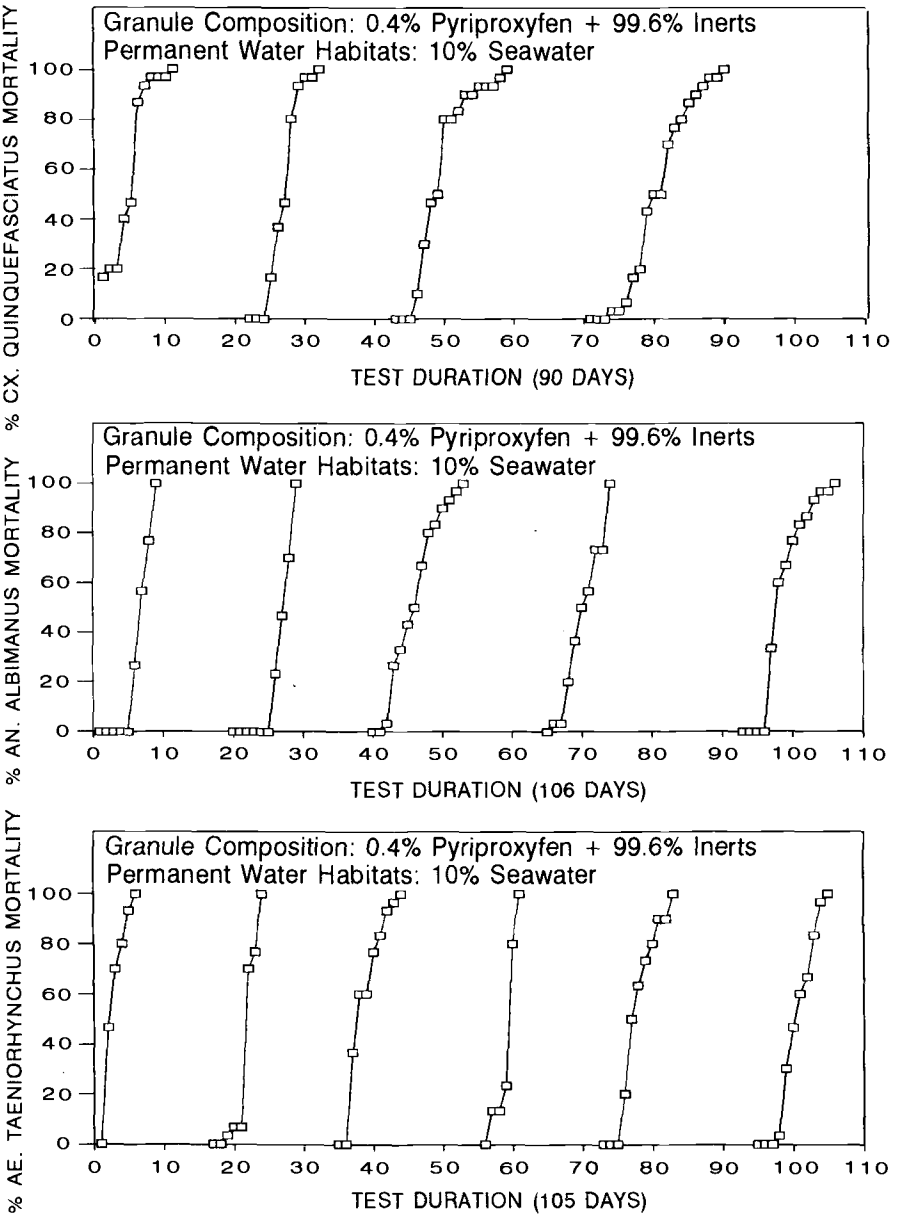
The long-term controlled-delivery potential of Nylar 10% EC from 43 to 44 day old granules composed of 95.6% Biodac, 0.4% pyriproxyfen, and 4.0% coating complex A/F (Fig. 16) or 96.2% Biodac 0.4% pyriproxyfen, and 3.4% coating complex A/B (Fig. 17) was evaluated against 1st or 2nd instar *Aedes, Anopheles*, or *Culex* larvae in shallow, permanent brackish water habitats (ca. 7 cm deep) at application rates of 5.6 kg/ha. Results of these tests showed some differences in species susceptibility to the controlled-delivery pyriproxyfen compositions. Differences in release rates were related to the biodegradation/hydrolysis of the coating complexes and decomposition of the Biodac granules

The comparative data showed that the submerged encapsulated granules could maintain controlled delivery of pyriproxyfen (specific gravity >1) to surface and subsurface areas of the water column to control *Anopheles, Aedes* or *Culex* larvae in their respective feeding/orientation zones.

Four larval broods of *Culex* were controlled with pyriproxyfen granules containing coating complex A/F or A/B over a 90 to 103 day test period, while 5 broods of *Anopheles* larvae were effectively controlled over a 105 or 106 day test period with pyriproxyfen granules encapsulated with coating complex A/F or A/B (Fig. 16 and 17). Six broods of *Aedes* larvae were completely controlled for 105 days with granules formulated with coating complex A/F (Fig. 16). A relatively consistent mortality trend was noted in all tests with both coating complexes and suggested that controlled delivery of pyriproxyfen from the Biodac granules followed a "pseudo" zero-order release profile that was similar to that observed for methoprene (Fig. 13 to 15).

Cockroach IGR Bioassays: Pyriproxyfen

A series of simulated terrestrial tests were also conducted against German cockroach nymphs with polymer-base controlled-delivery compositions of Nylar 10% EC (Fig. 18). Results of transfer bioassays with IGR-bait formulations composed of 99.0% polymer, 0.5% pyriproxyfen, and 0.5% bait complex indicated that the 3 types of bait stations (i.e., extruded chambers, continuous films, or coated vials) were effective in producing growth regulator effects (i.e., twisted wing/dark pigmentation abnormalities) in 100% of the cockroach populations for at least 9 months. Observations suggested that the slow cockroach-controlling efficacy was based on contact and/or ingestion of the IGR-bait matrix. Nymphs in each of the 5 broods were frequently seen resting on or in each type of bait station and eating portions of bait stations that were fabricated into extruded chambers and continuous films. Observations of IGR-induced abnormalities indicated that controlled-delivery of pyriproxyfen from each type of bait station followed "pseudo" zero-order kinetics, with a mild



Application Rate: 5.6 kg/ha

FIG. 16--Controlled delivery of Nylar 10% EC from Biodac granules encapsulated with coating complex A/F.

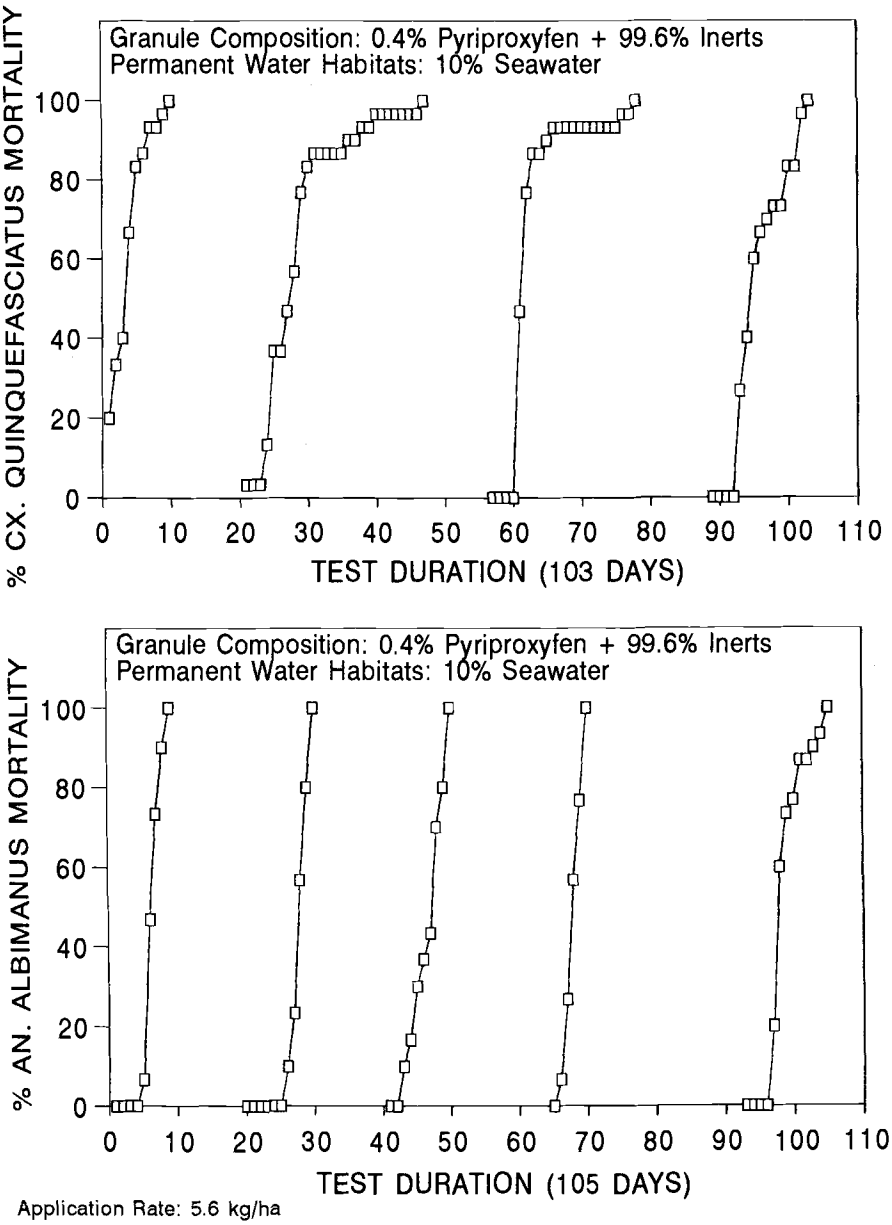


FIG. 17--Controlled delivery of Nylar10% EC from Biodac granules encapsulated with coating complex A/B.

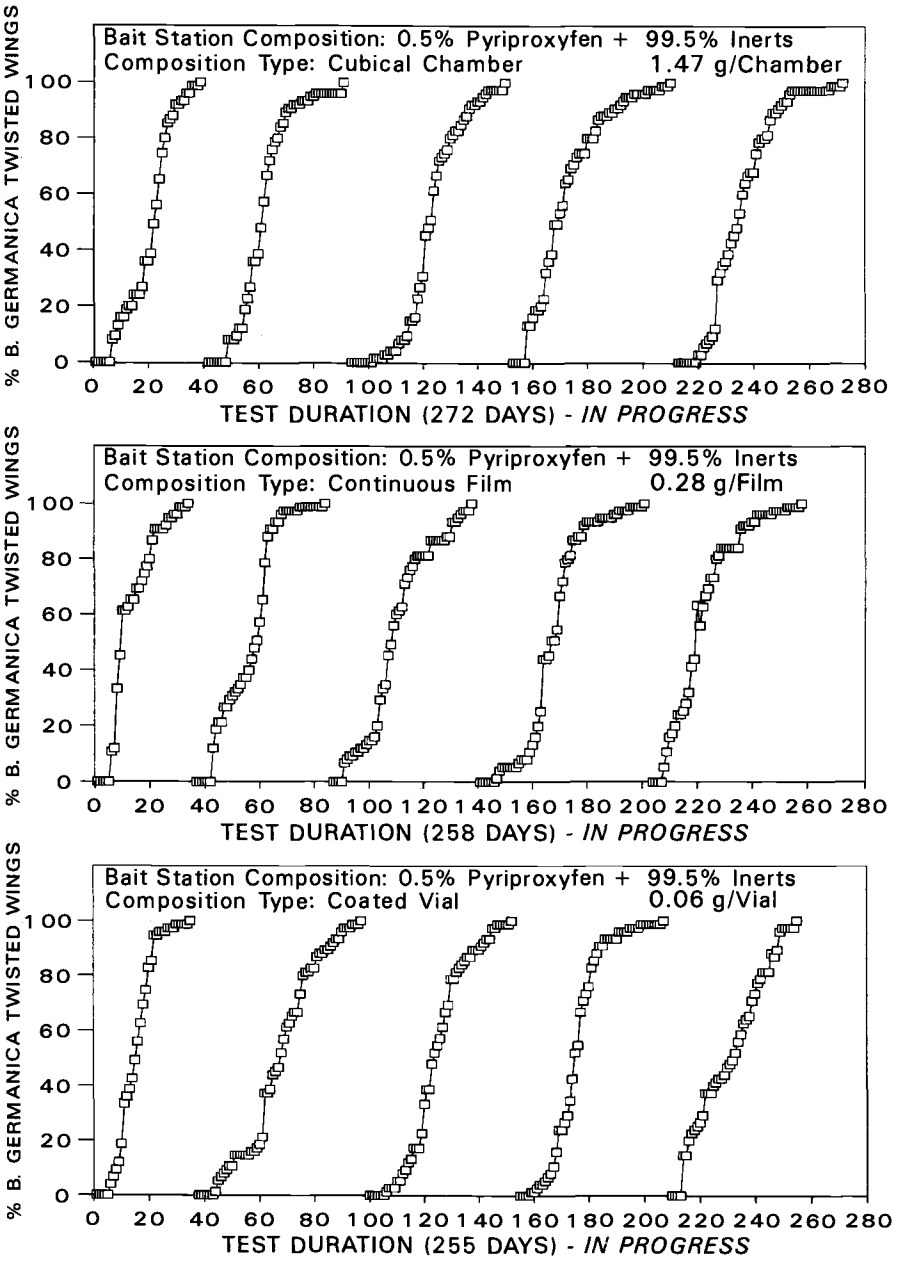


FIG. 18--Controlled delivery of Nylar 10% EC from polymer-base bait stations.

"burst effect" being observed in the initial test periods. Transfer bioassays with the 3 types of bait stations are in progress and are scheduled to be completed at the end of a one-year test period.

Mosquito-Controlling Granules: Operational Aspects

A Bell® 47/Soloy helicopter (Bell Helicopter, Fort Worth, TX) equipped with a Isolair® granule application system (Model 4500-47; Isolair, Rhododendron, OR) was used to evaluate the aerial application potential of Biodac and corn cob granules encapsulated with *B.t.i., B. sphaericus,* methoprene, or pyriproxyfen. Results of ground tests with ca. 27 to 91 kg of each coating complex-biopesticide or IGR formulation of corn cob or Biodac granules indicated that all encapsulated granular biopesticide and IGR compositions were flowable and dust free when applied at application rates of 5.6, 7.8, and 11.2 kg/ha.

Preliminary small-plot evaluations with the above indicated helicopter application system against 1st to 3rd instar *Ae. taeniorhynchus* and/or *Cx. nigripalpus* in impounded areas of black mangrove habitats of Lee County, Florida, with corn cob granules encapsulated with Vectobac TP (8000 ITU/mg) or Vectolex TP (5.6, 6.9 or 9.3% bacteria) and coating complex A/B or A/F at application rates of 5.6, 7.8 and 11.2 kg/ha are currently in progress. Corn cob granules were selected over Biodac granules for initial tests since corn cob granules could be encapsulated with higher levels of *B.t.i.* Small-plot field trials against several species of mosquitoes with corn cob or Biodac-base Vectobac TP or Vectolex TP encapsulated with coating complex A/B or A/F are also being conducted in several areas of the U.S. Initial results are promising and indicated the controlled-delivery potential of these encapsulated granular biopesticide or IGR compositions for extended control of mosquito larvae in a variety of habitats.

Scale-up manufacturing tests have indicated the transition from laboratory to commercial production of insecticide-encapsulated corn cob or Biodac granules would not be difficult with conventional equipment. E.P.A. registration of the coatings used in the coating complexes as inert ingredients for use with pesticides is in progress. It is also interesting to note that in addition to biolarvicides and growth regulators, corn cob and Biodac granules have been encapsulated with a variety of organophosphates (e.g., chlorpyrifos, diazinon, temephos), carbamates (e.g., propoxur), pyrethrins (pyrocide), and herbicides (e.g., endothall, glyphosate) used in aquatic or terrestrial pest control (Levy et al. Unpublished).

CONCLUSION

Bioassays have shown that Vectobac TP, Bactimos PP, Vectolex TP, Dianex EC, or Nylar 10% EC can be encapsulated via a variety of coating complexes on Biodac or corn cob granules for prolonged controlled delivery of *B.t.i., B.sphaericus,* methoprene, or pyripoxyfen for control of larvae of *Ae. taeniorhynchus, An. albimanus,* or *Cx. quinquefasciatus* for about 3 months in permanent or semipermanent, fresh water, brackish water or seawater habitats, or in pretreated semipermanent brackish water habitats. Comparable controlled-release profiles were also obtained in bioassays

against larvae of *Aedes, Anopheles,* and *Culex* species with encapsulated silicon dioxide, wood chips, and sand formulations of Acrobe TP.

Matricap controlled-delivery Biodac or corn cob compositions of *B.t.i., B. sphaericus,* methoprene, or pyriproxyfen were flowable and dust free, and were formulated with a specific coating complex and carrier to target mosquito larvae that orient and/or feed in surface or subsurface areas of a water column. The initial orientation of delivery of *B.t.i., B. sphaericus,* methoprene or pyriproxyfen at or below the surface of the water was a function of the specific gravity of the carrier matrix; however, the rate and duration of controlled targeted delivery of a bioactive agent from a solid carrier were shown to be functions of the type, concentration, specific gravity, and solubility of the 2 coating agents that were combined into a coating complex. Polymer and nonpolymer coating agents comprising a coating complex were selected on the basis of the type of bioactive agent, the type of matrix selected as a carrier, the specific orientation of the target mosquito in the aquatic habitat, and the proposed duration of delivery.

Our simulated pretreatment and direct treatment studies against *Aedes, Anopheles,* or *Culex* larvae with the coating complex-regulated granular compositions applied at 5.6 kg/ha to shallow fresh water, brackish water, or seawater habitats have suggested that prolonged submergence or intermittent submergence and drying did not inhibit the ability of the coating complexes from effectively regulating the release of *B.t.i., B. sphaericus,* methoprene, or pyriproxyfen from the solid carriers for ca. 3 months. Similar results were also obtained in deep water bioassays conducted against mixed populations of *Aedes* and *Anopheles* larvae in 10 and 50% seawater at 7.8 and 11.2 kg/ha.

The simplicity of the Matricap controlled-delivery formulation system has indicated that encapsulation of a biolarvicide or IGR can be accomplished on a District level as a tank mix as well as on a commercial basis. Simple mixing equipment (e.g., cement mixer) can be used to blend a granular carrier with a coating complex and bioactive agent formulation to produce dry flowable granules. It should be noted that the granular carriers and most of the coating complexes utilized in this study are registered as inert ingredients by the E.P.A. Inert registration applications have been filed with the E.P.A. for all unregistered coatings utilized in the coating complexes. Biopesticide products are E.P.A. registered for mosquito control while the active ingredients in the IGRs are registered for use in mosquito or cockroach control. Therefore, it is anticipated that E.P.A. approval of Matricap compositions consisting of regis- tered active and inert ingredients should be relatively easy to obtain. Preliminary helicopter system evaluations and initial reports from field trials have confirmed our laboratory results, and have indicated the operational mosquito-control potential of the encapsulated controlled-delivery biopesticide and IGR compositions.

Polymer-base controlled-delivery formulations of pyriproxyfen and a bait complex were also shown to provide long-term efficacy against nymphs of the German cockroach. Bait station components were shown to be capable of being extruded into cubical chambers, formed into continuous films or sheets, and coated on solid surfaces. The application of these solid compositions for the sustained control of other terrestrial pests such as ants, termites, and fleas is being considered.

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Thomas J. Wacek [1]

LIQUID FORMULATIONS OF NON-SPORE FORMING MICROORGANISMS

REFERENCE: Wacek, T. J., **"Liquid Formulations of Non-Spore Forming Microorganisms,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: Biological formulations have evolved from the use of organic based solids such as peat as a preservative and delivery system to water based carriers. Water based formulations provide more convenience for the end user. Innovations in media preparation, stabilizing agents and packaging have led to stable formulations of non-spore forming microorganisms.

Liquid media for biological formulations contain either minimal nutrient levels packaged in air impermeable containers, or very high nutrient levels packaged in oxygen permeable plastic. Soluble polysaccharides (particularly alginates) can be used as stabilizing agents. Also, concentrated pastes of cells which are kept frozen until use, lyophilized powders, and oil suspensions are available. Two important points for the successful liquid formulation of a biological are long term stability (1 to 2 years) and effectivity at the site of use.

KEYWORDS: biologicals, inoculants, Rhizobia, alginates, peat, water formulation

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This review of liquid formulations of microorganisms concerns non-spore forming microorganisms. Non-spore forming microorganisms do not have the survival mechanisms of spores and thus offer formulation challenges especially with regard to stability. Examples of non-spore forming microorganisms used as nitrogen fixing inoculants, as plant disease antagonists, and as plant growth promoters are *Rhizobium sp., Bradyrhizobium sp., Pseudomonas sp., Arthrobacter sp., Azospirillum sp., and Serratia sp.*.

Non-spore forming microorganisms have been traditionally formulated into diverse solid carriers. As an example, *Rhizobium species* nitrogen fixing inoculants have been in use for almost one hundred years. These formulations have been in solid carriers such as peat, charcoal, clay, bagasse, and just about any available high organic containing solid.

1 Dir. of R & D, Urbana Laboratories, St. Joseph, MO 64501

However, during the last ten years, most formulation development for *Rhizobium sp.* inoculants, and for other non-spore forming microorganisms, has been in liquid media - either aqueous or mineral oil. The main impetus for this is that liquid formulations are more convenient for many of the customers of the end products. The formulation objective is either to allow for slow, continual growth of the organism in the liquid or to suspend growth to a starved or survival level.

Liquid formulation types:

1. Frozen concentrates in water.
2. Water suspension packaged in anaerobic conditions
3. Water suspension packaged in oxygen permeable plastic.
4. Mineral or vegetable oil suspensions.

The concentration in the frozen concentrate is typically 10^{11} to 10^{12} viable cells per gram. Dilution rates of 1/100 to 1/1000 are used when the product is thawed. This formulation is the simplest if distribution channels are short. The formulation challenge is to find the proper cryoprotectant agents which will insure the greatest viability during freezing and thawing. Glycerol, dextran, mannitol, proteins, and powdered milk are amoung the cryoprotectant agents used at concentrations from 5 to 20% w/w (Gherna 1994). The type and concentration of cryoprotective agent needed will be specific for each particular microorganism.

In liquid aqueous formulations, the medium in which the microorganism is grown is of great importance. Generally a growth medium has a carbon source (e.g. glucose, sucrose, mannitol, glycerol) at a concentration of 1 to 5% w/v, a nitrogen source (e.g. yeast extract, KNO_3, NH_4NO_3) at 0.1 to 0.2% w/v and a mixed salt content generally with KH_2PO_4, K_2HPO_4, $MgSO_4$, and NaCl each at 0.03 to 0.2% w/v for pH buffering and nutrition. For starvation or survivial maintenance and/or anaerobic packaging, the concentration of components in the medium is generally decreased by a factor of 1/100 to 1/10,000 (Crist et al. 1984). For maximum aerobic growth, and oxygen permeable packaging, often the carbon source is increased up to 10% w/v in the medium.

Also, stabilizing agents have been added at the time of packaging. Examples are non-cross linked polysaccharides such as alginate and xanthan gums (Charley 1994, Dommergues et al. 1979; Jung et al. 1982). Xanthan gums have the advantage of thickening the formulation which is important for products which are applied directly to seed.

Liquid formulations need to have long term viability (traditionally thought of as viable for 1 year at normal storage temperatures) and effectivity at the time of use. With regard to viability most formulations will have approximately 10^9 viable cells per ml. At the end of one year of storage, formulations in oxygen permeable packaging will not have decreased in cell concentration while cells in anaerobic packaging will generally have decreased one log unit to 10^8 cells per ml. A decrease of one log unit (90%) is considered to be acceptable; or conversely, microbial cell concentrations at the time of formulation and production are typically one log higher than needed for optimum effectivity.

Oil suspensions (Kremer and Peterson 1983, Johnston 1962) have been used for Rhizobial inoculants to support increased viability in the product and when the product is

applied to seed. These oil suspensions make use of lyophilized cells. Oil suspensions have the advantage of longer viability when applied to the host or target area because of less dessication. Non-spore forming microorganisms need to maintain some level of moisture to remain viable even when in a 'dried' or lyophilized state. The disadvantage of oil suspensions is that they can be difficult to handle for the end user who is generally more accustomed to handling and spraying aqueous or emulsificable concentrates. Also, lyophilized cells of and by themselves can be used much like frozen concentrated cells -- i.e. a long term viable solid matrix which can be turned into a liquid suspension at the time of use.

The use of an invert emulsion for the application of a mycoherbicide (Amsellem et al. 1990) is a very good example of formulation for maximum effectivity at the time and place of use. It is important in this regard to realize that microorganisms, unlike chemicals, require an induction period. Specifically they need to be growing to be effective, and also may need specific conditions for infection.

As a further note for *Rhizobium* inoculants (be they liquid or solid), the point of application is often the seed and thus the seed is the carrier of the microorganism into the soil where the microorganism infects the root. Thus, there is another storage time for the product after it is out of the original container if it is applied to the seed more than several hours prior to planting. Liquid formulations have generally not worked as well as solid matrix formulations for these types of applications (Davidson and Reuszer 1978). This lack of effectiveness of liquid formulations on seed is primarily because of greater dessication, though the oil carriers do provide some protection for the microorganism from dessication in these types of applications.

Two final points to remember with regard to the liquid formulation of biological, non-spore forming microorganisms: One is that the formulations developed to date must only contain the microorganism desired and must not be contaminated with other organisms which either consume or out-compete the organism of choice. The second point is that the activity and survivability of the organism can depend greatly on what the microorganism is initially grown in. For example, Rhizobia grown in whey (Bissonnette and Lalande 1988) exhibited greater survivability when subsequently exposed to physical stress. Product formulation and storage is a 'stress' for these microorganisms. This serves as a reminder that microorganisms are 'what they eat' when it comes to their formulation stability and activity. Each genus and species will have their own particular requirements. There are no specific suggestions for media development other than that high levels of the carbon source (or a high C/N ratio) in the growth medium can lead to higher levels of internal bacterial storage compounds such as polyhydroxybutyrate (Dawes 1984).

SUMMARY

Liquid formulations of biologicals are becoming the product of choice for the consumer. However, non-spore forming microorganisms are more difficult to formulate than are spore forming organisms such as *Bacillus species.* Spores are natural survival vessels which can be readily dried and resuspended in liquid delivery systems at the time of

use. This drying and rehydration is not possible with non-spore formers, though two relatively simple methods of duplicating spore type survival are freezing and lyophilization.

The true liquid formulations of biologicals contain either aerobically grown cells packaged in oxygen permeable packaging, or cells grown in minimal media and packaged in non permeable containers.

Biological microorganisms are not like chemicals in that how they are grown prior to formulation is as important as what the final formulation contains. Particularly, the carbon source, the C/N ratio, and the concentration of nutrients in the growth medium are important for the viability and effectivity of the final biological formulation.

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Stefan T. Jaronski[1]

NEW PARADIGMS IN FORMULATING MYCOINSECTICIDES

REFERENCE: Jaronski, S. T., ''**New Paradigms in Formulating Mycoinsecticides,**'' Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: The advent of commercial mycoinsecticides – insect pathogenic fungi used as insecticides – creates new paradigms in formulating these organisms. The "active ingredient" (conidium, blastospore, or preserved mycelium) must be kept alive and infectious yet dormant in the formulation for a commercially acceptable shelf-life under ambient conditions. The inherent conidial hydrophobicity of most of the current candidate fungi must be overcome for many applications without killing the fungus. Formulation additives or spray adjuvants cannot interfere with the infection process. The fungal active ingredient must be kept alive as long as possible on the plant surface in the face of lethal solar irradiation.

KEYWORDS: Mycoinsecticides, *Beauveria, Metarhizium, Paecilomyces, Verticillium, Nomuraea, Aschersonia*, conidia, biological control, fungi, formulations.

INTRODUCTION

Mycoinsecticides–fungal pathogens of insects developed as insecticides–are finally coming into their own. There are at least 17 commercial fungal products in the world today. Three fungi, two strains of *Beauveria bassiana* and one *Metarhizium anisopliae*, have become registered products in the U.S. within the past two years.

The choice of fungal candidates is currently limited to seven species of fungi within the Deuteromycetes (Imperfect Fungi): *B. bassiana,*

[1] Senior Scientist and Manager, Biopesticides Research & Development, Mycotech Corporation, 630 Utah Ave., Butte MT 59701.

B. *brongniartti, Hirsutella thompsoni, M. anisopliae, M. flavoviride, Paecilomyces fumosoroseus, P. farinosus, Verticillium lecanii, Nomuraea rileyi,* and *Aschersonia aleyrodis.* Some of these have been well known for several hundred years (e.g., *B. bassiana* and *M. anisopliae*), others for only a few decades (*M. flavoviride*). The scientific literature is abundant with research reports about these fungi. One current biological database lists more than 700 papers concerning *B. bassiana* since 1970.

These mycoinsecticides present a new paradigm for the user. While they can be used in a manner similar to conventional pesticides, i.e., as foliar sprays, soil drenches, granules, baits, there are salient features unique to these microbial pest control agents. (1) The mycoinsecticides are *living, infectious* agents. (2) Since these fungi infect insects by penetrating through the insect cuticle, *direct contact* between the infectious unit (conidium or spore) and that cuticle is necessary. Thus, these mycoinsecticides act as classical contact insecticides. (3) Speed of action by entomogenous fungi is slower than most conventional chemicals. These fungi kill their insect hosts only after 3 to 7 days. Conceptual approaches to their use are analogous to the insect growth regulators. (4) These fungi have the potential to recycle after initial application, if environmental conditions are favorable.

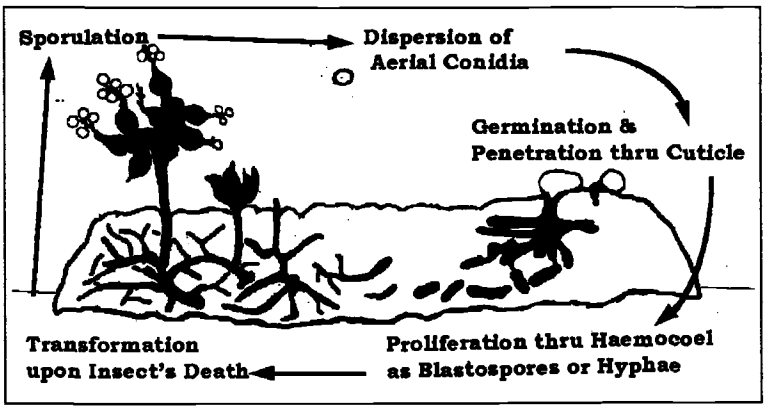

FIG. 1--Schematic life cycle of an insect pathogenic deuteromycete fungus, exemplified by *Beauveria bassiana.*

Understanding the life cycle of these entomopathogenic fungi is important for grasping not only how they should be used but also how these fungi need to be formulated. A typical deuteromycete life cycle is depicted schematically in Fig. 1. All the fungi listed above have life cycles that follow this general theme. In nature, the infectious unit is the aerial conidium ("spore"). It is ordinarily dispersed by rain and/or air currents and either directly lands on an insect's cuticle or is picked up by the insect from the insect's environment (soil or plant surface) during feeding or movement. Once on the cuticle, the spore responds to chemical cues present in the insect's waxy epicuticle and germinates within 8-16 hours. A germination hypha (and sometimes more

specialized structures) is produced during germination and the fungus penetrates the insect's cuticle using a combination of mechanical pressure and a mixture of enzymes (lipases, proteases, chitinases). Once the growing hyphae reach the haemocoel (body cavity) of the insect, usually within 24 hours of germination, the fungus rapidly proliferates through the insect. Growth can be in the form of mycelium or yeast-like blastospores. Some of the entomogenous fungi, e.g., *B. bassiana*, kill their hosts by depleting the insect's energy reserves ("physiological starvation"); others, such as *M. anisopliae*, produce a variety of toxic metabolites that act as neurotoxins (e.g., the destruxins) or general metabolic disruptors (the viridoxins) (Fargues et al. 1985; Gupta et al. 1993). The infected insect dies within 2-7 days. Immediately upon death of the insect and initial desiccation of the cadaver, the fungus differentiates into specialized reproductive structures, which in turn give rise to a new generation of conidia. In most cases sporulation of the fungus occurs on the exterior of the insect, giving the cadavers a distinctive appearance. In a few cases sporulation can be internal. A lethal dose of conidia for a susceptible insect can be anywhere from <10 for aphids [Vandenberg, J., personal communication) to several thousand for larger insects such as orthopterans (Mycotech, unpublished data). A more specific review of infection and pathogenesis by entomogenous deuteromycete fungi may be found in Zacharuk (1981).

Several different stages in the life cycle of these fungi are potential candidates for commercial mycoinsecticides. The aerial conidium is produced on erect structures arising from an insect cadaver, agar media or solid substrate. This spore is the agent of fungal dissemination and infection in nature. An insect cadaver can produce 10^7-10^8 conidia; commercial production has achieved 1-5 x 10^{13} conidia per Kg substrate (Bradley et al. 1992). The conidium has evolved to be the natural initiator of dispersal and infection, and as such is relatively resistant to environmental factors, particularly desiccation. Aerial conidia can maintain viability and infectivity for considerable lengths of time, especially at lower ambient temperatures.

The blastospore is the unit of a yeast-like phase of vegetative growth either inside the insect hemolymph or in submerged, liquid culture, particularly for *B. bassiana, M. anisopliae, M. flavoviride, Paecilomyces fumosoroseus*, and *P. farinosus*. It is infectious, germinating faster than an aerial conidium, but it is much more environmentally sensitive, particularly to desiccation (Bidochka et al. 1987; Kleespies and Zimmermann 1994). At present only a few months of shelf life, under refrigerated conditions, is possible [M. Jackson, unpublished data]. There is a report of successful bench scale spray drying of *M. anisopliae* blastospores [G. Zimmerman, personal communication], but on a larger scale this approach is still unproved.

The microcycle conidia are conidia-like structures produced in submerged, liquid culture under certain nutritional conditions (Thomas et al. 1986), and possibly only by certain isolates of *B. bassiana*. They are infectious but are somewhat morphologically and biochemically different from aerial conidia and they seem to be less environmentally resistant (Hegedus et al. 1992).

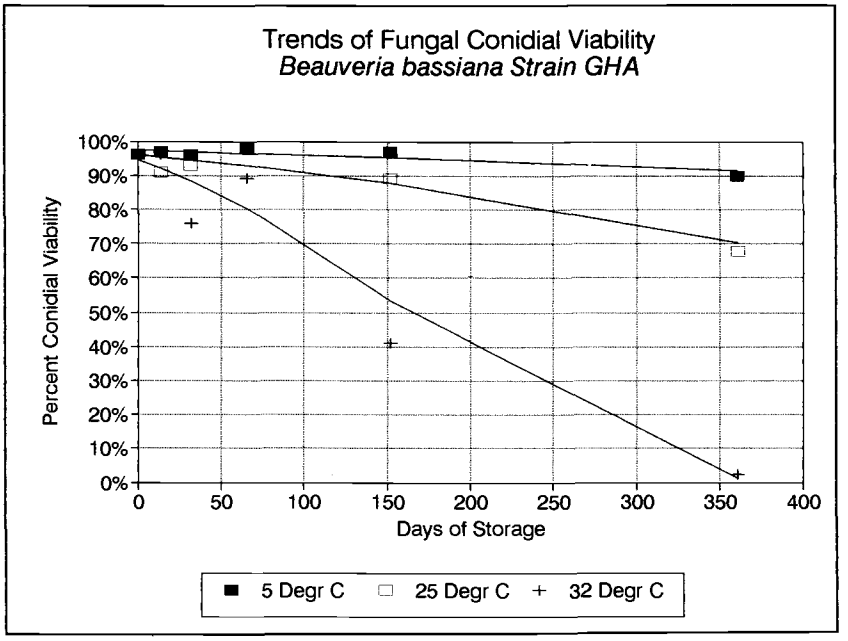

FIG. 2--Conidial viability trends for *Beauveria bassiana* Strain GHA stored as dry conidial powders at 5°, 25° or 32° C.

So how does one keep a fungal spore alive, yet dormant, for a satisfactory length of time? The answer lies in understanding the cues that initiate conidial germination (Fig. 3). A basic premise is that shortened shelf life is primarily due to spores slowly initiating germination, but dying as the succession of cues and requirements to complete germination are not fulfilled in the mycoinsecticide container.

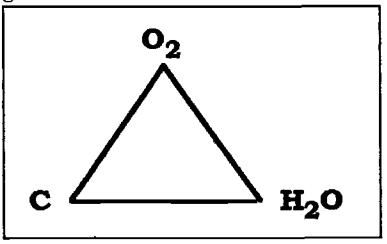

FIG. 3--Conceptual relationship among factors stimulating conidial germination for entomogenous fungi: oxygen (O_2), nutrients (C), and water (H_2O).

One concept of this phenomenon derives from the fire prevention triangle. Three things are necessary for combustion: fuel, oxygen, and an ignition source, linked conceptually to each other in a triangle. If you eliminate one corner of the triangle you can prevent fire. For conidial germination, the three require-ments for combustion, i.e., germination, translate to a nutrient source, oxygen and water. Eliminate one corner of the germination triangle and you can prevent germination. But one has to avoid killing the conidium at the same time. This is tantamount to keeping an ember glowing yet preventing a full fire.

Nutrients, the first leg of the germination triangle, are very dif-

ficult to exclude from the conidial powder. Even though these fungi have evolved as pathogens of insects, they still retain a saprophytic nature. Simple carbohydrates and inorganic forms of nitrogen are sufficient for conidial germination, mycelial growth and sporulation. As little as 6 nM glucose can stimulate and support conidial germination in *B. bassiana* (Smith and Grula 1982). Typical mass production harvest does not eliminate residual nutrients down to this level, much less below it. Given sufficient moisture and oxygen, spores will slowly initiate germination in the product package.

Excluding oxygen, the triangle's second leg, ignores the fact that spores are living; are metabolizing (albeit at a very low level), and require oxygen for prolonged survival. Measures that exclude oxygen, e.g., vacuum-packing or replacing the container head space with nitrogen or carbon dioxide, do not yield good shelf life, contrary to some opposite claims; shortened longevity is often the result (Jaronski, unpublished data).

The third leg of the triangle is moisture. Liquid water, at least on the level of a molecular film, is necessary to convey chemical cues to the conidium and initiate germination. Excluding water, or reducing the water activity (A_w) below a certain level, can prevent germination. This phenomenon has been reported for *B. bassiana* (Jung and Mugnier 1989), *V. lecanii* (Chandler et al 1994), and *M. flavoviride* (Hedgecock et al. 1995) and is the subject of at least one patent application. Of course, removing molecular water from a conidial powder can damage conidia and greatly shorten their longevity.

There is a complication. Intrinsic conidial longevity under even optimum conditions can be unique to a fungal species or even an isolate within a species. Examples of both situations is presented in Table 1. Considerable variability in the trends of viability over time at 25 °C. exists among 7 isolates of *Metarhizium flavoviride* (only two are shown in Table 1); 1 *M. anisopliae* isolate; 12 isolates of *B. bassiana* (only six shown) from Madagascar; and Strain GHA of *B. bassiana*. Our criterion of tolerable loss in viability is 23% (20% ± 3%). Within each species, isolates have distinct survival patterns reflective of inherent physiological differences. None of the *M. flavoviride* isolates or the *M. anisopliae* isolate had satisfactory shelf life as powders even at 25 °C.; several of the *Beauveria* isolates (e.g., MAD 12) also had a short usable shelf life, while a number of the other *B. bassiana* isolates (e.g., isolates MAD 4, MAD 14) maintained suitable viability for about three months. In contrast, Strain GHA maintained satisfactory viability for over 9 months. In Mycotech's Oil Flowable formulation, the *Metarhizium* isolates fared better but their conidial viabilities remained above 77% for only 1 or 2 months. Among the *B. bassiana* isolates, MAD11 was rapidly killed as an OF, and only MAD 14 and S2b1 of those shown still had satisfactory viabilities at 9 months. Strain GHA lost only 4% viability in that time.

Shelf life is also affected by the initial moisture of the conidial powder (Hedgecock et al 1995), the drying speed during harvest of conidia [Mycotech Corp., unpublished data], and the nutrition of the fungus during production (Hallsworth and Magan 199a, 1994b). This last factor

has only recently been identified. Manipulating polyol content within the conidia of *B. bassiana*, *M. anisopliae* and *P. farinosus* can extend the range of water availability over which fungal propagules can germinate. Polyol contents can be affected by fungal nutrition during vegetative growth *in vitro*. This phenomenon can have important implications for shelf-life (Hallsworth and Magan 1994b).

TABLE 1--Conidial viability of selected *Metarhizium flavoviride*, *M. anisopliae* and *Beauveria bassiana* isolates as either dry technical powders and oil flowable (OF) formulations at 25 °C.

		Conidial Viability of Dry Powders Days of Incubation						
Isolate	Species[1]	0	27	55	83	138	210	266
MAD 1	Mfv	97%	52%	15%	0%	...	...	...
MAD 5	Ma	92%	40%	12%	0%	...	...	...
MAD 9	Mfv	88%	28%	2%	0%	...	...	...
MAD 4	Bb	99%	98%	94%	85%	44%	51%	34%
MAD 11	Bb	99%	97%	96%	88%	74%	50%	35%
MAD 12	Bb	99%	95%	69%	23%	...	...	...
MAD 14	Bb	99%	94%	97%	95%	59%	60%	60%
MAD 19	Bb	98%	81%	59%	61%	22%	...	...
S2b1	Bb	93%	88%	81%	82%	76%	69%	37%
GHA	Bb	97%	95%	94%	96%	92%	90%	89%

		Conidial Viability in OF Oil Carrier Days of Incubation						
Isolate	Species[1]	0	28	56	83	138	210	266
MAD 1	Mfv	97%	96%	80%	41%	2%	...	...
MAD 5	Ma	97%	96%	48%	34%	0%	...	...
MAD 9	Mfv	96%	92%	38%	27%	2%	...	...
MAD 4	Bb	98%	98%	87%	89%	81%	72%	65%
MAD11	Bb	98%	12%	0%	0%	...	...	...
MAD12	Bb	97%	93%	73%	71%	61%	50%	37%
MAD14	Bb	98%	94%	85%	84%	90%	88%	79%
MAD19	Bb	88%	93%	86%	81%	80%	54%	40%
S2b1	Bb	93%	97%	98%	95%	86%	83%	85%
GHA	Bb	97%	96%	98%	95%	96%	95%	93%

[1] Abbreviations: Mfv, *Metarhizium flavoviride;* Ma, *M. anisopliae;* Bb, *Beauveria bassiana*

One implication of such data is that conidial longevity under standardized, controlled conditions has to be included in screens of candidate fungi, along with efficacy (virulence), and efficiency of conidial production.

There is a corollary to the need of keeping conidia alive in a formulation. That is the need to have minimal microbial contamination in the end product to satisfy EPA requirements, while avoiding use of

antimicrobials. While technical powders can be prepared relatively contaminant-free (contaminant number 10^{-1} of the conidial count), formulation ingredients can often introduce substantial numbers of microorganisms into the end product. This can be especially critical in aqueous formulations, but bacterial contamination can be present even in emulsifiable oils. Some dry inerts can also be heavily contaminated with diverse bacteria, *Aspergillus* spp. and *Penicillium* spp. With many *Bacillus thuringiensis* products, antimicrobial additives easily correct for adventitious contamination, to protect the key active ingredient, a protien. Almost all antimicrobials affect fungal conidia, however, particularly fungistats and fungicides. Use of pharmaceutical grade adjuvants and inerts is often cost prohibitive.

Paradigm 2: Adjuvants Need to Overcome Conidial Hydrophobicity Without Killing The Fungus

The second new paradigm derives from the fact that the fungal conidia of the most important fungi -- *Beauveria* spp., *Metarhizium* spp., and *Paecilomyces* spp. -- are extremely hydrophobic, yet for most uses, must be suspended in water carrier for application onto crops. Hydrophobicity is due to glycoprotein (hydrophobin) arranged in overlapping rodlets on the exterior of conidia (Bidochka et al. 1995). The result is that conidia are very difficult to suspend in water without the use of surfactants.

TABLE 2--Effect of selected spray tank adjuvants on the viability of conidia of *Beauveria bassiana*, *Metarhizium flavoviride*, and *Paecilomyces fumosoroseus*. The adjuvants were used at the concentrations indicated in a simulated tank mix with conidial powders. Viabilities were measured after four hours at 25 °C.

		Conidial Viability		
Adjuvant	Conc.	*B. bassiana* Strain GHA	*M. flavoviride* Strain MAD9	*P. fumosoroseus* Strain 612
Tween 80®	0.1%	98%	92%	95%
Silwet L77®	0.04%	98%	15%	93%
Li Combo®	0.38%	68%	n.d.	55%
Latron Ag 44M	0.5%	53%	n.d.	59
Plyac®	0.03%	91%	n.d.	87%

(Silwet L77 is a registered trademark of OSi Specialties, Li Combo, Plyac are registered trademarks of Loveland Industries, Latron Ag 44M is a registered trademark of Rohm & Haas.)

A surfactant may have deleterious effect on the conidium, however. It may promptly kill the conidium (Table 2). The different fungal species can have different susceptibilities to a wetting agent. Many of the organosilicone wetting agents are toxic to *M. flavoviride*, but not *B. bassiana* or *Paecilomyces fumosoroseus* (Table 2). The reasons for this differential sensitivity are not clear. While the mainstay wetting agents in the scientific community are Tween 80® (POE (20)sorbitan monoleate), ICI Surfactants), or Triton X100 ® (Octoxynol-9), Union

Carbide), many more agricultural wetting agents or spray tank adjuvants are available to the agricultural community, yet most have not been evaluated for their effect on fungal conidia.

Oil carriers have been a recent development (Prior et al. 1988; Bateman et al. 1993). Oil Flowables partially solve this dilemma. Such formulations have been developed for locust control campaigns in Africa and the commercial product, Mycotrol-GH® OF (Mycotech Corporation), for use against orthopterans in the U.S. Both vegetable and petroleum-based oils seem to enhance the efficacy of the entomogenous fungi. The oil may enhance physical and chemical contact of the conidia with the insect cuticle and may also partially solubilize hydrocarbons in the waxy epicuticle of the insect to stimulate germination. Certain oils, primarily petroleum-based paraffinics, also stabilize fungal conidia and provide good shelf-life, even at elevated (35-40 °C.) temperatures, while plant-derived oils provide only short shelf-life (Table 3). The effect of the latter may be due to the presence of short-chain fatty acids, which have been shown to be toxic to conidia.

TABLE 3--Conidial viabilities of *Beauveria bassiana* after six months of storage in various oils at 25 °C. and 40 °C. Initial viability of the conidia was 98%.

	Conidial Viabilities	
Carrier	25 °C.	40° C.
Dry Conidial Powder	90%	18%
Vegetable Oil	15%	0%
Peanut Oil	33%	0%
Cottonseed Oil	29%	0%
Mycotech OF Carrier Oil	94%	81%
Mycotech ES Carrier Oil	95%	86%

Oil formulations are generally designed for Ultra Low Volume ULV and undiluted applications. Normal agricultural practice, however, often necessitates dilution of typically a liter of oil formulation in water volume between 47 L and 1870 L per hectare on vegetable crops, and the equivalent of 3740 L per hectare in greenhouse applications. The potential for phytotoxic effects also encourages limiting the amount of oil to less than 1% (v/v).

This situation requires use of emulsifiers. Emulsifiers, however, can be toxic to conidia (Table 4). Here, one emulsifier quickly killed all the conidia within one month at 30 °C., a second caused a moderate but commercially significant viability loss, while a third had no effect. All three were harmless to conidia in a short duration screen.

Another challenge for fungal conidia in oils is their propensity to settle into dense, coherent sludges that are subsequently very difficult to resuspend. In some cases addition of carefully selected inerts alleviates this problem; in other cases unusual steps have to be taken.

Adjuvants can also affect conidial viability in dry, wettable powder formulations, shortening shelf life. Reduction in conidial longevity can

be temperature dependent (Table 5). In this example, formulations of a *B. bassiana* wettable powder containing three levels of a dry dispersant (WP9601, WP9602, WP9603) were placed on stability at 5, 25, 30, and 35 °C. The effect of the dispersant was strongly manifested only at 30° and 35 °C., when it was present at the two higher concentrations; at the lowest dispersant level, there was a smaller but still commercially significant loss in viability.

TABLE 4--Effect of proprietary emulsifiers on *Beauveria bassiana* Strain GHA conidial viability after one month at 30 °C.

Formulation	Conidial Viability
ES Carrier Oil	98%
Carrier Oil + Emulsifier 1	0%
Carrier Oil + Emulsifier 2	72%
Carrier Oil + Emulsifier 3	96%

The interactive effect of formulation components on conidial longevity must also be considered (Table 5). The level of the dispersant in formulation WP9603 was maintained in WP9604 and WP9605, but another propietary inert was added. The added inert at the higher concentration in WP9605 greatly slowed the viability loss at 35 °C. due to the dispersant.

TABLE 5--Effect of wettable powder formulation ingredients on conidial viability during storage. (For explanation of data see text.)

	Conidial Viabilities After 180 days at Indicated Temperature				
Temperature	WP9601	WP9602	WP9603	WP9604	WP9605
5 °C.	94%	94%	92%	94%	98%
25 °C.	86%	89%	94%	90%	91%
30 °C.	84%	9%	14%	76%	76%
35 °C.	75%	1%	10%	19%	81%

There are almost no data in the literature identifying the effects of most commercial emulsifiers, dispersants, and wetting agents on fungal conidia. Such data must be empirically derived for each fungal species and strain of interest.

Paradigm 3: Adjuvants Cannot Interfere with the Infection Process

The third paradigm in formulating mycoinsecticides is based on the complexity of the infection process. Initial stages of infection consist of a dynamic series of events involving the response of the conidium to distinct biochemical cues (Fig. 4), which are not well understood. Initial attachment of the spore to the cuticle seems to be mediated by electrostatic forces and the mutual hydrophobic nature of the conidial wall and the insect epicuticle (Boucias et al. 1988). Once the spore becomes attached to the cuticle, enzymes associated with the spore wall digest components of the waxy epicuticle and thereby provide the spore with the first biochemical cues for germination. Metabolic processes are

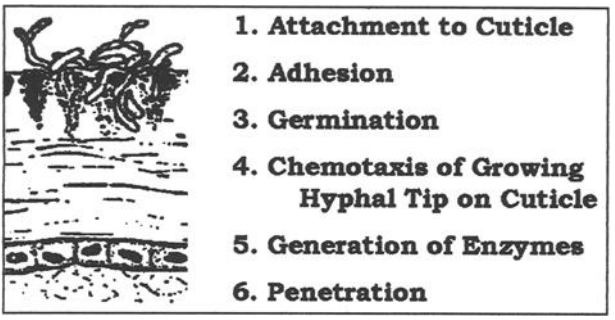

Figure 4. Stages during the infection process by entomogenous fungi potentially affected by formulation adjuvants.

initiated and the conidium begins to swell. Conidial attachment is reinforced during this phase by the secretion of adhesive materials. As the conidia germinate, some of the fungal species, e.g., *Metarhizium anisopliae,* produce a specialized anchoring structure, the appresorium, and a rapidly extending hypha. There is evidence that, at least with *N. rileyi* and *B. bassiana,* the growing tip of the hypha is influenced by the chemical nature of the cuticle surface. Some degree of host specificity is mediated at this point. In susceptible insects *Nomuraea rileyi* hyphae grow only a short distance on the cuticle before beginning penetration into the cuticle; in nonsusceptible insects hyphal growth is often "disoriented" and few hyphae begin penetration (Boucias and Pendland 1990). It is not yet clear whether this phenomenon extends to the other entomopathogenic deuteromycetes. As the penetration hypha begins its invasion of the cuticle it releases a mixture of lipases, proteases, and chitinases. These digest the cuticle and, coupled with mechanical pressure from the growing hypha, allow the fungus to invade the body of the insect within a few hours. During this initital infection process, which takes 2-12 hours, there is, in a sense, "communication" between the conidium and the insect's surface. It is quite possible that a wetting agent, or some other chemically active formulation component can affect the infection process without killing the conidium outright, by affecting the "communication." Detergents, solvents and high molecular weight proteins known to reduce hydrophobicity can affect the adhesion of conidia to insect cuticle (Boucias and Pendland 1991).

There is a general belief that high humidity is required for conidial germination on the insect. The requirement of *V. lecanii* conidia for high humidity has been documented, but conidia of this species, *A. aleyrodis,* and *V. lecanii* are different from the other fungi in that they have hydro*phil*ic conidia within a mucilage. Certainly an *in vitro* requirement of *B. bassiana* conidia for moisture (A_w > 0.935) has been documented (Hallsworth and Magan 1994b). However, this phenomenon may not be universal; there are examples where infection was independent of ambient relative humidity (Marcandier and Khachatourians 1987). This apparent anomaly arises because the microclimate of the spore – on the leaf surface, or on insect cuticle – often has much higher water vapor levels than the surrounding air, even reaching saturation at low ambient relative humidities. One can easily infect insects with these fungal agents at ambient humidities of 20-35%. High humidity (> 90% for at least 8 hours) is required, however, for spore production on the insect cadavers, but unless recycling of the fungus in the target habitat is highly desired, this limitation is immaterial.

Paradigm 4: The Fungus Must Be Kept Alive on the Plant

The fourth paradigm is that for good efficacy the conidia need to be kept alive on the plant surface as long as possible, particularly in situations where acquisition of the fungus by an insect is indirect, e.g., conidia adhere to the insect as it moves about on a sprayed surface. The major mortality factor on the leaf surface are UVA and UVB components of sunlight. The half-life of a population of conidia directly exposed to full sunlight is a matter of hours. Fortunately, in cases where the target insect inhabits the undersides of leaves, conidial half-life is greater, extending to several days in the case of *B. bassiana* Strain GHA [Jaronski, unpublished data]. Spores of *Aschersonia aleyrodis,* sprayed on cucumber leaves in the greenhouse, remained viable for 20+ days at 20 °C. and 10-12 days at 25 °C. (Fransen 1995). *Nomuraea rileyii* conidia on bean and cabbage were found to have a half-life of 3.6 hours on a sunny day, but when sunlight was physically filtered, the half-life was extended to over 40 hrs (Fragues et al. 1988). Formulation adjuvants can act as photosensitizers and greatly reduce conidial survival on the leaf (Fig. 5).

Potential UV protectants have been investigated by several authors (Inglis et al. 1995; Hunt et al. 1995). A number of materials have been found to have value. There are two caveats about this work: cost and human safety.

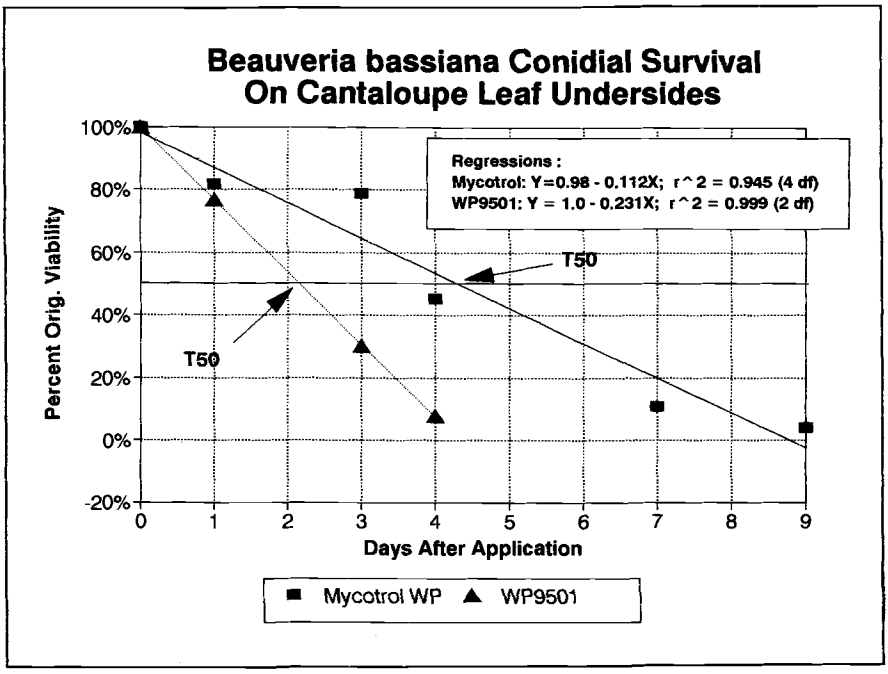

FIG. 5--Effect of formulation additives on *B. bassiana* Strain GHA conidial survival on the undersides of *Cucumis melo* L. (Cantaloupe) leaves in a trial conducted in Brawley CA, June 5-13, 1995.

For commercial success a mycoinsecticide has to be price competitive. Useful concentrations of many of these protectants are as or more expensive than the cost of the rest of the formulation. Furthermore, some identified protectants have dubious mammalian safety. Registration costs must also be considered. Few UV protectants are included in the U.S. Environmental Protection Administration List 4 of Inerts (compounds generally regarded as safe). As a result, a mycoinsecticide may have to undergo additional end product toxicological testing, from which it would otherwise be exempt (mycoinsecticide active ingredients do have to undergo acute pathogenicity/ toxicity, but these tests are much less expensive). The added registration costs and delays are strong disincentives.

While rainfastness is a frequent objective of chemical formulations, it can be self-defeating for mycoinsecticides. Conidia are *contact* insecticides. Anything that interferes with physical contact between conidium and insect, such as a layer of polymeric sticker, interferes with infection, and thus efficacy.

The formulations challenge reduces down into three components: (1) realizing that the active ingredient consists of living microorganisms rather than metabolites or synthetic chemicals, (2) acknowledging that little *a priori* information exists about the effects of formulation additives on fungi, and (3) realizing that inter- and intraspecies differences require that formulations be empirically devised for each specific fungal candidate. Despite all the challenges, the entomogenous fungi *can* be formulated; these fungi *are* being formulated into successful commercial end use products. As we learn more about infection processes and effects of adjuvants, the task will become easier.

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APPLICATION TECHNOLOGY

Roger A. Downer[1], Loren M. Kirchner[1], Franklin R. Hall[1], and Bert L. Bishop[2],

COMPARISON OF DROPLET SPECTRA OF FLUORESCENT TRACERS COMMONLY USED TO MEASURE PESTICIDE DEPOSITION AND DRIFT

REFERENCE: Downer, R.A., Kirchner, L.M., Hall, F.R., and Bishop, B.L., **"Comparison of Droplet Spectra of Fluorescent Tracers Commonly Used to Measure Pesticide Deposition and Drift,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: Five water soluble fluorescent tracers, (Rhodamine WT, Tinopal CBS-X, Eosin OJ, Brilliant Sulphaflavine, and Uvitex EC), and one non-ionic adjuvant, Induce, were studied to determine their effects upon atomization. The solutions were sprayed through an XR8004VS fan nozzle tip at 276 kPa (40 psi). The drop spectra were measured using an Aerometrics PDPA 100-1D with the nozzle positioned 30 cm above the probe volume.

Several differences in important droplet size distribution parameters were found between the tracers at various rates and between tracer/adjuvant combinations. The greatest difference occurred when comparing Tinopal CBS-X (6.5 g liter^{-1}) to Eosin OJ (3.5 g liter^{-1}), where there was a 200% and a 107% increase in the % volume of droplets < 100 μm and 150 μm diameter, respectively. There was also an approximately 61% and 35% increase in the % number of droplets < 100 μm and 150 μm, respectively. Similar results, although not as marked, occurred when comparing these two tracers both at 1 g liter^{-1}. The other tracers showed differences when compared to water and water/adjuvant mixtures. The results are considered in terms of their implications for quantification of drift and deposition studies.

KEYWORDS: Pesticides, Drift, Fluorescent Tracers, Atomization, Droplet Spectra.

[1]Research Associate, Research Assistant, Head and Professor respectively, Laboratory for Pest Control Application Technology, [2]Senior Statistician, Computing and Statistical Services The Ohio State University, Wooster, OH 44691.

Fluorescent tracers are popular and useful tools for measuring off-target movement and deposition (drift) of pesticide sprays as well as for quantitative and, in the laboratory, qualitative assessment of spray deposition. The strengths of, and confidence in, the use of fluorescence technology to track off-target movement and deposition is borne out by the numbers of examples found in the literature, and their use is likely to increase as researchers continue to address the problems (losses) associated with spray delivery. As yet, other quick, reliable, and safe analytical methods are not readily available although other workers have frequently used alternatives such as metallic salts as a result of frustration with e.g., photo-degradation of fluorescent tracers (Yates et al. 1976). Although tracer methods have been shown to have a number of practical limitations (Hall et al. 1991), most of these can be circumvented by thoughtful selection of tracers and attention to experimental procedures and analytical techniques.

In general, drift has been found to account for a relatively small percentage of the total amount of spray applied with different application systems. Captan deposits at locations outside tree canopies have been shown to account for less than 3.0 % of the total spray deposit when applied with an orchard air-blast sprayer (MacCollom et al. 1985). In aerial drift studies (fixed wing), deposits ranging from about 5.0 % of field spray rates at 10 m, 0.4% to 1.0 % at 100 m, and less than 0.02 % at 1000 m downwind have been reported (Hill 1989). However, in certain circumstances during aerial application to forest canopies as much as 50% of the sprayed material has been found to be still airborne 400m downwind of the canopy zone (Picot et al. 1986). Drift losses from ground spraying of glyphosate (8003 fan nozzles) have been found to be less than 1.0 % of total field spray rates at 25 m downwind (Yates et al. 1978). However, since only small amounts of material may cause serious crop damage and/or unwanted residues on non-target non-registered crops, drift continues to be a `high profile' problem.

To date, tracer selection criteria and tracer disadvantages have been discussed with regard to deposition and drift analysis (Hall et al. 1991; Salyani and Whitney 1988; Sharp 1974; Yates and Akesson 1963). More general discussions of fluorescence methods and limitations have been given from a laboratory analytical viewpoint by numerous authors, many of which provide excellent reviews (Bashford 1987; Ellis 1966; Guilbault 1985; Harris 1987; Hercules 1966, and Shipe 1984). However, although much attention has been paid to the laboratory analysis of samples containing fluorescent tracers, little attention has been paid to the possible rheological effect of tracers and their effect on spray delivery.

It is frequent practice, for reasons such as safety, to substitute a pesticide formulation with a tracer as a simulated agricultural spray mixture and assume that the results produced will be the same as if the pesticide were present. This is now known not to be the case and, in addition to the tracer in the spray tank, a blank formulation (placebo) is considered essential if a correct `mimic' of the atomization characteristics of the active pesticide is to be achieved. However, little attention appears to have been paid to the potential rheological modifications (e.g., surface tension and visco-elasticity) brought about by the use of a tracer and the effect upon the atomization characteristics and consequent effect on the droplet spectra produced. Since small amounts of the total spray volume make up 100 % of the drift, seemingly **small** changes in the `driftable'

portion of the spray cloud may have a **large** impact on the off-target deposition. Similarly, changes in the spray cloud droplet size and velocity composition may have consequences for the resultant deposit structure and retention characteristics of the spray at the target surface.

Therefore, the objectives of this study were to:

1. compare the droplet spectra of some fluorescent tracer dyes used in drift and deposition studies at this and other laboratories.
2. consider the data obtained in relation to drift and off-target deposition quantification and with respect to quantitative and qualitative measurement of spray deposits.
3. increase user awareness of some potential and hitherto little considered problems associated with the use of fluorescent tracers.

EXPERIMENTAL METHODS

Tracers

The tracers and other chemicals used in this study are shown in Table 1 along with their suppliers. All tracers were dissolved in tap water. Tinopal CBS-X (Tinopal), which is known not to dissolve well in 'hard' water, had 1 g liter^{-1} w/w of the Tetrasodium salt of ethylenediamine tetra-acetic acid (EDTA, a chelating agent) added to the solution to aid dissolution. Induce, a non-ionic wetter/spreader adjuvant, was included as the

TABLE 1 – List of tracers/adjuvants and their source

Material	Supplier	
	Name	Address
Tinopal CBS-X (Tinopal)	Ciba-Geigy	Greensboro, NC 27419
Uvitex EC	Ciba-Geigy	Greensboro, NC 27419
Eosin OJ	Keystone-Aniline Corp.	Chicago, IL 60612
Rhodamine WT	Keystone-Aniline Corp.	Chicago, IL 60612
EDTA - Tetrasodium salt of Ethylenediamine tetra-acetic acid (Sigma Grade)	Sigma Chemical Co.	St. Louis, MO 63178
Brilliant Sulphaflavine	Aldrich Chemical Co.	Milwaukee, WI 53233
Induce	Helena Chemical Co.	Memphis, TN 38137

adjuvant at 1 g liter^{-1} in selected test solutions (Table 3).

Initial experiments were set up to compare the droplet spectra of the tracers alone, all at 1 g liter^{-1}. The effect of tracer concentration was also investigated. Three rates of Eosin OJ, 0.1, 1.0 and 3.5 g liter^{-1}, and three rates of Tinopal CBS-X, 0.225, 1.0 and 6.5 g liter^{-1}, both without Induce, were compared. Rhodamine WT was only included in the initial experiments. All experiments included water as a standard treatment.

Droplet size Measurement

The mixtures were atomized through an XR8004VS fan nozzle at 276 kPa. The XR tip was chosen as a representative of nozzles typically used for application of pesticides through ground operated boom sprayers. Pressure was supplied by compressed air to a stainless steel pressure can. Water/solution temperature was 25 ± 2 °C.

Measurements were made using an Aerometrics PDPA-100 1D phase Doppler laser velocimeter (Aerometrics Inc. Sunnyvale, CA, USA). The PDPA was operated using a 495 mm focal length receiving lens and 1016 mm focal length transmission lens, and 160 mm collimating lens. Photomultiplier voltage was 325 volts; velocity offset was 20 m/s; measurement range was 25.7 - 900 µm. The nozzle was positioned 30 cm above the probe volume (the measuring point) of the PDPA. Auto high voltage and auto-intensity validation were turned off. The refractive index of all solutions was measured on a refractometer prior to any atomization measurements being made and the relevant figure entered into the Aerometrics program. All droplet sizing was carried out through the long (or 'x') axis of the spray cloud by traversing the nozzle across the probe volume by use of an xyz positioner (Fig 1). The methodology is in line with a technique previously standardized at The Laboratory for Pest Control Application Technology (LPCAT) (Chapple and Hall 1993). Each traverse yielded data for more than 10,000 droplets.

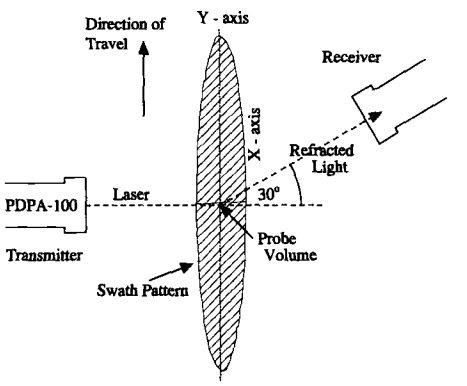

FIG 1 -- Relationship between the Aerometrics PDPA and traverse of spray cloud during drop spectra measurement.

For the comparison of tracers at 1 g liter^{-1}, each

replicate represents the mean of three `x' traverses and was made using a freshly prepared mixture. Replicates for all other comparisons are individual `x' traverses with no more than two replicates being done using any one treatment before a fresh mixture was prepared. Replicates were randomized such that no two successive traverses were the same treatment. To reduce the effects of residual contamination, the stainless steel spray can was thoroughly triple rinsed between each treatment and the cleanliness of the system checked (measurement made with PDPA) with plain water. The next spray solution sprayed for a minimum of thirty seconds in order to clear the spray lines.

The descriptors chosen for comparison are those considered to best describe the droplet spectra of the test solutions and to illustrate the most relevant aspects with regards to drift potential. These descriptors include the linear (arithmetic) mean diameter (D_{10}), volume mean diameter (D_{30}), volume median diameter ($D_{V0.5}$), number median diameter ($D_{N0.5}$) and % number and % volume <100 and 150 µm.

Statistical analysis was done using ANOVA (Statistics Analysis System, SAS Institute Inc.). Separation of the means was done using LSD's (alpha = 0.05 for all analyses). A comparison of the means of the tracers and water, treatments with and without EDTA, with and without Induce and the rates of Eosin and Tinopal as well as Eosin OJ at 1 g liter^{-1} with and without Induce against the means of Tinopal at 1 g liter^{-1} with and without Induce were done using contrasts (Hicks 1973).

RESULTS AND DISCUSSION

A comparison of the $D_{v0.5}$ and % by volume < 150 µm for all tracers at 1 g liter^{-1} is shown in Figure 2. Data for the other spray parameters measured is given in Table 2. Eosin OJ was found to be significantly different from all of the other tracers and from water alone for all reported droplet distribution parameters. In addition, Brilliant Sulphaflavine was significantly different from Eosin OJ and from Tinopal, with the

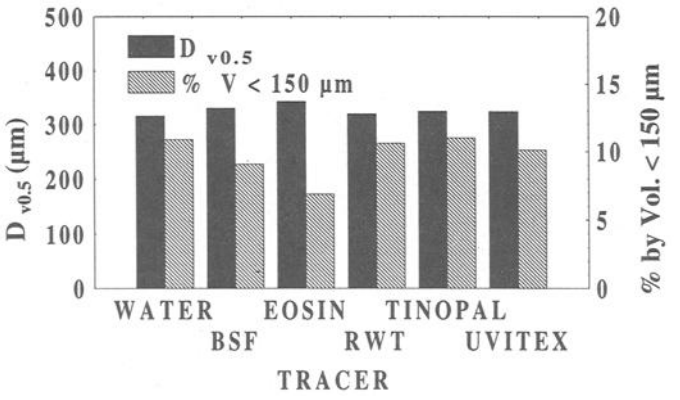

Figure 2 -- Comparison of $D_{v0.5}$ and % by volume < 150µm for all tracers

TABLE 2 -- Comparison of the arithmetic mean diameter (D_{10}), volume mean diameter (D_{30}), volume median diameter ($D_{V0.5}$), number median diameter ($D_{N0.5}$) and % Number and Volume <100 and 150µm for tracers at 1 g liter^{-1}. All data points are the means of three replicates.

Treatment	D_{10} µm	D_{30} µm	$D_{V0.5}$ µm	$D_{N0.5}$ µm	% No. <100 µm	% No. <150 µm	% Vol. <100 µm	%Vol. <150 µm
Water	102.2	167.1	315.5	65.1	68.8	81.9	4.4	10.9
Brilliant Sulphaflavine	116.3	183.6	330.0	77.5	61.6	77.0	3.3	9.1
Eosin OJ	137.4	206.0	342.8	99.1	51.0	68.1	2.2	6.9
Rhodamine WT	102.3	168.2	319.2	64.9	68.7	81.8	4.3	10.6
Tinopal + EDTA	96.5	163.0	323.6	60.0	71.9	83.9	4.7	11.0
Uvitex EC	105.9	172.4	323.2	68.2	67.1	80.5	4.0	10.1
LSD	13.1	13.8	8.5	12.8	6.8	5.2	0.8	1.5

exception of $D_{V0.5}$. Uvitex and Rhodamine WT were both very similar to Water and Tinopal and were not significantly different for any of the spray descriptors.

Table 3 shows a comparison of tracers with and without Induce as an adjuvant and, in the case of Eosin OJ, with and without EDTA. Tinopal and Eosin OJ are included at a range of rates. Overall, a separation of Tinopal from Eosin OJ is evident from the data. The largest differences occurred where Eosin OJ at 3.5 g liter^{-1} (no adjuvant) and Tinopal at 6.5 g liter^{-1} (plus EDTA) were compared. Although the two rates are different, they represent comparable field use rates for ground sprayer application, which makes comparisons between them meaningful. The data show that the increase in % number and % volume < 100 and 150 μm are: % number < 100 μm = 61% and < 150 μm = 35%; % volume < 100 μm = 200% and < 150 μm 107%. These changes in the region of small size drops are reflected as a 10% increase in the $D_{V0.5}$ and a 92% increase in the $D_{N0.5}$. Likewise the D_{30} increased by 38% and the D_{10} by 61%. The other tracer/rate/adjuvant combinations gave results similar to one another but different from the Tinopal and Eosin OJ combinations.

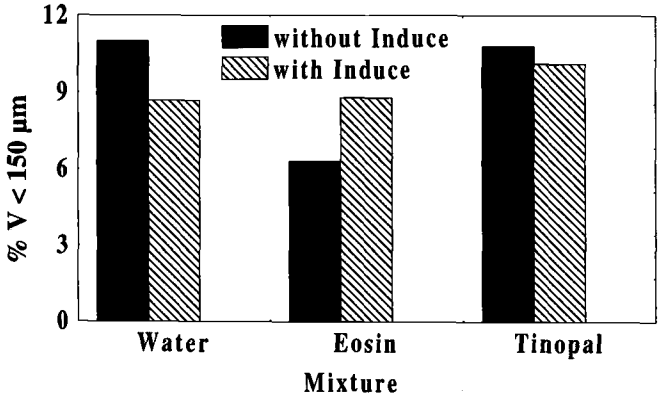

Figure 3 – Effect of Induce on % by volume < 150 μm

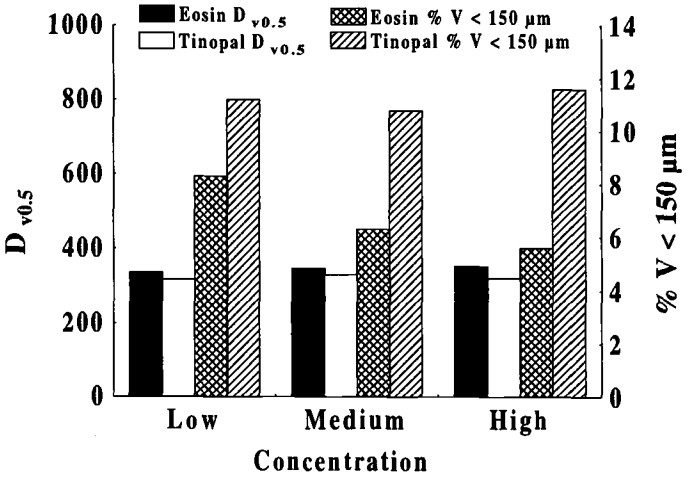

Figure 4 -- Effect of tracer rate on D v0.5 and % V < 150μm

TABLE 3 -- Comparison of D_{10}, D_{30}, $D_{V0.5}$, $D_{N0.5}$ and % Number and Volume <100 and 150µm for all tracers and tracer/adjuvant combinations. All data points are the means of four replicates. E = EDTA added. I = Induce added.

Treatment			Rate (g liter^{-1})	D_{10}	D_{30}	$D_{V0.5}$	$D_{N0.5}$	% No. <100µm	% No. <150µm	% Vol. <100µm	% Vol. <150µm
Water				100.8	165.4	312.5	63.6	69.6	82.3	4.5	11.0
Water	E			102.7	169.5	322.7	64.1	68.7	81.7	4.1	10.2
Water		I		123.0	190.0	330.2	84.6	57.7	74.4	3.0	8.7
Water	E	I		111.5	175.4	311.6	74.5	63.5	78.5	3.9	10.3
B. Sulph			1.0	114.9	183.3	331.4	75.3	62.4	77.4	3.3	9.0
B. Sulph		I	1.0	112.1	177.4	320.5	74.5	63.4	78.6	3.7	10.1
Eosin OJ			3.5	149.6	219.1	352.2	110.7	45.8	63.4	1.7	5.6
Eosin OJ	E		3.5	145.2	214.6	350.0	106.3	47.5	65.1	1.8	6.0
Eosin OJ	E	I	3.5	121.0	185.3	319.0	83.8	58.3	74.5	3.2	9.2
Eosin OJ			1.0	142.2	211.2	346.0	102.8	48.8	66.4	1.9	6.3
Eosin OJ		I	1.0	117.9	186.0	333.7	78.6	60.9	76.3	3.2	8.8
Eosin OJ			0.1	120.6	190.0	335.1	80.5	59.5	75.1	3.0	8.3
Eosin OJ	E		0.1	120.4	187.5	329.8	81.4	59.3	75.5	3.1	8.8
Eosin OJ	E	I	0.1	113.5	179.3	322.0	75.5	62.7	77.8	3.6	9.7
Tinopal	E		6.5	92.6	158.9	320.0	57.8	73.8	85.4	5.1	11.6
Tinopal	E	I	6.5	105.6	169.4	315.1	69.7	67.1	81.1	4.4	11.0
Tinopal	E		1.0	94.5	162.3	329.5	57.7	73.3	84.6	4.7	10.8
Tinopal	E	I	1.0	111.9	177.3	320.2	74.3	63.7	78.8	3.8	10.1
Tinopal	E		0.23	98.0	162.9	316.4	61.1	71.5	83.5	4.7	11.2
Tinopal	E	I	0.23	111.2	176.9	317.9	73.2	63.9	78.6	3.7	10.9
Uvitex EC			1.0	100.8	167.4	324.7	63.6	69.9	82.7	4.4	10.7
Uvitex EC		I	1.0	121.2	186.2	320.6	83.0	58.7	74.8	3.2	9.2
LSD				10.3	12.0	13.7	9.7	5.3	4.3	0.8	1.7

Contrast analyses carried out on the data (Table 4) showed that the inclusion of EDTA had no significant effect on any of the drop spectra parameters. The addition of Induce at 1 g liter^{-1} had some effect on atomization (Figure 3). There was some indication of an interaction between EDTA and Induce. Analysis of the data showed there to be significant differences between the highest and lowest rates for all parameters using Eosin but no differences using Tinopal (Table 4 and Figure 4).

Table 5 shows the results of contrast analyses carried out on the droplet spectra parameters of Eosin OJ and Tinopal, at 1 g liter^{-1}. The comparison was made based upon the means of four replicates and shows that the differences observed between the data sets were significant.

TABLE 5 -- Contrast analyses of comparative drop spectra data for Tinopal CBS-X and Eosin OJ at 1 g liter^{-1}. Tinopal includes EDTA at 1 g liter^{-1}. Each data point represents the mean of four replicates. Two each with and without Induce.

Drop Spectra Parameter	Treatment		F value	Pr > F	Percent Change from Eosin to Tinopal
	Eosin OJ	Tinopal			
D_{10}	135.3	98.1	58.37	0.0001	-27.5
D_{30}	203.3	163.9	49.89	0.0001	-19.4
$D_{v0.5}$	339.5	320.8	10.31	0.0042	-5.5
$D_{n0.5}$	96.9	61.7	56.56	0.0001	-36.3
% N <100µm	52.0	71.1	56.61	0.0001	36.7
% N <150µm	69.0	83.4	49.77	0.0001	20.9
% V <100µm	2.3	4.6	39.26	0.0001	100.0
% V <150µm	7.2	11.0	26.56	0.0001	52.8

Using a single nozzle at representative pressures to simulate a ground boom sprayer (with no added air velocity), the data presented show that there are differences in the atomization characteristics of tracer solutions. Two powder formulations, Eosin OJ and Brilliant Sulphaflavine, gave results that were, in general, different from the liquid formulation tracers and water alone, and different from each other. Tinopal, another powder formulation, was similar to water for most of the spray descriptors compared. In addition, the results show that tracer rate and the inclusion of an adjuvant may cause differences in atomization.

Equilibrium surface tension (EST) and $D_{v0.5}$ and kinematic viscosity were measured for all the mixtures tested. The data (not presented) showed that there was some correlation between EST and $D_{v0.5}$ (correlation coefficient R = 0.395), and EST and % by volume < 150 µm (R = 0.287) however, these correlations would seem to be too weak to answer the question why were the droplet size distributions different for certain tracers? There was little difference in the kinematic viscosity values for any of the mixtures. Comparison of the EST for mixtures containing Eosin and Tinopal showed that the EST was always greater for mixtures containing Eosin than for those containing Tinopal. It is likely that neither surface tension nor viscosity were responsible for the

TABLE 4 -- Contrast analysis of spray droplet parameters for various treatment combinations.

Parameter	Comparison	Tracers v Water	EDTA v No EDTA	Induce v No Induce	Eosin rates	Tinopal rates
D_{10}	F value	12.2	2.06	5.32	36.19	1.01
	Pr>F	0.002	0.17	0.03	0.0001	0.33
D_{30}	F value	12.5	2.18	1.22	27.84	0.48
	Pr>F	0.002	0.15	0.28	0.0001	0.49
$D_{v0.5}$	F value	11.5	1.45	12.02	6.89	0.17
	Pr>F	0.003	0.25	0.002	0.02	0.69
$D_{n0.5}$	F value	13.3	2.02	6.64	44.94	0.46
	Pr>F	0.002	0.17	0.18	0.0001	0.51
%N<100µm	F value	10.02	1.57	9.44	30.92	0.80
	Pr>F	0.005	0.22	0.006	0.0001	0.38
%N<150µm	F value	10.04	1.78	2.85	33.77	0.75
	Pr>F	0.005	0.196	0.11	0.0001	0.39
%V<100µm	F value	6.43	0.92	2.23	12.55	0.69
	Pr>F	0.019	0.35	0.15	0.002	0.42
%V<150µm	F value	6.78	1.12	0.37	11.85	0.44
	Pr>F	0.017	0.30	0.55	0.002	0.51

differences seen between droplet distributions. Induce, at typical use rates, has an EST of 30-35 mN m^{-1} however, the dynamic surface tension of Induce mixtures is close to that of water at anything less than 6 ms (sheet break up for a typical fan nozzle operated at 276 kPa occurs at around 5 ms). The same is likely for all the other mixtures tested. Some other property of the liquid must therefore be responsible for the differences seen, possibly extensional viscosity.

Of the differences seen in the data, the most significant in terms of drift are those in the % volume below 100 and 150 μm. Drop diameters below 150 μm are considered by some authors as the most important criterion in their becoming drift particles (Göhlich 1983). Comparisons have been made of the predicted downwind deposit maxima using the Porton model based on sedimentation theory (Gunn et al. 1948), and the Bache and Sayer model (Bache and Sayer 1975), based on sedimentation and turbulence, both at an emission height of 0.5 m and turbulent intensity of i = 0.1 (Parkin and Merritt 1988). This data would suggest that, at windspeeds of 1 and 4 m/s, droplets of 150 μm and under are the most vulnerable to off-target movement. Data generated using a computer simulation program (Fluent®) showed that water droplets as large as 200μm were influenced by initial droplet velocity and height of discharge (Reichard et al. 1992a). This data has been found to correlate well with data determined experimentally in a wind tunnel (Reichard et al. 1992b).

Although a simple consideration of the magnitudes of the values for the % volume < 100 and 150 μm may make their values appear trivial, from the standpoint of drift and off-target deposition, these small changes may lead to serious errors in drift estimation. For example, in a drift study carried out under similar meteorological conditions, using the same sprayer type, boom height, and the nozzle type and operating conditions used here, a simple change from, Rhodamine WT (1 g $liter^{-1}$) to Eosin OJ (1 g $liter^{-1}$) may result in a decrease in the % Volume < 150 μm (i.e. `driftable` portion) of about 35%. Similarly, a change from Tinopal to Eosin OJ as the tracking agent may result in a decrease in the % Volume < 150 μm of about 40%. Therefore, from this data, we would argue that the use of Eosin OJ in a dual tracer situation, for the measurement of drift from, e.g., two types of spray equipment, may lead to misinterpretation of the results and therefore drift potential of that equipment.

Likewise, pesticide/adjuvant comparisons that utilize various combinations and permutations of tracers, placebos, actives, and or adjuvants to determine drift potentials, may automatically bias the results simply due to small changes in the droplet spectra caused by the tracers. It would seem, therefore, that in order to generate data on off-target movement or placement of active pesticides which is truly representative, the tracer, as well as the placebo or adjuvant - or indeed any combination of these - must be correctly matched with the test substance in terms of its atomization characteristics. Failure to do so could lead to an incorrect assessment of the hazards and benefits of the pesticide under scrutiny. Thus, use of the actual pesticide may be the most accurate technique for assessing drift potential albeit more costly and difficult and fraught with the logistical problems of using actives. Furthermore, as researchers delve more deeply into the relationships between deposit **quality** and biological effect, similar mismatches may occur resulting in inappropriate conclusions as to the match between optimum sprayer

hardware configuration (nozzle, pressure, speed, application volume) and biological efficiency.

Therefore, as the assessment of deposition and drift using fluorescent tracers may on the whole be viewed as an analytical technique (itself a combination of other analytical methods), it is essential that systematic errors that affect the accuracy of the technique be identified and controlled, if not eliminated (Büttner and Hannes 1974). It must be remembered, however that the data presented herein is a limited data set based only upon a single fan nozzle with no interactions (between similar nozzles) included and without the introduction of air velocity (which interacts with the liquid physical properties and affects the way in which liquid sheets break up and droplets are formed, and could exacerbate the differences seen here). In addition, we have not addressed transport and/or impaction differences nor the influence of interacting multiple nozzles with tractor motion. Nonetheless, we believe the data does provide fundamental baselines for data sets being examined in an attempt to measure off-target movement and deposit quality of various pesticide formulation types as they relate to different physico-chemical properties.

Finally, in an effort to address the problems identified in this paper which may introduce serious and unnecessary variability into the results, the following are proposed:

1. Careful attention should be paid to the droplet spectra produced by tracer solutions, and these should be carefully matched to the droplet spectra produced by the pesticide spray solutions that they are intended to emulate.
2. If spray application equipment comparisons are to be made using tracers as the test material then the same tracer should be used for all the test equipment.
3. Standardized methods for drift analysis should be developed and agreed upon by those researchers involved in such programs, to facilitate meaningful data comparisons for realistic conclusions.

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H.E. Ozkan[1], A. Miralles[2], C. Sinfort[2], H. Zhu[1], D.L. Reichard[3], and R.D. Fox [3]

EFFECT OF SHIELDING SPRAY BOOM ON SPRAY DEPOSITION

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ABSTRACT: The effects of several spray-boom shield designs and "low-drift" nozzles on spray deposition are presented. Results are based on experiments conducted in a wind tunnel and computer simulations using the same experimental parameters. Performances of all experimental shields were evaluated under two spray pressures (0.15 and 0.3 MPa), and two air flow rates (2.75 and 4.80 m/s) in the wind tunnel. All nine shields tested during this study effectively reduced droplet deposition distance. Even the least effective shield design produced a 13% improvement in deposition of spray on the ground. A double-foil shield produced the best spray-deposit improvement of 59% compared to the same nozzles spraying without the shield. The shields were effective even when used with nozzles with higher flow rates (producing fewer small droplets). However, using larger capacity nozzles reduced droplet deposition distance more than using smaller capacity nozzles with even the most effective shield. Low-drift (LD) nozzles without a shield provided reductions in deposition distance ranging from 20 percent to 67 percent when compared to the deposition distance from a 0.61 L/min standard flat-fan (SFF) nozzle operating under identical conditions. The 0.61 L/min SFF nozzles operating with Shield 2 (the best shield) was twice as effective in reducing droplet deposition distance as the same capacity LD nozzles operating without a shield. However, the low-capacity LD nozzles without a shield were twice as effective in reducing drift as the SFF nozzles of the same capacity operating with Shield 5/1 (the shield with the worst performance). Without a shield, LD nozzles at higher flow rates are no more advantageous in reducing droplet deposition distance than SFF nozzles of similar flow rate.

KEYWORDS: shield, drift, wind tunnel, droplet, flat fan nozzle, spray, patternator

[1] Professor and Post-doctoral Research Associate, respectively, Department of Food, Agricultural, and Biological Engineering, The Ohio State University, Columbus, OH 43210.

[2] Head, and Assistant Professor, respectively, CEMAGREF, The Crop Protection Program, 361 rue J.F. Breton, BP 5095, 34033 Montpellier Cedex 1, France.

[3] Agricultural Engineers, USDA-ARS Application Technology Research Unit, OARDC, Wooster, OH 44691.

INTRODUCTION

Although current chemical application methods and equipment have improved application accuracy considerably, chemical spray application remains an inefficient operation. In some cases only a small portion of the intended chemical dose actually reaches the target and contributes to the desired biological effect.

The problem of spray drift, i.e., movement of a pesticide to a site other than the intended site of application, remains a serious health and safety problem. Variables affecting drift are discussed in detail by Smith et al. (1982). The most important application factor influencing drift is the size of droplets sprayed. Spray drift, target deposit, and coverage depend largely on the range of droplet sizes produced by the atomizer (Bode and Butler 1983). Research has shown that for typical applications with boom type sprayers, droplets of 100 μm or less often drift out of the intended swath, and 50 μm or less diameter droplets, completely evaporate before reaching the target (Zhu et al. 1994).

Since most of the drift problems are created by the movement of small droplets outside the application area, research has been conducted to reduce the volume of spray contained in small droplets. Recently, manufacturers have introduced nozzles that are designed to reduce the number of small, drift-prone droplets. Other companies have developed chemical products ("drift retardants") to achieve the same result: reducing the volume of spray contained in small droplets. Research has shown the significance of spray mixture properties on spray droplet size and drift when these products are added to the spray mixture (Richardson 1974; Bode et al.1976; Haq et al. 1983; Bouse and Carlton 1985; Bouse et al. 1988; Akesson et al. 1989; Hall 1989; Akesson and Gibbs 1990; Bouse et al. 1990; and Ozkan et al. 1994).

Several recent developments have been aimed at modifying existing equipment to increase deposition efficiency of the more effective small droplets while reducing the potential for drift. In general, this has been accomplished by using either air-assist technology or some kind of shield or shroud to overcome the drift-producing air currents and turbulence that occur around the nozzle during spraying. Although air-assist technology has been proven to be effective in increasing deposition while reducing drift, commercially available equipment using this technology currently has not been widely adopted by the applicators yet because of relatively high cost of the equipment.

Shields have been considered as economically viable alternatives to expensive air-assist sprayers. Most of the studies conducted to evaluate effectiveness of shields indicate that most of these devices reduce off-target spray drift (Furness 1991; Cenkowski et al. 1994; Smith et al. 1982; Maybank et al. 1990; Ford 1984; Wolf et al. 1993). However, the results vary considerably from one study to the other. In addition, experiments conducted in the field are subject to errors due to varying atmospheric conditions. Therefore, a more precise way to compare shield designs such as those discussed in the literature, would be to test them under controlled environmental conditions.

OBJECTIVES

The main objective of this research is to study the effect of several types of spray boom shields on spray deposition and drift. The secondary objective was to compare effectiveness of "low-drift" nozzles without a shield with that of standard flat-fan nozzles with a shield.

EQUIPMENT USED

Experimental setup

The experiments were conducted in a wind tunnel that is 10 m long, 1.5 m wide, and 1.0 m in height. A 3 by 3 m spray patternator table is an integral part of the tunnel floor (Fig. 1), with 0.05 m collector channels perpendicular to the tunnel. Half of the channel length is inside and half outside the tunnel to allow space for the mechanism used for positioning and dumping the graduated collection tubes. More information about the wind tunnel is given by Miralles and Bogliani (1993). Nine shield designs, shown in Fig. 2 and described below were installed over the patternator and covered the entire width of the wind tunnel, leaving no open space between the shield and the wind tunnel walls on both ends. However, no shield blocked more than 1/3 of the vertical dimension of the wind tunnel. The horizontal distance from the shields to the nozzle boom varied slightly with shield type, but was about 0.1 m (see Figs. 3-5). A boom with three nozzles spaced 0.5 m apart and 0.4 m above the patternator was installed 0.4 m downwind from the leading edge of the patternator for all experiments. Flat fan nozzles (Albuz, APE) with 110 degrees of spray angle and 0.6 L/min nominal flow rate (at a pressure of 0.3 MPa) were used for most experiments. However, limited tests were conducted with same type (Albuz, APE) of nozzle but with a higher nominal flow rate (1.71 L/min at 0.3 MPa), and with "low drift" flat-fan nozzles (Albuz, ADE) with flow rates of 0.61 and 1.71 L/min at 0.3 MPa. The Volume Median Diameter ($D_{v.5}$) reported by the manufacturer for these nozzles are given in Table 1.

Shields tested

Although all the shields tested in this study were designed and fabricated at CEMAGREF (Montpellier, France), they are similar in concept to shields that are commercially available. A brief description of the nine shields follows.

Shield 1: (Single Circular). Shield No. 1 is circular, with a radius of 0.3 m, and an angle of inclusion of 90 degrees (Fig.2).

Shield 2: (Double Circular). As shown in Fig. 3, this shield consists of Shield 1 and a second shield with a larger radius (0.5 m) and a smaller angle of inclusion (75 degrees) mounted directly above and behind Shield 1.

Shield 3: (Flat 45). This is a 0.42 m wide, 1.5 m long flat shield constructed of sheet metal (Fig. 2). It was positioned in the wind tunnel at a 45 degree angle from horizontal. The vertical distance between the leading and the trailing edges of the shield was 0.30 m, and the distance from the leading edge of the shield to the patternator was 0.55 m, the same as for Shield 1.

Shield 4: (Flat 60 Metal).This is a 0.35 m wide, 1.5 m long flat shield constructed of sheet metal (Fig. 2). It is positioned in the wind tunnel at a 60 degree angle from horizontal. The vertical distance between the leading and the trailing edges of the shield was 0.30 m, and the distance from the leading edge of the shield to the patternator was 0.55 m.

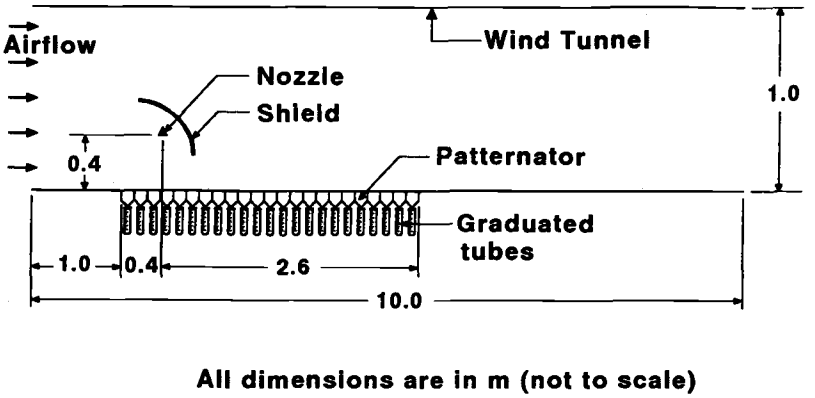

Figure 1: The wind tunnel and the patternator used to test shields

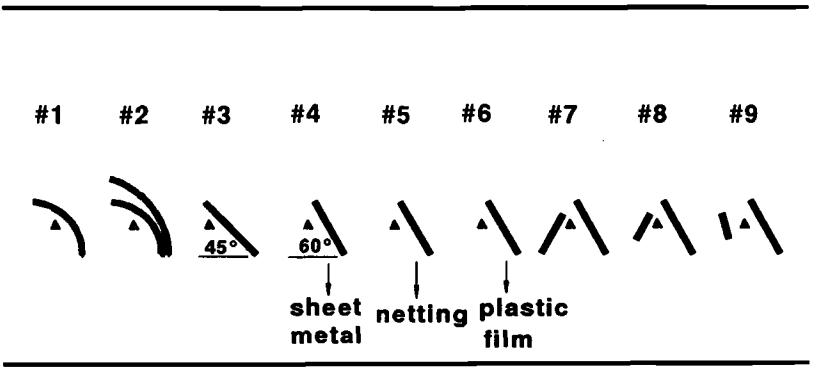

Figure 2: Shields used in the wind tunnel.

TABLE 1. Droplet size characteristics of nozzles *.

Nozzle**	Pressure MPa	Droplet size, μm			% Spray Volume	
		Dv.1	Dv.5	Dv.9	% <60 μm	% <100 μm
SFF 0.61 L/min	0.15	84	184	390	3	20
SFF 0.61 L/min	0.30	68	139	295	7	33
SFF 1.71 L/min	0.15	103	276	567	2	10
SFF 1.71 L/min	0.30	85	221	436	4	17
LD 0.61 L/min	0.15	95	255	490	2.5	10
LD 0.61 L/min	0.30	78	209	387	5	15
LD 1.71 L/min	0.15	133	330	515	2	4
LD 1.71 L/min	0.30	110	297	500	3	7

* Data for spraying water using a Malvern Particle Size Analyzer as reported by the manufacturer.

** Standard Flat Fan (SFF) Albuz APE 110° and Low Drift (LD) Albuz ADE 110°

Shield 5: (Porous 1-layer; Porous 2-layers). The dimensions of this shield are the same as those of Shield 4, except, this shield was porous; it was constructed of a netting type material with holes that were of approximately 1 mm^2. Tests were conducted placing both one layer and two layers of netting on the shield frame. The second layer was added to reduce open area of the shield by approximately 50%.

Shield 6: (Flat 60 Plastic). The dimensions of this shield are the same as those of Shields 4 and 5. However, Shield 6 was constructed of plastic (0.2 mm thick).

Shield 7: This shield consists of Shield 6 (plastic), plus a smaller (0.12 m x 1.5 m) sheet metal shield, placed in front of the nozzles, and tilted backward at an angle of 60 degrees from horizontal (Fig. 4).

Shield 8: This shield is similar to Shield 7 except the small shield in front of the nozzles is much smaller (0.08 m x 1.5 m). This change in design was made to allow more open area in the front top section of the shield assembly for providing more air to flow between the shields and downward behind the nozzles (Fig. 2).

Shield 9: The same two shields as in Shield 8 were used, but the front shield was tilted forward at an angle 75 degrees from horizontal (Fig. 2).

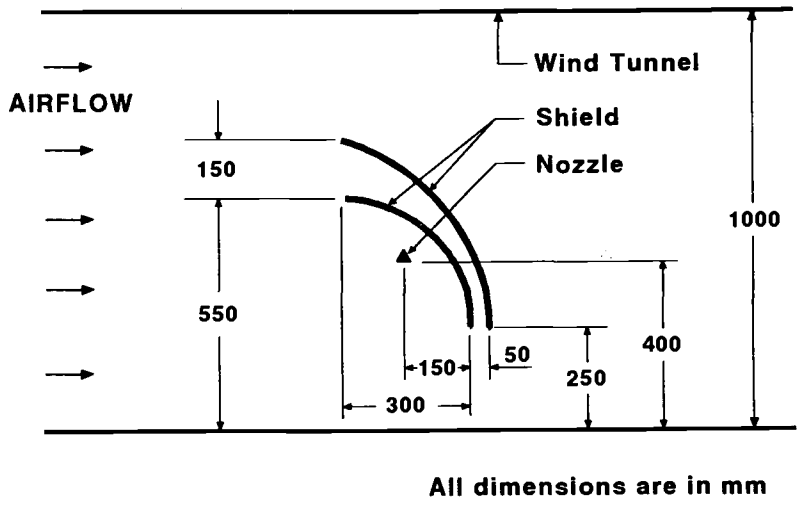

Figure 3. Dimensions of the Double Circular shield.

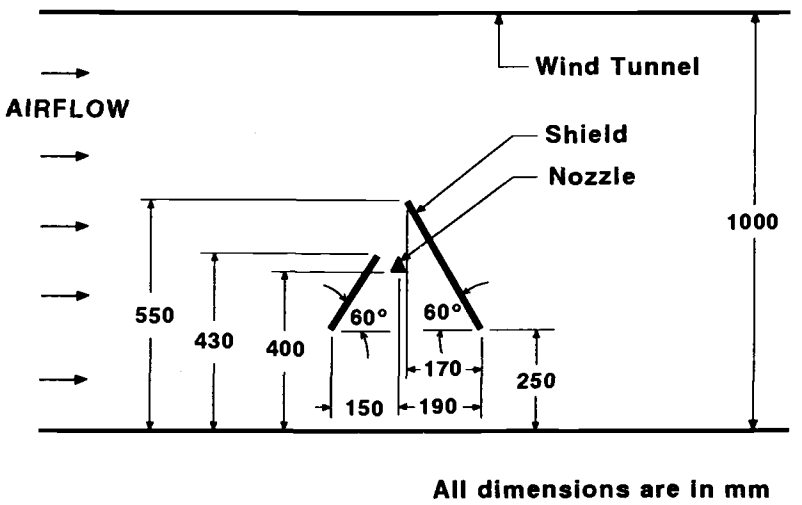

Figure 4. Dimensions of Shield 7.

PROCEDURE

Performance of all experimental shields were evaluated under two spray pressures (0.15 and 0.3 MPa), and two air flow rates (2.75 and 4.80 m/s) in the wind tunnel. In addition to the tests with shields, one set of tests at both pressures and air flow rates was conducted without shield. Each experiment was conducted three times and the mean values were used to compare shields. All 59 of the patternator collection channels were wetted using a hand gun nozzle when starting experiments each day, and when the patternator was set idle for a considerable length of time between experiments conducted in the same day. The procedure followed was:

1. With no shield in the wind tunnel, the spray pressure and air flow were set to 0.15 MPa and 2.75 m/s, respectively. Next the pump was started and spraying began. After the steady state conditions were reached, graduated cylinders of the patternator were lowered to collect spray liquid. The graduated cylinders were kept at this position until the liquid collected in any one cylinder reached approximately 90% of its rated volume. Next, the cylinders were elevated, and the volume of liquid collected in all 59 cylinders was recorded. Then, the tubes were emptied and the procedure was repeated two more times using the same time of liquid collection. Next, using the same nozzle pressure (0.15 MPa), air flow was increased to 4.80 m/s, and the liquid levels in graduated cylinders were recorded 3 times. Later, similar measurements were taken at a nozzle pressure of 0.30 MPa for wind speeds of 2.75 and 4.80 m/s.
2. Shield No.1 was placed in the wind tunnel, and the measurements explained in Step 1 were taken. This procedure was repeated for each of the remaining eight shields.
3. Mean values were used to determine, "the Distance to Center of Mass" ($\mathbf{D}_c$) of the spray distribution. Using $\mathbf{D}_c$ as a means to characterize spray distribution has been explained by Miralles and Bogliani (1993). The equation used to determine $\mathbf{D}_c$ was,

$$D_c = \frac{\sum_{i=1}^{59} V_i \, d_i}{\sum_{i=1}^{59} V_i}$$

where:

D_c : Distance to Center of Mass (m)
i : Number of the patternator channel (i=1,...,59)
v_i : Liquid volume at i^{th} channel of the patternator
d_i : Distance to midpoint of i^{th} channel where liquid volume is measured (m)
$d_i = 0.05\ (i-0.5)$ [0.05 is the channel width]

Limited tests were conducted using another 110 degree flat-fan nozzle (Albuz APE series) with a higher nominal flow rate (1.71 L/min at 0.3 MPa), to determine if using

a shield would be equally effective with higher capacity standard flat-fan nozzles. Tests were conducted with no shield in the wind tunnel and only with the Shield 1 at position 2.

Tests were also conducted using "low drift" nozzles (Albuz ADE series) with nominal flow rates of 0.61 and 1.71 L/min at 0.3 MPa operating pressure to determine if low-drift nozzles, which normally produce fewer drift-prone droplets, are as effective without a shield as standard flat-fan nozzles with a shield. Low-drift nozzles also have flat-fan pattern with 110° spray angle. Droplet size data for these nozzles are given in Table 1.

RESULTS AND DISCUSSION

Results with 0.61 L/min standard flat-fan nozzles

$\mathbf{D}_c$ values were used to evaluate shields for their effectiveness against spray drift. The shield with the smallest $\mathbf{D}_c$ value was considered to be the most effective for reducing spray droplet drift. Fig. 5 illustrates the percent reduction in $\mathbf{D}_c$ with nine shields in comparison to the $\mathbf{D}_c$ when no shield was used. The reduction in $\mathbf{D}_c$ varied from 7.9% with Shield 5 (with one layer of porous netting) at 4.80 m/s air flow and 0.3 MPa pressure, to 65% with Shield 2 (double foil) at 4.80 m/s air flow and 0.15 MPa. It is obvious that the Shield 5/1, made with only one layer of the porous netting material, did not perform adequately. However, the same shield with two layers of netting (Shield 5/2) performed adequately at the low spray pressure.

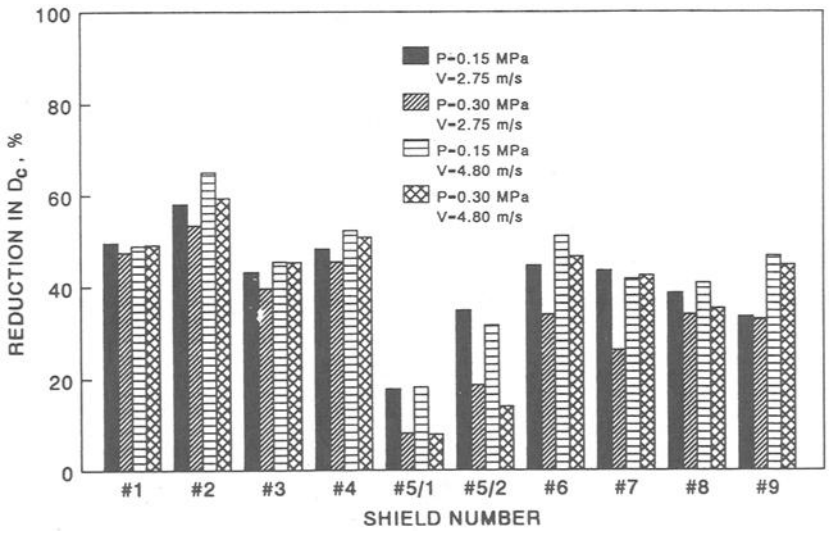

Figure 5. Percent reduction in Mean Distance to Center of Mass ($\mathbf{D}_c$) with different shields under two air flow rates in the wind tunnel and two nozzle pressures in comparison to the Mean $\mathbf{D}_c$ with no shield (with 0.61 L/min SFF nozzles).

Fig. 5 shows how different combinations of air velocity and spray pressure affect $\mathbf{D}_c$ with different shields in the wind tunnel. As expected, the combination of high pressure and high air velocity (the combination with the greatest potential for drift) always produced the highest values of $\mathbf{D}_c$ regardless of the shield used. Other observations that can be drawn from the data presented in Fig. 5 are as follows:

1. For the velocities and spray pressures selected for this study, when no shield was used, $\mathbf{D}_c$ was affected more by the increase in air flow than by the change in spray pressure. [This was also observed by Miralles and Bogliani (1983) in an earlier study].
2. Experiments with all of the shields, except Shield 1, indicated that the combination of high pressure and low air flow produced higher $\mathbf{D}_c$ values than the combination of high air flow and low pressure. This may be interpreted as, when a shield is used, the change in droplet size due to change in spray pressure affected $\mathbf{D}_c$ more than the change in air velocity.
3. When the pressure was changed from 0.15 to 0.3 MPa under high air flow conditions, shields were not as effective in reducing $\mathbf{D}_c$ as it was when the air flow was low.
4. At high spray pressure conditions (0.3 MPa), a change in air flow from 2.75 to 4.80 m/s did not influence $\mathbf{D}_c$ values (an increase in this case), as much as it did when the spray pressure was low (0.15 MPa). This implies that the shields were less effective at controlling drift with greater wind velocities when $Dv_{.5}$ was smaller.

To determine which one of the shields tested has the best overall performance, the $\mathbf{D}_c$ values obtained from tests under two spray pressure and two air flow rates were averaged and the mean $\mathbf{D}_c$ was determined for each shield. By using DNMRT, at 0.05 level of significance, $\mathbf{D}_c$ values of shields 1 and 4, and 2 and 6 were statistically the same. The differences between $\mathbf{D}_c$ values from all other shields were statistically significant at 0.05 level of significance. As illustrated in Fig. 6, when ranked based on reduction in $\mathbf{D}_c$ values, Shield 2 had the best performance followed by Shields 4 and 1, Shields 6 and 3, Shield 9, Shield 7, and Shield 8. As expected, Shields 5/2 and 5/1 gave the worst performance of all the shields tested.

Results with 1.71 L/min Standard Flat-Fan Nozzle

Results of tests with 1.71 L/min nozzles indicate that a reduction in $\mathbf{D}_c$ values ranging from 33.1 to 42.1 percent were realized as a result of using a shield. Fig. 7 illustrates performances of "low-capacity" (0.61 L/min) nozzles and relatively "high-capacity" (1.71 L/min) nozzles under identical operating conditions. Tests conducted under all four combinations of operating conditions (2 different pressures, 2 different air flow rates), using the same shield (Shield 1 at position 2), the high-capacity nozzles always had smaller $\mathbf{D}_c$ values compared to those for low-capacity nozzles. However, this was expected because high-capacity nozzles without a shield always had smaller $\mathbf{D}_c$ values than for low-capacity nozzles using the shield. This indicates that, if applicable, choosing a nozzle with a larger nominal flow rate may be as effective at reducing drift as constructing a costly shield around the spray boom.

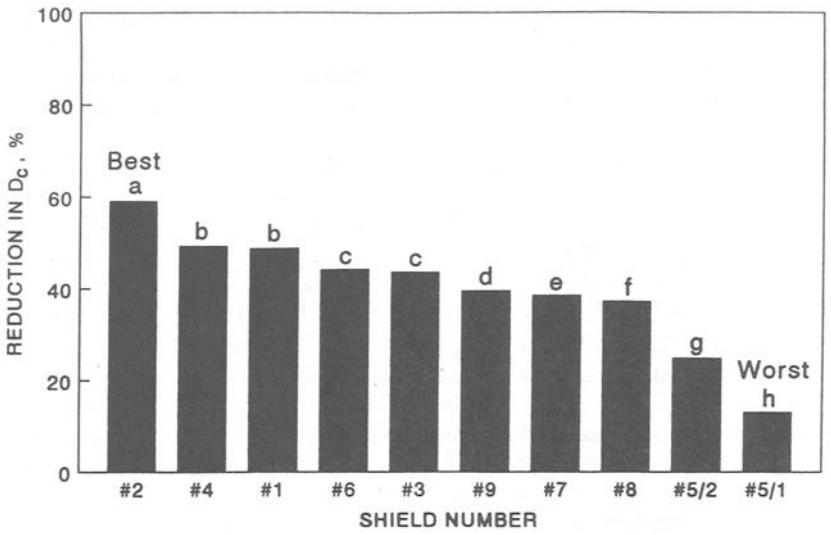

Figure 6. Overall ranking of shields tested. Values are mean of four treatments shown in Figure 5. (Bars with similar letters are not significantly different at P=0.05, DNMRT).

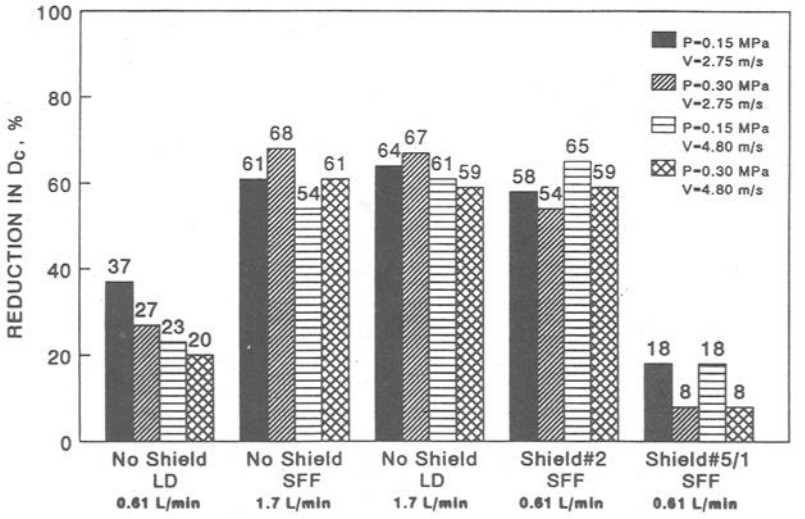

Figure 7. Percent reduction in Mean Distance to Center of Mass ($\mathbf{D}_c$) with the best shield (shield 2) and the worst shield (Shield 5/1) using 0.61 L/min Standard Flat-Fan (SFF) nozzles; and with no shield, using 0.61 and 1.71 L/min low-drift (LD) nozzles and 1.71 L/min SFF nozzles (as compared to 0.61 L/min SFF nozzles operating with no shield).

Results with 0.61 L/min and 1.71 L/min "Low-Drift" Flat-Fan Nozzles

As illustrated in Fig. 7, using 0.61 and 1.71 L/m in low-drift (LD) nozzles without a shield at 0.15 and 0.30 MPa pressures and at air velocities of 2.75 and 4.80 m/s provided reductions in $\mathbf{D}_c$ ranging from 20 percent (with 0.61 L/min nozzle at high pressure, high air velocity) to 67 percent (with 1.71 L/min nozzles at high pressure, low air velocity) when compared to the reduction in $\mathbf{D}_c$ from a 0.61 L/min standard flat-fan (SFF) nozzle operating under identical conditions. The 0.61 L/min SFF nozzles operating with Shield 2 (the shield with the highest reduction in $\mathbf{D}_c$), was twice as effective in reducing $\mathbf{D}_c$ as the same capacity LD nozzles operating without a shield. However, the low-capacity LD nozzles without a shield was twice as effective in reducing $\mathbf{D}_c$ as the SFF nozzles of the same capacity operating with Shield 5/1 (the shield with the worst performance). On the other hand, the high-capacity LD nozzles without a shield were as effective in reducing $\mathbf{D}_c$ as the low-capacity SFF nozzles with Shield 2. Percent reductions in $\mathbf{D}_c$ from 1.71 L/min LD and SFF nozzles without a shield were nearly the same. This indicates that the LD nozzles at higher flow rates were no more advantageous in reducing drift of droplets than the SFF nozzles of similar flow rate.

SUMMARY AND CONCLUSIONS

The problem of spray drift remains a serious health and safety problem. Among the strategies recommended for combating drift resulting from field sprayers are using a shield assembly that partially or completely covers the spray boom . The effect of several spray-boom shield designs and "low-drift" nozzles on spray drift are presented in this study. Performance of all experimental shields were evaluated under two spray pressures (0.15 and 0.3 MPa), and two air flow rates (2.75 and 4.80 m/s) in the wind tunnel. In addition to the tests with shields, one set of tests at both pressures and air flow rates was conducted without shields. A spray patternator table half of which lies inside the wind tunnel was used to determine deposition distance of droplets. The Distance to Center of Mass ($\mathbf{D}_c$) was used to characterize the spray distribution from each experiment. Major conclusions from this study are:

1. All of the nine shields we tested effectively reduced spray drift from 0.61 L/min capacity nozzles by directing more of the small, drift-prone spray droplets toward the ground. Even the least effective of the shield designs (shields made of a porous material) produced a 13% improvement in deposition of spray on the ground. The double-foil shield produced the best performance; it improved $\mathbf{D}_c$ by 59% in comparison to same nozzles spraying without the shield.
2. The shields were effective even when used with nozzles with higher flow rates (producing fewer small droplets). However, using larger capacity nozzles reduced drift more than using smaller capacity nozzles with the most effective shield.
3. As expected, the combination of high pressure and high air velocity (the combination with the greatest potential for drift) always produced the highest values of $\mathbf{D}_c$.

4. For the velocities and spray pressures selected for this study, when no shield was used, $\mathbf{D}_c$ was affected more by the increase in air flow than spray pressure. Experiments with all the shields, except Shield 1, indicated that the combination of high pressure and low air flow produced higher $\mathbf{D}_c$ values than the combination of high air flow and low pressure. This may be interpreted as, when a shield is used, the change in droplet size due to change in spray pressure affected $\mathbf{D}_c$ more than the change in air flow.
5. If applicable, choosing a nozzle with a larger nominal flow rate may be as effective at reducing drift as constructing a costly shield around the spray boom.
6. Low-drift (LD) nozzles without a shield at 0.15 and 0.30 MPa pressures and at air velocities of 2.75 and 4.80 m/s provided reductions in $\mathbf{D}_c$ ranging from 20 percent to 67 percent when compared to the reduction in $\mathbf{D}_c$ from a 0.61 L/min standard flat-fan (SFF) nozzle operating under identical conditions. The 0.61 L/min SFF nozzles operating with Shield 2 (the shield with the highest reduction in $\mathbf{D}_c$), was twice as effective in reducing $\mathbf{D}_c$ as the same capacity LD nozzles operating without a shield. However, the low-capacity LD nozzles without a shield was twice as effective in reducing $\mathbf{D}_c$ as the SFF nozzles of the same capacity operating with Shield 5/1 (the shield with the worst performance).
7. Without using a shield, the SFF nozzles at 1.71 L/min flow rate were as effective in reducing drift of droplets as the LD nozzles of the same flow rate.

Acknowledgement

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F. Nelson Keeney[1], Kent P. Steele[2], and Grant A. Von Wald[3]

EFFECT OF SURFACE CHARGE/PARTICLE SIZE OF A LATEX PARTICLE ON TRANSPORT THROUGH SOIL

REFERENCE: Keeney, F. N., Steele, K. P., and Von Wald, G. A., **"Effect of Surface Charge/Particle Size of a Latex Particle on Transport Through Soil,"** Pesticide Formulations and Applications Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: The role of particle size and surface charge on the ability of a particle to migrate in a soil water column was investigated utilizing carboxylated polystyrene latexes. Experimentally, the only latex particle that was stable and mobile in the soil was the highly charged (519 μ eq/g) 0.19 micron diameter latex. Hydrodynamic chromatography (HDC) was effective in determining both concentration and size distribution of the latex particles in the soil water matrix where there was high surface charge (519 μ eq/g) and small particle size, (< 0.2 microns) or low surface charge (7-11 μ eq/g) particles between 0.166 and 0.507 microns stabilized by a polyoxyethylene-polyoxypropylene block copolymer.

Size played a role, with the movement of the 0.166 micron latex equivalent to that of the 0.19 micron latex. Both were significantly more mobile (less sorptive) than the 0.507 micron latex. Soil also had an influence as 0.19 micron particles were seen to be less mobile in the high organic carbon (OC) soil (Catlin) versus a moderate OC soil (Cecil).

A compartmental model based only on sorption described the data reasonably well. The parameter estimates from the model for the sorption constant, void volume in the soil column, and initial latex concentration were a close approximation to those observed and consistent throughout the study.

KEYWORDS: surface charge, particle size, latex particle, transport, soil

[1]Senior Research Scientist, Formulation Science and Technology, DowElanco, Indianapolis, IN 46268

[2]Research Scientist, GAMMA-MISL, DowElanco, Indianapolis, IN 46268

[3]Research Leader, Dow Chemical, Midland, MI 48667

Recently Lahav and Tropp (1992) studied the movement of synthetic microspheres in saturated soil columns. Utilizing microspheres negatively charged via carboxylate groups, they observed that 1) retention of microspheres took place mainly near the entrance site of the suspension and increased considerably with decreasing flow rate; 2) consecutive runs on the same column and the addition of electrolyte $CaCl_2$ resulted in a small but steady increase in the retention of microcapsules on the column, and 3) latex particles below 1 micron were mobile in both heavy and light soils and were similarly affected by $CaCl_2$ concentration.

Several deficiencies were noted in this study. Flow rates were artificially high relative to a rain event or soil hydrodynamics under a rain event (range 3.6-9 mL/min). No actual data were presented on particle mobility as a function of size (or distribution), density, or surface charge. Little experimental detail was given. Generally the microcapsules utilized in the study were not well characterized.

Since the mobility of microcapsules in porous media depends on their surface charge, size and density (Yao et al.1971) and not on the contents within the microcapsule, we decided to investigate the role of particle size and surface charge on the ability of the latex particle to migrate in a soil water column under agronomic conditions. Carboxylated polystyrene latexes were used in this study.

LATEX MICROSPHERES

The latex microparticles were obtained from Bangs Laboratories, Inc. (979 Keystone Way, Carmel, IN 46032-2823). The polymer was a polystyrene vinyl carboxylic acid copolymer. The latexes were received as 10 wt % aqueous dispersions. Properties of the microparticles used in this study are shown in Table 1 and were determined by the manufacturer.

TABLE 1--Latex Properties
Polystyrene Vinyl Carboxylic Acid

Particle size (microns)	0.166	0.507	1.009	0.19	0.44	0.945
Surface titration (μ eq/g)	11.0	7.0	4.0	519	172	114
Parking Area ($Å^2$/Surface)	515	265	233	9.5	12.4	8.7
Stock Code	P0001660CN	P0005070CN	P0010090CN	P0001900CN	P0004402CN	P0009450CN

AQUEOUS SOIL EXTRACT

To an 8 oz glass bottle was added soil (20g) and deionized water (160g). The slurry was shaken on an Ebenbach shaker for 30 minutes followed by centrifugation at 2000 and 2700 rpm for 10 minutes each in a Damon/IEC Centrifuge. The supernatent liquid was filtered through fluted filter paper. The filtrate was identified as aqueous soil extract.

SOILS

Three characterized soils were used in this study. The soil type, texture and characteristics are shown in Table 2. They were provided by the Environmental Chemistry Laboratory.

TABLE 2--Soil Characteristics

Soil Type	Soil Texture	% Sand	% Silt	% Clay	% Organic Carbon(OC)	% Organic Matter(OM)	Bulk density g/cm^3
Sea Sand	Sand	96	2	2	<0.01	<0.01	1.67
Cecil	Sandy Loam	78	13.6	8.4	0.33	0.55	1.68
Catlin	Sandy Clay Loam	11.2	60.0	28.8	2.17	3.87	1.24

APPARATUS

The soil sorption apparatus is shown in Figure 1. The glass flask was a modified 500 mL three-neck flat bottom flask fitted with a septum port on one side and a stopcock outlet on the bottom. The glass column measured 2.5 cm (ID) x18cm with a usable space for the soil of approximately 5 cm above the supporting glass rods. The soil was supported on the glass rods by two disks of 316 stainless steel mesh wire placed on top of each other, one 170 mesh and the other 325 mesh. The edges of the wire were wrapped with Teflon tape. The soil was carefully poured into the glass column and lightly tapped. A positive displacement pump, Altex model 110A, with a flow rate range of 0.1 to 9.9 mL/minute was used in this study. This pump proved to be very reliable and reproducible. The pump flow rate was calibrated and delivered a measured volume of deionized water over a prescribed time. The line void volume between the stopcock at the bottom of the flask and the entrance at the top of the column measured approximately 2.6 mL.

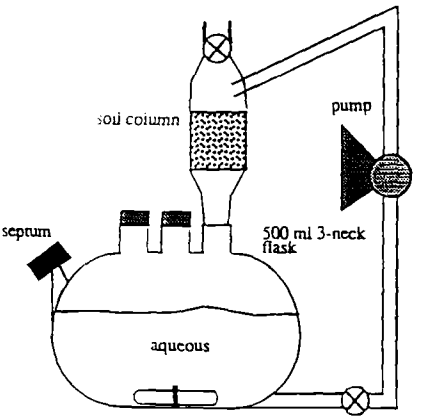


Figure 1

LATEX SOIL SORPTION STUDY

Deionized water (160 mL) was circulated through 20g soil in the column for 12-15h. The flow rate varied depending on soil type. For sand a rate of 9.9 mL/min maintained a slight water puddle on top of the soil. Catlin and Cecil soil required 0.3-0.5 mL/min to attain the same conditions. The soil column was then allowed to drain for 2-3h. The soil water in the flask was discarded and the flask washed thoroughly followed by an acetone rinse. The soil column was weighed to ascertain the weight of the wet soil. Typically, sand, Cecil and Catlin gained 5, 5-5.5, and 8 g of water respectively.

Deionized water was added to the assembled flask [160 mL minus volume of latex stock solution needed to make a final concentration of 3.2 micrograms latex solids/mL (corresponding to 1.0 pound latex solids/acre)]. The pump was turned on and the rate was established such that the pump rate was just slightly faster than the percolation rate through the column. Flow rates are shown in Table 3. Flow rate decreases with Cecil and Catlin soils may have been caused by further soil compaction or pore blockage. Flow rate was recorded over the coarse of the experiment. Equilibration required from 45 min to 1.5 h. The latex stock concentrate was added and stirred for 30 sec. At this point the initial sample was taken.

TABLE 3--Typical Pump Flow Rates for Soil Column, mL/min

	Sea Sand	Cecil	Catlin
Initial	9.9	0.4-0.5	0.3
Final	9.9	0.1	0.1

The sample size was approximately 1 mL. Periodically additional samples were withdrawn. Over the course of an experiment, generally less than 48 h, sixteen to eighteen samples were withdrawn for analysis. The flow rate was adjusted over the course of the experiment to maintain a slight water puddle.

ANALYTICAL METHODOLOGY

Hydrodynamic Chromatography

Hydrodynamic chromatography (HDC) is a liquid chromatographic method where colloids, generally latex, are separated in a packed bed of impervious, non-porous particles. It was developed by Dow in the 1970's and is used routinely to analyze latexes in the size range of 0.02 to 1.0 micron (Small 1976). Samples were filtered through a 10 micron polyester filter and added to the HDC eluent. Typical conditions utilized in this study are listed below:

Column	15 micron diameter packed with solid polystyrene divinylbenzene copolymer beads
Eluent	2mM NaH_2PO_4 pH=3.2 0.2%(w/v) BRIJ 35 [(lauryl ether ethoxylate(23 EO's)] (ICI) 0.05%(w/v) sodium lauryl sulfate
Detection	UV detector at the wavelength of 210 nm with delayed marker injection

A typical chromatogram from one sample taken from an experiment using 0.19 micron latex is shown in Figure 2. Quantitation information for the three highly charged latexes is shown in Table 4. This method worked very well for the highly charged latexes. Soil colloids did not appear to interfere with peak resolution or detection.

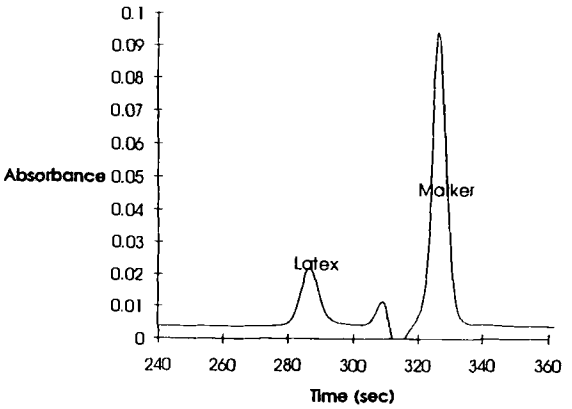


Figure 2—Chromatogram for 3.23 µg/mL 0.19 µm Latex from Soil Sorption Experiment

TABLE 4--LOD, LOQ and Reproducibility for Latex Quantitation by HDC

Latex Diameter	Limit of Detection	Limit of Quantitation	Reproducibility (r.s.d. %)
0.19μm	0.009μg/mL	0.03μg/mL	1.1
0.44μm	0.03μg/mL	0.1μg/mL	0.95
0.945μm	0.25μg/mL	0.8μg/mL	1.7

Pyrolysis/Gas Chromatography(Py/GC)

Py/GC is a technique used to characterize the composition of polymers. Polymer chains are de-polymerized back to monomer and other fragments by heating to 700° C in a helium atmosphere. The pyrolysis products are separated by gas chromatography and detected by Flame Ionization Detection. (FID). The 0.19 micron latex stock dispersion was diluted into soil extract to make 100 micrograms/mL concentration. Just prior to analysis methyl methacrylate (MMA) latex was added as an internal standard. Ten (10) microliters of suspension was dried onto the pyrolysis ribbon and pyrolyzed. Standards of known ratio of MMA to polystyrene latex were used to calibrate the Py/GC. A typical pyrogram is shown in Figure 3. The LOD for this procedure was approximately 1.0 microgram/mL over the latex size ranges investigated in this study. No interferences were observed when the latex was dispersed in soil extract.

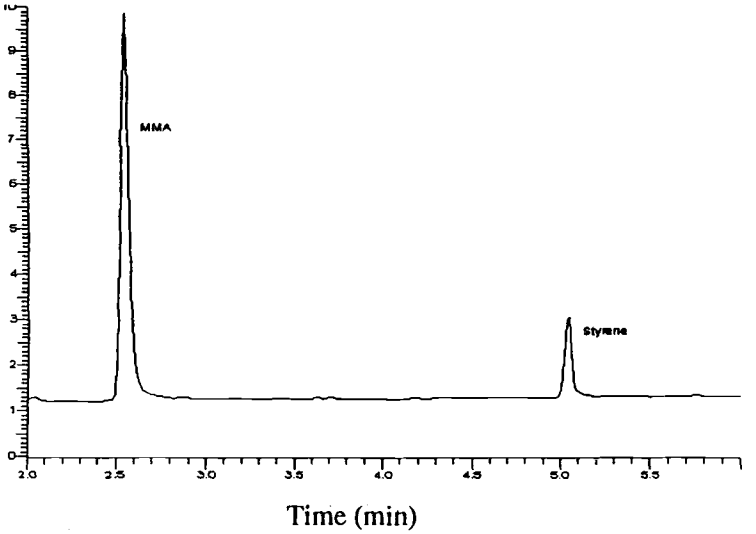


Figure 3—Pyrogram of 100 μg/mL MMA Latex and 10 μg/mL PS Latex

RESULTS AND DISCUSSION

Certain latexes could not be studied utilizing HDC to quantitate their concentration on dilution in the aqueous soil matrix because they were found to be unstable due to particle flocculation on aggregation to particles larger than 1 μm, the upper limit of detection for HDC. No evidence was observed for particle sedimentation or late decomposition. For details of the difficulties in utilizing HDC for direct analysis of latex particle in soil matrices, see Appendix I.

Allowing for the limitations of HDC, this technique was utilized for profiling the soil sorption of those latex suspensions that were found to be stable. Namely, the high surface charge 0.19 μm alone and the two low surface charge latexes, 0.166 and 0.507 μm, stabilized with 0.25 volume % BASF's Pluronic P-105, a polyoxyethylene-polyoxypropylene block copolymer. The schematic shown in Figure 1 was used. The flow technique was chosen over a batch system because it closely represents transport of pesticides in soil. Although the flow technique is unsuitable for chemical kinetics it does provide apparent rate laws and kinetic parameters which are of interest in this study.

The disappearance of latex concentration from the bulk solution over time was monitored. Figure 4 shows the influence of particle size on soil sorption with the Catlin soil. The two smaller particles were significantly less sorptive than the larger 0.507 μm particle. Experimentally, the only latex particle that was stable, and thereby mobile in the soil, was the high surface charged 0.19 μm, leading to the conclusion that high surface charge and a very small particle are essential elements for particle mobility in the soil. These findings are consistent with model predictions and the research of previous investigators (Yao et al. 1971, Lahav and Tropp 1980) who found that soil mobility of microparticles in porous media depends on their surface charge, size, and density.

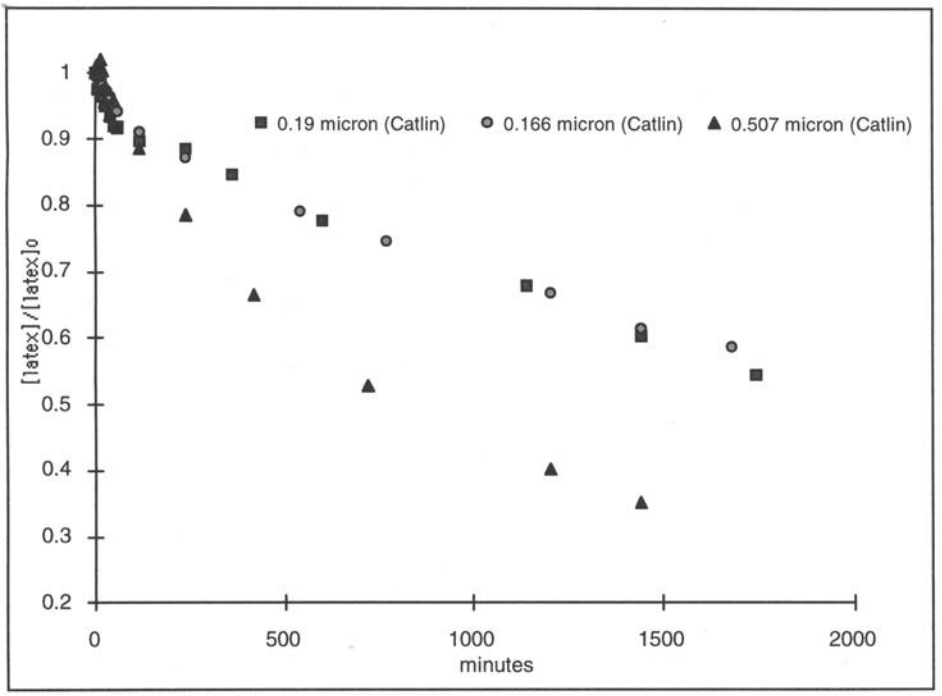


Figure 4
Sorption (Disappearance) of Latex Particles of Different Size on Catlin Soil

The influence of soil organic content (OC) on soil sorption for the high surface charge 0.19 μm latex was investigated. The data is shown in Figure 5. The two soils vary significantly in their organic carbon content with Catlin being considerably higher . The soil sorption profile for the two soils was quite similar for the first several hours. However, as the experiment continued, the differences between Catlin and Cecil soil became apparent. This is consistent with nonionic surfactant soil sorption where Urano et al.(1984) found that at sub-CMC surfactant sorption was proportional to the organic carbon content of the soil. The higher the organic carbon content the more sorptive the soil.

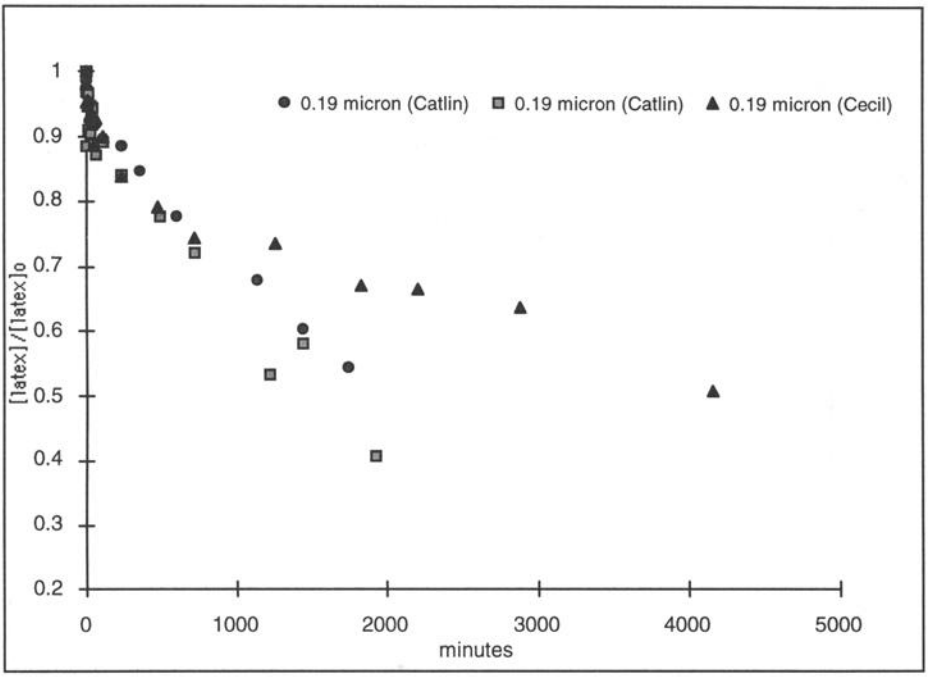


Figure 5
Sorption (Disappearance) of 0.19 micron Latex Particles on Catlin and Cecil Soils

Figure 6 shows a comparison of particle surface charge and soil sorption. The surfactant stabilized low surface charge 0.166 μm and the high surface charge 0.19 μm latex were identical in terms of soil sorption profile over the course of experiment. This suggests that a significant portion of the nonionic surfactant was associated with the low surface charge particle, allowing it to move through the porous soil without being strongly bound to the soil (or flocculated). This is consistent with the findings of Liu (1992) and Edwards et al. (1992,1994) who found that hydrophobic organic compounds solubilized within the micellular pseudophase are not sorbed to soil. By analogy, a low surface charge latex stabilized by non-ionic surfactant, is potentially mobile in soil under hydrodynamic conditions.

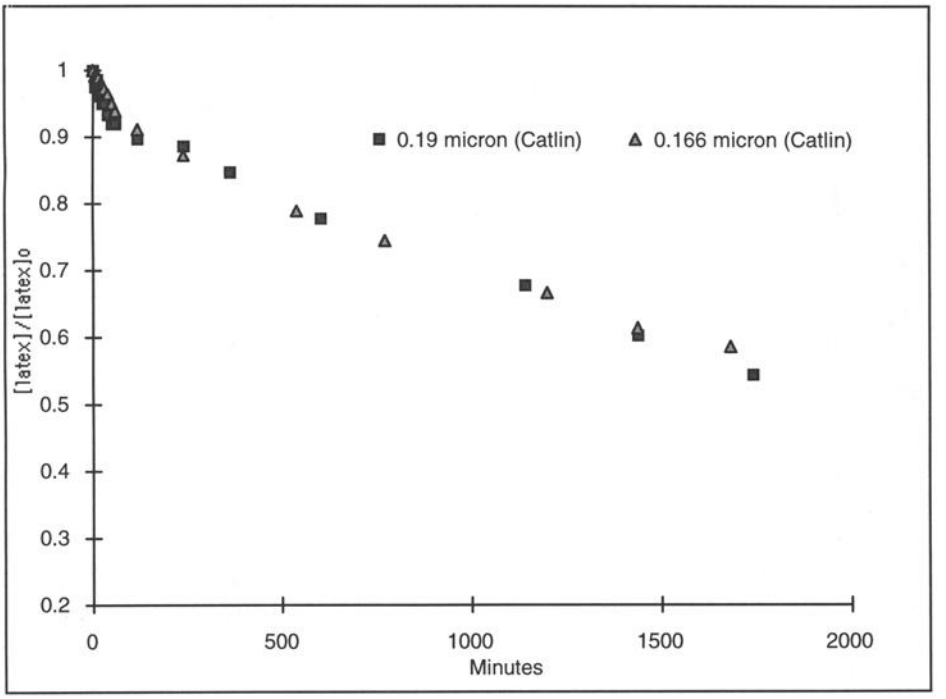


Figure 6
Sorption (Disappearance) of Latex Particles of Different Surface Charge on Catlin Soil

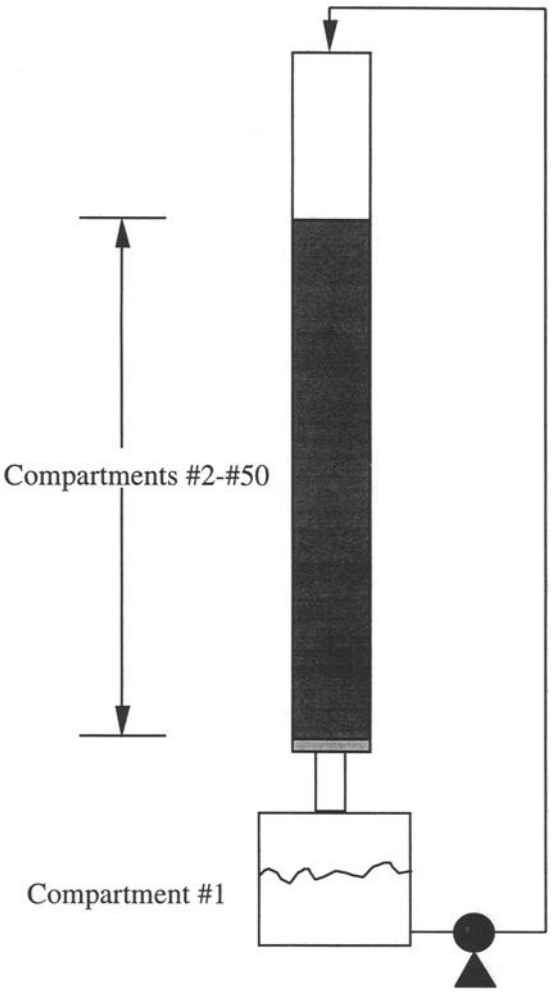
Numerous models were investigated in an effort to fit the data generated from the latex soil sorption studies. The models are shown in Table 5.

TABLE 5--Model Formulations for Latex Particle Sorption Data

Model Name	Mathematical Form
Mth order sorption and Nth order desorption	$d[latex_{liq}]/dt = - k_1*[Latex_{liq}]^m + k_2*[Latex_{soil}]^n$
Time Power Law	$[Latex_{liq}] = [Latex_{liq}]_0 - k_1*Time^m$
Gamma: (Connaughton et al., 1993)	$[Latex_{liq}] = [Latex_{liq}]_0 * (b/(b + Time))^a$
Compartment Model - Sorption only	$d[Latex(J)_{liq}]/dt = ([Latex(J-1)_{liq}]-[Latex(J)_{liq}])*Flow - k_1*[Latex(J)_{liq}]$
Compartment Model - Sorption & Desorption	$d[Latex(J)_{liq}]/dt = ([Latex(J-1)_{liq}]-[Latex(J)_{liq}])*Flow - k_1*[Latex(J)_{liq}] + k_2*[Latex_{soil}(J)]$

All models were implemented in SimuSolv* Modeling and Simulation Software (Tradename of The Dow Chemical Company). The simplest forms were investigated first. No single simple model form fit all of the data sets. Either the residuals were not randomly distributed about zero, or the model parameters were not consistent with the physical set up. Part of the initial modeling problem was due to the instability of the latex suspensions, but also it was not clear that the experimental design and sample collection would yield data that could be modeled by these simple kinetic expressions. In particular, the variable flow rate over time could not be taken into account in any of the first three model formulations. Therefore, a model was developed to reflect the physical reality of the experimental design and to obtain the kinetic parameters to describe the sorption and desorption of the latex particles.

The result was a compartmental model shown schematically in Figure 7. In this model formulation, the water reservoir is the first compartment, and the soil column was divided into forty-nine compartments of equal volume. There is a separate mass balance relationship for each compartment such that the amount incoming minus the amount outgoing is equal to the amount sorbed onto the soil in that compartment. The compartmental model makes use of the flow rate information. A similar model containing a desorption term gave no better fits to the data, so the simpler sorption only model was used.



For each compartment, material balance is modeled as
incoming - outgoing - sorbed

Figure 7
Schematic for Latex Soil Column Compartment Model

Parameters generated for the model are presented in Table 6. Numbers in parentheses after the parameters are one standard deviation of the parameter estimate. The standard deviations of the parameter estimates are between 7% and 25% of the expected values for the estimates. Although the uncertainty is high, all of the estimates are significantly different than zero. As the sorption parameters show, the larger 0.507 micron particles have the largest sorption coefficient, and the 0.19 micron particles on Cecil soil had the smallest sorption coefficient. Overall, the parameter estimates reflect the trends seen in Figures 4 to 6.

TABLE 6--Parameter Estimates for a Fifty Compartment Model

Soil	Size (microns)	k_{sorp}	$Latex_0(1)$ (mg/mL)
Catlin	0.19	8.13E-3(1E-3)	3.01(0.01)
Catlin	0.507	2.64E-2(5.4E-3)	3.48(0.02)
Catlin	0.166	4.39E-3(3E-4)	3.46(0.01)
Cecil	0.19	1.68E-3(4E-4)	3.1(0.05)
Catlin	0.19	9.1E-3(7E-4)	3.11(0.06)

Further development of the mathematical model for the movement of latex particles in soils was not pursued because of the difficulty of obtaining experimental data for latexes of other sizes or surface charges. The data set described in this report is too small to warrant further model development. The objective of developing a general tool to predict the movement of latex particles in any soil was not realized.

SUMMARY

Carboxylated polystyrene latexes were used to model the role of particle size and surface charge on the ability of a particle to migrate in a soil water column. Experimentally, the only latex particle that was stable and mobile in the soil was the highly charged (519 μ eq/g) 0.19 micron latex. Other latex particles were not stable in the soil water column without the addition of additives. Hydrodynamic chromatography (HDC) was effective in determining both concentration and size distribution of the latex particles in the soil water matrix where there was high surface charge (519 μ eq/g) and small particle size, (< 0.2 microns) or low surface charge (7-11 μ eq/g) particles between 0.166 and 0.507 microns stabilized by a polyoxyethylene-polyoxypropylene block copolymer. Potential particle stabilization of a highly charged 0.44 micron latex with a polyoxyethylene-polyoxypropylene nonylphenol formaldehyde condensation product was suggested.

Experimental results showed that the surfactant stabilized 0.166 and 0.507 micron latexes were mobile in the soil column. The movement of the 0.166 micron latex was equivalent to that of the 0.19 micron latex. Both were significantly more mobile (less sorptive) than the 0.507 micron latex. With the 0.19 micron latex the soil organic content (OC) influenced soil sorption. The 0.19 micron particles were less mobile in the high OC Catlin soil versus a moderate OC soil like Cecil.

Numerous mathematical models were evaluated to fit the experimental data. A compartmental model based only on sorption reasonably described the data. The parameter estimates from the model for the sorption constant, void volume, and initial latex concentration were a close approximation to those observed and consistent throughout the study.

ACKNOWLEDGMENTS

The authors wish to thank Steve Cryer and Jeff Wolt of Environmental Fate for technical support and assistance in the design and establishment of the apparatus as well as fruitful discussions and critique of the study protocol. Special thanks to Joe Winkle , whose idea it was to do the project, and for his encouragement along the way. The expertise of Steve Wilson and Dennis Wujek in the area of latex polymers and surfactants used in their stabilization was invaluable during the course of this experiment.

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APPENDIX I

Critical to the success of this study was the ability to measure the decrease in concentration of the carboxylated polystyrene latex when exposed to soil and water soluble soil colloids at concentrations in the range of 0.3 to 3 μg/mL and for latexes in the size range of 0.1 to 1 μm. For the high surface charge latexes (0.19, 0.44, and 0.945 μm) HDC quantitated the latex concentration when dispersed in the HDC eluent. Figure 2, representing 3.23 μg./mL 0.19 μm latex from a soil sorption experiment, shows a typical chromatogram. Calibration curves for the 0.19 and 0.945 μm latexes were linear over the concentration range of 0.03 to 6 μg/mL. Quantitation data for these three latexes are shown in Table 4. The upper size limit of HDC is 1.0 μm.

During the course of this investigation it was discovered that the 0.44 and 0.945 μm high surface charge latex showed a decrease in concentration on dilution in deionized water, Catlin soil water extract and 0.01M $CaSO_4$, used to mimic the ionic strength of the Catlin soil extract (Von Wald,1995). Flocculation of the latex was suspected.

Data for the 0.44 μm latex supporting this conclusion are shown in Table 7. All showed a decrease in latex concentration over time. It was not possible to make HDC measurements for the $CaSO_4$ sample because the 0.44 μm latex eluted at a shorter time indicating partial flocculation of this latex in 0.01 M $CaSO_4$. The 0.945 μm latex showed a similar trend (data not shown). The 0.19 μm latex was quantifiable by HDC in deionized and Catlin soil extract waters. Further investigation showed that all three of the low surface charge latices (0.166, 0.507, and 1.009 μm) were unstable in deionized water and Catlin soil extract water at the concentration range for this study (3-4 μg/mL). This is illustrated by the chromatograms for the 0.507 μm latex suspended in water and the HDC eluent (Figure 8). The shift to a shorter retention time indicates a larger particle size for the latex suspened in water. This is indicative of particle flocculation or aggregation. An identical phenomenon was observed with the 0.166 μm latex (data not shown). For the larger 1.009 μm latex the cause for non-detection was not determined. No evidence could be found for flocculation of this latex in water. Because the upper size limit of HDC is 1 micron, any flocculated 1 micron latex may have been too large to detect.

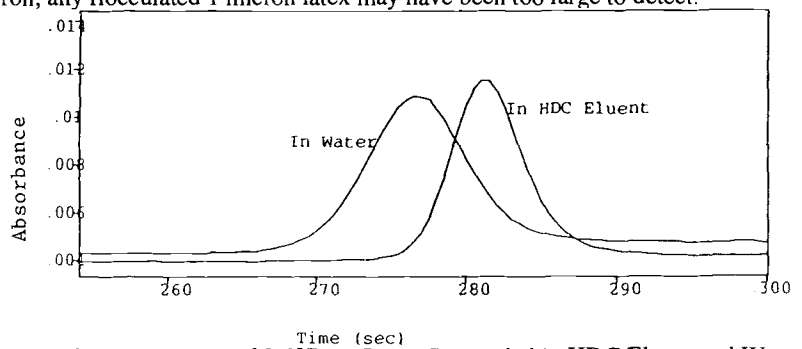

Figure 8—Chromatograms of 0.507 μm Latex Suspended in HDC Eluent and Water

TABLE 7--Determination of 0.44 μm Diameter PS Latex Using HDC

	5.04 μg/mL in water	5.01 μg/mL in filtered Catlin Soil Extract	4.41 μg/mL in Catlin Soil Extract
Day	HDC	HDC	HDC
0	4.77	4.98	3.73
1	4.72	5.07	3.58
3	3.91	4.67	3.40
4	3.53	3.18	3.32
7	2.21	0.33	3.12
9	1.74	0.31	3.06
11	1.31		3.06
14	1.00		3.04

The concentration of nonionic surfactant in the latexes was thought to be in the range of 0.25 to 0.5 volume %. At the initial use concentration for the latex solid of 3-4 μg/mL the level of nonionic surfactant would be in the range of 0.08 to 0.16 μg/mL in the bulk solution. This concentration is well below the critical micelle concentration (CMC) for the type of nonionic surfactants commonly used (Rosen 1989, Keller and Rickabaugh 1992). At levels sufficiently below the surfactant CMC, stabilization of the latex particle would be minimal at best, since competing equilibria would also be occurring between the water soluble organic colloids in the bulk solution and the soil solid phase (Edwards et al. 1992,1994). Therefore, stabilization of the low surface charge latexes was investigated by adding BASF's Pluronic P-105, a polyoxyethylene-polyoxypropylene block copolymer, to the bulk solution. At 0.25 volume % in the bulk solution Pluronic P-105 successfully stabilized the 0.166 and 0.507 μm latexes with the Catlin soil but not the larger 1.009 μm latex as evidenced by HDC. That is, the concentration of latex remained constant over several days (data not shown).

In an effort to determine whether latex concentration detected by HDC decreases due to flocculation, especially in soil water extract, a size independent technique was investigated. Pyrolysis/Gas Chromatography (PyGC) was chosen (Von Wald 1995). Using this technique the latex concentration is determined from the peak area of the styrene monomer pyrolysis product. Although this technique is not as sensitive as HDC, (LOD was ~0.5 μg/mL), this level of detection would allow one to investigate the soil sorption through two half-lives.

The 0.44 μm latex was suspended in a number of different solution matrices and measured using both techniques. The data is shown in Table 8. It was not possible to make HDC measurements for the $CaSO_4$ solution for the reason mentioned previously. On the other hand Py/GC detected the expected concentration within the reproducibility anticipated for the method. As expected from previous studies the concentration of the

0.44 μm latex decreased significantly over time. However, Py/GC detected a much smaller or insignificant change in latex concentration over time for all samples. The most rapid decrease in concentration detected by HDC was observed for the filtered Catlin soil extract where the latex concentration decreased to 6 % of the starting value after only seven days. This result suggests that other factors besides the ionic strength and colloid content may be involved.

TABLE 8--Determination of 0.44 μm Diameter PS Latex Using Py/GC and HDC

	5.04 μg/mL in water		5.01 μg/mL in filtered Catlin Soil Extract		4.48 μg/mL in 0.01 M CaSO4		4.41 μg/mL in Catlin Soil Extract	
Day	HDC	PyGC	HDC	PyGC	HDC	PyGC	HDC	PyGC
0	4.77	5.19	4.98	5.09	...	4.10	3.73	3.60
1	4.72	4.78	5.07	4.80	...	4.32	3.58	3.35
3	3.91	4.40	4.67	5.31			3.40	4.20
4	3.53	4.43	3.18	4.46			3.32	3.61
7	2.21		0.33	~3			3.12	
9	1.74		0.31				3.06	
11	1.31						3.06	
14	1.00						3.04	

These measurements confirm that formation of flocculated larger particles which cannot be detected by HDC contribute to the observed decrease in latex concentration as measured by HDC. However, the observation of a decrease by Py/GC suggests that if flocculation is the cause of the observed decrease in measured concentration, then either the latex is forming aggregates or adhering to the wall of the vial so that it cannot be reproducibly sampled.

Previously, it was mentioned that polyoxyethylene-polyoxypropylene block copolymers stabilized the 0.166 and 0.507 μm low surface charge latexes in the soil water column, but not the higher particle size 1.009 μm latex. Additional experiments (data not shown) were performed in Catlin soil extract utilizing ICI's comb polymers, ATLOX 4913 and 2350. ATLOX 4913 contains a methyl methacrylate (MMA) backbone which is ethoxylated. ATLOX 2350 is an ethoxylated nonylphenol formaldehyde condensation product. Both have HLBs in the range of 11-12. Utilizing these polymers at 0.25 volume %, dispersions with the 0.44 μm latex were prepared in Catlin soil extract water. The data presented in Table 9 suggested that the ATLOX 2350 stabilized the latex, whereas evidence for stabilization from ATLOX 4913 is unclear. The reproducibility from these preliminary studies was reduced over that shown from earlier studies and the initial concentration of latex in the ATLOX 2350 was lower. Although several possible explanations could be argued for these observations further studies were not pursued with the comb polymers.

TABLE 9--Stability of 0.44 μm PS Latex Suspended in Catlin Soil Extract with ATLOX Surfactants
Measured by HDC (Concentrations in μg/mL)

Date	0.25% ATLOX 4913	0.25% ATLOX 2350	Catlin Soil Extract
3/6, Day 0	5.7	4.4	5.4
3/7, Day 1	5.9	5.1	5.6
3/10 Day 4	6.2	5.4	4.7
3/14 Day 8	5.1	4.7	4.2
3/20 Day 14	6.0	4.8	4.0
3/20 Day 14	6.2	4.6	4.0
3/27 Day 21	6.1	4.6	3.6
3/27 Day 21	7.2	4.6	3.7
4/3 Day 28	5.4	4.7	3.7
4/3 Day 28	5.6	4.4	3.8

REVIEWS

Norman R. Pallas[1]

A Review of the Measurement of Wettability for Agricultural Applications

REFERENCE: Pallas, N. R., **"A Review of the Measurement of Wettability for Agricultural Applications,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT:
This review addresses issues important to the estimation of wetting, spreading, and adhesion in the development and use of agricultural formulations. Beginning with a thorough review of the physical principles underlying the phenomena, methods for evaluation of the wetting properties of smooth and rugous solids, consolidated and unconsolidated porous media, powders, and fibers are described with some examples of typical data. Only brief mention is made of the importance of dynamics and the measurement of dynamic properties.

KEYWORDS: wetting, spreading, adhesion, contact angle, line tension, adsorption, Draves, Wilhelmy, immersion, penetration

Introduction:

The concept of wettability is at some point of practical importance to nearly every industry and process. Whether one desires to maximize the efficiency of a distillation column, improve the performance of a protective coating, or formulate a wettable powder, the same physico-chemical properties need be considered. As application scientists we each have a seemingly instinctual understanding of what is 'wet' and 'not-wet' which frequently transcends and occasionally contradicts the physical science underlying the reality.

[1]Team Leader Agricultural Technical Development, Rhône-Poulenc Surfactants and Specialties, 1900 Prospect Plains Road, Cranbury, NJ 08512

Wetting properties are of great import throughout the conceptualization, formulation, delivery, and activity of many agricultural formulations. The choice of additives to WP, WDG, SC, and even EC's , to improve dispersion, grind efficiency, stability, spray deposition, soil penetration, and in many cases bio-efficacy depends upon the appropriate choice of a method for evaluating the relative effects in a practical application.

While it is of obvious importance to have practical measures of wettability, the measurement of wettability for agricultural applications is not subject to any standard. Often, the choice of method used to estimate the effect of an additive to a formula is inappropriate for the system being studied and may lead to incorrect conclusions.

It is therefore the purpose of this paper to elucidate the basic phenomena, present popular methods and review some methods less well known which may find utility in the formulation and delivery of agricultural materials.

First, taking in the interest of brevity a somewhat less than rigorous thermodynamic approach, the basic physico-chemical concepts underlying wetting are reviewed, then experimental methods are described along with a discussion of their use in formulation science.

Basic Principles:

The three essential components of the general phenomenon of wetting are identified as; spreading, adhesion, and immersion, though immersion may be considered a special case of spreading. Such a neat demarcation does not apply well to agricultural systems. In almost every application two or all three phenomena are in play. It is then very important that we have an understanding of the basic underlying issues.

To begin with a few essential definitions, let's consider a 'simple' system of two immiscible, non-volatile liquids a and b, in thermal and hydrostatic equilibrium with an insoluble gas. Taking surface tension to be defined as the amount of work necessary to extend an interface 1 unit area, then at constant temperature and pressure;

$$dF = \Sigma \gamma dA \qquad \text{Eq. (1)}$$

Where F represents the sum of the reversible work for all interfaces, γ is surface tension, and A is area. Cohesion may be defined as the amount of work required to separate a column of a single component liquid into two surfaces each of unit area. Similarly, adhesion may be defined as the amount of work required to separate a liquid/liquid interface, a/b, of unit area composed of two different liquids a and b, into two vapor liquid interfaces each of unit area, a and b;

$$W_{co} = 2\gamma_{a,b} \qquad W_{ad} = \gamma_a + \gamma_b - \gamma_{ab} \qquad \text{Eq. (2)}$$

The expression for the reversible work of adhesion was first derived by Dupré in 1869. The difference between the work of adhesion and the work of cohesion defines the so-called Harkin's spreading coefficient:

$$S = W_{ad} - W_{co} \qquad \text{Eq. (3)}$$

While equations (2) and (3) are explicitly written for liquid/liquid and liquid/vapor interfaces, they may also be expressed for a liquid in contact with vapor and a solid.

It was Cooper and Nuttall who in 1915 while investigating the spreading of insecticides on leaves described the condition for the spreading of a liquid on a solid or another liquid. If S is positive, then it is expected that spontaneous spreading will occur, if negative then spontaneous spreading will not occur. Experimentally, the accurate determination of spreading coefficients is more difficult than implied above. If, for example, the two liquids have any mutual solubility, then it is insufficient to simply use surface tensions for the fluids in equilibrium with their own vapors, or if any water vapor is present its potential to adsorb to one of the liquids must also be considered. These complications also apply to spreading of a liquid on a solid. While these experimental difficulties do limit the practical utility of spreading coefficients, the ideas are conceptually useful. A more detailed historical perspective and discussion of these concepts and the practical limitations and difficulties can be found in the writings of Ford and Furmidge (1967), Zisman (1963), Adamson (1982), and Rusanov (1996).

In a practical sense, there is only one system which must be considered in some detail, that of a liquid in equilibrium with its own vapor and in contact with a non-deformable, non-volatile, insoluble solid. (Figure 1)

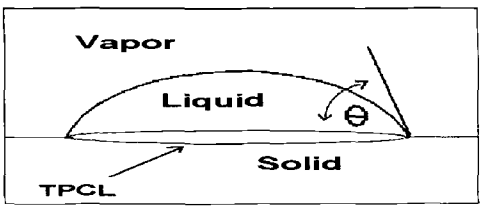

Figure (1)
A drop of liquid on a solid showing the contact angle, three phases, and the Triple Phase Contact Line.

The equilibrium between the solid, liquid, and vapor phases was described by Young (1805) and can be written, employing the concept of virtual work as we have done earlier, as;

$$\gamma^{lv} \cos\theta = \gamma^{sv} - \gamma^{sl} \qquad \text{Eq. (4)}$$

From equation (2) the work of adhesion can now be given by;

$$W_{ad} = \gamma^{sv} + \gamma^{lv} - \gamma^{sl} \qquad \text{Eq. (4a)}$$

then with equation (4) $W_{ad} = \gamma^{lv}(\cos\theta + 1)$ Eq. (4b)

Where the superscripts refer to the l - liquid, v - vapor, and s - solid phases. The contact angle θ, is always defined through the denser phase, regardless of whether a drop of liquid in vapor sits on a solid or a bubble of vapor is trapped under a solid surrounded by liquid. It is important to note that the interfacial tensions are in equilibrium with all phases and do not represent free energies.

The form of equation (4) may not be entirely correct for some circumstances. Following previous work (Pethica 1977)

$$dF = \Sigma \gamma dA + \tau dL \qquad \text{Eq. (5)}$$

which, at constant temperature and pressure, represents the sum of the work terms involved in the extension of the area covered by the drop and where;

$$\tau = (\delta F/\delta L)_{TVA} \qquad \text{Eq. (6)}$$

denotes the line tension, and L is the length of the triple phase contact line (TPCL) at constant temperature, T, volume V, and area A. The length of the TPCL is equivalent to the perimeter of the circle subtending the area of the solid covered by the drop shown in Figure (1). Recognizing from Figure (1) that;

$$dA^{sl} = - dA^{sv} = RdL \qquad \text{Eq. (7)}$$

$$\text{Then, } \gamma^{sv} = \gamma^{sl} + \gamma^{lv} \cos\theta + \tau/R \qquad \text{Eq. (8)}$$

Where R is the radius of the solid/liquid interface. It is immediately clear from equation 8 that the effect of line tension becomes important only as the radius of the drop becomes quite small.

The original form equation (4) as expressed by Young made use of stress tensors, it was recast by Sumner (1937) in its current form, but it was Gibbs (1961) who foresaw the effects of line tension. It has been pointed out that the original expression by Young is completely general, if somewhat less than useful in a practical sense, but the form as given by equations (4) or (8) introduce implicit restrictions not present in the original work (Gray 1967). A more detailed, rigorous thermodynamic discussion of these concepts is given by Morra et al. (1990), including a discussion of the theory of solid surface free energies as described by the Good-Girifalco and Fowkes treatments.

Critical Surface Tension for Wetting:

In order for equations (4) or (8) to be valid, thermal, chemical, and mechanical equilibrium must be attained in addition to the solid being a non-deformable, homogeneous, flat surface, in contact with pure fluids. For systems where the radius of curvature is greater than about 1mm, all possible effects due to line tension are negligible (Aveyard and Clint 1996 , de Gennes 1985). So, we then expect from equations (3) and

(4) that in order for a fluid to spread fully upon a solid or a liquid, giving a zero contact angle, a small value for the liquid/vapor tension is desired. This apparent necessity formed the basis for the concept of 'critical surface tension' (Samson 1964, Dann 1970) which should not be confused with surface tension on approach to a critical point and is not related to the specific surface free energy of the solid. Zisman described in his 1964 review that by plotting the measured cosine of the contact angle vs. the surface tension of a homologous series of fluids all on the same solid, a straight line was formed providing a value for the maximum surface tension needed to obtain a zero contact angle. The value of this critical tension was found to be independent of the chemical nature of the liquids used.

Adsorption to the solid may, however, change the value of the critical tension. So, as Rosen points out (Rosen 1978) 'a solution whose surface tension is below the critical tension for the substrate may or may not produce complete wetting'.

Wetting and Adsorption:

Zisman found that there are systems which have the requisite low tension but do not spread due to the formation of 'auto-phobic' bilayers. The phenomenon of the formation of autophobic layers emphasizes the importance of adsorption and the state of the adsorbed film to the relevant interfaces on spreading and wetting (Hu and Adamson 1977, Vogler 1992, Smolders 1961, Sasaki et al. 1957). Seimiya et al. (1969) demonstrated experimentally the relationship between the work of adhesion and adsorption to the solid/liquid interface, apparently unaware of the work by Smolders. The work of Haidara et al in 1996 is of particular interest in that they firmly established the existence of changes of state in adsorbed films on solids using contact angle as the marker. The presence of a surface phase transition on a solid would have great impact on the stability of suspensions of solids. The concept of surface aggregation to form bilayers or to form small aggregates akin to micelles is hardly new (Adamson 1982) but the use of changes in wettability makes easier their detection.

Surface Roughness and Heterogeneity:

We have ignored, so far, the effects of surface rugosity or heterogeneity on spreading and wetting. For most any solid of practical interest, the contact angle found upon advancing a liquid front is larger than that found upon retracting that same TPCL. This defines the advancing and receding contact angles. This phenomena of contact angle hysteresis is believed due to surface roughness or compositional heterogeneity of the solid surface which is easily produced by contamination. Of particular importance to many systems of practical interest is the equilibration of a hydrophobic solid with water vapor (Zhu et al 1994). Tiberg and Cazabat (1994) proposed that both the formation of bi-layers and high relative humidity, 30-50% R.H., may explain the so-called super-spreading phenomenon of certain surfactants. As early as 1980 (Fowkes et al. 1980) it was recognized that the presence of a small number of a hydrophilic sites on a hydrophobic surface, such as Teflon, can radically alter the contact angle and spreading due to the adsorption of water.

Equations to account for the effect of surface roughness have been proposed by Wenzel and Cassie and Baxter, though tests of these equations have been less than successful (Dettre and Johnson 1964, Gray 1967, Li and Neumann 1992, Morrow 1975). Attempts to account for the effects of heterogeneity theoretically have met with modest success (Johnson and Dettre 1964 , Drelich et al 1996) Much of the difficulty lies in the evaluation of real solids for rugosity and heterogeneity. It is then important when estimating contact angles to measure both the advancing and receding angles, and to apply them appropriately.

The presence of small amounts of high energy, hydrophilic impurities, on a generally hydrophobic solid, or surface roughness can certainly affect the measurement of contact angle and spreading and has also been shown to affect the dependence of contact angle on drop size bringing into doubt not only the magnitude but existence of line tension (Drelich and Miller 1993, Shanahan 1995).

Line Tension:
From equation 8 it can be seen that if the magnitude of the line tension term is large enough, or the radius of curvature of the TPCL small enough, there may be an effect on the value of the contact angle and wetting. Depending upon the sign of the line tension, the contact angle and indeed the apparent wetting of a solid may either increase or decrease. Estimates of the magnitude of the line tension vary widely; from $+10^{-5}$ to -10^{-5} dyne! Good and Koo (1979) investigated the variation of the apparent contact angle on hydrophobic solids as a function of drop size and found large values of line tension, but ascribed this to corrugation of the TPCL caused by hydrophilic sites on the solids. They coined the term pseudo-line tension to describe the effect. Work by Li et al. (1990) tend to support this contention.

More recently, Gu et al. (1996) found values of line tension to be positive and about 0.1dyne for four hydrocarbons on a hydrophobic fluorocarbon surface by examining the shape of the interface around a conic cylinder. They postulate that all line tensions are positive. Aveyard and Clint (1996) in examining the wettability of particles at the water/vapor interface using an unique method not based upon observing changes in the meniscus with particle size, conclude that 'line tensions do in reality span the range of values reported in the literature'. Rusanov (1996), on a theoretical basis and citing experimental evidence, also asserts that positive or negative line tensions do exist. In as much as the so-called line-tension is due to the interaction of two dissimilar surfaces and the resulting changes in adsorption in the TPCL region may be positive or negative, it seems likely that the line-tension may also be of either sign.

While it seems unlikely that the magnitude and sign of line tensions will not be determined unambiguously any time soon, it is certain that the phenomena involved can either promote or inhibit wetting. This fact becomes especially important when the dimensions of the system become small, such as can be found for typical powders, pores of plants, or even during the deposition of a spray. An effect of spray droplet size of an acaricidal treatment on the mortality of citrus rust mites has been noted by Salyani and

McCoy (1989). It was found that in general small droplets have higher mortality than larger droplets at constant surface coverage. Shanahan (1995) has even described effects which may occur on larger scales when the contact angle is close to zero. He has described theoretically the manner in which spreading may be promoted by the presence of a small heterogeneity not unlike those mentioned above which would produce a 'crawling' drop. This result also emphasizes the importance of the care with which a solid is prepared or handled before conducting any wetting measurements.

Wetting Kinetics:

Similar 'fingering' instabilities have been studied and explained as the result of the Marangoni effect surface tension gradients (Sternling and Scriven 1959, Troian et al. 1989). Such dynamic effects may be induced not only by evaporative concentration or thermal gradients at the edges of a spreading front, but also by slow diffusion of surfactants. To the extent that such concentration gradients may play a role in the dynamics of spreading, dynamic surface tension may be important.

While dynamic effects on wetting due to diffusion of surfactants has seen relatively little work, the dynamics of wetting of pure fluids has been examined. The kinetic effect on advancing contact angles tends to be small, around 5°or less (Elliot and Riddiford 1962) though Morrow and Nguyen (1982) found for 8 liquids with surface tensions ranging from about 19 to 71mN/M and static contact angles of 22° to 108°, that no effect of interfacial speed from 0 to 0.02 cm/sec. could be detected. It was concluded that there is no effect of impressed motion on the TPCL in the absence of adsorption and when the interfacial velocities are low enough that viscous forces are negligible.

The kinetics of spontaneous capillary wetting expressed as the rate of penetration of a liquid into a tube were studied by Joos et al. (1990). They found good agreement with the predicted dependence of the advancing contact angle with viscosity and surface tension expressed as the capillary number derived from hydrodynamics. Similar work published at the same time by Foister (1990) has provided a more universal correlation by accounting for slippage of the TPCL. The dependence upon the capillary number was verified for spontaneous wetting of a fiber by Quéré and Di Meglio (1994). However, Brochard-Wyart and de Gennes (1992) have shown that under some circumstances hydrodynamics alone are insufficient to describe the kinetics. They also considered the phenomenon of de-wetting; the rate of growth of a dry patch for a non-wettable surface.

Clearly, since it is currently impossible to completely describe even the simplest wetting systems in sufficient detail for practical applications, we can only describe in general terms the relationship between contact angle, surface tension, capillarity, and any of the performance criterion in agricultural formulations or delivery systems. Hence there is great need for practical, meaningful methods for the evaluation of wettability for each application.

For practical purposes, the problem of estimating wettability can be separated into two systems; those related to single phase solids, and multi-phase solids. In the above discussion we have consider a number of confounding factors related to the apparent wettability of single phase solids as given by contact angle measurements. So, we will begin with measurement techniques for single phase solids. However, many practical systems are not single phase solids. We also need to have methods for the evaluation of powders, considered multi-phase as they include vapor, and packed multi-phase solids such as soil.

Methods for Practical Applications:
As all practical methods for the estimation of wetting involve at some point values of the contact angle, it is appropriate to begin with a description of a few of the methods which directly measure contact angles.

Direct observation of a sessile drop of a small amount of liquid, typically 10 - 20 μl, expressed from a syringe onto a solid surface illuminated with a light source and viewed with a telescopic goniometer is the oldest and still popular method for estimating contact angles (Figure 2). With the exception of a goniometric telescope, little specialized equipment is needed. Several inexpensive commercial instruments of this sort are available.

The contact angles, advancing or receding, may be recorded manually, or the entire drop photographed for latter analysis. More recently, computer based image analysis has automated these measurements (Pallas and Harrison 1989). The ASTM method D 724-94 describes the use of this technique for the estimation of surface wettability of paper. The main difficulty with this method is to control or determine whether the advancing or receding angle is measured. If the drop is allowed to fall, it may rebound somewhat and produce an angle which is neither advancing nor receding. Similarly, if a drop at the end of the syringe tip is gently lowered into contact with the solid and the tip withdrawn, the drop may vibrate and again produce a contact angle of indeterminate approach to the solid. The use of a hydrophobic tip, when aqueous solutions are studied is recommended. In this manner the drop may be placed upon the solid with little disturbance. Alternatively, the syringe tip may be an integral part of the solid and the test liquid introduced from below via a small hole. Perhaps the best routine is to never detach the drop from the syringe but maintain contact with the solid forming a liquid bridge. Of course, a vapor bubble may be used on a solid completely immersed in solution.

Preparation of the solid for measurement is critical. Any treatment which will alter the surface structure or composition, such as the transfer of skin oils during handling, must be avoided. Adventitious contamination via the vapor phase or even from laboratory dust can be problematic. As discussed above, whether the sample is desiccated or fully equilibrated with water vapor will affect the data. The choice depends upon the application. Full equilibration with respect to temperature and other phases is as critical.

Other, similar, methods for the direct estimation of contact angle have been reviewed by Neumann and Good (1979).

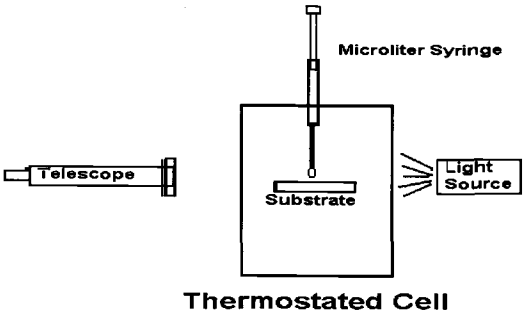

Figure (2)
Diagram of Goniometric Contact Angle Apparatus

Direct measurement of contact angle has not been used much in agricultural applications due to the difficulty in dealing with relevant solids: i.e. leaves, soils, chitin of insect bodies.

Direct measurement has been used in the evaluation of surfactants as adjuvants. Chung and Han reported (1993) values for contact angles of various formulations of atrazine on crabgrass. The efficacy data collected strongly suggested that the best wetting formulas give the best performance. A good correlation was also found for efficacy with the so-called adhesion tension; the product of surface tension and cosine of the contact angle.

While many compilations of measurements of the wetting properties of surfactants can be found, it is somewhat rare that they are performed on the target organism (Wicke et al 1993, Vollhardt and Wicke 1993, Sun and Foy 1995) , and even more rare that an active pesticide is present (Sun and Foy 1995, Brumbaugh et al. 1995) More frequently, evaluations are performed on substitute solids, such as parafilm, PTFE, or polyethylene (Singh et al 1984). Considering the myriad possible interactions of the various components of a typical agricultural formula, it is essential that the measurements be conducted with both surfactant and active on the appropriate solid.

The wettability of a formula also greatly impacts the degree of bounce or reflection and retention of a sprayed material on a leaf surface. Crease et al. (1991) found that both large sized drops and high dynamic surface tension promote bounce, but they did not investigate the effect of wettability. This conclusion is somewhat counterintuitive. As a drop impacts a surface, in an inelastic collision, the deformation of the drop caused by the reversal of momentum is countered by the restoring forces of viscosity and surface tension. There is, however, a trade-off between high surface tension and good wetting

properties; for a series of mixtures with the same viscosity and wetting properties, the higher tension material should bounce less. While it may be generally true that lower tensions usually provide better spreading, it is the adhesion tension which is probably more indicative of reduced reflection. Johnstone (1973) considered these problems in some detail, but did not consider effects due to time dependent adsorption. The tendency of a drop to roll or slide off a leaf after deposition is highly dependent upon the wetting properties.

Furmidge (1962) derived an equation for the retention of spray droplets after impact. The so called retention factor is given by;

$$F = \theta_M \, [\gamma^{LV} (\cos \theta_R - \cos \theta_A)/\rho]^{1/2} \qquad \text{Eq. (9)}$$

where θ_M is the average of the advancing, θ_A , and receding, θ_R, contact angles, and ρ is the density. It was demonstrated that the amount of liquid retained on a leaf surface is proportional to the retention factor. The relative dynamics of the adhesion and spreading process have not been considered.

The extent of spreading or spreading coefficients has often been used (whether valid or not) to attempt to predict the performance of a particular adjuvant. The most often used method for the evaluation is simply the so-called spreading ratio. In this method a fixed amount of a solution, usually 10 - 100 μL, is placed upon the test solid, (usually polyethylene is substituted for a biological material) , and the extent or area of spreading is calculated. The ratio of the spreading area in the presence of the test material divided by the area found without the test material is reported. Here again, control of relative humidity, temperature, and especially the preparation and handling of the test solid are of great impact to the results. The dimensionless spreading ratio also has been found to be very dependent upon concentration, volume of the drop, and relative humidity, as well as the preparation of the solid. Variations in excess of 100% are not uncommon.

The utility of spreading ratio measurements as a predictor of the efficacy of an adjuvant is questionable, even when performed on a biological surface. Uptake data of radio-labeled deoxyglucose into three different plant types has, been shown not to correlate well with either contact angle or spreading by Zabkiewicz et al. (1988).

Arguably, the most popular indicator of wettability is Draves wetting times also referred to as the skein test as described by ASTM method D2281 or the cotton tape method as described by CIPAC method MT 53.1 . The use of this method for agricultural applications was first proposed by McWhorter (1963). Essentially, in this method the time required to just sink a cotton skein in a test solution is determined (Figure 3).

A small weight attached to a 5g skein of naturally waxed cotton by a strong thread and the skein are dropped into a 500ml graduate cylinder which has been filled with the test solution. A stop watch is used to determine the amount of time until the bottom of the

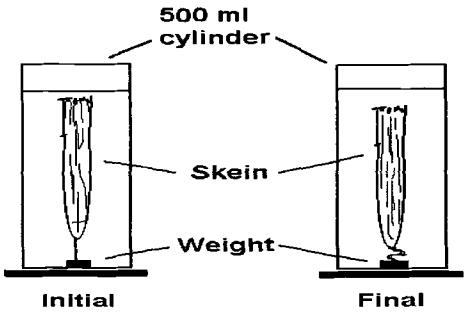

Figure (3)

buoyant skein begins to sink toward the bottom of the cylinder. This can be seen as a relaxation of the thread attaching the weight to the skein.

The skein test relies, essentially, upon the imbibition of the test fluid into the air-filled interstices of the cotton threads which comprise the skein. The skein is held floating in the test fluid by the buoyant force equal to the weight of the fluid displaced. The sinking of the skein via imbibition of fluid with the concomitant expulsion of the trapped air is then achieved by decreasing the work of adhesion. So, to great extent the wettability is proportional to the adhesion tension, $\gamma^{lv} \cos \theta$. Of course, it is the advancing angle which dominates the process. When sufficient trapped air is released, the skein drops.

The skein test is useful to the extent that surface tension at the liquid/vapor interface is one important component of wetting and spreading in general, but not only is the process of wetting a cotton skein very different from wetting a leaf, more importantly so is the surface composition. Clearly, the use of the skein test is not an appropriate choice for agricultural formulas. Given in Table (1) below are some comparative data for a variety of different materials. The order of performance at the 0.1%wt concentration given in the last column demonstrates that while a low value of surface tension is important, it does not fully explain the results; clearly the contact angle and adsorption and rate of adsorption, to the cotton needs to be considered as well as.

An alternative to the Draves method using plant material directly is also described in the CIPAC method MT53.2 . To evaluate the wetting of a surfactant solution on a leaf surface, one determines the minimum concentration of the surfactant needed to completely wet the leaf. The 'January King' variety of cabbage leaves are recommended, as they are difficult to wet. The leaf is completely immersed in the test solution, withdrawn, and after allowing 5 seconds for drainage of excess liquid, the coverage is examined. The average value is recorded for that concentration which produces complete wetting of 4/5 of the leaves. At least 20 leaves should be tested. The main difficulty with this method lies in the choice and availability of the leaves and the condition of the plant.

Table (1)
Draves Wetting Times (seconds)
Surface Tension in parenthesis (mN/M)

Substance	0.025% wt.	0.05%wt.	0.1% wt.	Order
Na DOS	32 (30)	8.3 (27)	3 (26)	1
Na LS	72 (44)	11.5 (35)	4 (30)	2
NP10	88 (31)	34.5 (31)	13.5 (30)	4
Taurate	>180 (28)	83 (30)	41 (31)	6
EO12 LA	186 (32)	101 (32)	59.5 (32)	8
TDA 9	124.5 (27)	25.5 (27)	8 (27)	3
DSB 85	>240 (35)	>240 (33)	200 (32)	9
Sticker/Spreader	>240 (32)	50 (31)	20 (30)	5
TDA 15	>240 (31)	87.5 (31)	37.5 (31)	7

(Note: The substances in Tables 1-3 are identified in order as: Na Dioctyl sulfosuccinate, Na Lauryl Sulfate, Nonyl phenol 10EO, Na Oleyl n-Methyl Taurate, Lauryl Alcohol 12EO, Tri-decyl alcohol 9 EO, Di Na Dodecyl Diphenyl oxide Disulfonate, a blend, Tri-decyl alcohol 15EO)

Certainly, the wettability of leaves varies widely with plant species. Very hydrophobic plant leaves, such as cabbage may produce contact angles with water as high as that for paraffin of 110°, 89° for barley, or 57° for turnip (Wicke et al 1993).

Variations depending upon the season, growing conditions, and age of the plant may all contribute to irreproducibility of the measurements. As cabbage leaves are in general very hydrophobic, the variation of the coverage with pure water precludes establishing a control. In as much as the wetting properties can be radically altered by a variety of chemical materials, it is recommended that the full formula, including any actives, be tested as well, if any correlation to a performance criterion is expected.

A more sophisticated method which has been in general use since the late 1950's is a variation on the Wilhelmy plate method and is sometimes referred to as the wetting balance (Guastalla 1957). This method has been found useful in the evaluation of the wettability of porous oil bearing reservoir rock (Mennella and Morrow 1995) and has been generally reviewed (Martin and Vogler 1991). Buckton (1990) has shown how the method may be used to estimate the surface free energies of powders. Computer-operated commercial instruments have been available for several years.

The Wilhelmy or Wetting Balance essentially, measures the mass of the meniscus acting on a solid object of known perimeter. This is accomplished by suspending the test solid from one end of a micro-electro balance as shown in Figure (4) . The test solid may be of any shape, provided the perimeter in contact with the fluid may be accurately estimated.

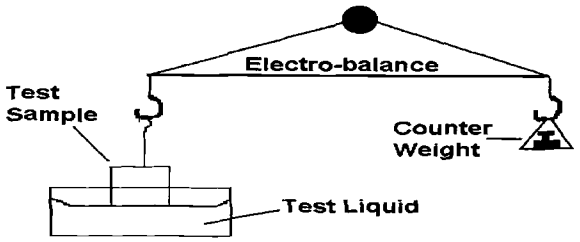

Figure (4)
Diagram of Wilhelmy / Wetting Balance

Then the adhesion tension is given by;

$$\gamma \cos \theta = m\, g / P \qquad \text{Eq. (10)}$$

Where m is the apparent mass of the meniscus, g is the acceleration due to gravity, and P is the perimeter of the test solid. Of course the mass of the test solid is tared or counter-weighted.

Provided that the balance is properly calibrated and that the perimeter is accurately known, the method is absolute, requiring no correction factors. Equation (10) applies only when the bottom edge of the test solid is precisely at the vapor/liquid interface. The exact position of the interface is easily judged by observing the reflection of the test solid off the liquid surface, or by monitoring the mass reading as the test solid and liquid approach each other and noting when the mass reading jumps upon contact of the solid with the liquid. Having the test solid exactly leveled with respect to the liquid is important to the extent that P in equation (10) correctly represents the perimeter of the solid in contact with the liquid. Measurement of surface tension only depends upon using a solid, such as slightly roughened platinum or microscope cover-slip, which will be fully wetted by the test liquid. As shown in Figure (5), there is little to no hysteresis present for a liquid which fully wets the test solid.

When the test solid is properly cleaned and prepared the surface tension of a liquid such as water is easily determined (Pallas and Harrison 1990).

Then knowing the surface tension of the test liquid, the test solid of unknown contact angle is put on the balance and put into contact with the test solution. The apparent mass as a function of depth of immersion is recorded, as shown in Figure (6). Then with knowledge of the surface tension of the test liquid, and appropriate buoyancy corrections for the depth of immersion, the contact angle is calculated by use of equation (10). Both the advancing and receding angles are obtained.

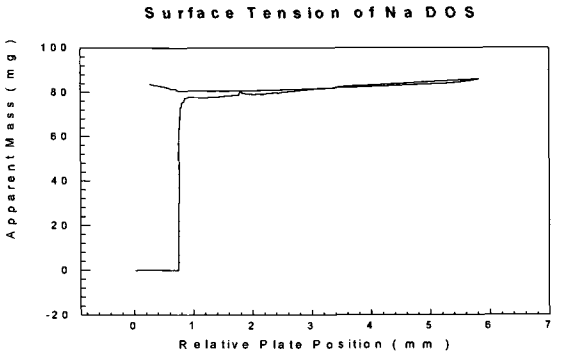

Figure 5
Surface Tension Scan using Wetting Balance: Note minimal hysteresis

Alternatively, the adhesion tension may be recorded, then measurement of the surface tension separately is unnecessary. Sun and Foy (1995) used this method to examine the wettability of velvetleaf. While no values of adhesion tension were reported, they did note that the apparent mass of the leaf increased, more or less, depending upon the test solution studied, after removal from the test liquid. It was supposed that this might indicate differences in ad/absorption. It is not clear, however, whether or not the changes were due simply to adhering liquid.

But, as they point out, in a separate experiment, that there was no discernible difference between test solution's spreading pattern on the leaves, it seems reasonable to suppose that the amount of adhering solution might be the same for each test liquid independent of ad/absorption, assuming good wetting was obtained for each test solution. While this method seems potentially very useful for the evaluation of the wettability of plant material, or chitin, preparing a sample of known, or at least consistent, perimeter is challenging.

The perimeter of a test solid may be estimated indirectly by measuring the apparent mass of the meniscus of a test liquid of assumed adhesion tension. Low energy fluids, such as ethanol or methanol, are useful in this respect as their surface tensions are well known, they are easily obtained in high purity, and are much less subject to adventitious surface contamination than a high energy fluid such as water. On the other hand a surfactant solution may be used, in order to assure good wetting, by measuring the tension using a solid of known perimeter. In this manner the apparent wettability of a powder may be examined.

A glass microscope cover slip may be coated with a powder, such as diuron, by use of a spray adhesive. Alternatively, a packed form as described by Buckton et al. (1995). The main difficulty in the use of a packed powder lies in the accurate estimation of the perimeter and in assuring that the test solid is returned to a pristine state before reuse.

This complication is not present when the powder is adhesively applied to a glass plate. Figure (6) shows a hysteresis loop for a diuron coated glass slide in water after having determined the perimeter using a surfactant solution which provides a zero contact angle and no hysteresis. Contact angles of about 112° advancing and 32° receding are indicated. The reproducibility of these measurements is about ±2°.

Table (2) presents some comparative data on a variety of surfactant systems. Crowl and Woolridge (1967) demonstrated the relationship between liquid grind efficiency and adhesion tension for a variety of materials.

By comparing the performance of the Draves times given in Table (1) to the contact angles, or indeed the calculable adhesion tensions, basing the choice of a good wetting agent for the production of, for example, a suspension concentrate on a Draves time alone may be very misleading. Variations on the method have been used to judge imbibition rates into porous solids and even wicking rates into textile materials as a variant of the Draves method (Chwastiak 1973). Certainly, even a single fiber such as a hair or a

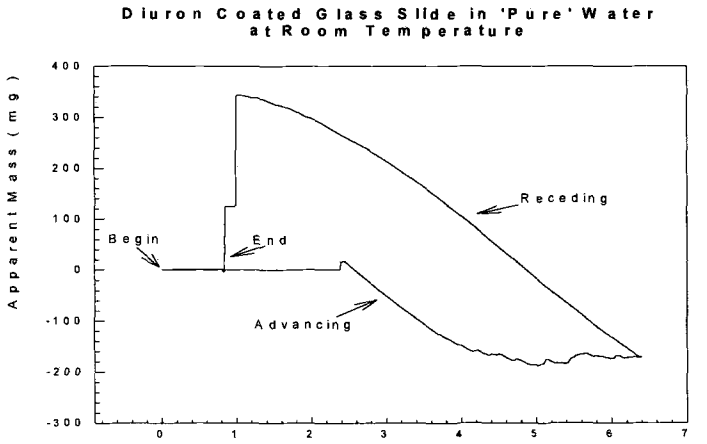

Figure (6)

thread may be studied in this manner. While the wetting balance method is clearly a powerful tool, due to its expense and the necessary effort to produce good quality data, other simpler methods are more popular.

Clearly, the wettability of powders is important to the formulation of suspensions and solid formulations in general. The tendency of a single particle, or collection of particles

denser than water to float on the surface of water is due not only to their hydrophobicity, but also to the surface area to mass ratio.

Table (2)
Powder Advancing Contact Angles for Diuron

Substance	0.025%wt.	0.05%wt	0.1%wt	Order
Na DOS	0	0	0	1*
Na LS	99	54	28	5
NP10	30	15	0	4
Taurate	0	28.3	31	6
EO 12 LA	39	37	35	8
TDA 9	0	0	0	1*
DSB 85	38	26	8	2
Sticker/Spreader	23	8	0	3
TDA 15	27	31	34	7

So, it is easy to conceptually understand how water-fowl can float, while an animal whose coat is similarly hydrophobic but lacks the great surface area imparted by feathers, will sink. We encounter the same problem in trying to wet an agricultural powder.

One very simple test for the wettability of powders is described by the ASTM method C979-82 for pigments to be incorporated into concrete. If 10g of a powder added to 150ml of deionized water in a 250ml beaker readily mixes when stirred with a spatula, then the powder is considered water wettable. This is not suitable for screening in agricultural formulation, as it does not allow for the relative evaluation of additives.

A variation on this method, potentially more suitable for agricultural formulas, similar to CIPAC MT53.3 is simply to note the amount of time needed to for 0.1g of a powder to sink in a 0.1 wt % solution of a surfactant. This simple test belies the complexity of the process which involved adhesion to the interface, immersion through the interface, and spreading of the liquid onto the particles. It is also assumed that inter-particle interactions are negligible. It should be recognized that a good dispersant is not necessarily a good wetting agent and vice versa.

Comparative data for the wetting time and sinking time of diuron in several different surfactant solutions are presented in Table (3). The wetting time is simply that time when visually all of the powder appears wet with liquid, but still is adhering to the interface. The sinking time is that time when subjectively 99% of the powder has sunk below the interface.

The ordering is presented as wetting time : sinking time. No cross-correlation with static surface tension, Draves time or contact angle is evident for either wetting or sinking nor do wetting and sinking times correlate. As mentioned above, the mechanisms are more complex than any single, simple measurement would predict.

Table (3)
Powder Sinking and Wetting Times for Diuron
0.1%wt surfactant with 0.1g powder

Substance	Wetting Time (sec.)	Sinking Time(sec.)	SurfaceTension	Order
Na DOS	9.0	89	25.6	3 : 5
Na LS	71	130	30.2	5 : 7
NP 10	5	64	30	2 : 4
Taurate	97	100	30.6	6 : 6
EO 12 LA	18	48	30	4* : 1
TDA 9	3.5	54	27	1 : 2
DSB 85	>240	>240	31.6	7 : 8
Sticker/Spreader	>240	>240	30.4	7 : 8
TDA 15	18.5	55	31	4* : 3

The evaluation of the wetting of unconsolidated media, such as soil or sand represents perhaps the greatest challenge to our methodology. The importance to the performance of a formula can lie in its ability to penetrate a heavy thatch, wet into the soil and contact the target organism, as well as simply alter the soil wettability to provide better infiltration of subsequent waterings. While in principle it would seem appropriate to use the sinking time method described above, the tortuous path a fluid must follow through a soil bed is poorly mimicked in that test.

While contact angle goniometry has been used on packed powders and could certainly be applied to soils, the compaction process would alter the native wettability and certainly invalidate the method, aside from other difficulties. A method for the quantitative estimation of the contact angle of soil unconsolidated media relies upon the Washburn equation (Washburn 1921);

$$L = \gamma^{lv} \cos\theta rt/2\eta \qquad \text{Eq (11)}$$

which gives the distance of penetration, L, of the fluid front in a porous bed at time, t, equal to the surface tension times the radius of the tube, r, divided by twice the fluid viscosity, η. Good (1973) examined the method and rewrote the equation to explicitly account for the tortuosity of the porous bed and spreading pressures. The method has been criticized due to the fact that it is by nature dynamic and cannot account for changes in the tension due to adsorption with time. The method was refined by Bartell as described by Dunstan and White (1986). In the Bartell method, a back-pressure is applied which is sufficient to just stop the fluid penetration. Then the contact angle may be calculated from;

$$\Delta P = 2\gamma^{lv} \cos\theta / r_{eff} \qquad \text{Eq (12)}$$

$$r_{eff} = 2(1-\phi)/ \phi\rho A \qquad \text{Eq (12a)}$$

where ϕ, is the volume fraction of the solid, ρ is the density of the solid, and A is the specific surface area per gram of the solid. This method has been used successfully to estimate the wettability of soils and to demonstrate the effectiveness of wetting agents (Letey et al. 1962 and Pelishek et al. 1962)

An innovative and much simpler method which is essentially a derivative of the Washburn method is that developed by Mane et al. (1993). Clear plastic tubes folded crisply in half and held in place with cellophane tape have one half filled with the soil to be tested and the end plugged with cotton. The filled straws are laid on a rack so that the empty arm was raised at about a 25° angle. Then the test solution is introduced into the empty arm. A stop-watch is used to determine the amount of time needed to penetrate 8cm of the soil. Replicate tests provide a measure of accuracy.

For materials which do not pack well, such as thatch or peat-moss, the percolation test described by Tepleton and Rodriguez (1992) is useful. In this method 200cc of test solid are placed in a vertically held tube fixed with a screen on one end. Then 200 cc of test solution are poured into the tube. The amount of liquid absorbed is determined by difference and reported as a percent.

Summary:
From a thorough review of the basic principles underlying wetting phenomenon, the need for practical methods applicable to agricultural applications is clear. Much more extensive work is still needed before wetting properties can be predicted from 'simple' measurements, let alone chemical structures. Methods applicable for the screening of surfactants for the formulation of dry formulas, as well as performance systems for adjuvants, stickers, and soil wetting agents, among others have been briefly described. Comparative data for several of the methods has demonstrated that no single measurement alone is sufficient for all practical purposes. It is hoped that this discussion will help guide application scientists in their work.

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R. Scott Tann[1]

A REVIEW OF SURFACTANTS USED IN NOVEL AGRICULTURAL APPLICATIONS

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ABSTRACT:. A review of recent literature found several novel or new applications of surfactants in the agrochemical business. This review of the literature is intended to highlight some of the most recent developments in the industry. Structured liquids and vesicle technology has led to more stable formulations. These formulations generally were not possible several years ago. The development water based systems is emerging technology which has served to breathe new opportunities with the use of surfactants. Water dispersible granule technology has led to several interesting applications of surfactants as well. From heat activated binders to the synergistic role surfactants play in some formulations, the use of surface active materials in water dispersible granules is expanding. Surfactants also are beginning to emerge as an ingredient which can effect the basic biology of the active ingredient. During the review of the literature several citations were found illustrating uses of surfactants in roles such as: reducing eye irritation of the formulation, reducing the phytotoxicity of the formulation, as well as reducing the odor of the formulation. Another important aspect of surfactants has found a new use of foaming agents in insecticide formulations. Several references will be cited where the formulation is formulated to foam upon application of the pesticide.

KEYWORDS: surfactants, structured liquids, water dispersible granules, biological modifiers, foam formulations

[1] Scientist, Research and Development, Witco Corporation, 3200 Brookfield St., Houston, TX, 77045.

Surfactants have been utilized for many years in the agricultural industry. From the early days of fatty acid soaps to the more sophisticated technology of today's designer surfactants, the role of surfactants has been ever changing. No longer are surfactants simply the emulsifiers or dispersants in your pesticide formulation. Surfactants are currently utilized in new or novel approaches once reserved only for the idealistic formulator. The formulations of today still use traditional surfactants but activity has grown in several areas. Some of these areas discussed further in this review are: structured liquid formulations, novel applications of surfactants in water dispersible granules and surfactants acting in biological enhancement areas.

Various formulation types are found throughout the industry. Liquid formulations currently marketed include: Emulsifiable concentrates (EC), suspensions concentrates (SC) and soluble liquids (SL). Dry formulations currently marketed include wettable powder (WP), and water dispersible or soluble granules (WG). The figure (Fig. 1) below represents some of the common formulation types in today's industry throughout the US and Europe.

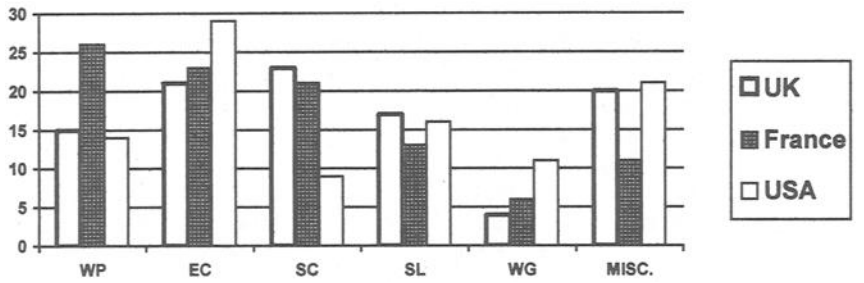

FIG. 1 -- Total Formulations registered in 1995 (Knowles 1995).

In most of the formulation types in Fig. 1 the surfactants role is very traditional. In the case of the EC formulation the surfactants are emulsifiers whereas the WP and WG formulations use surfactants as: dispersants, wetting agents and compatibility agents. While the majority of surfactants are being used in these roles certain other formulations are being developed combining the traditional role of the emulsifier and novel applications.

STRUCTURED LIQUID FORMULATIONS

Structured liquid formulations have been present in industry for many years. Applications of this technology can be found in the personal care industry as shampoos and bath oils. Another area the technology appears is in the laundry or cleaning applications. Fabric softeners are often formulated as structured liquids in water. The surfactant or softener is allowed to form spherulites which give structure to the liquid

softener formulation. In the area of agricultural formulations this type of product is generally reserved to a research laboratory. A hybrid of this technology includes microencapsulated formulations. In the agricultural industry these formulations maintain a structure as the capsules formed during the polymerization often are not rigid particles and may include an external surfactant layer around the polymerized capsule. Still a third type of structured liquid formulation may include those formulations using a polymer to stabilize a suspension. The polymer swells in the presence of the aqueous phase giving an outward appearance of a structured liquid.

In two of the cases stated earlier, the microencapsulated formulation and the polymer stabilized suspension, a key ingredient to a true structured liquid formulation is missing. The surfactant in a true structured liquid formulations exists in small spherulites resembling a particle. A structured surfactant formulation (SSF) is a suspension of an insoluble solid pesticide in an aqueous structured surfactant (Newton et al. 1991). These formulations typically demonstrate a number of key characteristics including:

1. The surfactants form organized structures (spherulites) larger than conventional micelles.
2. The pesticide particles are suspended between the spherulites.
3. The formulations pour easily because the spherulites are deformable.
4. The formulations demonstrate high viscosity at low shear and low viscosity at high shear.

Recently novel applications have been found for this technology. A formulation has been reported using a non-polar phase base. This is unique in the sense that most prior formulations have been developed in aqueous media. It has now been found that stable vesicles in a non-polar phase can be prepared (De Vringer 1991). Several ingredients are key to the development of this formulation type. These formulations typically involve the following (De Vringer 1991):

1. Surfactants such as glycerol monoesters of fatty acids, sorbitan esters and POE alkyl esters.
2. Lipophilic stabilizing factors including: sterols, branched fatty alcohols, fatty acids and esters of dicarboxylic acids.
3. The non-polar phase often is composed of mineral oil and/or silicone oil.
4. Hydrophilic stabilizing factors including ethanol and ethanolamine.

The preparation of these vesicles can involve a number of different approaches. From simple mixing to evaporation techniques the complexity of the formulation can vary. Some of the typical preparations are cited below:

1. Simple mixing of all components under low shear (De Vringer 1991).
2. Evaporation involves the dissolution in a polar media and addition to a non-polar phase. The polar phase is allowed to evaporate at a high temperature leaving behind the non-polar vesicles (De Vringer 1991)

3. Evaporation of the solvent in a thin film using an organic solvent (De Vringer 1991).

The development of a structured liquid formulation utilizing a non-polar media marks an important development in this technology. No longer will formulators need to limit their thoughts to the aqueous environment. The use of these formulations is expected to continue to generate new formulations and opportunities.

Although microencapsulation based formulations are not by definition structured liquids, many of the properties observed with SSF type materials are present in the microencapsulated formulation. A microencapsulated product is generally formed by the following process:

1. The active ingredient is dispersed with a monomer of choice and a surfactant.
2. The polymerization of the monomer occurs at the interface of the reactants forming microcapsules.
3. The microcapsules are suspended by the post addition of a dispersant unless the surfactant used in the polymerization process is an adequate dispersant.

Common surfactants associated with the microencapsulation process can be divided into two basic categories. The first category being those surfactants used during the polymerization step. The second category being those used as suspension stabilizers after the polymerization is complete. During the polymerizations common surfactants include: alkylphenol ethers, EO/PO block co-polymers, sulfosuccinates and monomeric surfactants (acrylamidoalkyl sulfonic acid). Upon completion of the polymerization surfactant choices change to include: condensed naphthalene sulfonates, lignin sulfonates, nonyl phenol sulfates and styryl phenol ethoxylates. It should be noted however, the use of any or all of these systems depends on the choice of the monomer and initiator. A stable emulsion is necessary for the polymerization to occur. Microencapsulated formulations have had similar issues as any suspension concentrate but the weak shell wall necessary for the release of the active ingredient often causes a variety of packing and storage stability issues. It is these weak shell walls which allow us to compare the SSF with the microencapsulated formulations. As technology in these areas mature these issues will be resolved and it is likely surfactants will be used to solve them.

DEVELOPMENTS IN WATER DISPERSIBLE GRANULES (WDG)

Water dispersible granules have used surfactants since the beginning of the development of these formulations. Surfactants have provided the wetting, dispersing and compatibility so often necessary with these formulation types. In the past few years however, the use of surfactants in different areas of these formulations has begun to be seen. The role of the surfactant as a synergist is one area of growth. Another such area is the use of surfactants to modify the crystal growth and structure in the dry WDG area. The use of surfactants as heat activated binders for sensitive active ingredients also has been explored in this field.

It has been noted often the conversion of a liquid based pesticide to a solid based pesticide can result in some loss of biological activity of the active ingredient. To address this issue surfactants have been chosen to enhance the biological activity of the water dispersible granule. The addition of a synergist selected from among various surfactants to a pesticide has been examined in order to sufficiently bring out the activity of the latter (Iwasaki et al. 1987). Synergistic surfactants can represent many classes of products. Examples of synergistic surfactants include: quartenary ammonium chlorides, betaines, organic amino acids, amine oxides, and imidazolines (Iwasaki et al. 1987).

Since surfactants generally act upon the surface; modification of crystal properties is an area of application investigated in WDG technology. The crystalline nature of the active ingredient can directly impact the milling and granulation of the material. In some cases of low melting technicals silica has been incorporated to aid in the milling of a soft malleable material. In other cases such as in the xylidine chemistry crystal morphology can be a source of trouble. As an example, " Wettable powders of N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine stored for extended time periods lost dispensability" (Dudkowski 1977). In this case the granules were losing dispersability and forming hard packed material upon storage. The root cause of the problem was determined to be the crystal morphology of the active ingredient. The herbicide exists at two distinct polymorphs: a yellow microcrystalline form and an orange macrocrystalline form (Dudkowski 1977). The yellow polymorph slowly converts to the more stable orange polymorph at ambient temperature (Dudkowski 1977). The remedy to this problem was found in the use of a surfactant to stop the conversion of the yellow form to the orange form. When 1 to 2 % wt./wt. of ethoxylated beta diamine is melted together with the xylidine and a molecular solution is formed the undesirable polymorph is prevented (Dudkowski 1977). A generic structure of this chemistry is given in Fig. 2.

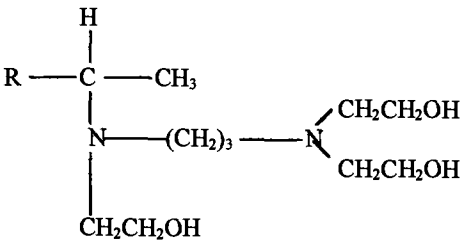

FIG. 2--Ethoxylated β-diamine structure

The use of binding agents in WDG formulations has been widely known for many years. The common binders such as: cellulose, starches and polymers have their drawbacks in formulation. Many of these binders fail to disperse well in the spray tank or cause other precipitate problems in the field. Recently a patent was issued using surfactants as binders for these formulations. In this patent high molecular weight solid surfactants were used to bind the individual agglomerates. This invention comprises a WDG or SG which is comprised of agglomerates of solid pesticidal particles banded

together by solid bridges of a water soluble heat activated binder (HAB) (Geigle et al. 1991). Heat activated binders (HAB) are applied by melting the HAB and applying to the particles as they are being agglomerated . The common HAB mentioned in the specific reference include: EO/PO Co-polymers where 80% is ethylene oxide and 20% is propylene oxide, and dinonyl phenol ethoxylate with 150 ethylene oxide units (Geigle et al. 1991). Although these are the two specific examples in the citation, many other surfactants can be chosen. Suitable criteria for the application of the surfactants for heat activated binders include:

1. Melting point of 40 - 120° C.
2. Water solubility with an HLB of 14-19.
3. Dissolution in mildly agitated water in 50 minutes or less.
4. Melt viscosity of at least 200 cps.
5. The surfactant must have a difference of 5° C or less between the softening point and the onset of solidification (Geigle et al. 1991).

Heat activated binders may be necessary when a high level of silica is present or if the material is a low melting active ingredient. The use of these materials allows the granule to maintain it's particle size through out handling and will minimize dust. This application of surfactant differs from most WDG roles as dispersant and wetting agent. Often times the HAB may allow the formulator to impart added characteristics of dispersion by inclusion of the HAB.

BIOLOGICAL IMPACT OF SURFACTANTS

In many formulations surfactants have been functioning in their traditional role as emulsifiers or dispersants while imparting biological benefits not actually designed. In recent years formulators have been investigating the effect of surfactants on the basic biology of the pesticide. In a number of studies the surfactant chosen was based on both the traditional roles of surfactants as well as the biological function of the surfactant. Surfactants have been used in roles such as reduction of pytotoxicity, reduction of eye irritation, reduction of dermal toxicity and recently to enhance the growth of plants.

Often in the formulation of active ingredients one wishes to reduce the phytoxicity of the surfactants. In the area of fungicides and insecticides minimizing the phytotoxicity of the formulation is often key to it's commercial use. In 1983 Dellicolli reported the reduction of phytotoxicity with a surfactant. A water - insoluble non-sulfonated alkali ligin based spray tank additive is provided which when mixed with the pesticide prior to application reduces the phytotoxic effect of the pesticide (Dellicolli 1983). The application of this particular surfactant was found to be very beneficial in reducing the phytotoxicity of the triazine family of herbicides and several fungicides.

In recent years the desire to reduce the exposure hazards of the formulation has become a greater concern. One area of exposure for the applicator of pesticides is the eye. Reduction of eye irritation has led to several patents in the area of pesticides.

Surfactants also have been found which reduce or ameliorate the irritation of the formulation. Listed below are some of the examples found in this review.

1. Amine oxides have been used to reduce the eye irritation of water soluble active ingredients (Nguyen 1992).
2. Alkylamine alkoxylate in combination with a C6,22 saturated or unsaturated alkyl mono or di carboxylic acid (Berk and Kassebaum 1995).
3. Propylene glycol present in the range of 25-35% by weight has been shown to reduce eye irritation (Tocker 1988).

Efforts to reduce the eye irritation while successful in the above references often times will be tied directly to the formulation reported. In most cases a general reduction of irritation by a surfactant can not applied to all pesticides. The formulator is well advised to establish the irritation of the specific formulation with and without the surfactant chosen even if the surfactant has been reported to lower irritation.

Another area of concern for worker exposure is in the handling of dry formulations. Placing a dermally toxic pesticide on a dry carrier may reduce the risk of a spill but may not reduce the toxicity of the pesticide. A technique has been reported which utilizes a surfactant to reduce the exposure hazard of a dermally toxic active ingredient. The invention relates to dermally toxic pesticide compositions consisting of the pesticide and a dry inert carrier, that have been additionally safened for handling by addition thereto of a nonionic surfactant having an HLB of from about 17 to about 20 (Sher et al. 1987). The surfactant attaches to the outer layers of the granule thus providing a barrier to the active ingredient on the clay. When added to the spray tank the surfactant easily dissolves in water to allow the release of the active ingredient from the carrier. Surfactants found to act in this manner are listed in Table 1 below: (Sher et al. 1987)

TABLE 1-- Surfactant found to be useful in reducing dermal toxicity

Surfactant	HLB
POE (50) Stearate	17.9
POE (100) Castor Oil	18.0
Alkoxylated Lanolin	18.0
POE Coco mono glyceride	18.0
POE (20) Castor Oil	18.1
2° Alcohol PEG ether	18.0
PEG400 monooleate	18.3
POE (100) stearyl ether	18.8
PEG monostearate	18.8

Surfactants have been shown to be effective in soil penetration in many references. By modifying the surface tension of the water the surfactant allows water to penetrate

easier. Recently a study indicated a surfactant not only can modify the surface tension but can enhance the growth of plants. The invention is a method of enhancing the growth of plants comprising applying a nonionic surfactant to soil to protect the plant seeds and to enhance the subsequent growth of the plants (Browning 1995). The use of secondary alcohol ethoxylates were shown to enhance the root length of cotton seedlings. Fig. 3 illustrates this effect of the secondary alcohol ethoxylate at two usage rates.

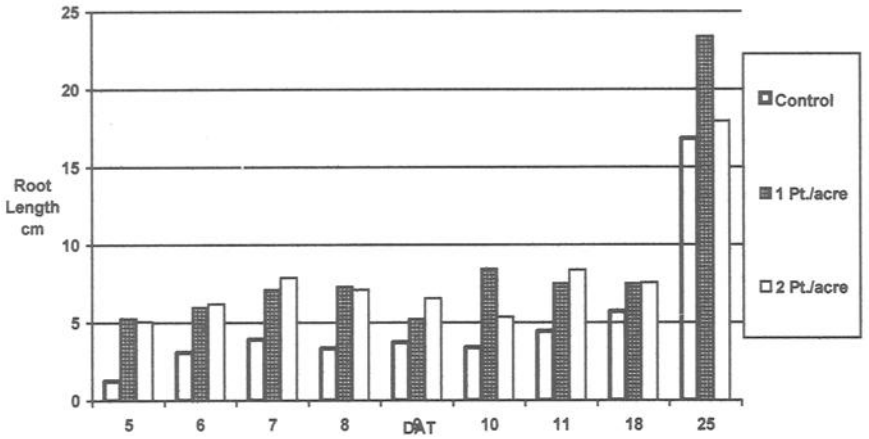

FIG. 3 -- Effect of 2° Alcohol ethoxylate on cotton seedling

Furthermore the secondary alcohol ethoxylate seems to aid in the germination of the plant seeds. It has been determined that when Tergitol® 15-S-9 is applied to soil, it protects plant seeds and enhances their germination (Browning 1995).

FOAMING APPLICATIONS

For many years formulators have gone to extensive means to eliminate or minimize foam in the application of surfactants in agriculture. Antifoams and defoamers have been widely accepted practices. Recently developments in the area of producing formulations which foam is beginning to surface. Termaticides for home use and marking formulations have been patented in recent years. The present invention relates to low expansion rapidly absorbed pesticidal or herbicidal foams (Barnett 1990). This invention was formulated for use as a home use insecticide which provides better coverage than the traditional liquid product. Another application has appeared in the use of foam for marking fields and other treated areas. An object of the present invention is to provide a herbicidal foam composition which has the advantage that already treated areas can be visually distinguished (Sakamoto et al. 1993). Several surfactants have been identified as useful in foaming applications. The following surfactants have been classified as useful:

1. Anionic surfactants - calcium dodecylbenzene sulfonic acid, fatty acid salts, triethanolamine alkyl sulfonates, and alpha olefin sulfonates.
2. Nonionic surfactants - alkyl polyoxyethylenes, sorbitan monooleate, PEG fatty acid esters.

MISCELLANEOUS APPLICATIONS

Several applications of surfactants have been reported which when investigated for this review did not fit well in a particular category. These applications include a method for treating seeds as well as the use of a surfactant for odor reduction. In 1994 a patent was issued to provide for the coating of seeds with a polyoxyethylene glycol. It has been found that when certain water soluble, film forming polymers are combined in definite proportions, the resulting polymer mixture possesses improved properties when used for seed coating applications (Akhtar et al. 1994). Odor reduction is a concern with certain pesticides. In 1995 a patent was issued utilizing a surfactant to reduce the odor of 2,4-dichlorophenoxy acetic acid. The present invention relates to using a mixture of a nonionic surfactant blend having an acidulated soybean soapstock component with a compatible herbicide to reduce an objectionable odor of the herbicide (Gednalske and Herzfeld 1995).

CONCLUSIONS

The use of surfactants is expanding continuously. Traditional surfactants are currently being expanded into non-traditional areas. Surfactants in a particular formulation can no longer be chosen based on surface modification effects. Added value can be achieved when choosing surfactants for a particular application. This will allow further differentiation of product lines and markets. The active ingredients of tomorrow will not only achieve the desires of the producer but the overall formulation will be integrated with the inert ingredients. Surfactants will play different roles as inerts and possibly even challenge the meaning of a surface active agent.

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SURFACE ACTIVE AGENTS/ADJUVANTS

Basic Chemistry

Dennis G. Anderson[1], William J. Eberle[2] and David R. Stubbs[2]

HYDROLYTIC STABILITY OF PHOSPHATE ESTER SURFACTANTS

REFERENCE: Anderson, D. G., Eberle, W. J., and Stubbs, D. R., "Hydrolytic Stability of Phosphate Ester Surfactants," Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: The unique properties of phosphate ester surfactants make them highly functional components in a multitude of formulations throughout the agricultural industry. Synthetic routes leading to the formation of phosphate esters are relatively well understood. The formation of mono-, di-, tri- and pyro-phosphate ester species, however, varies considerably with reaction conditions and stoichiometry. Historically, the analysis of these mixtures has centered around the potentiometric titration of acidic species to determine the concentration of the various phosphate ester species present. The information obtained using this approach can be misleading and fails to provide the comprehensive characterization possible using through the application of state-of-the-art analytical methodology; including ^{31}P nuclear magnetic resonance spectroscopy, high performance liquid chromatography and capillary electrophoresis. These techniques will be compared relative to the examination of phosphate ester surfactants. Specifically prepared phosphate esters were studied for hydrolytic stability at ambient and elevated temperature. Little evidence for hydrolysis was observed past seven days exposure, with minor effects noted due to temperature and water concentration.

KEY WORDS: analysis, phosphate ester, anionic surfactant, chromatography, spectroscopy, electrophoresis, hydrolysis

[1]Manager of Analytical Sciences, Oleochemicals/Surfactants Group, Witco Corporation, Chicago, IL 60638-1395

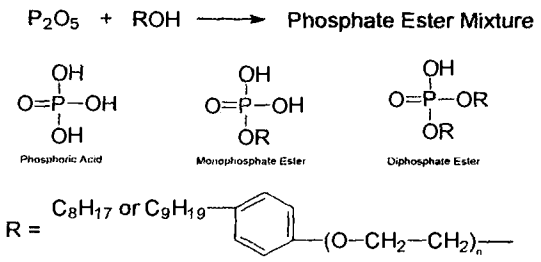
[2]Scientist and Scientist II, respectively, Ciba Crop Protection, Ciba-Geigy Corporation, Greensboro, NC 27419-8300

INTRODUCTION

Phosphate ester type anionic surfactants are used in a variety of application areas: including emulsifiers in agricultural formulations, dispersants for dyes and pigments, polymerization systems and plating baths. This surfactant class enjoys this wide application range due to:

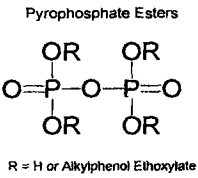
1. Extremely good electrolyte tolerance (particularly important in agricultural applications where hard water conditions are encountered)
2. Outstanding alkali stability and wide range in surfactant properties
3. Good emulsifiability and detergency over a wide pH range
4. Excellent coupling and solubilizing ability due to high solubility in electrolyte solutions
5. Solubility in both water and organic solvents

Phosphate esters are generally formed through the reaction of an alcohol or alkylphenol ethoxylate and a phosphorylating agent. This is shown schematically for an alkylphenol ethoxylate in Equation 1.



Equation 1

The concentration of alcoholic species and phosphorous pentoxide determine the relative amounts of phosphoric acid, monophosphate and diphosphate generated. Normally, little if any, triphosphate is formed. If insufficient alcohol is used, pyrophosphates (Equation 2) may be formed which are hydrolytically stable and may possess undesirable surfactant properties.



Equation 2

The structure of the alkylphenol ethoxylate has a considerable effect on the behavior of the phosphate monoester/diester mixture. A shorter ethylene oxide chain and longer hydrophobe results in a material with lower water solubility, while an increase in the hydrophilic character (longer ethylene oxide chain and shorter hydrophobe) will increase water solubility. By proper choice of hydrophobe and ethylene oxide chain a product with the proper Hydrophile-Lipophile Balance (HLB) can be readily synthesized.

EXPERIMENTAL

A series of phosphate ester surfactants were synthesized to provide a significant variation in monoester, diester and free phosphoric acid content. A number of synthetic routes were employed, depending on the desired composition in the finished product. The composition of the surfactants subjected to hydrolysis are listed in Table 1.

Table 1 - Comparison of Phosphate Ester Surfactants

Sample	Wt. % Free Nonionic	Wt. % Monophosphate Ester	Wt. % Diphosphate Ester	Wt. % Phosphoric Acid
A	3.1	45.8	50.9	0.2
B	5.1	44.6	49.6	0.7
C	10.4	57.1	30.1	2.4
D	2.6	80.9	4.7	11.8

Hydrolysis studies were performed using phosphate ester mixtures containing 10 and 25% water. To reduce reaction mixture viscosity, and to insure complete miscibility, 12-14% acetonitrile was added. Samples were then stored at 25 and 50 °C for varying lengths of time. Aliquots were periodically withdrawn and stored in a freezer at 0 °C prior to analysis.

CLASSICAL ANALYSIS

Traditional methods for the characterization of phosphate esters generally involve the determination of total phosphorous content and titration of residual acidity. In the former case, the sample is generally wet or dry ashed, followed by hydrolysis to phosphate ion and subsequent spectrophotometric determination following color development as described by Cullem.

Titration with caustic can provide a wealth of information regarding the composition of phosphate esters, since the strength of acid groups remaining following esterification is greater than the acid groups that were replaced. Samples containing approximately 1.3 milliequivalents of total acid are dissolved in 60 mL of a 3:1 mixture of isopropanol:water mixture. The resulting solution is titrated with 0.100 N aqueous sodium or potassium hydroxide using a Brinkman 636 Titroprocessor, E635 Dosimat and E649 Stirrer until two equivalence points are observed. At this point, 30 mL of aqueous 40% calcium chloride solution is added and allowed to react for a minimum of five minutes prior to resuming titration until a third equivalence point is observed as shown in Figure 1.

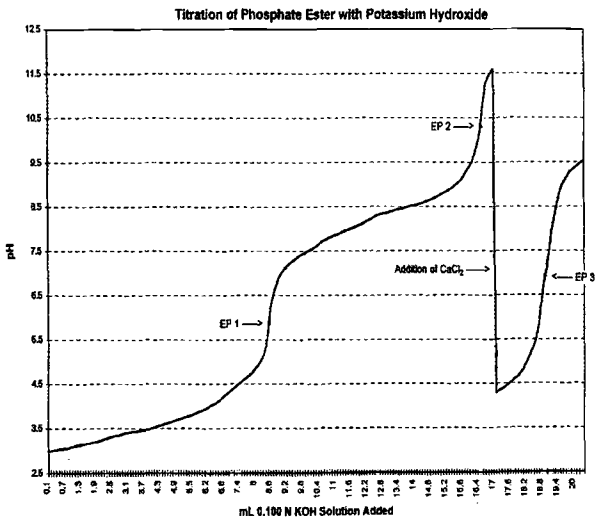


Figure 1 - Potentiometric Titration of Phosphate Ester

The first equivalence point corresponds to:

$$ROPO(OH)_2 \text{ --------> } ROP(OH)O_2^{-1}$$

$$(RO)_2PO(OH) \text{ -----> } (RO)_2PO_2^{-1}$$

$$H_3PO_4 \text{ ------> } H_2PO_4^{-1}$$

while the second equivalence point corresponds to:

$$ROP(OH)O_2^{-1} \text{ -----> } ROPO_3^{-2}$$

$$H_2PO_4^{-1} \text{ -----> } HPO_4^{-2}$$

The remaining proton on HPO_4^{-2} is too weak an acid to titrate with base. To determine this species, calcium chloride solution is added, which reacts with HPO_4^{-2} to liberate hydronium ions, causing the sharp drop in pH observed in Figure 1. The liberated acid is then titrated with base until the final equivalence point is observed (EP3).

Using the following equations, the data generated during titration can be used to calculate the concentration of phosphoric acid, monophosphate and diphosphate ester present in the sample:

$$\% H_3PO_4 = \{[(EP3)-(EP2)][N\ Base][98.1][100]\} / [g\ sample]$$

$$\% Monoester = \{(EP2)-(EP1)][N\ Base][MW\ Monoester][100]\} / [g\ sample\} - \% H_3PO_4$$

$$\% Diester = \{[(2)(EP1)-(EP2)][N\ Base][MW\ Diester][100]\} / [g\ sample]$$

While these calculations offer excellent precision and provide useful quality control data for phosphate ester surfactants, they fail to account for the presence of unreacted alcohol and triester in finished products. In addition, the unreacted acid groups present in pyrophosphate esters titrate in the regions noted within equivalence points 1 and 3. This complicates matters considerably and limits the determination of monophosphate and diphosphate ester level when a significant concentration of pyrophosphate is present.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Frazier et al. described high performance liquid chromatography (HPLC) in general and Aboul-Kassim and Simoneit used ion exchange in particular for the analysis of anionic surfactants. The use of weak ion exchange chromatography (WAXLC) has not, to our knowledge, been applied previously to the characterization of alkylphenol phosphate esters. In an effort to find a technique with higher specificity for the mixture of oligomeric phosphate ester anions than potentiometric titration, WAXLC was examined.

HPLC analyses were conducted using a Hewlett-Packard HP-1050 pump and autosampler. A Perkin-Elmer LC-235 diode array detector was used for detection and an HP Lab Automation System for data reduction and storage. Constant column temperature was maintained using an Eldex CH-150 column oven. Analysis conditions included a 0.50 mL/min. eluant flow rate, 5.0 μL injection volume and 220 nm detection wavelength. The mobile phase was a 50:50 v:v mixture of acetonitrile:aqueous pH = 3.0 sodium phosphate monobasic buffer. Ionic strength was adjusted between 0.50 and 5.0 mM to achieve maximum resolution of sample species. The analytical columns used included Exsil NH_2 and Spherisorb NH_2. Typical column dimensions were: 100 mm in length, 3 mm internal diameter and 3 micron particle size.

Analyses at pH = 3.0 maintained the phosphate esters in the ionic form and were found to provide optimum ion exchange performance with the tertiary amine stationary phase. Detection wavelengths between 200 and 280 nm were found to yield equivalent analytical data. A typical chromatogram is given in Figure 2 and indicates excellent separation of sample components.

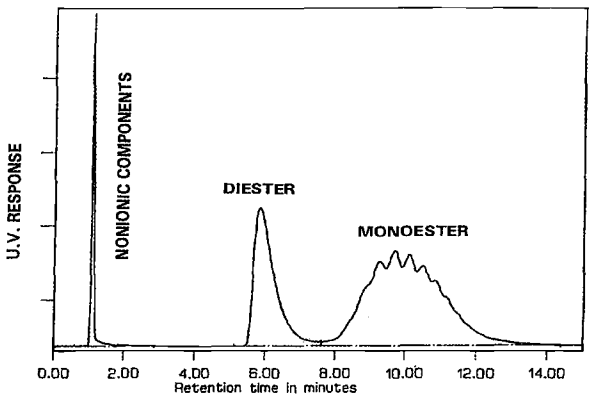


Figure 2 - HPLC Separation of Phosphate Ester Species

The unreacted alkylphenol ethoxylate elutes near the void volume of the column as expected for an unionized species. The diphosphate esters elutes second and the monophosphate ester [strongest acid] elutes last. Measurement of peak areas allowed the calculation of area percentages, which Scott found to be essentially equivalent to weight percentages. This is not surprising, since each species contains a single UV absorbing moiety (the phenol substituent) per alkyphenol ethoxylate chain.

The monophosphate/diphosphate ratio can be calculated and used for product comparisons along with the concentration of unreacted alkylphenol ethoxylate. Since pure standards are not readily available or feasible to prepare for most commercial products, direct calculation of weight percentages is not possible. In addition, the HPLC data do not account for the presence of water, H_3PO_4 or pyrophosphate esters. Chasin and Vandegrift indicated the data provide an excellent indicator of product characteristics. This is particularly true when comparing products from laboratory scale and commercial sources.

A number of process variables exist in the manufacture of phosphate esters which produce titratable species which can interfere with the accurate determination of monoester and diester species. Evidence of this can be seen in Table 2, where data from titration, HPLC, capillary electrophoresis (CE) and nuclear magnetic resonance spectroscopy (NMR) are compared.

Table 2 - Alkylphenol Ethoxylate Phosphate Ester Chacterization

Sample	1	2	3	4	5	6	7
Monoester / Diester Ratio							
Phosphorous NMR	1.9	1.4	2.1	13.3	2.4	1.5	2.4
High Performance LC	2.0	1.3	2.0	12.7	2.4	1.6	2.3
Capillary Electrophoresis	2.1	1.3	2.2	11.8	2.1	1.2	1.4
Potentiometric Titration		1.9	15.9	20.7	2.8	1.2	1.4
Unreacted Nonionic	19.6	6.5	2.2	2.1	7.4	1.4	19.6
Phosphoric Acid							
Phosphorous NMR	1.4	2.3	12.2	12.4	2.5	3.1	1.3
Potentiometric Titration		1.0	14.4	13.4			1.2
Pyrophosphates							
Phosphorous NMR	1.2	2.8	11.5	2.6	< 0.2	2.3	< 0.2

The ratio of monoester/diester from HPLC, CE and NMR compare very favorably and are likely to reflect the actual concentration of these species in the samples examined. In addition, HPLC is the only technique which provides information concerning the residual alkylphenol ethoxylate in the phosphate ester. This is further demonstrated in Table 3, where a single sample was examined over a 31 month period with excellent precision.

Table 3 - Reproducibility of Phosphate Ester HPLC Data

Analysis Date	Monoester/Diester Ratio	% Unreacted Nonionic
02/16/94	2.2	9.0
03/31/94	2.4	9.6
03/17/95	2.3	10.8
12/18/95	2.4	9.0
04/22/96	2.4	8.9
09/23/96	2.4	9.5
Average	2.4	9.5
Rel. Std. Dev.	4%	8%

CAPILLARY ELECTROPHORESIS

Free solution capillary electrophoresis (CE) is well suited for the analysis of anionic and cationic surfactants. Separations are based on the mass to charge ratio of sample species under the influence of an electric field. Bech, Goebel et al. and Chen and Pietrzyk discussed the limited application of CE in industrial quality control laboratories to date, therefore, primary emphasis was placed on the use of HPLC. In the characterization of phosphate ester surfactants, however, CE was found to provide unique information relative to mono and diester species present.

Free solution capillary electrophoretic separations were performed using a Thermal Separations Spectraphoresis 1000 instrument with a high speed scanning ultraviolet detector. Uncoated fused silica columns, 63 mm effective length and 50 μM internal diameter from J&W Scientific, were used throughout. Instrument parameters included a constant voltage at normal polarity of 25 kV, a field strength of 357 V/cm, an average current of 36 μA, 1.0 second hydrodynamic injection and a 220 nm detection wavelength. Sample preparation was identical to WAX LC. The capillary was filled with a run buffer consisting of a 50:50 mixture of 50% acetonitrile in water:aqueous pH = 7.5 borate/phosphate buffer. The catholyte solution was 25% acetonitrile in 50 nM NaH_2PO_4. The final pH of this solution was adjusted to pH = 3.0 with H_3PO_4. Data collection, storage and reduction were accomplished using a Thermal Separations PC 1000 data system and a Hewlett-Packard Lab Automation System. When comparing peak areas with difffering retention times [such as ethoxylate distribution], spatial areas [area/retention time] were used to maintain response equivalence. This procedure corrects for increases in peak area caused by decreasing peak velocity at the detector as a function of migration time.

Analyses performed near a pH of seven and at relatively high ionic strength were found to result in maximum resolution of the components present in phosphate esters. Figure 3 indicates the level of resolution possible. The electropherogram demonstrates the early elution of unreacted alkylphenol ethoxylate; where migration is caused primarily by electroosmotic flow. These species serve as a convenient neutral marker for migration time measurements. The presence of early eluting species, in addition to unreacted alkylphenol ethoxylate, limits the use of this region of the electropherogram for quantitative measurements; therefore, estimation of nonionic content using WAX LC is expected to have greater accuracy. Diphosphate anions elute directly after the nonionic species, followed by monophosphate anions. In each of these regions, resolution into individual ethylene oxide oligomers is observed. Within each of these regions, the fewer the number of ethylene oxide units, the longer the migration time. This occurs since anions migrate based on charge to mass ratio in a direction opposite to the detection point (cathode).

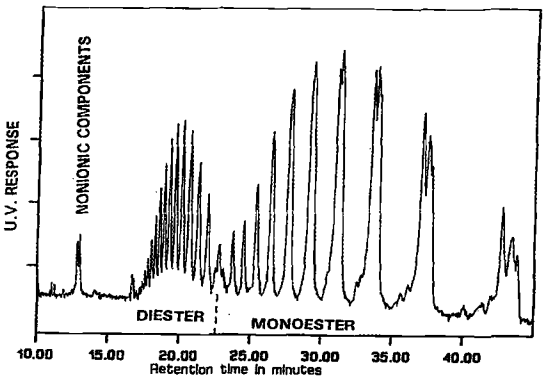


Figure 3 - Electropherogram of Phosphate Ester Species

To obtain the ratio of monoester/diester, all the oligomeric peaks are summed first. The respective total areas are then used to obtain the ratio. Results obtained in this manner are in good agreement with data obtained from WAX LC and NMR. Additionally, migration times permit estimation of moles of ethylene oxide in the species present as well as estimation of the average degree of ethoxylation within the phosphate ester surfactant.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Phosphate esters are difficult to analyze using ^{31}P nuclear magnetic resonance spectroscopy due to broad and ill defined resonances (Figure 4).

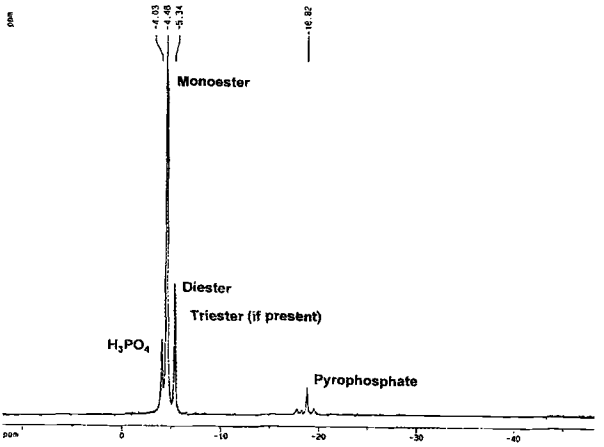


Figure 4 - ^{31}P NMR Spectrum of Phosphate Ester

The conversion of phosphate esters to their corresponding trimethylsilyl (TMS) esters significantly improves the spectra generated by greatly increasing chemical shift differences between phosphoric acid, mono, di and triester species and sharpening all resonances considerably.

Samples were initially dissolved in deuterochloroform, followed by addition of excess N,O-bis (trimethylsilyl) trifluoroacetamide. Spectra were obtained using a Bruker AMX-400 NMR spectrometer operating at 161.98 MHz . Proton decoupled ^{31}P spectra (32k data points) were obtained using a pulse width of 2.5 μsec, sweep width of 10,000 Hz and a delay of 10 seconds between transients. Chemical shifts were recorded relative to a solution of 85% H_3PO_4 in CD_3CN. The addition of trimethyl silyl groups to phosphate esters causes an upfield shift of approximately 9 ppm for each group added, as demonstrated in Figure 5.

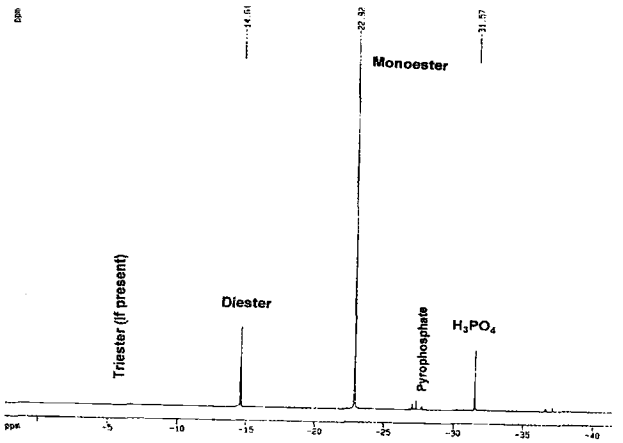


Figure 5 - ^{31}P NMR Spectrum of Derivatized Phosphate Ester

A diphosphate ester would form a mono-TMS derivative, resulting in an upfield shift of 9 ppm from the underivatized ester. In the case of monophosphate, two TMS groups would be added and a shift of 18 ppm would be observed. Similarly, no shift would be observed for a triphosphate ester and 27 ppm for H_3PO_4. Integration of the resonances from the derivatized samples provides a ready mechanism to calculate the molar concentrations of H_3PO_4, mono, di and triphosphate esters in the phosphate ester surfactant. The pyrophosphate resonances are unique in that they exhibit coupling between phosphorous atoms, with each ^{31}P resonance being observed as a doublet. Resonances from symmetric pyrophosphates give rise to singlets.

COSY (COrrelation SpectroscopY) experiments were noted by Murray for the examination of proton interactions, however, with pyrophosphate esters,

COSY can be used to study ^{31}P - ^{31}P interactions. A COSY experiment (Figure 6) generated cross peaks between two doublets [J = 12.7 Hz] at -27.12 and -27.15 ppm, thereby demonstrating a coupling interaction between the two resonances.

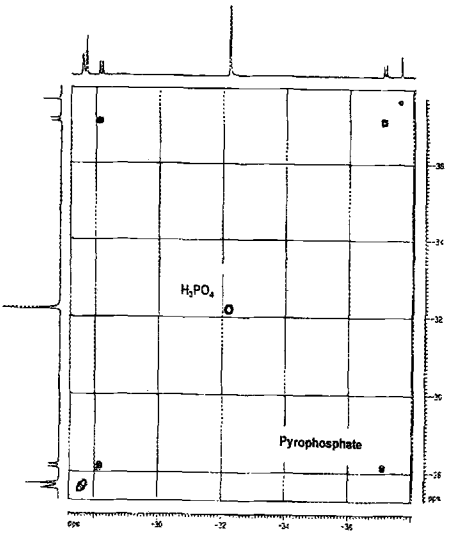


Figure 6 - COSY Spectrum of Phosphate Ester

This COSY experiment provided a simple method of detecting pyrophosphates by observing any crosspeaks in the spectrum. One can then infer the nature of the pyrophosphates by the relative upfield shifts observed for the ^{31}P doublets.

HYDROLYSIS STUDIES

Hydrolysis data was generated from the phosphate ester samples described in Table 1. The concentration of free nonionic, monoester and diester were obtained using HPLC data; while the level of phosphoric acid was obtained from ^{31}P nuclear magnetic resonance spectra.
Species concentration for Sample "A" containing 10% water and stored at 25 °C are shown below:

Table 4 - Hydrolysis of Sample "A"

Time (Days)	% Free Nonionic	% Monophosphate Ester	% Diphosphate Ester	% Phosphoric Acid
0	3.1	45.8	50.9	0.2
7	3.4	48.2	48.2	0.2
14	3.3	48.3	48.2	0.2
21	3.3	48.2	48.2	0.3
28	3.3	48.2	48.2	0.3
42	3.3	50.4	45.8	0.3

Additionally, data from Sample "D" with 25% water and 50 °C are given below:

Table 5 - Hydrolysis of Sample "D"

Time (Days)	% Free Nonionic	% Monophosphate Ester	% Diphosphate Ester	% Phosphoric Acid
0	2.6	80.9	4.7	11.8
7	3.0	79.7	5.0	12.3
14	3.2	79.4	5.0	12.4
21	3.4	79.2	4.9	12.5
28	3.7	78.9	4.8	12.6
42	4.3	78.1	4.9	12.7

To make this information easier to interpret, the plots shown in Figure 7 and 8 were prepared.

Figure 7 - Hydrolysis of Sample A (10% Water at 50 Celsius)

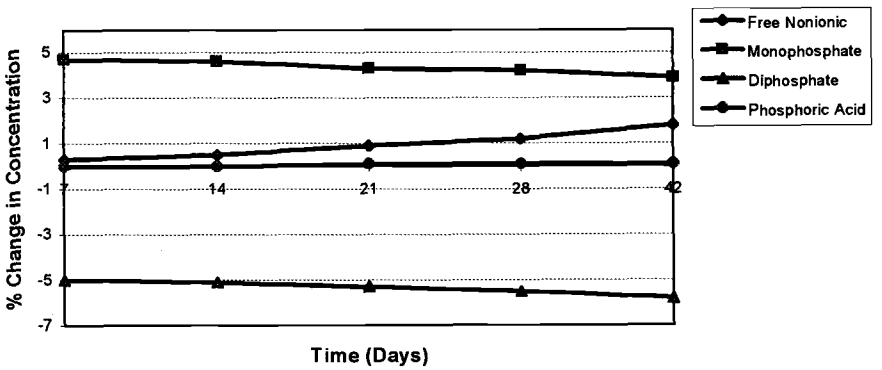
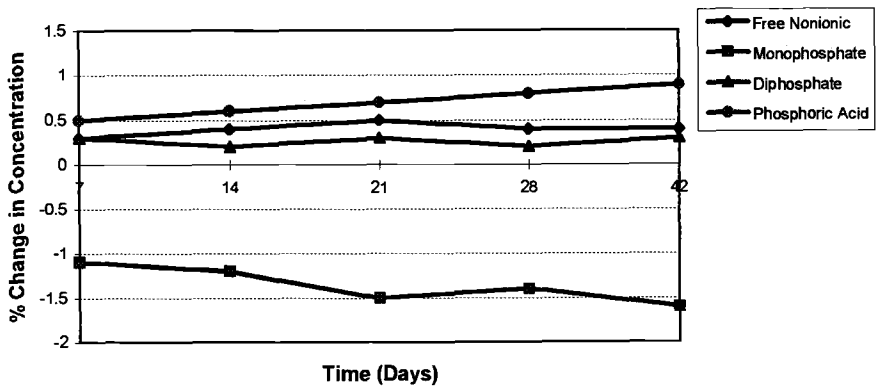


Figure 8 - Hydrolysis of Sample D (25% Water at 25 Celsius)



These plots show only minor changes in the concentration of species present. In addition, for Sample "A", the increase in monophosphate, free nonionic and phosphoric acid is generally equivalent to the loss in diphosphate. In contrast, Sample "D" (containing the very high initial monophosphate concentration) lost monophosphate and gained small amounts of phosphoric acid, nonionic and diphosphate.

A summary of compositional changes for all model phosphate esters is given in Tables 6 - 9.

Table 6 - Summary of Compositional Changes Within Sample "A"

% Water	Temperature	Time (Weeks)	% Free Nonionic	% Monophosphate Ester	% Diphosphate Ester	% Phosphoric Acid
10	25	1	0.3	2.4	-1.7	0.0
10	25	6	0.4	4.6	-5.1	0.1
10	50	1	0.3	4.7	-5.0	0.0
10	50	6	1.7	3.9	-5.8	0.1
25	25	1	0.4	0.0	0.0	0.1
25	25	6	0.3	2.5	-2.6	0.1
25	50	1	0.4	4.5	-5.0	0.1
25	50	6	1.1	4.2	-5.4	0.1

Table 7 - Summary of Compositional Changes Within Sample "B"

% Water	Temperature	Time (Weeks)	% Free Nonionic	% Monophosphate Ester	% Diphosphate Ester	% Phosphoric Acid
10	25	1	-0.9	3.1	-2.1	-0.1
10	25	6	-0.9	5.2	-4.3	-0.1
10	50	1	-0.9	0.5	0.5	-0.1
10	50	6	0.3	6.6	-6.9	-0.1
25	25	1	1.5	7.8	-9.2	-0.1
25	25	6	1.5	9.5	-10.6	-0.1
25	50	1	1.5	9.5	-10.9	-0.1
25	50	6	2.3	9.1	-11.3	-0.1

Table 8 - Summary of Compositional Changes Within Sample "C"

% Water	Temperature	Time (Weeks)	% Free Nonionic	% Monophosphate Ester	% Diphosphate Ester	% Phosphoric Acid
10	25	1	-1.3	4.4	-6.2	0.3
10	25	6	-1.4	4.6	-4.4	1.3
10	50	1	-0.9	4.3	-3.4	0.1
10	50	6	-0.6	3.2	-4.9	1.1
25	25	1	-1.9	7.0	-6.4	1.5
25	25	6	-1.4	6.8	-7.3	1.5
25	50	1	-1.1	5.8	-5.9	1.5
25	50	6	-0.5	6.3	-7.5	1.7

Table 9 - Summary of Compositional Changes Within Sample "D"

% Water	Temperature	Time (Weeks)	% Free Nonionic	% Monophosphate Ester	% Diphosphate Ester	% Phosphoric Acid
10	25	1	1.1	-1.5	0.2	0.5
10	25	6	1.2	-1.5	0.2	0.7
10	50	1	1.1	-1.5	0.2	0.7
10	50	6	1.4	-1.5	0.2	0.9
25	25	1	0.3	-1.1	0.3	0.6
25	25	6	0.4	-1.6	0.3	0.9
25	50	1	0.4	-1.2	0.3	0.5
25	50	6	1.7	-1.7	0.3	0.9

CONCLUSIONS

The applications and capabilities of several analytical tools have been explored in terms of characterizing phosphate ester surfactants. Classical titrations with aqueous caustic provide quick, low cost analyses with excellent precision. This technique does not, however, provide information relative to the presence of unreacted alkylphenol ethoxylate or triester in the final product. The presence of pyrophosphate esters also has an influence on the distribution of species calculated from the titration data, limiting the accuracy on monoester and diester measurements.

High performance liquid chromatography provides a quick, specific measure of the relative proportions of unreacted alkylphenol ethoxylate [unique to HPLC], diester and monoester. The accuracy of these values has been verified by agreement with two complimentary techniques, CE and NMR. HPLC also does not provide information on phosphoric acid content. Capillary electrophoresis provides a highly specific measure of relative monoester and diester concentrations and a unique estimation of ethoxylation content and species distribution.

Nuclear magnetic resonance spectroscopic data produces a definitive measure of all phosphorous containing species in the phosphate ester surfactant, including triester and pyrophosphates. Although NMR spectrometers are not widely available in quality control laboratories, this technique is extremely useful for validating information generated with more conventional analytical methodology. The broad spectrum of phosphate ester performance and useful qualities can be described and reproducibly assured through the use of these techniques.

A number of model phosphate ester surfactants were synthesized to contain a wide variation in free nonionic, monoester, diester and free phosphoric acid. In the worst case, storing these materials at ambient and elevated temperature, with ten and twenty five percent moisture, produced only minimal hydrolysis. When hydrolysis was observed, most took place within the first seven days; with only minor compositional changes during the remaining 35 days of exposure. Hydrolysis rates increased only slightly when the storage temperature was increased from 25 to 50 °C and the water content increased from 10 to 25%.

Overall, these studies have confirmed the good hydrolytic stability generally attributed to phosphate ester surfactants.

ACKNOWLEDGMENTS

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George A. Policello and Kalman Koczo[1]

FOAM CONTROL IN TRISILOXANE ALKOXYLATE SYSTEMS

REFERENCE: Policello, G. A. and Koczo, K., **"Foam Control in Trisiloxane Alkoxylate Systems,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: The properties that make trisiloxane alkoxylates (TSA) so desirable, such as superspreading and spray coverage, also present some challenging issues with regard to their use in the field. Since TSAs are highly surface active, they can produce an extremely stable foam. Traditional antifoam compounds, based on polydimethylsiloxane oils (PDMS) have proven to be ineffective in controlling foam generated by TSA surfactants. It is believed that the antifoam oil droplets must enter the air/water interface of the foam film in order for foam rupture to occur. If the oil droplet forms a stable pseudoemulsion film at the interface, and the droplet does not enter the surface, foam control is not achieved. Unfortunately this is the case with traditional foam control agents.

Oils based on novel siloxane propoxylates have been found to form unstable pseudoemulsion films in trisiloxane alkoxylate solutions, thereby overcoming the low efficiency associated with traditional foam control agents in TSA systems.

KEYWORDS: trisiloxane alkoxylate, foam control, TSA, trisiloxane, pseudoemulsion film.

Trisiloxane based surfactants are well known for their ability to reduce the aqueous surface tension of spray solutions to values below 22 mN/m. In part this low surface tension is responsible for the superspreading properties associated with these unusual wetting agents (Goddard et al. 1992). However, because trisiloxane alkoxylates (TSA)

Scientists at Witco Corporation, OSi Specialties Group, Research and Development; 777 Old Saw Mill River Road Tarrytown, NY 10591.

are such efficient surfactants, they can produce an abundance of foam. Normally this is not a problem since the applicator can tame foam with a commonly used silicone defoamer. Unfortunately, this is not the case with foams generated by trisiloxane based surfactants. In most situations the addition of a silicone based defoamer has little impact on foam destabilization.

Currently with TSA foams, the user needs to add the defoamer in excess of up to 30 times more than commonly used for conventional surfactants in order to subdue the foam to a manageable level. The destabilization of such a foam is not quick (minutes) and may require additional attention (gentle agitation) to help attenuate the foam to an acceptable level. The addition of the TSA at the end of the filling cycle appears to lessen the dilemma. But even then, the foam that is generated, at the end of filling, is persistent.

MATERIALS AND METHODS

Foam control - Inversion method

Foam control was determined by adding 50 mL of surfactant solution, with and without a foam control agent, to a 250 mL graduated cylinder, and agitated by inverting ten times. Foam volume was measured immediately, and at various time intervals after agitation.

Foam suppression - Sparge method

Surfactant solution (100 mL) was added to a 1L graduated cylinder. The solution was sparged with 0.15 L/min. nitrogen, using a fritted pipette. The time required to fill the cylinder with foam is relative to the foam control efficiency. Foam volume (mL) vs time is monitored for 4 hours, or until the foam volume is sufficient to fill the cylinder.

Pseudoemulsion film stability

Surfactant solutions were prepared in deionized water that was additionally treated with a Millipore filtration system. The surfactant solution (75 mL) was placed in a petri dish and covered to avoid evaporation. Using a glass pipette, a droplet of silicone antifoam (SAG® 100) was released under the surface of the desired surfactant solution. The time required for the oil droplet to enter the surface and form a lens is relative to the stability of the pseudoemulsion film. It is important not to contaminate the surface with oil when introducing the glass pipette. Therefore, the pipette should be filled after submersion into the surfactant solution.

Surface tension

Surface tension was measured by the Wilhelmy Plate method, using a sand blasted platinum blade as the sensor. All solutions were prepared in 0.005M NaCl (equilibration aid).

Surfactants and foam control agents used in this study are described in Table 1.

TABLE 1--Description of surfactants and foam control agents.

Reference	Product	Description
Surfactants:		
TSA	Silwet L-77®	Trisiloxane ethoxylate, 8 EO, methyl capped (Witco Corporation).
OPE	Triton® X-100	Octylphenol ethoxylate, 10 EO (Union Carbide Corporation).
Foam Control Agents:		
AF-1	SAG® 100	Polydimethylsiloxane based defoamer
SP-1	...	Siloxane propoxylate (30% PO)
SP-2	...	Siloxane Propoxylate (40% PO)

RESULTS AND DISCUSSION

Traditional foam control agents (FCAs) are extremely efficient at defoaming conventional surfactants, such as OPEs. Generally, low levels of an FCA (ppm) is all that is needed to get foam to a manageable level. This is not the case when the foam is generated by a TSA surfactant. The traditional FCAs have relatively little impact on controlling foam from a TSA solution. Table 2 demonstrates that high levels of AF-1 are required to destabilize the TSA foam (> 3000 ppm). This is a significant amount of FCA since the surfactant is only used here at 1000 ppm. At times we have observed that as much as 7000 ppm AF-1 was needed to control the TSA foam. By comparison AF-1 is commonly used at 20 ppm in conventional surfactant systems.

Table 2-- Comparison of TSA and OPE for resistance to defoaming

Surfactant	Foam Volume (mL) Initial	1 Minute
TSA (w/o AF-1)	50	42
TSA + AF-1 (100 ppm)	46	36
TSA + AF-1 (1800 ppm)	40	30
TSA + AF-1 (3000 ppm)	40	26
OPE (w/o AF-1)	70	54
OPE + AF-1 (100 ppm)	40	4

Mechanisms for defoaming

Polydimethylsiloxane oils are commonly used for foam control because of their low nonaqueous surface tension (20-22 mN/m) and their insolubility in water. Generally it is believed that the surface tension, of the neat foam control agent (nonaqueous surface tension), should be below that of the aqueous solution being defoamed.

Koczo *et al* (1994) describe one possible mechanism for defoaming, which suggests that oil droplets (FCA) move into the plateau borders of the foam film. The oil droplets can adsorb near the air water interface and form a pseudoemulsion film (Figure 1). If the pseudoemulsion film is stable then foam control will not be achieved (Wasan et al. 1994, Koczo et al. 1992). On the other hand, if the pseudoemulsion film is unstable, then the oil droplet will enter the surface of the film and form a lens.

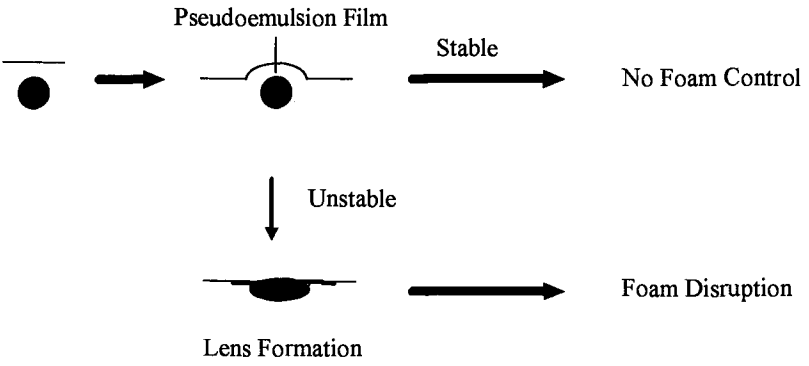

FIGURE 1--Suggested mechanism for foam control

Figure 2 illustrates the proposed mechanism for the defoaming process. Oil droplets are forced into the plateau borders of the foam bubbles during film drainage. The droplet forms a pseudoemulsion film, which if unstable, enters the surface to form a lens. As the film continues to thin, the droplet can enter the opposite surface forming a bridge. The capillary pressure established by the bridge causes local thinning around the lens. The film then pinches off from the drop, resulting in bubble disruption (Koczo et al. 1994).

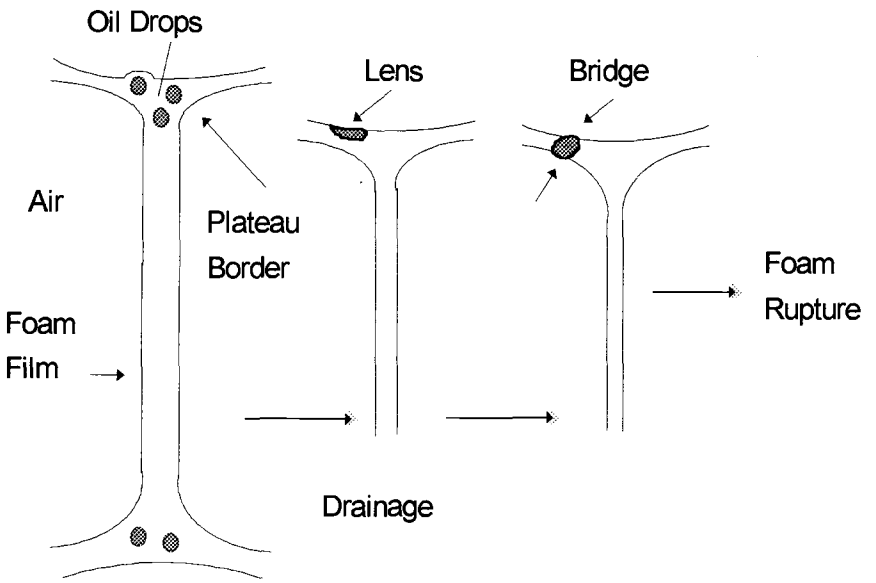

FIGURE 2--Suggested mechanism for antifoaming

Poor control of the TSA foam is the result of the FCA oil droplet forming a stable pseudoemulsion film. Table 3 shows the relationship between surface tension and stability of the pseudoemulsion film. Here a drop of polydimethylsiloxane oil was released under the surface of the TSA solution. The time required for the oil droplet to enter the surface and form a lens is relative to the stability of the pseudoemulsion film. If the oil droplet does not enter the surface quickly, then foam control is low or nonexistent. The surface tension of the oil in AF-1 is similar to that of the TSA solution (both ~ 21 mN/m). This is a possible reason that the oil droplets form stable pseudoemulsion films. As the surface tension of the TSA increases, with a decrease in concentration, the rate at which the FCA oil enters the bubble film also increases (destabilization of the pseudoemulsion film).

TABLE 3--Influence of surface tension on pseudoemulsion film stability.

Wt % Surfactant	Surface Tension mN/m	Stable Film? Y/N	Time to Enter Surface
0.1	21	Yes	> 5 min.
0.01	21	Yes	> 5 min.
0.003	24	No	80 s
0.001	34	No	15 s

Table 4 illustrates the relationship of surface tension and foamability for TSA (Inversion method). It is no surprise that foam stability decreases with a decrease in surfactant concentration. However the addition of AF-1 to the solution has minimal effect on foaming when the surface tension is < 24mN/m. Once the surface tension rises above this point the FCA has a marked effect on foam stability. This is in agreement with what was presented in Table 3, where the pseudoemulsion film is unstable in TSA solutions with a surface tension ≥ 24 mN/m, which corresponds to the foam control observed in Table 4.

TABLE 4--Influence of AF-1 on foamability of TSA.

		1 min. Foam Volume (mL)	
Wt % Surfactant	Surface Tension mN/m	Alone	+ 100 ppm AF-1
0.1	21	28	26
0.01	21	28	26
0.003	24	22	10
0.001	34	---	0

Traditional FCAs are based on a polydimethylsiloxane oil containing hydrophobic particles, such as silica. As demonstrated above the traditional FCA (AF-1) forms a stable pseudoemulsion film in TSA solutions of ≥ 0.01 %. The result is poor foam control efficiency, even when the FCA is used at excessively high concentrations (up to 3000 ppm - Table 2).

Novel siloxane propoxylates (SP-1 and SP-2) do not form stable pseudoemulsion films in TSA solutions, but the droplets quickly enter the air/liquid interface of the foam film. The time required for either the traditional FCA or the SPs to enter the surface of an OPE solution is relatively quick (Table 5). However, in a TSA solution, AF-1 forms a stable pseudoemulsion film, and does not enter the surface even after 48 h. The SPs on the other hand do not form a stable pseudoemulsion film and quickly enter the surface.

TABLE 5--Influence of antifoam type on pseudoemulsion film stability (0.1 wt% surfactant solution)

Surfactant	Pseudoemulsion Film Stability	
	OPE	TSA
AF-1	5 s	> 48 h
SP-1	5 s	2 s
SP-2	5 s	2 s

The stability of the pseudoemulsion film is a key to the performance of FCAs. As mentioned earlier, if the film is stable, than foam control does not occur. When the pseudoemulsion film is unstable, the oil droplet enters the surface, and results in foam control. The foam control efficiency of an FCA can be demonstrated by introducing a nitrogen sparge into a solution of the TSA. The rate at which the foam fills a 1 L graduated cylinder is relative to antifoam efficiency. Generally, the longer the fill time the greater the foam suppression provided by the FCA. Figure 3 illustrates that the addition of 200 ppm AF-1 to a solution of TSA does not control foam production, but gives a foam profile (Foam volume vs time) that is essentially identical to TSA without AF-1. The foam produced is fairly stable and does not readily collapse even after the sparge is removed (Table 6). The foam profile for TSA + 200 ppm SP-1 or SP-2 shows that good foam suppression is achieved (Figure 3), and the foam is easily destabilized when the sparge is removed from the surfactant solution (Table 6).

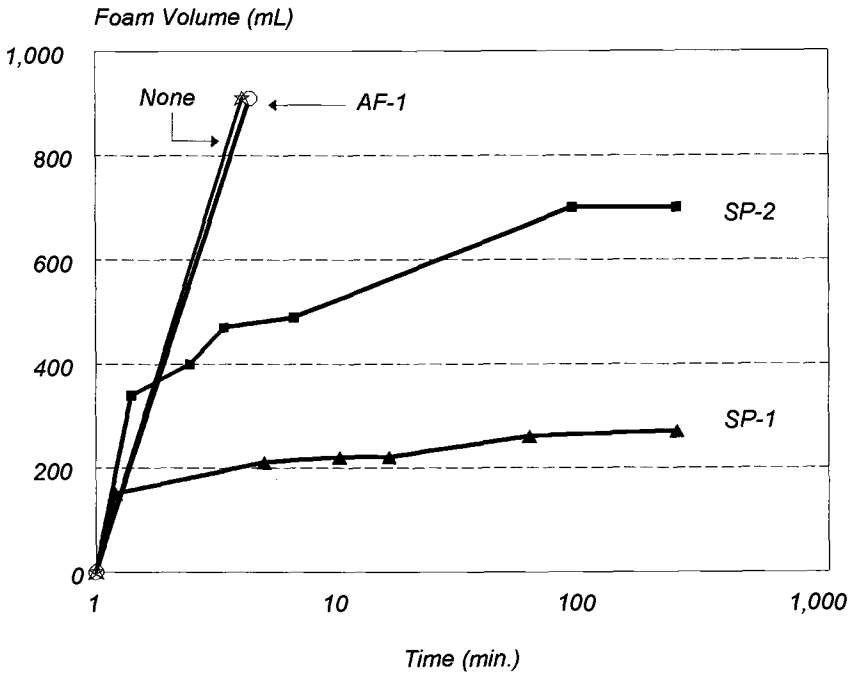

FIGURE 3--Influence of FCA (200 ppm) on foam control of TSA (0.1 wt%)

TABLE 6--Impact of FCA on foam collapse for TSA (0.1 wt%)

FCA	Foam Volume (mL) Initial	5 min.
None	935	910
AF-1	910	650
SP-1	120	10
SP-2	535	40

Traditional FCAs are easily deactivated because of the emulsification of the polydimethylsiloxane oil during the defoaming process. The disruption of the foam film breaks the antifoam droplet into smaller droplets that can be stabilized or emulsified by the surfactant solution (Racz et al. 1996). As a result it is necessary to replenish the FCA to keep foam in check.

The durability of the SPs is quite impressive, since they control foam for extended periods of time (> 24 h without deactivation). This indicates that the SPs are not easily emulsified by the TSA, thus obviating the need to continually add defoamer.

SUMMARY

Traditional foam control agents based on polydimethylsiloxanes plus a hydrophobic particle (silica) are ineffective at controlling foam generated by trisiloxane alkoxylates, such as Silwet L-77® surfactant. Droplets of the PDMS based foam control agent dispersed in a TSA solution form stable pseudoemulsion films, which results in poor foam control properties. Siloxane propoxylates are a new class of foam control agents designed specifically for TSA surfactants. Droplets of the SPs quickly enter the surface of the foam film, leading to effective foam control.

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Randal M. Hill[1] and Richard F. Burow[2]

WHY ORGANOSILICONE ADJUVANTS SPREAD

REFERENCE: Hill, R. M. and Burow, R. F., **"Why Organosilicone Adjuvants Spread,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT:

The use of certain organosilicone surfactants as agricultural adjuvants has come about as a result of their unusual ability to promote the rapid spreading of dilute solutions on hydrophobic leaf surfaces. The reason for this unique spreading is not well understood.

The spreading of surfactant solutions, including the organosilicone surfactants, has recently been shown to exhibit a maximum in spreading rate as the surfactant concentration is increased, and as the substrate is made more hydrophobic. Certain organic surfactants promote spreading equally as rapid as the organosilicones on slightly less hydrophobic surfaces. Organosilicone surfactants, on the other hand, exhibit rapid spreading even on very hydrophobic surfaces. Some have attributed the rapid spreading of the organosilicone surfactants to their unique molecular shape, but the evidence does not support this. A correlation has been found between solubility or turbidity and spreading—those materials which form turbid dispersions seem to provide the most rapid spreading. In this case turbidity is due to the presence of a dispersion of bilayer vesicles. The presence of vesicles contributes to the low dynamic surface and interfacial tensions at short time scales, leading to the ability to spread more rapidly over more hydrophobic substrates.

KEYWORDS: organosilicone, adjuvant, spreading, trisiloxane. surfactant

[1] Interface Expertise Center, Central R&D, Dow Corning Corporation, Midland, MI 48686-0994

[2] Performance Chemicals TS&D, Dow Corning Corporation, Midland, MI 48686-0994

INTRODUCTION

The use of organosilicone surfactants as adjuvants to enhance the efficacy of foliar applied agrochemicals such as herbicides, fungicides, and foliage applied nutrients has recently been extensively reviewed (Roggenbuck 1990, Stevens 1993, Knoche 1994, Tadros 1995). Such adjuvants allow agrochemicals to be used at the lowest recommended label rates which is desirable for both economic and environmental reasons.

In discussing the function of the surfactant, these reviews have focused on such properties as equilibrium and dynamic surface tensions, contact angles on hydrophobic surfaces, and spread areas relative to water (after some fixed time period), and the correlation of these surface properties with greenhouse and field performance, and with studies of foliar uptake and the penetration of certain agrochemicals into plant leaf surfaces. Many siloxane surfactants have been studied, but the greatest benefits have been seen with the trisiloxane surfactants. In the nomenclature used by Bailey (1967) and Noll (1968), the trisiloxane polyoxyethylene surfactants are denoted M(D'E_nOR)M, where

M stands for $(CH_3)_3SiO_{1/2}$—,

D' stands for —$(CH_3)R^1SiO$—,
with the R^1 being some non-methyl group, in this case the attached polar group,

E_n stands for the polyoxyethylene group, —$CH_2CH_2CH_2(OCH_2CH_2)_n$—,
where n is the number of oxyethylene segments, and

R stands for an end-capping group, usually —H, —CH_3, or —$OC(O)CH_3$.

The molecular structure of M(D'E_nOR)M is shown in Figure 1.

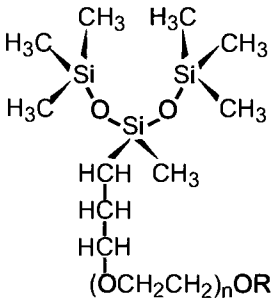

Figure 1. Molecular structure of the trisiloxane superwetting agents.

Surfactant effects on foliar uptake and field performance of agrochemicals are species and compound specific. Knoche (1994) tabulates the effect of siloxane surfactants on the foliar uptake of a variety of agrochemicals by different plant species, as well as field and greenhouse performance. It is believed that certain trisiloxane surfactants enhance transport of spray solutions into plants, either through stomatal pores or by way of cuticular penetration (Roggenbuck 1990, Stevens 1993). Performance in field trials and greenhouse studies parallels these considerations. The best way to understand the seemingly confusing effects on different species is to recognize that efficacy of foliage

applied agrochemicals generally requires penetration of the active ingredient into the plant. The efficacy of agrochemicals such as contact fungicides which do not need to penetrate the plant is directly improved by enhancing spreading. For other types of agrochemicals, spreading is only useful if it correlates with penetration. Highly efficient spreading of the aqueous vehicle over the leaf surface could lead to rapid evaporation and even roll-off thereby inhibiting penetration (Knoche 1994).

FACTORS CONTRIBUTING TO RAPID SPREADING

Dilute aqueous solutions of certain siloxane surfactants are able to rapidly wet quite hydrophobic surfaces such as polyethylene or Parafilm® or waxy leaf surfaces (Ananthapadmanabhan *et al.* 1990, Murphy *et al.* 1991, Bahr *et al.* 1992, Ekeland *et al.* 1992, Goddard and Padmanabhan 1992, Zhu 1992, Zhu *et al.* 1994, Hill *et al.* 1994, Lin *et al.* 1994, Lin *et al.* 1996, Svitova *et al.* 1996, Stoebe *et al.* 1996). Kanner *et al.* (1967) stated that siloxane surfactants based on siloxane groups containing 2-5 silicon atoms, and having a limited but finite solubility in water were the "best" wetting agents on polyethylene. This unusual phenomenon has been termed "superwetting" or "superspreading" and appeared to be unique to a very small group of siloxane surfactants (Zhu *et al.* 1994).

The efficient wetting of hydrophobic leaf surfaces by aqueous pesticide formulations containing organosilicone surfactants has been related to four factors:

1. the unusual "hammer" shape of the trisiloxane surfactant (Ananthapadmanabhan *et al.* 1990, Murphy *et al.* 1991, Goddard *et al.* 1992),
2. moisture effects (Zhu 1992, Zhu *et al.* 1994),
3. surfactant aggregation or solubility/turbidity (Kanner *et al.* 1967, Hill *et al.* 1994), and
4. surface and interfacial tension lowering (Murphy *et al.* 1991, Svitova *et al.* 1996).

We will discuss each of these factors, and show that molecular shape is not a factor, but that the unusual wetting of organosilicone surfactants is due to a combination of low dynamic surface and interfacial tensions.

MOLECULAR SHAPE

The most widely used organosilicone surfactants consist of a heptamethyltrisiloxane group coupled to a polyglycol polar group as shown in Figure 1. The unique "hammer" shape of the organosilicone hydrophobe has been given as part of the reason for the unusual wetting and spreading properties of organosilicone surfactants (Murphy *et al.* 1991, Goddard *et al.* 1992, Murphy *et al.* 1993, Stevens *et al.* 1993b). This is supposed to lead to "molecular zippering action" (Ananthapadmanabhan 1990, Stevens 1993a), a kind of tractor-tread motion due to a mechanical rolling over of the surfactant molecules at the edge of a spreading droplet. Conventional hydrocarbon surfactants , on the other hand, are said to not spread rapidly because they supposedly tend to lie flat on the surface thus "jamming the zipper" (Ananthapadmanabhan 1990).

However, Hill *et al.* (1994) has shown that a "linear" trisiloxane surfactant, which lacks the "hammer" shape of the conventional superwetter organosilicone surfactants, spreads

just as well. In addition, Stoebe *et al.* (1996) has recently shown that a linear alkyl ethoxylate ($C_{12}E_3$) exhibits a similar rate of spreading to the organosilicone surfactants on slightly less hydrophobic surfaces.

Estimates of molecular lengths of trisiloxane surfactants may be calculated from bond lengths and bond angles as described by Hill *et al.* (1994). Molecular volumes can readily be calculated from the molecular weight and density. The cross-sectional area can then be estimated by dividing the volume by the length. Such a procedure to estimate molecular shapes of surfactants at interfaces is widely used by surfactant technologists (Mitchell *et al.* 1983, Evans and Wennerstrom 1994). Molecular areas may also be derived from the slope of the surface tension *vs.* log(concentration) curve below the critical aggregation concentration (CAC). The "best" values of cross-sectional areas are derived from small angle X-ray scattering (SAXS) measurements of lamellar phase liquid crystal (Mitchell *et al.* 1983). In the case of the trisiloxane surfactants, the calculations and the two experimental measurements give consistent results (He *et al.* 1993).

These calculations and measurements indicate that the shape of the heptamethyltrisiloxane group is somewhat different from a conventional organic hydrophobe group, for example a dodecyl group. The trisiloxane group is shorter (9.7 Å vs. 15 Å) and wider (cross-sectional area of 55 $Å^2$ vs. 23 $Å^2$). But the molecular volume of the trisiloxane hydrophobe is actually larger than the organic—530 $Å^3$ vs 350 $Å^3$. These numbers demonstrate that describing the trisiloxane group as "small" or "compact" in comparison with conventional hydrocarbon surfactants as is often done (Ananthapadmanabhan 1990, Stevens 1993a)is not correct.

The cross-sectional area of an E_{7-8} group is also about 55 $Å^2$ (Mitchell 1983). Based on these quantities, the correct proportions of the organosilicone surfactant, $M(D'E_7OH)M$ and a conventional hydrocarbon surfactant, $C_{12}E_7$ are shown in Figure 2. The hydrophobic and hydrophilic portions of $M(D'E_7OH)M$ have essentially the same cross-sectional area, causing it to form bilayer structures in water, even at low concentrations. Although its shape is different from conventional hydrocarbon surfactants, it does not have an "inverted" shape, and is not "T-shaped" or "hammer-shaped".

Thus, neither experimental measurements of spreading rates, nor a correct visualization of the molecular shape of the organosilicone surfactants supports attributing their unique properties to their unusual shape.

THE EFFECT OF HUMIDITY

Zhu (1994) found that spreading of dilute solutions of organosilicone surfactants on Parafilm® was sensitive to humidity—spreading was much faster above a threshold relative humidity of about 50%, and was reduced to nil under very dry conditions. This sensitivity to humidity has also been reported by Gaskin and Stevens (1993) and seen in field use of organosilicone adjuvants[3]. Zhu *et al.* (1994) interpreted his results to mean that a pre-existing water film on the surface was required for rapid spreading.

[3] Personal communication to the authors from a number of agrochemicals dealers.

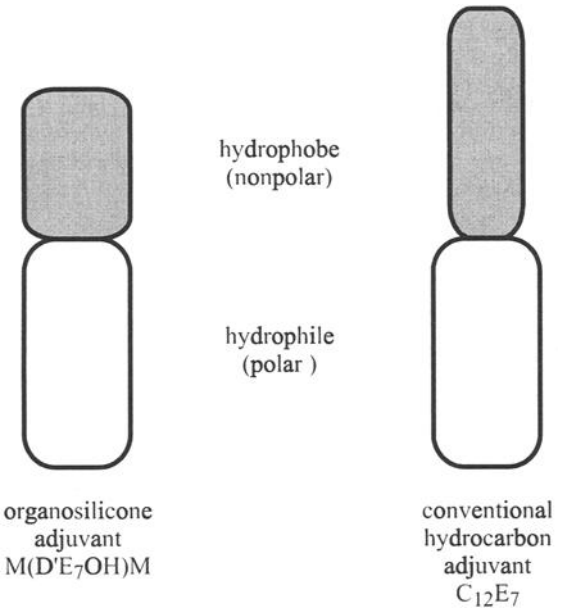

Figure 2. Diagram comparing the molecular shape of trisiloxane and hydrocarbon surfactants.

However, Stoebe *et al.* (1996) has recently shown that the sensitivity to humidity depends very much on the particular surface one is dealing with—on Parafilm® and other similar rough paraffinic surfaces, spreading is much faster above a threshold humidity, but on other types of surfaces (for example, some metallic surfaces) the effect is much weaker. Thus, a pre-existing water film is not a general requirement for rapid spreading, but leaf surfaces are rough and contain wax crystals and should show the same sort of humidity dependence as Parafilm®.

SOLUBILITY OR TURBIDITY

Various investigators have reported on the apparent relation between solubility (or turbidity) and wetting by organosilicone surfactants (Kanner *et al.* 1967, Zhu 1992, Hill *et al.* 1994). Marginal solubility, wherein surfactants form stable cloudy dispersions, seems to give the best wetting. Many nonionic surfactants become insoluble with increasing temperature, phase separating at a temperature called the cloud point or cloud temperature (T_c). Above T_c the surfactant is present as a dispersion of oil droplets. Hill *et al.* (1994) has shown that the organosilicone surfactant M(D'E_7OH)M[4] forms cloudy dispersions over a wide temperature range due to the presence of a dispersion of bilayer vesicles (shown in Figure 3) rather than being above the surfactant's cloud point.

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[4] The structure of Dow Corning 211 Silicone Surfactant is M(D'E_7OH)M.

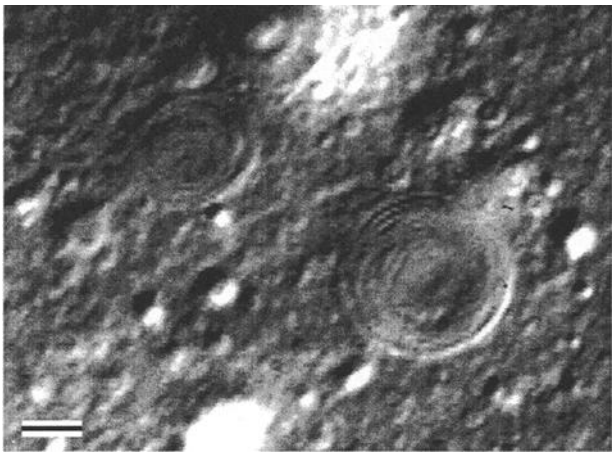


Figure 3. Photograph taken with a light microscope (using differential interference contrast) of a 1% dispersion of M(D'E_7OH)M showing bilayer vesicles. The bar is about 5 microns.

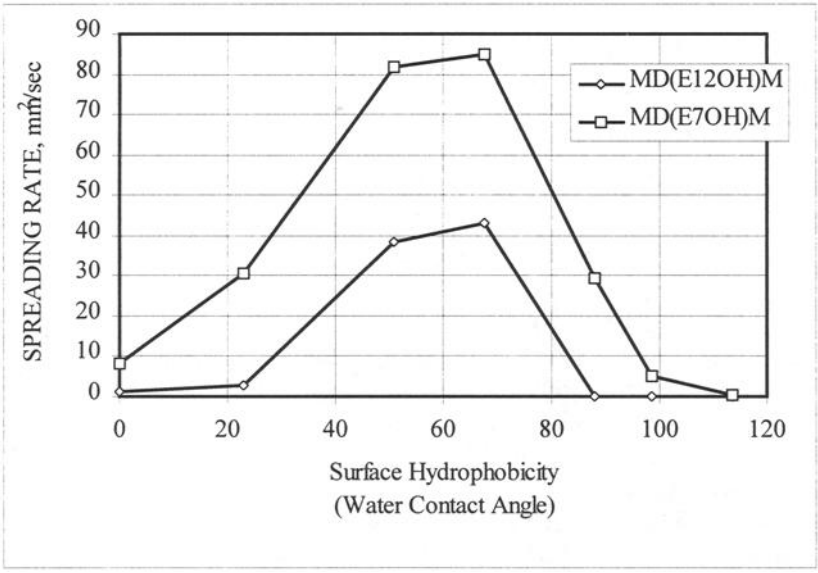


Figure 4. Spreading rates of M(D'E_7OH)M and M(D'E_{12}OH)M[5] as a function of substrate surface energy. Re-plotted from Stoebe *et al.* (1996).

[5] The structure of Dow Corning 212 Silicone Surfactant is M(D'E_{12}OH)M.

Recent work by Stoebe *et al.* (1996), re-plotted in Figure 4 and Figure 5, has demonstrated that while vesicle dispersions tend to produce the most rapid spreading, rapid spreading is also observed for non-turbid (that is, micelle forming) surfactant solutions of both trisiloxane and hydrocarbon surfactants. Zhu (1992) observed that sonication of turbid dispersions of organosilicone surfactants renders them nearly transparent and increases the spreading rates. He interpreted this as due to a reduction of the particle size—the system remained a dispersion of bilayer vesicles.

The water contact angle plotted in Figure 4 and Figure 5 is the contact angle observed for pure water on the substrate and is a measure of the surface energy of the substrate. High contact angles represent hydrophobic surfaces. The experimental methods involved in these measurements are fully described by Stoebe *et al.* (1996).

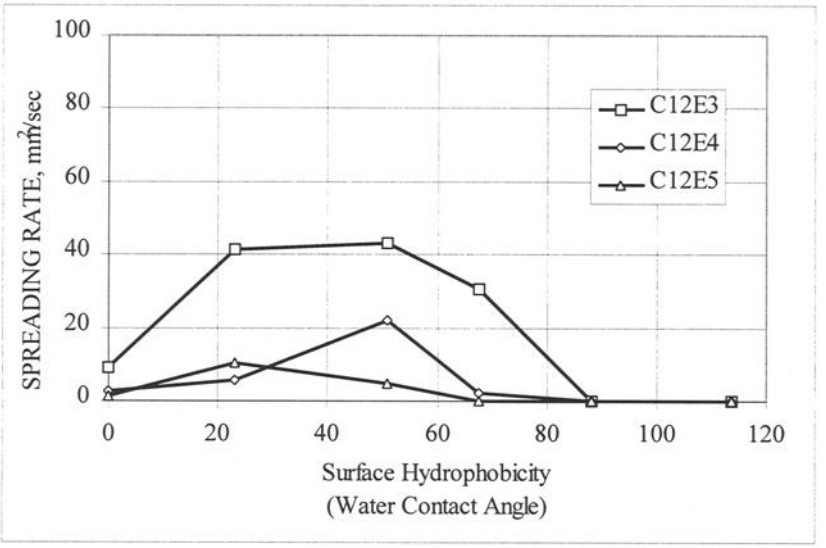
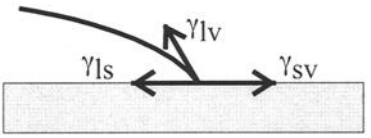


Figure 5. Spreading rates of a series of alkyl ethoxylate nonionic surfactants as a function of substrate surface energy. The turbidity decreases from $C_{12}E_3$ (very turbid) to $C_{12}E_5$ (clear). Re-plotted from Stoebe *et al.* (1996).

The maximum in spreading rate as a function of the substrate surface energy shown in these figures, as well as the maximum in spreading rate as a function of surfactant concentration found earlier by Zhu *et al.* (1994) are general characteristics of surfactant enhanced spreading (Stoebe *et al.* 1996). Rapid spreading and both maxima have now been found for a variety or organosilicone and hydrocarbon surfactants. These measurements mean that a particular type of surfactant aggregate (such as a bilayer vesicle), or turbidity/solubility are not necessary for an organosilicone surfactant to function efficiently.

SURFACE AND INTERFACIAL TENSION

Whether a liquid will spread over a solid substrate such as a leaf surface is determined by the sign of the spreading coefficient, S:



$$S = \gamma_{sv} - (\gamma_{ls} + \gamma_{lv})$$

where γ_{sv} is the solid / vapor (solid / air) surface tension, γ_{ls} is the interfacial tension between the liquid and the solid, and γ_{lv} is the liquid / vapor (liquid / air) surface tension.

If S is positive, the liquid will spread over the leaf. This equation shows that spreading not only requires a low surface tension for the surfactant solution, but also a low interfacial tension between the solution and the leaf surface. This is why solutions of fluorocarbon surfactant solutions, which have even lower surface tensions than do solutions of siloxane surfactants, do not spread over hydrocarbon hydrophobic surfaces—the interfacial tension between a fluorocarbon surfactant solution and a hydrocarbon substrate is relatively large. This equation indicates whether the liquid will spread or not, but not how fast the liquid will spread.

Unfortunately, neither the surface tension of solid surfaces such as that of waxy leaves or polyethylene, nor the interfacial tension between solid surfaces and surfactant solutions can be measured experimentally. This means that we can only guess at whether the spreading coefficient is positive or negative in most practical situations involving solid surfaces. In order to get around this problem, Svitova *et al.* (1996) measured dynamic surface and interfacial tensions using hydrophobic liquids such as mineral oil and liquid paraffins.

The equilibrium interfacial tension between normal alkanes and 0.5 w% solutions of $M(D'E_7OH)M$ varies linearly between 0.025 dynes/cm for hexane to 0.4 dynes/cm for hexadecane (Svitova *et al.* 1996). These small values indicate that the organosilicone surfactants are very effective at lowering interfacial tensions against low energy hydrocarbons, which is consistent with their ability to cause spreading over such substrates. However, surface tensions of surfactant solutions do not correlate very well with wetting time or degree of spreading (Vick 1984, Ananthapadmanabhan *et al.* 1990, Murphy *et al.* 1991). One must consider the ***sum*** of quantities represented by the spreading coefficient, S, rather than just the surface tension. Even S does not correlate very well with spreading unless ***dynamic*** surface and interfacial tensions are used in its calculation.

We have recently extended the work of Svitova *et al.* (1996) and present here some of our results. Dynamic surface and interfacial tensions were measured using the drop volume method (Joos and Van Ufflen 1995, Faour *et al.* 1996) on an instrument we constructed. Solutions of $M(D'E_7OH)M$ were prepared using de-ionized water, hand-shaken to mix,

and measured within one day. Flow rates were varied using a Harvard Instruments syringe pump to achieve drop intervals varying from about 0.3 to 5 seconds. The drop interval is related to the surface age—the shorter the interval, the more rapidly the surface is being stretched.

The surface tension of organosilicone surfactants falls rapidly with surface age toward the equilibrium value of about 20 dynes/cm, as is shown in the following figures.

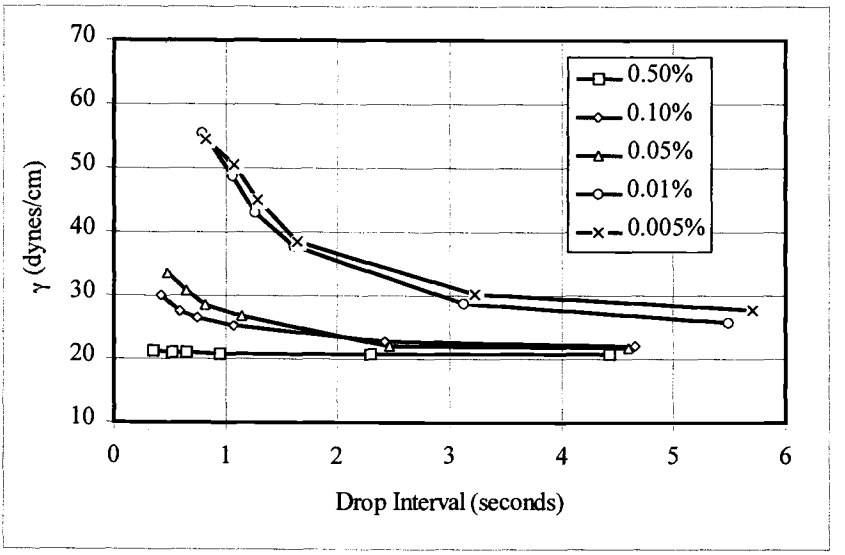


Figure 6. Dynamic surface tension of M(D'E_7OH)M solutions measured using the drop volume method.

Figure 6 shows that the surface tension of more concentrated solutions of M(D'E_7OH)M (above 0.5 %) reach the equilibrium value of about 20 dynes/cm at the shortest time scales we could reach using this method. The corresponding interfacial tensions are shown in Figure 7.

Dynamic surface tensions reflect the rate at which surfactant moves to a freshly made interface. The more rapidly the surface tension decreases with time, the more rapidly the surfactant is adsorbing at the interface. Although the surface tension fall rates shown in Figure 6 and Figure 7 are rapid, especially at the higher concentrations, it is the combination of rapid adsorption and the low surface and interfacial tensions given by the organosilicone surfactants which explains their rapid spreading. Turbidity due to the presence of a dispersion of bilayer vesicles appears to lead to faster adsorption of surfactant (Svitova *et al.* 1996) and therefore more rapid spreading.

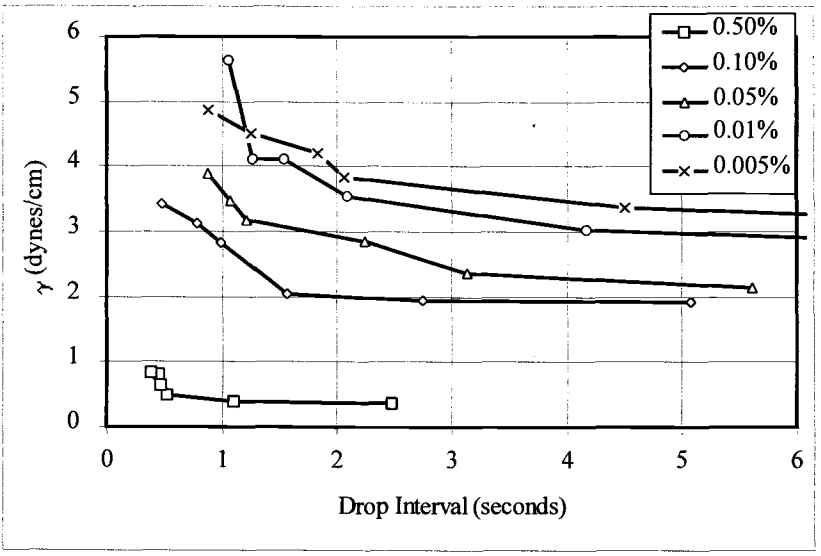


Figure 7. Dynamic interfacial tensions of M(D'E_7OH)M solutions against tetradecane measured using the drop volume method.

Using dynamic surface and interfacial tensions such as those illustrated in Figure 6 and Figure 7, and working on liquid alkane surfaces, we have confirmed the conclusion of Svitova *et al.* (1996) that if the spreading coefficient, S, is calculated from the values of the dynamic surface and interfacial tensions at short time-scales (about 1 second and less), then an excellent correlation exists between the dynamic spreading coefficient and rapid spreading. When the dynamic spreading coefficient is positive at short time scales, then rapid spreading is observed. A positive spreading coefficient calculated from the equilibrium surface and interfacial tensions does not predict spreading. Only if the value remains positive to short time scales is rapid spreading observed. Although this result was obtained working on liquid surfaces, we are confident that the conclusion applies to solid surfaces as well—rapid spreading by surfactant solutions is due to a combination of low dynamic surface and interfacial tensions.

CONCLUSION

Organosilicone surfactants effectively wet waxy leaf surfaces, and enhance the penetration of certain herbicides into the plant. A variety of mechanisms and factors have been proposed to explain the apparently unique ability of certain trisiloxane surfactants to function in this way, including the unique molecular shape of the organosilicone hydrophobe, turbidity or solubility, and low surface tensions. None of these factors satisfactorily explains the features of the unusual wetting. We have shown that the organosilicone surfactants have the same overall wetting behavior as conventional hydrocarbon based surfactants. The organosilicone surfactants are able to wet slightly more hydrophobic surfaces because of their lower dynamic surface and interfacial tensions. The spreading coefficient calculated from the dynamic values at relatively short

time scales (about 1 sec) correlates well with rapid spreading of dilute surfactant solutions over hydrophobic liquid surfaces such as mineral oil. This insight can be applied to solid surfaces also—rapid spreading by surfactant solutions is due to a combination of low dynamic surface and interfacial tensions, not the molecular shape of the surfactant. Turbidity, due to the presence of a vesicle dispersion contributes to rapid spreading by accelerating transport of surfactant to the interfaces.

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Efficacy

Kolazi S. Narayanan[1] and Michael Tallon[2]

SOLID ADJUVANT SYSTEMS -- FORMULATIONS, STABILITY, AND EFFICACY

REFERENCE: Narayanan, K. S. and Tallon M., **''Solid Adjuvant Systems-Formulations, Stability, and Efficacy,''** Pesticide Formulations and Applications Systems: 17th Volume, ASTM 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: Adjuvants are increasingly used as a means of reducing pesticides in the environment by providing biological enhancement. Incorporation of adjuvants with the active ingredients can produce added value and product differentiation. Examples of solid forms of adjuvants that can provide multiple benefits are not very common. Solid adjuvants have several advantages like elimination of containers (using water dispersible/soluble bags), capability of direct incorporation with solid formulations (wettable powders, water soluble/dispersible granules), and use as tank mix additives.

Formulated value added multibenefit adjuvant systems (Agrimax 3™) are described in the literature [Narayanan, 1993]. These are optimized microemulsions containing alkyl pyrrolidones, anionic surfactants and water insoluble copolymers derived from vinyl pyrrolidones. Agrimax systems are designed to provide increased spreading, penetration and rainfastness. A second type of adjuvants is copolymers of vinyl pyrrolidones containing positive pendant groups (Restrict™). Restrict™ polymers are shown to reduce leaching of pesticides from the soil [Narayanan, et. al., 1993 a].

The above adjuvant systems were converted to value added solid forms by the use of specific complexing agents like urea. Prototype compositions derived from the above adjuvant systems, preparative methods, stability, physical properties, and biological performance are discussed. The role of urea as a complexing agent is also discussed.

KEYWORDS: Adjuvants, solid adjuvants, Agrimax 3™, N-alkyl pyrrolidones, anionic surfactants, alkyl grafted polyvinyl pyrrolidone, microemulsions, urea complexation, Restrict™, dimethyl aminoethyl methacrylate vinyl pyrrolidone copolymers, starch complexation, stability, dispersion, biological performance

INTRODUCTION

Use of adjuvants to provide several added benefits to pesticides in general is gaining importance (Foy, C. L., 1992; Farm Chemicals 1990-92; Weed Control Manual, 1992). Some of the benefits derived from the proper use of adjuvants are: enhanced biological activities, increased

[1]Dr. Narayanan is Research Fellow, Agricultural Products, and [2]Dr. Tallon is Research Scientist, NMR Spectral Analysis, Analytical Chemistry at International Specialty Products, Inc., ISP Management Company Inc., 1361 Alps Road, Wayne, New Jersey 07470.

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rainfastness, improved penetration, and better wetting, spreading, and protection against UV radiation for the active ingredients. Since these effects are specific to the nature of the active ingredients, often it is required to use more than a single adjuvant composition in a single application. A product that offers multiple benefits would be of great value as an adjuvant. Agrimax™ systems showed multiple benefits. Agrisolve™ [Adjuvant A] and Agrimax 3™ [Adjuvant B] are proprietary multipurpose adjuvant compositions for pesticide formulations. Adjuvant A is an optimized single phase composition incorporating water-insoluble long chain pyrrolidones in an aqueous system, designed to enhance wetting, spreading, and penetration of active ingredients. Adjuvant A can be diluted in water without phase separation [Narayanan, et al, 1993 b]. This composition can be formulated with commercially available concentrates for several active ingredients or can be used as a tank-mix additive. Adjuvant B is an optimized formulation containing pyrrolidone-based water insoluble polymers microdispersed in aqueous media as a homogeneous single phase consisting of mixed alkylpyrrolidones and anionic surfactants. Adjuvant B is dilutable at all concentrations without separation. This formulation can impart rainfastness particularly for water soluble active ingredients. Further, several UV protectant molecules can be solubilized in the Adjuvant B system. These systems containing microdispersed UV protectants were reported to be very stable and produced stable miniemulsions on dilution [Narayanan, et. al, 1995 a]. The Restrict™ [Adjuvant C] class of polymers (copolymer of vinyl pyrrolidone and dimethyl amino ethyl methacrylate) showed reduced leaching of several pesticides in the soil [Narayanan, et. al., 1993 a].

This paper describes solid formulations derived from liquid forms of pyrrolidone-based adjuvants (Adjuvant A, Adjuvant B and Adjuvant C) previously described in the literature [Narayanan , 1993; Narayanan, et. al., 1993 a, 1993 b, 1995 a]. Solid forms of the adjuvants were made via complexation with urea/starch and freeze-drying slurries containing appropriate quantities of the adjuvant compositions, water, and the complexing agents.

EXPERIMENTAL METHODS

Sample Preparations

Adjuvant compositions were prepared by weighing appropriate quantities of the ingredients in a one-liter polymer-reaction flask. All ingredients used were commercially available. Homogeneous compositions were obtained by stirring with a mechanical stirrer for a period of one hour. All compositions were prepared from commercially available sources. Solid adjuvants were prepared as shown in the following Examples. Physical properties, stability, applications, and biological enhancement from some of the solid adjuvants are described in the Results and Discussion Section.

Example 1 - Adjuvant B/Urea (1:1)

A one-necked 1 L round bottom flask equipped with a magnetic stirrer and thermometer was charged with 250 mL water, 250g urea, and 250g Adjuvant B (liquid), in the order listed. The charge was completely dissolved at room temperature by stirring for about 30-60 minutes. The solution was transferred in equal parts to six freeze drying flasks, each of 600 mL capacity. The flasks and contents were placed in a commercial freeze drier unit and subjected to 500 millitorr at -90°C for a period of 24 hours. The materials were scrapped from the flasks and blended into a fine powder in a commercial blender. The total solid recovered was 350g, (recovered yield = 81%), the balance was left on the sides of the flask and blender. The

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sample was stored in a vacuum desiccator. The sample was analyzed for it's homogeneity and found to have 56.5-58.0% urea (theoretical value = 57.8%, Adjuvant B contains 28% water). The residual water on the dry solid was ~ 0.5%.

Example 2 - Adjuvant B/Urea (3:1)

In a 16 Oz bottle, 25g urea was dissolved in 50g water via sonication, and the solution was then mixed with 75g Adjuvant B (liquid). The mixture was freeze dried as in Example 1. The resulting product was a white powder. The recovered yield was 95.8g or 96%, the balance being the loss from transfer and work up.

Example 3 - Adjuvant B/Urea (1:3)

Example 2 was repeated except 75g urea was used in the place of 50g, and 25g Adjuvant B was used in the place of 50g. The final product was a white solid.

Products of Examples 1 through 3 were homogeneous with urea content + 1.5% of theoretical values. Dissolution at 0.5% in water occurred with less than 100 inversions.

Example 4 - Granulation of Example 1

A 200g sample from Example 1 was charged in a 2 ft diameter Ferrotech pan granulator kept at an inclined angle of 40^0. The pan was rotated at a speed of 20 RPM. A total of 18.2 mL water was sprayed during granulation over a period of ~ 8 minutes. The granules were dried in a laboratory fluid/bed/drier with maximum air-flow setting at 40^0C for 25 minutes. The granules were cooled to room temperature. The dried granules were sized to 10/40 mesh via an automatic sieve shaker with 10 mesh and 40 mesh sieves. 26% of the total granulated product passed through 10 mesh and was retained by 40 mesh screen at 4 minutes sieving set at intensity 3. The granules showed acceptable hardness and friability and dissolved in water at 0.5% in less than 100 inversions. The granules can be easily pressed into tablets in the presence of additional binders like Agrimer 30.

Example 5 - Adjuvant A/Urea

Solid versions of Adjuvant A adjuvant with urea/adjuvant ratio at 1:3,1:1, and 3:1 were similarly prepared. The products were free flowing powders.

Example 6 - Adjuvant C/Urea (1:1)

Solid Adjuvant C with urea/polymer ratio at 1:1 was similarly prepared. The product was a free flowing powder

Example 7 - Adjuvant C/Starch

Solid Adjuvant C with corn starch at 0%, 2%, 10%, 25%, 50%, 75%, and 95%, the balance being the polymer, were prepared by the process essentially described in Example 1. The resulting products were free flowing powders with residual water less than 2%.

Example 8 - Incorporating Solid Adjuvant With Active Ingredients

Following example is shown as a typical case to incorporate the solid adjuvant with active ingredients. A twin shell blender was charged with 400g ammonium salt of phosphonomethyl glycine, and 123g of solid composition of Example 1 in the dry blender. The solid charge was blended for 10 minutes. The charge was transferred to a 2 liter Hobart planetary

mixer. 92g liquid Adjuvant B was added to the charge over a period of 5 minutes and the contents were mixed at a speed setting of 2 for 15 minutes, followed by additional mixing at a speed setting of 3. The wet pasty mass was then transferred to a LCI (LCI Corp) Benchtop Granulator, a basket type extruder with adjustable speed and interchangeable screens. Granules were made at an operating speed set at 10 (maximum) and the screen opening at 1 mm. After extrusion, the sample was dried in a Retsch TG1 fluid bed drier for 30 minutes at 40°C. After cooling for up to 24 hours at ambient temperature (23°C), the granules were sized to 10/40 mesh as in Example 4.

The yield was ~ 85%. Average rate of dissolution for 0.5g sample to dissolve in 50 mL 342 ppm standard hard water was < 100 inversions. Friability index [Fu et. al., 1995] was > 90%(% granule retained in 40 mesh screen after subjecting 10g sample with 25 PFTE balls of 0.6 cm diameter for 400 rotations in a Roche drum in a laboratory Vanderkemp Friambilator).

Particle Size Measurements

Emulsion droplets (5-50 microns) were analyzed via an optical microscope, model Nikon S-Kt at 250 X magnification. Macroemulsion range particle size distribution (1-100 microns) for aqueous dispersions and emulsions was measured using a Microtrac particle size analyzer. Microemulsion range particle size distribution (0.01-0.1 microns) was measured using Leeds Northrup, Microtrac ultrafine particle analyzer, containing software package for data analysis (Narayanan, et. al., 1993 c).

Surface Properties Measurements

Surface tension was measured using the ring method (Weser, 1980) with a Fisher surface tensiomat. Contact angle was measured with droplets of average size ~ 5 mm diameter, using Kruss droplet analyzer, Model ACAMS - 40 with attached computer software for image analysis (Kruss USA, 1990). Wetting time was measured using Drave's cotton skein test (ASTM, 1990)

Viscosity, Conductance, and pH Measurements

Viscosity of the appropriate compositions was measured by weighing the required quantity of the formulation to produce 250g of final sample at the required dilution. The samples were stirred by a magnetic stirrer for one hour, and the viscosities and conductance were measured after one additional hour standing for equilibration. The viscosities were measured with a Brookfield digital viscometer Model # RVT DV-II, using a RV spindle # I. The conductance was measured with a YSI Model 35 digital conductance meter, and a platinized Pt glass mounted electrode with a precalibrated cell constant of 1 cm^{-1}. The pH of the aqueous samples was measured using a Corning Research pH meter and a combination glass electrode. The pH meter was calibrated with three standard buffers of different values: 4.0,7.0, and 10.0.

Evaluation of Dispersion Quality

Freeze-dried Adjuvant C/Starch solid forms were evaluated for quality of aqueous dispersions. 2.5g of the freeze-dried solid was stirred in 50 mL water (deionized water or water of appropriate hardness) for one hour. The dispersion was quantitatively transferred into several 50 mL Nessler tubes and allowed to stand for several hours. Samples were analyzed at 0, 0.5, 2 ,3 ,4, and 5 hours. At each time interval, the top and the bottom half were separated by pipetting out the top 25 mL dispersion. The two halves were separately centrifuged for 30 minutes at 6000 RPM, and the solid was separated by decantation. The separated solid was washed and centrifuged three times and then dried to constant weight. Theoretical percent solid (starch) was computed and the results were plotted. A complete homogeneous dispersion should show 50% recovery from both halves

for the duration of the study.

Thermal Analysis

Glass transition temperature (T_g) for appropriate samples was determined using a Perkin-Elmer DSC 7 differential scanning calorimeter and 7500 professional computer. The onset of decomposition and maximum rate of decomposition were determined using a Perkin-Elmer DSC 7 Thermogravimetric analyzer and a 7500 professional computer.

NMR Analysis

NMR spectra were acquired on a Varian XL-300 MHz spectrometer operating at 300 MHz for proton analyses. Solid Adjuvant B (of Example 1) was dissolved in either D_2O or DMSO-d_6 at a concentration of 5% by weight for proton NMR analyses. 2D-Dipolar-coupled NMR spectra were recorded in the hypercomplex mode, with a spectral window of 4000 Hz utilizing 2048 complex data points and 256 T1 increments. Mixing times used in these 2D-analyses were 100 msec; 200 msec; and 300 msec for ROESY experiments in DMSO-d_6, whereas 300 msec; 400 msec; and 500 msec mixing times were used for NOESY spectra of SDS/N-alkyl pyrrolidone in water. Post-acquisition delays of one second were used for all 2D-NMR analyses described. Post processing of 2D-NMR spectra included apodization of the raw data with a phase-shifted exponential sine-bell multiplication to both dimensions of the 2D-data matrix, prior to Fourier transformation.

Rainfastness Evaluations

General methodology described in the literature was used for quick laboratory screening for rainfastness (Narayanan, 1993, Lopez and Hua, 1994).

Field Trials

Results of several field trials and greenhouse evaluations with Adjuvant A and Adjuvant B were reported earlier [Narayanan 1993, Narayanan, et. al., 1993 b, 1995 a]. Field trials using solid Adjuvant B as a tank mix additive for the herbicide, Dicamba (2-methoxy-3,6-dichlorobenzoic acid), on triazine resistant pigweed, and in 'no till' corn were conducted by independent investigators [Foy et. al., 1995 and Bewick et. al., 1994]. Preliminary results of leaching inhibition using liquid form of Adjuvant C and starch modified solid form (75% solid polymer + 25% starch) in Midwestern soil for commercial Broadstrike (Flumetsulam) were reported ineffective [Narayanan et al., 1995 b].

RESULTS AND DISCUSSIONS

Physical properties of liquid forms of Adjuvant A, Adjuvant B and Adjuvant C are summarized in Table 1.

Stability on Storage and Dilution

The adjuvants shown in Table 1 showed no appreciable change in composition, physical properties, or performance on storage at ambient temperature for > one year. Adjuvant A is an aqueous system designed to enhance wetting, spreading, and penetration of active ingredients. This formulation can be diluted in water in all proportions without separation. The particle size of the polymer was well below 200 Å. Adjuvant B is also a water based thermodynamically stable microemulsion (wherein a water-insoluble polymer is microdispersed). On dilution with water at: 1/10,1/50,1/100, and 1/1000 the polymer did not separate. At appropriate dilutions, the particle size distribution was centered around 200-300 Å, well within the microdispersion range. Adjuvant C is an aqueous solution

containing 20% polymer.

TABLE 1--Properties of Liquid Adjuvants

System/properties	Adjuvant A	Adjuvant B	Adjuvant C
Physical State	clear liquid	clear liquid	clear liquid
Boiling Point (deg C)	> 100	> 100	> 100
Freezing Point (deg C)	< 0	< 0	< 0
Flash Point (deg F)	> 200	> 200	> 200
Specific Gravity, 25 deg C	1.00	0.975	1.05
pH (as is) and [1/10]	9.0 [7.8]	8.7 [8.71]	6-8 [6-8]
Brookfield viscosity, cps	19.0	84.5	20,000-50,000
Est. HLB	14.9	12.2	...
Description	SPREADER/ ACTIVATOR/ PENETRANT	SPREADER/ STICKER	LEACHING INHIBITOR/ PENETRANT

Stability at low pH

Adjuvant A and Adjuvant B were evaluated for stability at low pH by monitoring phase separation and particle size distribution at both elevated and cold temperature on dilution in the presence of externally added acids. These systems were stable at pH $\geq$ 3, especially if the acid used has hydrophobic counter ion such as ethoxylated phosphate esters [Narayanan, 1994].

Surface properties

Table 2 summarizes the surface properties of Adjuvant A and Adjuvant B and a commercial spreader/activator(Activate plus) in aqueous solutions as a function of dilution. At the recommended use concentration of 0.25%, Adjuvant A and Adjuvant B showed wetting times < 6 sec, < 10 sec., surface tension < 26 dynes/cm, < 30 dynes/cm., and contact angle ~ 35 degrees, ~ 56 degrees, respectively. The values at full dilution would determine the surface activity/compatibility with several commercial formulations as tank mix additives. If we take into account droplet dry-down effect (assuming a reasonable estimate of 50% evaporation during flight), the effective values for the surface properties for Adjuvant A and Adjuvant B are: instantaneous wetting time ; surface tension, < 28 dynes/cm; and contact angle ~ 46 degrees. The calculated HLB for Adjuvant A and Adjuvant B are: 14.9, and 12.2 respectively.

Dicamba is a trademark of Sandoz, and Broadstrike is a trademark of Dow Elanco

Properties, Stability of Solid Adjuvants

Physical properties of some of the solid adjuvants are summarized in Table 3. The surface properties of the urea complexed solid Adjuvant A and solid Adjuvant B were similar to those of the liquid forms described above. This observation is consistent with other urea complexed surfactants (urea-tridecyl ethoxylates) reported in the literature [Davis, 1995]. There was no change in the compositions or in the physical properties on storage. The urea complexed Adjuvant C with 50% polymer was used to incorporate the polymer into a certain sulfonamide, along with a proprietary combinations of dispersing agents, wetting agents, and clay fillers. The resulting powder produced an excellent dispersion. Use of liquid Adjuvant C, as 20% aqueous solution or Adjuvant C/Starch products produced polymer-incorporated solids with poor dispersion.

TABLE 2--Surface Properties of Aqueous Adjuvant A and Adjuvant B

Adjuvant A

Concentration,%/ Dilution	Surface Tension, dynes/cm	Contact angle[1], degrees	Wetting Time, seconds
1.0, 1/100	27.1 ± 0.1	35.5 ± 1	< 1
0.75, 1/133	27.2± 0.2	40.8 ± 4	< 1
0.50, 1/200	27.0± 0.6	37.1 ± 2	3.3 ± 0.2
0.25, 1/400	26.0 ± 0.09	34.6 ± 5	5.5 ± 0.4
0.15, 1/666	25.8 ± 0.08	35.7 ± 4.4	15.5 ± 1.3
0.10, 1/1000	25.7 ± 0.09	33.9 ± 5	49.6 ± 9.3

Adjuvant B

Concentration,%/ Dilution	Surface Tension, dynes/cm	Contact angle[1], degrees	Wetting Time, seconds
1.0, 1/100	26.5 ± 0.1	41.6 ± 5	0
0.75, 1/133	26.1 ± 0.1	42.6 ± 4	0
0.50, 1/200	27.9 ± 0.1	46.2 ± 7	0
0.25, 1/400	29.7 ± 0.08	55.6 ± 7	8.0 ± 1
0.15, 1/666	30.6 ± 0.08	65.1 ± 7	45.6 ± 11.5
0.10, 1/1000	32.5 ± 0.09	79.3 ± 5	573 ± 185

Commercial Activator - Activate Plus

Concentration,%/ Dilution	Surface Tension, dynes/cm	Contact angle[1], degrees	Wetting Time, seconds
1.0, 1/100	23.4 ± 0.09	46.7 ± 5	8.4 ± 0.4
0.75, 1/133	22.3 ± 0.1	44.4 ± 3.5	9.9 ± 1
0.50, 1/200	22.7 ± 0.1	45.4 ± 8	13.3 ± 1.5
0.25, 1/400	22.7 ± 0.04	45.0 ± 7.6	21.7 ± 2
0.15, 1/666	26.2 ± 0.04	42.5 ± 7.5	38.7 ± 2
0.10, 1/1000	27.9 ± 0.09	45.5 ± 7.7	80.0 ± 11

[1]contact angles reported here are on a parafilm surface

The ease of dispersion of the Adjuvant C/starch system was evaluated as described under "Evaluation of dispersion Quality". Figure 1 summarizes the results. The vertical distance between the forked curve shows percent undispersed solid on standing for the given time period. The stat material (corn starch) or freeze-dried starch (100% starch) showed poor dispersion in water. In one hour only about 10% was dispersed. Incorporation of Adjuvant C polymer improved the dispersibility of starch, depending upon the amount of the polymer. Freeze-dried Adjuvant C with 25% starch produced excellent dispersion in the first one hour, with > 90% of the starch remaining dispersed.

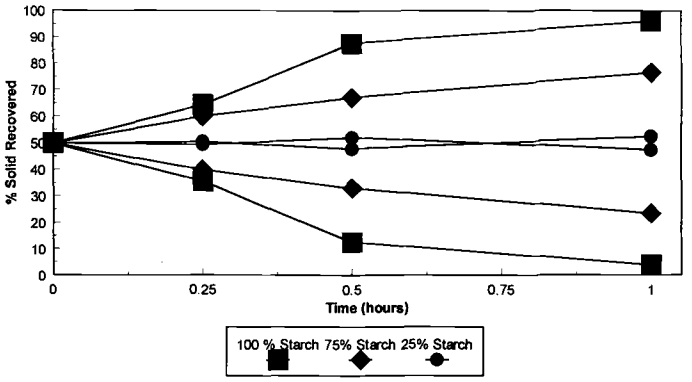

FIG. 1 -- Dispersion stability (% solid recovered) as a function of time for 5% dispersions in water prepared from solid Adjuvant C/Starch compositions.

TABLE 3--Properties of Solid Adjuvants

Compositions	Adjuvant A/Urea (1:1)	Adjuvant D Adjuvant B/urea (1:1)	Cornstarch/ Adjuvant C (1:3)
Physical State	free flowing powder	free flowing powder	free flowing powder
Melting Pt./Tg (^{0}c) Decomp. Temp. (^{0}C)	> 100 > 130	> 100 > 175 > 130 > 300	
Solubility/ Dispersibility in water	single phase	single phase	dispersible (>90%/1 hr at 1%)
a.i. incorporated	dicamba(Na salt) carbaryl	dicamba/phosphono-methyl glycine (Na salt)	dicamba (Na salt)/ sulfonamide
Field trials	...	yes	yes

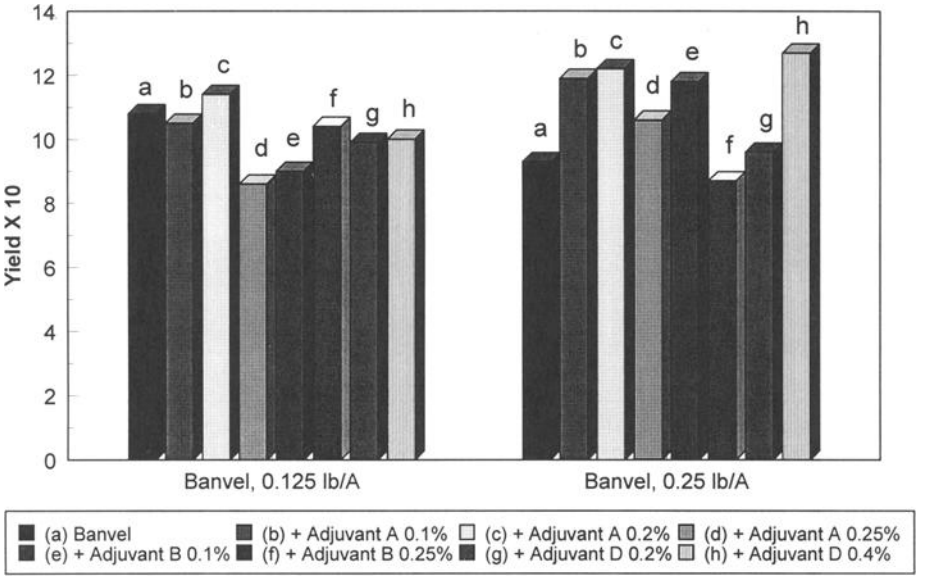

FIG. 2 -- Yield of corn (Kg/Sq.Meter) treated with Banvel/Adjuvants [no-till], Foy et al.,1994

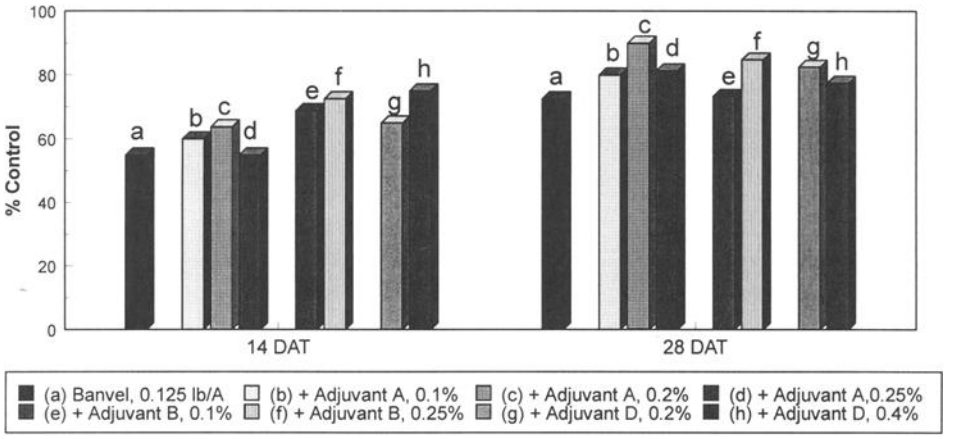

FIG. 3 -- Triazine resistant smooth pigweed control in corn with Banvel/ Adjuvants [no-till], Foy et. al., 1994 - DAT: days after treatment.

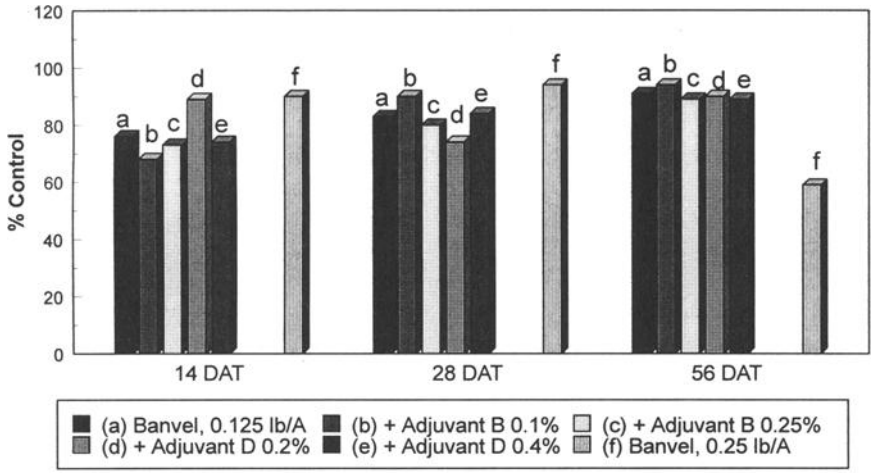

FIG. 4 -- Smooth pigweed in corn with Banvel/Adjuvants, Bewick et. al., 1994 - DAT: days after treatment.

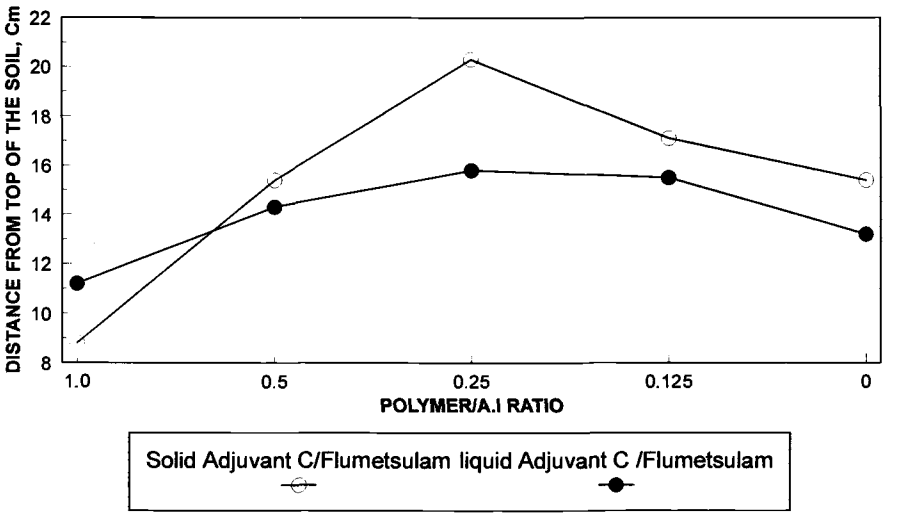

FIG. 5 -- Effect of polymer formulation and polymer-herbicide ratio on the herbicide movement through Midwestern soil

Results of Field Trials

Figure 2 summarizes the overall yield of no-till corn after harvest from trial plots with various treatments [Foy et al, 1994]. The yield of corn was increased 20%, when solid adjuvant (Agrimax 3S™ [Adjuvant D]) was added as a tank mix at 0.4% with 0.28 kg Dicamba/ha. Figure 3 [Foy et al, 1994]and Figure 4 [Bewick et al, 1994] summarize the efficacy of Dicamba on triazine-resistant pigweed in corn with and without adjuvants conducted at two different locations. The efficacy of the solid adjuvant on the control of triazine-resistant pigweed is evident.

Results on Rainfastness

A greenhouse trial (Foy et. al, 1995) with a laboratory rainfall simulator developed on the principle of droplet formation from needle tips showed increased rainfastness of Dicamba based on 'shoot fresh weights of 'velvetleaf' 28 days after treatment. Dicamba without adjuvant was not rainfast. Simulated rain at the rate of 12 mm rain/30 min was generated at 15 min, 30 min, 60 min, and 120 min after treatment. The lowest dose used for Dicamba + Solid Adjuvant D (Dicamba at 92g a.i./ha + 0.2% Solid Adjuvant added as tank mix in the spray solution) and the simulated rain at 15 min after treatment resulted in 1/2-2/3 less fresh weed weight compared to treatment without adjuvant. Results were similar with delayed applications of rain (30 min,60 min, after treatment)or with increased dose of the solid adjuvant at 0.4%. Results were less effective when rainfall was applied 120 min after treatment.

Leaching Inhibition

Figure 5 summarizes the overall comparative results obtained using solid Adjuvant C (75% polymer-25% starch) and an aqueous 20% solution of liquid Adjuvant C as a tank mix additive with Broadstrike in Midwestern soil [Hall and Wolf, 1995]. The solid Adjuvant C showed relatively lower movement of the active ingredient through the soil. Results with Dicamba were not conclusive.

Ramifications

Solid adjuvant (Adjuvant D) is prepared by freezedrying liquid Adjuvant B composition with high concentration of urea ($\geq$ 25%) to produce a high melting solid (m. pt > 100 deg. C). The urea acts as a complexing agent with the components of Adjuvant B and thereby stabilize the solid state. The solid adjuvant is used in the spray solution at concentration $<$ 5%. In the presence of large excess of water, urea molecules would be independently hydrated and complexation effect of urea would be insignificant in the spray solution. In aqueous solution the surface and interfacial properties of the solid Adjuvant D system would be identical with those of the liquid Adjuvant B (without urea). It would be appropriate to examine the surface properties and biological ramifications of the liquid system, which have been studied earlier.

Biological enhancement produced via Adjuvant A and Adjuvant B systems could arise from the following factors:

- faster wetting
- faster spreading
- cuticular penetration /desorption leading to faster/increased translocation
- formation of protective coating via molecular orientation

Faster wetting

Adjuvant A, Adjuvant B systems (at > 0.1%)showed low dynamic surface tension. The dynamic surface tension at the initial rate of formation

(bubble frequency $\geq$ 5), was comparable or lower for Adjuvant B and Adjuvant A compositions when compared to Silwet L77[1] (44, 47; 46, 51 and 45, 54 dynes/cm at frequency of 5 and 10 bubbles/sec, respectively). Drave's wetting time for Adjuvant A and Adjuvant B were comparable or lower when compared to Activate Plus at concentrations $\geq$ 0.15%.

Faster spreading

Faster spreading is accomplished by virtue of lower surface energy (dynamic surface tension and dynamic contact angle should be low) on the leaf surface. When Adjuvant A/Adjuvant B samples were introduced to commercial formulations, spreading area of droplets increased 10 fold on parafilm surface [Narayanan, 1993]. Parafilm surface was chosen as an 'in vitro model' to simulate leaf surfaces [Chambers et al., 1992].

Enhanced Cuticular Penetration

Increased uptake/translocation of trichloropyr ester when used with N-dodecyl pyrrolidone was reported [Buick, 1990]. Cuticular desorption studies using isolated citrus leaf cuticles showed an increase in desorption rate constant by 2 orders of magnitude for 2,4 D (2,4-dichloro phenoxy acetic acid), when C_8/C_{12} N-alkyl pyrrolidones was introduced [Schonherr, 1993; Schonherr et al., 1992].

Formation of Protective Coating via Molecular Orientation

Synergy between C_8/C_{12} N-alkyl pyrrolidones and anionic surfactants have been studied in detail [Rosen et al., 1988; Zhu et. al., 1989]. Adjuvant A/Adjuvant B systems are examples of microemulsions prepared taking advantage of the synergistic interaction between sodium dodecyl sulfate (SDS) and pyrrolidone systems. Adjuvant B in the concentrated form contain ~ 28% water. This system is believed to be reverse micelles with hydrophobic components as the continuous phase, with the polymer oriented in the core in their coiled state with hydrophobic groups outside. During dilution with water the reverse micelles would open up going through a lamellar phase in which the surfactants would orient in a head-to-head and tail-to-tail configuration. The polymer molecules open up to an uncoiled state with its minimum energy conformation. On high dilution, the lamellar state would further reorient to form regular micelles with water as the continuous phase, the polymer assuming once again a coiled conformation with hydrophilic groups preferably pointing outside. The above changes were monitored via viscosity and conductance as a function of dilution. A maximum region of viscosity was observed, corresponding to the lamellar phase in which the polymer has the maximum tendency to uncoil and spread out.

The maxima in viscosity and conductance for Adjuvant B was observed around 40% added water corresponding to 56.7% total water content taking into account the initial amount of water present. Adjuvant A system also showed similar behavior. Solid forms of Adjuvant A and Adjuvant B showed similar characteristics on dilution. During the dilution, systems developed slight haziness in the ranges: 20-60% added water for Adjuvant B. These changes can be attributed to uncoiling of the polymer. The hypothesized oil-out-micelle initial state for Adjuvant B would explain their capacity to hold in solution high concentrations of certain hydrophobic actives [Narayanan et al., 1995 a]. Polymer film formation during dry down, with the active ingredient contained under the film is a possible mechanism for enhanced biological activity and rainfastness observed. Further work is required to understand the mode of action via cuticular penetration, diffusion across the cuticular membranes, and translocation by means of radiotracer studies. Deliberate design of adjuvants to form liquid crystals

[1]Silwet L77 is a trademark of OSI

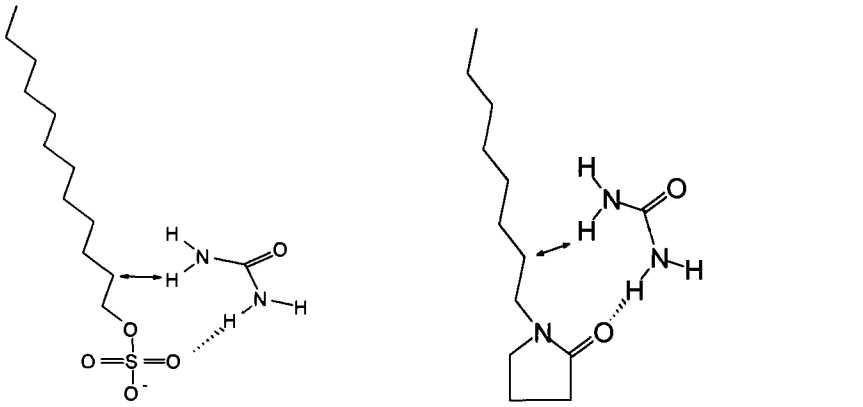

FIG. 6 -- Depiction of through space proton interaction, based on 2D-Dipolar-coupled NMR (in DMSO)

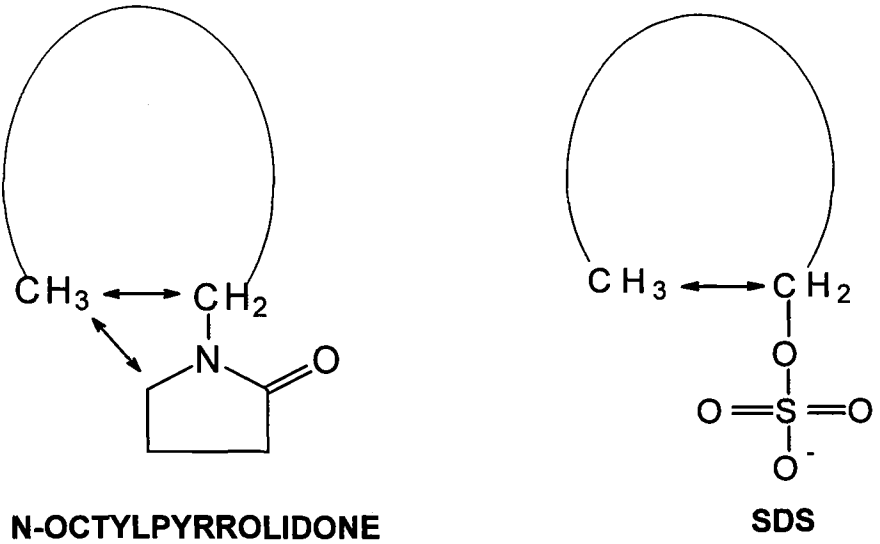

FIG. 7 -- Depiction of through space proton interaction, based on 2D-Dipolar-coupled NMR (in water)

is a recent approach to provide rainfastness, reduced volatility and increased stability of a.i,s [Rogiers, 1995]

Function of Urea

Formation of inclusion complexes between urea and aliphatic hydrocarbons, and urea/aliphatic carboxylic acids are described in the literature [Davis, 1995]. In these complexes the minimum stoichiometric ratio of urea/guest molecules was found to be: 5 for C_6 and 10 for C_{12} compounds. The cross section of the urea bound inclusion hydrocarbon compounds is described elsewhere [Smith, 1952]. The solid systems described here required a much lower concentration of urea. The driving force for complexation in the condensed phase or in concentrated systems could arise from H-bonding between urea and the pyrrolidone carbonyl moiety. 2D-dipolar-coupled NMR experiments were performed with some model systems to investigate the presence of through space interaction between urea and the component molecules present in the solid adjuvant systems.

2D-Dipolar-coupled NMR experiments can elucidate through space interactions of multicomponent systems. Preliminary NMR analyses on Solid Adjuvant B (Example 1)in anhydrous media such as DMSO indicate that the urea amide protons are in close spacial proximity to the central methylene protons of the long hydrocarbon chain within the SDS molecule and/or N-alkyl pyrrolidone as shown in Figure 6. This study was carried out in triplicate with three different mixing times to ensure the reproducibility of this particular through space interaction. The results obtained were identical in all three experiments and reveal a preferential through space interaction between Urea and SDS and/or N-alkyl pyrrolidone. In contrast, 2D-dipolar NMR analyses on SDS and N-alkyl pyrrolidone in water indicate that the long hydrocarbon chains of SDS and N-alkyl pyrrolidone fold back on themselves as evidenced by long-range through space interactions between the terminal methyl groups and the methylene protons attached to the sulfate moiety within SDS, or to the ring methylene proton in the N-alkyl pyrrolidone as shown in Figure 7. These results support that a microemulsion of a micellar type exists under aqueous conditions. Future NMR studies are planed to fully investigate these aforementioned preferential interactions in both anhydrous and aqueous environments.

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Johnnie R. Roberts[1], Allen K. Underwood[1], Anthony Clark[2], Robert E. Mack[1], James M. Thomas[1], and Greg C. Volgas[1]

DRY CONCENTRATE (DC) SPRAY ADJUVANTS

REFERENCE: Roberts, J. R., Underwood, A. K., Clark, A., Mack, R. E., Thomas, J. M., and Volgas, G. C., **"Dry Concentrate (DC) Spray Adjuvants,"** Pesticide Formulations and Applications Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: Research was conducted to determine the effectiveness of three dry concentrate (DC) adjuvants. COHORT® DC^3 (organic nonionic surfactant) and KINETIC® DC^3 (silicone-based nonionic surfactant) were as effective or more effective than conventional liquid formulation surfactants. NXS™ DC^3 buffering agent was more effective at maintaining spray solution pH than the liquid buffering agent BUFFER P.S.™[3]

KEYWORDS: dry concentrate spray adjuvant, nonionic surfactant, silicone-based nonionic surfactant, buffering agent, COHORT DC, KINETIC DC, NXS DC

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[1] Director, Development & Registration; Director, Proprietary Products, Research & Development; Supervisor, Research & Development; Manager, Product Development, Helena Chemical Company, Memphis, TN 38119

[2]Supervisor, Research & Development/Environmental Affairs Manager, Helena Chemical Company, Fresno, CA 93727

[3]COHORT, KINETIC, NXS, and BUFFER P.S. are trademarks of Helena Chemical Company, Memphis, TN

A review of U.S. Environmental Protection Agency (EPA) registered pesticide labels revealed that there are no recommendations for dry concentrate (DC) formulations of spray adjuvants (Anonymous 1995; Meister 1995). One of the major trends in the U.S. pesticide industry is a shift towards "dry" formulations from conventional "liquid" formulations for various reasons, including container disposal, worker exposure, and other environmental concerns. It would be ideal to use "dry" spray adjuvants with these pesticides, to coincide with some of these issues. In addition, these adjuvants may have improved characteristics compared to their conventional liquid counterparts, such as removal of flammable components, improved shipping, handling, and storage characteristics, and a reduction/elimination of volatile organic constituents and free ethylene oxide.

The labels for new dry pesticide formulations still require the use of conventional liquid spray adjuvants. However, the labels may be updated provided that research demonstrates the effectiveness of the dry adjuvants. Development work was begun to develop a series of DC spray adjuvants that have equal or superior physical and/or chemical properties compared to their conventional liquid counterparts. In addition, the DC formulations would have to provide equal biological enhancement of pesticide activity in comparison to the conventional liquid spray adjuvant. This biological enhancement should result in an economic benefit to the end user.

METHODS AND MATERIALS

Adjuvants

Physical and chemical properties for four major types of spray adjuvants were determined by a review of major U.S. pesticide labels. Products meeting those properties were developed by Helena Chemical Company in conjunction with co-operating basic manufacturers of pesticides and spray adjuvant active ingredients.

Liquid adjuvants

INDUCE®[4] (organic nonionic surfactant [NIS]; proprietary blend of alkyl aryl polyoxy-alkane ether and free fatty acids).

KINETIC (organosilicone-based surfactant [OS]; proprietary blend of polyalkylene-oxide modified polydimethylsiloxane and nonionic).

BUFFER P.S. (buffering/conditioning agent; proprietary blend of alkyl aryl polyethoxy ethanol phosphates and organic phosphatic acids).

Dry Concentrate adjuvants

COHORT DC (organic nonionic surfactant [NIS]; proprietary blend of polyethyoxylated hydroxy alkyl surfactants encapsulated in organic nitrogen).

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[4]INDUCE is a trademark of Helena Chemical Company, Memphis, TN

KINETIC DC (organosilicone-based surfactant [OS]; proprietary blend of polyalkyleneoxide modified polydimethylsiloxane, nonionic surfactants, and polymerized ethoxylates).

NXS DC (Buffering/conditioning agent; proprietary blend of inorganic and organic acid salts).

Chemical and/or physical property determination

Static surface tension of the solutions was determined using the Du Nuoy Surface Tension Method. Dynamic surface tension was measured using the Sugden bobble pressure Method. The contact angle was measured using a goniometer at room temperature, 30 seconds after a 4 microliter droplet is placed onto a Parafilm M substrate. The droplet radius was measured at room temperature 30 seconds after a 20 microliter droplet was placed onto a Parafilm M substrate. From the observed droplet radius, the droplet area was calculated. These surface chemistry measurements were performed for the four surfactant-type spray adjuvants (INDUCE, KINETIC, COHORT DC, and KINETIC DC) (Roberts, J.R. 1992). The pH buffering ability of BUFFER P.S. (1.0% v/v) or NXS DC (10 g/L) was determined via the addition of 0.1 N sodium hydroxide to a 100 mL solution containing the adjuvant.

Field testing

Third party researchers were selected to conduct replicated trials to determine the biological effects of the surfactants with major pesticides. All trials included the use of a pesticide without any adjuvant, with a liquid adjuvant as the standard, and the comparable dry concentrate adjuvant of the same type. In some trials, pesticides were used at sublethal doses to magnify the adjuvant effect. Details for each trial are included in the results and discussion.

RESULTS AND DISCUSSION

Physical properties of surfactants

COHORT DC had comparable static surface tension but improved dynamic surface tension properties compared to the conventional liquid nonionic surfactant INDUCE (Table 1). This indicates that COHORT DC may have improved droplet retention on plant surfaces compared to INDUCE. No differences were observed for either contact angle or droplet spread between COHORT DC and INDUCE. KINETIC DC had a higher dynamic surface tension compared to the liquid organosilicone-based nonionic surfactant KINETIC. However, contact angle was similar between these two adjuvants although droplet spread was less with KINETIC DC compared to KINETIC. Overall, the new DC adjuvants have comparable physical properties compared to their conventional liquid counterparts.

TABLE 1--Effect of surfactant on physical properties of spray droplet.

Surfactant spread	Rate	Static surface tension (mN/m)	Dynamic surface tension* (mN/m)	Contact angle (degrees)	Droplet Area (mm^2)
INDUCE	0.25% v/v	33	57.1	42	5
COHORT DC	1.2 g/L	32	44.7	45	3
KINETIC	0.125%	24	40.2	0	123
KINETIC DC	1.2 g/L	24	54.0	0	5.5

* Measured at 200 msec

Organic nonionic surfactants

The efficacy of pyrithiobac sodium (sodium 2-chloro-6-[(4,6-dimethoxy pyrimidin- 2-yl)thio]benzoate) plus MSMA (monosodium methanearsonate), chlorimuron (2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid) and nicosulfuron (2-[[[[(4,6-dimethoxy-2-pyridinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide) was enhanced by an organic nonionic surfactant added to the spray mixture (Table 2). In many cases, weed control went from unacceptable control to very good to excellent control. COHORT DC at 1.2 g/L spray solution enhanced chlorimuron efficacy similar to or greater than that achieved when INDUCE was added to the spray solution. When the COHORT DC at 1.5 g/L was added to the spray solution, pyrithiobac sodium + MSMA efficacy on common cocklebur *(Xanthium strumarium* L.) was enhanced. Nicosulfuron control of redroot pigweed *(Amaranthus retroflexus* L.) and fall panicum *(Panicum dichotomiflorum* Michx.) was

TABLE 2--Effect of organic nonionic surfactant on pyrithiobac + MSMA, chlorimuron, or nicosulfuron efficacy.

Surfactant	Rate	Pyrithiobac+ *	Chlorimuron		Nicosulfuron	
		Common cocklebur	Redroot pigweed	Common ragweed	Redroot pigweed	Fall panicum
			Control (%)			
None	0%v/v	51	71	33	30	18
COHORT DC	1.2g/L	-	87	73	85	77
COHORT DC	1.5 g/L	65	93	64	86	78
INDUCE	0.25% v/v	51	90	70	56	52

* pyrithiobac (Staple SP; DuPont Agricultural Products) at 56 g/ha + MSMA at 1.46 L/ha chlorimuron (Classic; water dispersible granule; DuPont Agricultural Products) at 6 g/ha nicosulfuron (Accent; water dispersible granule; DuPont Agricultural Products) at 17.5 g/ha

enhanced more by COHORT DC than by INDUCE. These results indicate that the dry concentrate organic surfactant COHORT DC is equally or more effective than its corresponding liquid counterpart INDUCE. In addition, COHORT DC was used at one-half the use rate of INDUCE, reducing the amount of adjuvant required. At 9 and 26 days after treatment, sulfosate (N-phosphonomethylglycine trimethylsulfonium salt)(Touchdown; ZENECA Ag Products) control of annual ryegrass (*Lolium multiflorum* Lam.), wild oats (*Avena fatua* L.), yellow foxtail (*Setaria glauca* (L.) Beauv.), barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) johnsongrass (*Sorghum halepense* (L.) Pers.), annual morningglory (*Ipomoea purpurea* (L.) Roth), lambsquarters (*Chenopodium album* L.), purslane (*Portulaca oleracea* L.), wild mustard (*Barassica kaber* (DC.) L.C.Wheeler), groundcherry (*Physalis ixocarpa* Brot. ex Hornem.), hemp sesbania (*Sesbania exaltata* (Raf.) Rydb. ex A.W.Hill), velvetleaf (*Abutilon theophrasti* Medicus), and rough pigweed (*Amaranthus retroflexus* L.) was enhanced when either INDUCE or COHORT DC was added to the spray solution (Tables 3 - 8). In all cases, COHORT DC enhanced sulfosate control to a level similar to or greater than when INDUCE was added to the spray solution.

TABLE 3--Effect of organic nonionic surfactant on sulfosate* efficacy at 9 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control				
Surfactant	Rate	Annual ryegrass	Wild Oats	Yellow Foxtail	Barnyard grass	Johnson grass
None	0.0% v/v	4.3	2.7	3.3	1.0	2.3
COHORT DC	1.2 g/l	6.0	5.7	4.7	3.0	4.7
INDUCE	0.25%v/v	5.3	4.3	4.3	2.3	4.0

***sulfosate (Touchdown; ZENECA Ag Products) at 0.5%v/v.**

TABLE 4--Effect of organic nonionic surfactant on sulfosate* efficacy at 26 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control				
Surfactant	Rate	Annual ryegrass	Wild Oats	Yellow Foxtail	Barnyard grass	Johnson grass
None	0.0% v/v	-	2.3	5.0	0.3	2.3
COHORT DC	1.2 g/l	-	5.7	5.0	1.3	5.7
INDUCE	0.25%v/v	-	3.7	5.0	0.7	4.0

***sulfosate (Touchdown; ZENECA Ag Products) at 0.5%v/v.**

TABLE 5--Effect of organic nonionic surfactant on sulfosate* efficacy at 9 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control				
Surfactant	Rate	Annual morningglory	Lambs-quarters	Purslane	Wild mustard	Ground-cherry
None	0.0% v/v	2.0	3.0	1.0	4.0	2.0
COHORT DC	1.2 g/l	3.0	5.0	3.3	5.7	2.7
INDUCE	0.25%v/v	3.0	4.7	2.7	5.0	2.7

***sulfosate (Touchdown; ZENECA Ag Products) at 0.5%v/v.**

TABLE 6--Effect of organic nonionic surfactant on sulfosate* efficacy at 26 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control				
Surfactant	Rate	Annual morningglory	Lambs-quarters	Purslane	Wild mustard	Ground-cherry
None	0.0% v/v	-	2.3	1.0	2.7	3.0
COHORT DC	1.2 g/l	-	2.7	2.3	3.7	3.0
INDUCE	0.25%v/v	-	3.0	2.3	3.3	2.7

***sulfosate (Touchdown; ZENECA Ag Products) at 0.5%v/v.**

TABLE 7--Effect of organic nonionic surfactant on sulfosate* efficacy at 9 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control		
Surfactant	Rate	Sesbania	Velvetleaf	Rough pigweed
None	0.0% v/v	0.0	1.0	2.3
COHORT DC	1.2 g/l	2.0	2.0	3.7
INDUCE	0.25%v/v	2.3	2.3	3.3

***sulfosate (Touchdown; ZENECA Ag Products) at 0.5%v/v.**

TABLE 8--Effect of organic nonionic surfactant on sulfosate* efficacy at 26 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control		
Surfactant	Rate	Sesbania	Velvetleaf	Rough pigweed
None	0.0% v/v	0.0	1.3	3.3
COHORT DC	1.2 g/l	1.3	1.3	3.7
INDUCE	0.25%v/v	1.0	2.0	4.0

***sulfosate (Touchdown; ZENECA Ag Products) at 0.5%v/v.**

Organosilicone-based surfactants

At 3 and 28 days after treatment paraquat (1,1'-Dimethyl-4,4'-bipyridinium ion; present as the dichloride salt (ZENECA)) control of annual ryegrass (*Lolium multiflorum* Lam.), wild oats (*Avena fatua* L.), yellow foxtail (*Setaria glauca* (L.) Beauv.), barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), johnsongrass (*Sorghum halepense* (L.) Pers.), annual morningglory (*Ipomoea purpurea* (L.) Roth), lambsquarters (*Chenopodium album* L.), purslane (*Portulaca oleracea* L.), wild mustard (*Barassica kaber* (DC.) L.C.Wheeler), groundcherry (*Physalis ixocarpa* Brot. ex Hornem.), hemp sesbania (*Sesbania exaltata* (Raf.) Rydb. ex A.W.Hill), velvetleaf (*Abutilon theophrasti* Medicus), and rough pigweed (*Amaranthus retroflexus* L.) was enhanced when either KINETIC or KINETIC DC was added to the spray solution (Table 9-13). In some instances, weed control was increased from an unacceptable level to an excellent level. In all cases, KINETIC DC enhanced paraquat control to a similar level to that when KINETIC was added to the spray solution.

TABLE 9--Effect of organosilicone nonionic surfactant on paraquat * efficacy at 3 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control				
Surfactant	Rate	Annual ryegrass	Wild Oats	Yellow Foxtail	Barnyard grass	Johnson grass
None	0.0% v/v	5.0	7.3	5.7	7.3	6.0
KINETIC DC	0.6 g/L	7.0	9.3	6.0	8.0	8.0
KINETIC	0.125%v/v	7.0	10.0	7.0	7.0	7.3

***paraquat (Gramoxone; ZENECA Ag Products) at 2 .3 L/ha**

TABLE 10--Effect of organosilicone nonionic surfactant on paraquat * efficacy at 3 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control				
Surfactant	Rate	Annual morningglory	Lambs-quarters	Purslane	Wild mustard	Ground-cherry
None	0.0% v/v	4.0	3.7	5.7	7.3	7.3
KINETIC DC	0.6 g/l	5.0	6.3	7.0	7.0	8.0
KINETIC	0.125%v/v	4.7	5.0	7.0	8.0	8.7

***paraquat (Gramoxone; ZENECA Ag Products) at 2 .3 L/ha**

TABLE 11--Effect of organosilicone nonionic surfactant on paraquat * efficacy at 28 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control				
Surfactant	Rate	Annual morningglory	Lambs-quarters	Purslane	Wild mustard	Ground-cherry
None	0.0% v/v	-	4.0	3.7	5.7	4.3
KINETIC DC	0.6 g/l	-	7.0	4.3	7.0	5.7
KINETIC	0.125%v/v	-	6.7	4.7	7.7	6.7

***paraquat (Gramoxone; ZENECA Ag Products) at 2 .3 L/ha**

TABLE 12--Effect of organosilicone nonionic surfactant on paraquat * efficacy at 3 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control		
Surfactant	Rate	Sesbania	Velvetleaf	Rough pigweed
None	0.0% v/v	6.3	5.7	6.3
KINETIC DC	0.6 g/l	7.7	6.3	7.3
KINETIC	0.125%v/v	8.0	7.0	7.7

***paraquat (Gramoxone; ZENECA Ag Products) at 2 .3 L/ha**

TABLE 13--Effect of organosilicone nonionic surfactant on paraquat * efficacy at 28 days after treatment.

		Control (0-10) ; 0=No Control, 10=Complete Control		
Surfactant	Rate	Sesbania	Velvetleaf	Rough pigweed
None	0.0% v/v	5.3	4.3	3.3
KINETIC DC	0.6 g/l	6.7	5.0	4.3
KINETIC	0.125%v/v	7.7	5.3	4.7

***paraquat (Gramoxone; ZENECA Ag Products) at 2 .3 L/ha**

Buffering/conditioning agents

The two buffering/conditioning agents differed in their response to the addition of sodium hydroxide to the solution (Fig. 1). The pH range where the majority of pesticide chemistries are positively effected is in the range of 5-7.

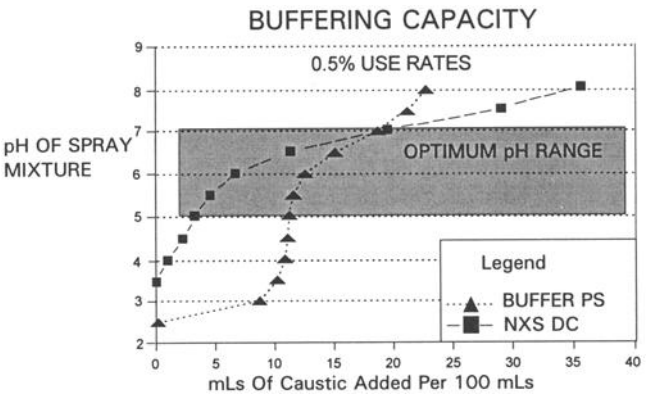

Fig. 1--Effect of NXS DC vs BUFFER PS for holding the pH of a mixture in the optimum pH range.

BUFFER P.S. initially reduced pH to a lower value than when NXS DC was added to the solution. However, NXS DC has a greater buffering capacity than that of BUFFER P.S., as evidenced by the lack of increase in pH when more than 10 mL of sodium hydroxide was added to the solution. In contrast, the BUFFER P.S. liquid solution changed rapidly with the addition of more than 4 mL of sodium hydroxide. This indicates that the dry buffering agent NXS DC can effectively adjust and maintain a spray solution to within an optimum pH range. In addition the dry concentrate formulation lacks the corrosion hazards associated with the liquid product.

CONCLUSIONS

The dry concentrate formulations, COHORT DC, KINETIC DC, and NXS DC, represent an effective alternative to liquid formulations. In some instances, such as buffering of spray solution, the new formulations have improved characteristics over their conventional liquid counterparts. However, EPA registered pesticide labels will need to be changed to reflect the use of weight of the DC adjuvants versus the current volume recommendations for the liquid adjuvants.

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Frank A. Manthey,[1] Edward F. Szelezniak,[2] and John D. Nalewaja[1]

LIPOPHILIC CHEMISTRY AFFECTS SURFACTANT PHYTOTOXICITY AND ENHANCEMENT OF HERBICIDE EFFICACY

REFERENCE: Manthey, F. A., Szelezniak, E. F., and Nalewaja, J. D., **''Lipophilic Chemistry Affects Surfactant Phytotoxicity and Enhancement of Herbicide Efficacy,''** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: Nonionic surfactants are comprised of a lipophilic and a hydrophilic moiety. Experiments were conducted to determine the effect of the lipophilic moiety on droplet spread; surfactant phytotoxicity, and surfactant enhancement of herbicide phytotoxicity. Six different lipophilic moieties and two herbicides were evaluated on four plant species. Each lipophilic moiety was represented by two surfactants: one with a low hydrophilic:lipophilic balance (HLB) value, near 12.0, and one with a high HLB value, near 16.0. Droplet spread was greater with low than high HLB surfactants and was greatest with trimethylnonanol ethoxylate (TMN); intermediate with secondary alcohol ethoxylate (SAE) and octylphenol ethoxylate (OPE), and least with linear alcohol ethoxylate (LAE), nonylphenol ethoxylate (NPE), and oxysorbic (TWN). Surfactant enhancement of droplet spread was less on redroot pigweed (*Amaranthus retroflexus*) than on barley (*Hordeum vulgare*), green foxtail (*Setaria viridis*), or kochia (*Kochia scoparia*). Isopropylamine salt of glyphosate [*N*-(phosphonomethyl)glycine] and the ammonium salt of imazethapyr {2-[4,5-dihydro-4-methyl-4-(1-methylethyl-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid} had little effect on droplet spread. Lipophilic chemistry and HLB affected surfactant phytotoxicity to green foxtail, kochia, and redroot pigweed but not to barley. Foliar injury ranged from 1 to 23% with green foxtail, 0 to 17% with kochia, and 0 to 14% with redroot pigweed. Injury to barley was similar for all surfactants and ranged from 3 to 8%. Injury generally was greater with low than high HLB surfactants. However, SAE and OPE caused greater injury to green foxtail at high than low HLB. Green foxtail was the only plant species injured more than 8% by high HLB surfactants. Droplet spread did not correlate with surfactant phytotoxicity regardless of plant species. Glyphosate phytotoxicity generally was enhanced most by high HLB LAE to barley, green foxtail, and kochia and by high HLB TWN to redroot pigweed. Imazethapyr phytotoxicity was generally greatest when applied with high HLB SAE to barley and redroot pigweed, with high HLB LAE to green foxtail and with low HLB LAE to kochia. Neither droplet spread nor surfactant phytotoxicity correlated with glyphosate or imazethapyr efficacy.

KEYWORDS: Adjuvants, droplet spread, glyphosate, herbicide efficacy, HLB, imazethapyr.

[1]Research Scientist and Professor, respectively, Department of Plant Sciences, North Dakota State University, P.O. Box 5051, Fargo, ND 58105.

[2]Visiting Scientist, Institute of Plant and Soil Sciences, Pulawy, Poland.

Nonionic surfactants are comprised of a lipophilic and a hydrophilic moiety. For most nonionic surfactants, the hydrophilic moiety is an ethylene oxide chain. Lipophilic moieties vary from simple aliphatic to complex alkyl phenoxy moieties. The hydrophilic and lipophilic moieties determine water and lipid solubility properties of the surfactant.

Differences in phytotoxicity of herbicide-surfactant mixtures have been associated with variation in hydrophilic and lipophilic moieties on the surfactant molecule (Jansen 1964; Manthey et al. 1995a, 1995b). For maximum activity a balance between lipophilicity and hydrophilicity of the surfactant and herbicide must be achieved (Van Valkenburg 1982).

Surfactant HLB is important for droplet spread (Manthey et al. 1996a, 1996b); surfactant phytotoxicity (Helenius and Simons 1975; Manthey et al. 1996b); and enhanced herbicide efficacy (Green and Green 1993; Nalewaja et al. 1995; Manthey et al. 1995a, 1995b). The HLB required for maximum enhancement of herbicide efficacy varies with herbicide and plant species.

Surfactants having similar HLB values but different lipophilic chemistry vary in efficacy (Jansen 1964; Manthey et al. 1995b). For example, glyphosate reduced wheat fresh weight 84% when applied with Tween® 20, HLB 16.1, compared to 65% when applied with Triton® X165, HLB 15.8 (Nalewaja et al. 1995).

The HLB value can be determined analytically, but is often calculated mathematically. The HLB value for a surfactant is calculated by dividing the weight percent of ethylene oxide in the surfactant by five (Rosen 1989). Mathematical determination of HLB values ignores the inherent properties of the lipophilic moiety. Thus, surfactants with similar HLB values should be expected to differ in their enhancement of herbicide efficacy.

This research was conducted using a set of surfactants that differed in their lipophilic chemistry but had similar HLB values. Experiments were conducted to determine the effect of lipophilic chemistry on droplet spread on the leaf surface, surfactant phytotoxicity, and surfactant enhancement of herbicide phytotoxicity.

EXPERIMENTAL METHOD

General procedure

Barley, green foxtail, kochia, and redroot pigweed were seeded in 0.5 L plastic pots containing a 50:50 mix of peat and sandy loam soil. Plants were thinned to six per pot one week after emergence. Plants were watered and fertilized as needed for healthy growth. Natural daylength was supplemented for a 16 h photoperiod with metal halide lamps with a plant level intensity of 450 $uE/m^2/s$. The greenhouse was maintained at 20 $\pm$ 5 °C at night and 30 $\pm$ 5 °C during the day.

Chemical description and HLB values of the surfactants evaluated are presented in Table 1. Herbicides evaluated were glyphosate (Roundup®) and imazethapyr (Pursuit®). Both herbicides are formulated as soluble liquids, but differ in their water solubility and octanol:water partition coefficient, K_{ow} (Table 2). Treatments were applied in a 160 L/ha spray volume. Fargo municipal water (56 ppm Ca/Mg, pH 8.2) was used as the spray carrier.

Experiments were conducted in a randomized complete block design with four replicates and each experiment was repeated. Means were separated using Fisher's Protected LSD test at the 0.05 probability level.

Droplet Spread

Surfactants at 0.25% (v/v) were evaluated for spreading ability with and without herbicide. Glyphosate was applied at 5.45 mL/L (310 g ae/ha) and imazethapyr was at 1.38 mL/L (52 ae g /ha) based on 160 L/ha

spray volume. Droplet spread was determined using the second leaf of 3-leaf barley, third leaf of 4-leaf green foxtail, the youngest fully expanded leaf of 3- to 5-cm tall kochia, and the fourth leaf of 5- to 6-leaf redroot pigweed. Leaves were excised and held horizontally on the laboratory bench. A 1 uL droplet was placed in the middle of the adaxial leaf surface, avoiding major leaf veins. Droplet drying was observed 60 s after application. Therefore, droplet spread was measured 45 s after application using a metric ruler. The 1 uL droplet is larger than a typical spray droplet, but allowed for measurement of droplet spread without the use of a dye. Several water soluble dyes were evaluated but were found to affect droplet spread on the leaf surface.

Table 1 -- Chemical description and HLB values of surfactants evaluated.

Trade name[a]	Chemical description[b]	Abbreviation	HLB[b]
Alfonic® 1412-60	C_{12-14} linear alcohol ethoxylate	LAE	12.0
Alfonic® 1412-80	C_{12-14} linear alcohol ethoxylate	LAE	16.0
Igepal® CO630	Nonylphenoxypoly(ethyleneoxy)ethanol	NPE	13.0
Igepal® CO887	Nonylphenoxypoly(ethyleneoxy)ethanol	NPE	17.2
Tergitol® 15-S-7	C_{11-15} secondary alcohol ethoxylate	SAE	12.1
Tergitol® 15-S-20	C_{11-15} secondary alcohol ethoxylate	SAE	16.3
Tergitol® TMN6	Trimethylnonanol ethoxylate	TMN	11.7
Tergitol® TMN10	Trimethylnonanol ethoxylate	TMN	16.1
Triton® X114	Octylphenoxy polyethoxyethanol	OPE	12.4
Triton® X165	Octylphenoxy polyethoxyethanol	OPE	15.8
Tween® 85	POE(20)sorbitan trioleate	TWN	11.0
Tween® 20	POE(20)sorbitan monolaurate	TWN	16.7

[a]Alfonic® surfactants from Vista Chemical Co.; Igepal® from Rhone-Poulenc; Tergitol® and Triton® from Union Carbide Chemicals and Plastics Co.; and Tween® from ICI Americas.

[b]McCutcheon's Emulsifiers and Detergents, Volume 1, 1993, MC Publishing Co., Glen Rock, NJ.

Table 2 -- Formulation and water solubility of herbicides.

Herbicide[a]	Trade name	Formulation[b,c]	Water solubility[b] g ae/100 ml	K_{ow}[b]
Glyphosate	Roundup®	360 g ae/L SL, IPA salt	90.0	0.0006
Imazethapyr	Pursuit®	240 g ai/L SL, NH_4 salt	0.14	31.0

[a]Glyphosate from Monsanto; and Imazethapyr from American Cyanamid.

[b]Herbicide Handbook, 1994, 7th Edition, Weed Science Society of America, Champaign, IL.

[c]SL is soluble liquid; IPA is isopropylamine.

Surfactant phytotoxicity

Surfactants at 0.25% (v/v) were applied to 2.5-leaf barley, 3-leaf green foxtail, 3- to 5-cm tall kochia, and 4-leaf redroot pigweed. Surfactants were applied using a moving nozzle pot sprayer that delivered 160 L/ha spray volume through a flat fan 650067 nozzle tip at 276 kPa. Foliar injury was determined 24 h after application using a scale of 0 = no injury to 100 = complete kill.

Herbicide Efficacy

Glyphosate was applied at 200 g ae/ha to barley, 90 g ae/ha to green foxtail, 180 g ae/ha to kochia, and 40 g ae/ha to redroot pigweed. Imazethapyr was applied at 40 g/ha to barley, 15 g/ha to green foxtail, 20 g/ha to kochia, and 5 g/ha to redroot pigweed. Surfactants were at 0.25% (v/v) in a 160 L/ha spray volume with each herbicide. Treatments were applied to 2.5-leaf barley, 3-leaf green foxtail, 3- to 5-cm tall kochia, and to 4-leaf redroot pigweed using a moving nozzle pot sprayer that delivered 160 L/ha spray volume though a flat fan 650067 nozzle tip at 276 kPa. Shoot fresh weight was determined 14 d after treatment. Data were converted to percent shoot fresh weight reduction based on shoot fresh weight of untreated plants.

RESULTS AND DISCUSSION

Droplet spread

Droplet spread on adaxial leaf surface was similar for barley, green foxtail, and kochia (Table 3). Droplets with low HLB surfactants spread more than those with high HLB and spread was greatest with TMN, intermediate with SAE and OPE; and least with LAE, NPE, and TWN. Low HLB LAE, NPE, and TWN caused little or no increase in droplet spread compared to water without surfactant. These results clearly indicate the importance of lipophilic chemistry in determining droplet spread.

Of the high HLB surfactants, only TMN increased droplet spread beyond that of water alone. Spread of droplets containing high HLB TMN was much less than droplets that contained low HLB TMN. However, droplets containing high HLB TMN spread more than droplets containing low LAE, NPE, or TWN on barley, green foxtail, and kochia. Thus, droplet spread is less with high compared to low HLB within a surfactant chemistry, but droplet spread can be greater with high than low HLB surfactants of different chemistry.

While all surfactants enhanced droplet spread on redroot pigweed, the HLB and lipophilic chemistry had less affect on spreading on redroot pigweed than on barley, green foxtail, or kochia (Table 3). The range between least and most droplet spread was 1.0 to 21.6 mm for barley and green foxtail, 1.0 to 24.5 mm for kochia, and 2.1 to 2.8 mm for redroot pigweed. The amorphous wax structure may account for the lesser spread on redroot pigweed than on the fine microcrystalline structure of barley, green foxtail, and kochia (Manthey et al. 1996b). Surfactants typically enhance droplet spread less on leaves with amorphous leaf wax than with microcrystalline leaf wax (Knoche and Bukovac 1993; Manthey et al. 1996b).

The inclusion of glyphosate or imazethapyr had little effect on spread of droplets containing low or high HLB surfactants on barley, green foxtail, or kochia (Table 3). However, spread on redroot pigweed was less with droplets containing both glyphosate and high HLB surfactants, except for TMN, compared to droplets containing only glyphosate. All surfactants enhanced spread of droplets containing imazethapyr on redroot pigweed, except for high HLB NPE.

Droplet spread was influenced by surfactant HLB and chemistry of lipophilic moiety, and plant species (Table 3). Plant species differ in chemical composition of leaf wax and in surface wax structure. Lipophilic chemistry and HLB affect the affinity of surfactant to the leaf wax which would affect droplet spread. Droplet spread is greater with low than high HLB surfactants as the more lipophilic surfactant would have greater affinity for lipophilic leaf waxes.

TABLE 3 -- Droplet spread with and without herbicide on the adaxial leaf surface of barley, green foxtail, kochia, and redroot pigweed as influenced by surfactant lipophilic chemistry and HLB.

Herbicide	Surfactant[a]	Barley		Green foxtail		Kochia		Redroot pigweed	
		Surfactant HLB							
		Low	High	Low	High	Low	High	Low	High
		mm							
None									
	LAE	1.8	1.3	2.0	1.1	2.2	1.0	2.4	2.1
	NPE	2.3	1.5	2.1	1.0	2.3	1.0	2.4	2.1
	SAE	6.3	1.4	4.0	1.4	12.6	1.5	2.8	2.3
	TMN	21.6	5.6	21.6	4.1	24.5	8.6	2.8	2.4
	OPE	3.4	1.4	3.1	1.3	9.0	1.7	2.4	2.1
	TWN	1.4	1.0	1.6	1.5	1.4	1.3	2.1	2.1
	None	1.0		1.0		1.0		1.0	
LSD (0.05)		-- 1.3 --		-- 1.1 --		-- 1.4 --		-- 0.3 --	
Glyphosate									
	LAE	2.3	2.0	2.3	1.8	1.8	1.1	3.0	1.8
	NPE	2.6	1.3	2.9	1.4	2.0	1.0	3.3	1.4
	SAE	3.8	2.0	3.8	2.0	10.9	1.1	3.4	2.0
	TMN	16.6	2.5	17.1	2.8	21.9	3.0	3.8	2.8
	OPE	3.9	1.9	3.0	1.8	3.4	1.2	3.0	1.8
	TWN	2.0	1.5	1.9	1.8	1.1	1.0	2.9	1.8
	None	1.6		1.6		1.1		2.6	
LSD (0.05)		-- 2.3 --		-- 1.4 --		-- 1.4 --		-- 0.5 --	
Imazethapyr									
	LAE	2.8	1.9	2.1	1.0	1.9	1.4	2.0	2.1
	NPE	2.1	1.0	2.0	1.0	2.4	1.8	2.0	1.4
	SAE	7.3	2.0	4.6	1.0	15.9	1.8	2.1	1.9
	TMN	27.5	4.5	21.1	3.3	22.0	7.1	2.4	2.0
	OPE	4.6	2.0	3.5	1.5	10.6	2.0	2.4	2.0
	TWN	2.0	2.0	1.3	1.1	1.5	1.9	2.1	1.9
	None	1.0		1.0		1.0		1.1	
LSD (0.05)		-- 2.7 --		-- 1.3 --		-- 1.7 --		-- 0.4 --	

[a]LAE is C_{12-14} linear alcohol ethoxylate; NPE is nonylphenol ethoxylate; SAE is C_{11-15} secondary alcohol ethoxylate; TMN is trimethylnonanol ethoxylate; OPE is octylphenol ethoxylate; and TWN is oxysorbic.

Surfactant phytotoxicity

Lipophilic chemistry and HLB affected surfactant phytotoxicity to green foxtail, kochia, and redroot pigweed but not to barley (Table 4). Barley injury was similar from all surfactants and ranged from 3 to 8% regardless of surfactant HLB or chemistry. Kochia and redroot pigweed were injured greater than 8% by low HLB LAE and NPE. Little or no injury to kochia or redroot pigweed occurred with high HLB surfactants, regardless of lipophilic chemistry, or with low HLB TMN, OPE, and TWN. Green foxtail was the only plant species that was injured more than 8% by high HLB surfactant. Low HLB SAE, NPE, and LAE and high HLB LAE, SAE, and OPE caused greater than 8% injury to green foxtail. SAE and OPE caused greater injury to green foxtail at high than at low HLB.

Droplet spread did not correlate with surfactant phytotoxicity to barley, green foxtail, kochia, or redroot pigweed. Phytotoxicity is strongly correlated with surfactant uptake (Silcox and Holloway 1989). Rapid uptake led to appearance of discrete necrotic areas that corresponded to site of droplet application.

TABLE 4 -- Foliar injury to barley, green foxtail, kochia, and redroot pigweed as influenced by surfactant lipophilic chemistry and HLB.

	Barley		Green foxtail		Kochia		Redroot pigweed	
	Surfactant HLB							
Surfactant[a]	Low	High	Low	High	Low	High	Low	High
	% injury							
LAE	7	4	11	13	16	5	11	1
NPE	3	5	22	4	17	0	14	0
SAE	5	8	12	23	4	2	8	1
TMN	5	6	1	2	4	6	1	3
OPE	4	7	5	13	3	8	6	6
TWN	5	6	1	6	0	1	0	1
LSD (0.05)	- NS -		- 2.0 -		- 1.0 -		- 2.0 -	

[a]LAE is C_{12-14} linear alcohol ethoxylate; NPE is nonylphenol ethoxylate; SAE is C_{11-15} secondary alcohol ethoxylate; TMN is trimethylnonanol ethoxylate; OPE is octylphenol ethoxylate; and TWN is oxysorbic.

Droplet spread is often associated with lipophilic surfactants. Lipophilic surfactants that result in large droplet spread could be "trapped" or move more slowly through the cuticle (Silcox and Holloway 1989) because the concentration of surfactant/area is less than those that do not spread. Surfactants with high HLB values often contain long ethylene oxide chains that result in large molecules. A large molecular size and a hydrophilic nature make the penetration of a surfactant through the waxy leaf cuticle difficult. Thus, to reduce surfactant phytotoxicity, a balance is needed between surfactant HLB and lipophilic chemistry.

Herbicide efficacy

All high HLB surfactants, except for TMN, enhanced glyphosate efficacy regardless of plant species (Table 5). Except for TMN, glyphosate efficacy was greater when applied with high than low HLB surfactants. TMN was the least effective high HLB surfactant with glyphosate. Low HLB surfactants did not enhance glyphosate efficacy on green foxtail and low HLB OPE and TWN reduced glyphosate efficacy on green foxtail. All low HLB surfactants, except LAE, reduced glyphosate efficacy to kochia. The greater effectiveness of high than low HLB surfactants in enhancing glyphosate efficacy has been well documented (Manthey et al. 1996a; Nalewaja et al. 1995). The poor enhancement of glyphosate activity by low HLB surfactants relates to absorption (Gaskin and Holloway 1992; Stock et al. 1993). Glyphosate absorption by foliage was 19% when applied with LAE C_{12-14} HLB value of 12 compared to 46% when applied with LAE C_{12-14} HLB value of 16 (Nalewaja and Matysiak 1995).

HLB had less effect on imazethapyr than glyphosate efficacy (Table 5). All low HLB surfactants enhanced effectiveness of imazethapyr but not glyphosate. Enhancement of imazethapyr was similar by both low and high HLB SAE and TMN for barley, LAE, NPE, TMN for kochia, and SAE, TMN, and TWN for redroot pigweed. However, imazethapyr phytotoxicity to green foxtail was greater when applied with high than low HLB

surfactants. Previous research indicated that the optimum HLB for surfactant enhancement of imazethapyr on green foxtail was 14.9 (Manthey et al. 1995b).

The uptake of highly water soluble compounds (log K_{ow} = -3) is enhanced by high HLB surfactants, while uptake of water insoluble compounds (log K_{ow} = 3) is enhanced by low HLB surfactants. HLB has less affect with compounds of intermediate polarity (log K_{ow} = -1 to 1) (Stock et al. 1993). Glyphosate is very water soluble (log K_{ow} = -3.2) (Table 1) and is enhanced most by high HLB surfactants. HLB was less important with imazethapyr which has an intermediate polarity (log K_{ow} = -1.5).

TABLE 5 -- Fresh weight reduction of barley, green foxtail, kochia, and redroot pigweed by glyphosate and imazethapyr as influenced by surfactant lipophilic chemistry and HLB.

Herbicide	Surfactant[a]	Barley		Green foxtail		Kochia		Redroot pigweed	
		Surfactant HLB							
		Low	High	Low	High	Low	High	Low	High
		% fresh weight reduction							
Glyphosate									
	LAE	92	98	35	78	75	84	37	69
	NPE	...	...	34	68	45	74	27	72
	SAE	48	98	32	65	19	74	26	51
	TMN	49	51	35	21	8	26	25	22
	OPE	41	80	18	44	29	74	31	67
	TWN	38	86	18	68	51	82	48	77
	None	14		33		59		16	
LSD (0.05)		-- 4 --		-- 7 --		-- 6 --		-- 5 --	
Imazethapyr									
	LAE	71	79	80	85	80	78	69	77
	NPE	...	...	80	83	73	78	...	...
	SAE	81	84	77	81	76	76	70	78
	TMN	82	81	78	82	74	73	71	72
	OPE	66	76	75	81	71	77	66	71
	TWN	38	80	67	80	60	77	69	71
	None	4		32		13		27	
LSD (0.05)		-- 4 --		-- 2 --		-- 6 --		-- 4 --	

[a]LAE is C_{12-14} linear alcohol ethoxylate; NPE is nonylphenol ethoxylate; SAE is C_{11-15} secondary alcohol ethoxylate; TMN is trimethylnonanol ethoxylate; OPE is octylphenol ethoxylate; and TWN is oxysorbic.

Lipophilic moieties differed in their effect on surfactant enhancement of glyphosate and imazethapyr efficacy (Table 5). For example, TMN was not effective with glyphosate, even with high HLB, but was effective with imazethapyr. Although rankings varied with plant species, glyphosate phytotoxicity generally was enhanced most by LAE with species having microcrystalline wax (barley, green foxtail, and kochia); while TWN was the most effective lipophilic moiety with species having amorphous wax (redroot pigweed). TMN was the least effective with glyphosate on species having microcrystalline or amorphous wax.

The effect of surfactant lipophilic chemistry on imazethapyr phytotoxicity varied with plant species but did not relate to surface wax structure. Imazethapyr phytotoxicity was greatest when applied with

high HLB SAE to barley (microcrystalline wax) and redroot pigweed (amorphous wax); with high HLB LAE to green foxtail (microcrystalline wax); and with low HLB LAE to kochia (microcrystalline wax). Low HLB TWN was least effective with imazethapyr on barley, green foxtail, and kochia (microcrystalline wax), and low HLB OPE was least effective with imazethapyr on redroot pigweed (amorphous wax). A greater number of plant species with microcrystalline and amorphous wax structure need to be tested before a definitive conclusion can be made concerning the effect of surface wax structure and surfactant lipophilic chemistry on the efficacy of glyphosate and imazethapyr. However, these data indicate the importance of lipophilic chemistry on surfactant enhancement of glyphosate and imazethapyr phytotoxicity.

HLB and lipophilic chemistry contributed to surfactant effects on droplet spread, surfactant phytotoxicity, and herbicide efficacy (Tables 3, 4, and 5). Glyphosate efficacy was greatest with little or no droplet spread. However, efficacy varied greatly with surfactants that caused little or no droplet spread. Spread of droplets containing imazethapyr did not correlate with imazethapyr efficacy. Surfactants enhanced glyphosate and imazethapyr efficacy at concentrations greater than needed for droplet spread. Thus, factors other than droplet spread had a greater effect on the efficacy of these herbicides.

Surfactants applied with herbicides affect deposit distribution, thickness, and contact with the leaf surface (Bukovac et al. 1995; Nalewaja et al. 1992). Spray deposits generally are either an annulus ring or a uniform deposit. The affinity of the herbicide to the surfactant can affect spray deposit and more importantly the deposit of the herbicide relative to the surfactant. Bukovac et al. (1995) reported that if polarity of a compound and surfactant was markedly different then they may deposit on the leaf in separate domains. If polarity was similar, the compound may be solubilized by surfactant micelles and the two components may occupy a common domain in the deposit. Clearly, the surfactant would have little or no effect on herbicide absorption if the surfactant and herbicide deposit separately on the leaf. The polarity and chemistry of active ingredient and surfactant determines their affinity for each other. Thus, the lack of glyphosate enhancement with low HLB surfactants or high HLB TMN may be due to a lack of affinity between glyphosate and surfactant and, subsequently, an unfavorable deposition pattern on the leaf surface.

Surfactant phytotoxicity did not correlate with glyphosate or imazethapyr reduction of fresh weight of green foxtail, kochia, or redroot pigweed. Since surfactants did not differ in their phytotoxicity to barley (Table 4), correlation between surfactant phytotoxicity and herbicide efficacy on barley was not determined. Phytotoxicity and herbicide efficacy experiments were not conducted simultaneously. The amount of apparent herbicide efficacy caused by surfactant phytotoxicity is not known.

Slight injury from enhanced membrane permeability may have no effect or be beneficial to herbicide efficacy. Surfactants may facilitate herbicide movement into the cell by increasing the permeability of the membrane (St. John et al. 1974; Watson et al. 1980). Gaskin and Holloway (1992) reported that localized injury from high concentrations of surfactant did not reduce glyphosate uptake or translocation. However, injury that results in cellular death would prevent foliar absorption into and translocation out of the injured tissue which would reduce efficacy of systemic herbicides.

CONCLUSION

The effect of surfactant HLB and the chemistry of the lipophilic moiety on droplet spread, surfactant phytotoxicity, and herbicide efficacy varied with plant species. Droplet spread and surfactant phytotoxicity did not relate to surfactant enhancement of glyphosate or imazethapyr phytotoxicity. Neither droplet spread nor surfactant

phytotoxicity explained or predicted surfactant effectiveness with glyphosate or imazethapyr. Surfactant HLB and lipophilic chemistry were both important to enhancement of herbicide efficacy. These experiments clearly indicate that specific characteristics of the lipophilic moiety are important to efficacy of surfactants used as adjuvants with herbicides. The specific physical and chemical properties of lipophilic moieties that are important in determining surfactant enhancement of herbicide efficacy need to be identified. This information could be used to create models to predict surfactant efficacy for various herbicides.

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John D. Nalewaja[1] and Robert Matysiak[1]

LINEAR ALCOHOL ETHOXYLATES AFFECT GLYPHOSATE AND FLUAZIFOP-P DEPOSITS

REFERENCE: Nalewaja, J. D., and Matysiak, R., **"Linear Alcohol Ethoxylates Affect Glyphosate and Fluazifop-P Deposits,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: Scanning electron micrographs were taken of spray droplet residues from glyphosate (Honcho®) and fluazifop-P (Fusilade 2000®) applied with linear alcohol ethoxylates (LAE) having linear alcohol chains with C_{8-10}, C_{12-14}, and C_{16-18} and ethylene oxides (EO) at 40-46, 60-62, and 80% of surfactant molecular weight. Glyphosate spray applied with high HLB, 80% EO, LAE gave a uniform thick deposit. LAE which formed these distinct deposits with close contact to the leaf related to previously reported high glyphosate phytotoxicity to wheat. Fluazifop-P spray residual characteristics did not relate closely to efficacy. However, LAE that gave more uniform deposits were associated with the greatest fluazifop-P phytotoxicity. Fluazifop-P formulants may have reduced the LAE effect on spray droplet deposit. Glyphosate was a formulation without surfactant. Generally, fluazifop-P residuals were almost not discernable when applied with LAE C_{8-10} and C_{12-14} with 40-46% EO, the least effective LAE for enhancement of fluazifop-P phytotoxicity to wheat. Surfactant affect on spray droplet deposit in addition to previously reported solubility characteristics appear important to enhancement of glyphosate and fluazifop-P phytotoxicity.

KEY WORDS: adjuvants, scanning electron micrographs, spray deposit.

Surfactants are often added to postemergence herbicide spray mixtures to enhance efficacy. Herbicides differ greatly in physical and chemical properties and in formulants used to facilitate application (Ahrens 1994). Glyphosate

[1]Professor and Visiting Scientist, respectively, Plant Sciences, North Dakota State University, P.O. Box 5051, Fargo, ND 58105.

(N-(phosphonomethyl)glycine) is water soluble and formulations are available with and without surfactant. Fluazifop [(±)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid] has low water solubility and is formulated as an emulsifiable concentrate. Surfactant adjuvants that lower spray solution dynamic surface tension enhance spray droplet retention by leaves (Matysiak 1995). Surfactant concentrations beyond that required to minimize surface tension have often further enhanced herbicide phytotoxicity indicating that the surfactant in addition to increasing spray retention is important to herbicide absorption (de Ruiter et al. 1992).

Spray droplet spreading has often been used to demonstrate adjuvant efficacy. Droplet spreading probably relates to contact with the leaf and should be positive to spray retention. However, droplet spread does not relate to adjuvant efficacy with herbicides (Stock and Holloway 1993). Hydrophilic surfactants enhanced and hydrophobic surfactants reduced glyphosate absorption (Gaskin and Holloway 1992) and phytotoxicity (Nalewaja et al. 1995). The uptake of lipophilic compounds is generally favored by lipophilic surfactants (Stock et al. 1993). Lipophilic fluazifop-P absorption was greater for linear alcohol surfactants with C_{8-10} than C_{16-18} alcohols and 40 to 46% than 80% ethylene oxide (Nalewaja and Matysiak 1995). Fluazifop-P absorption data did not always relate to efficacy, especially when applied at higher spray volume.

Herbicide uptake data indicates that surfactants may function as solvents for herbicides or provide a microenvironment for penetration of the leaf surface. Surfactants that cause cuticle hydration enhance absorption of hydrophilic glyphosate but surfactants that increase cuticular wax fluidity enhance lipophilic chlorotuluron absorption (Coret and Chamel 1995).

Information on the influence of spray droplet deposit on herbicide efficacy is not conclusive. Glyphosate and chlorotuluron uptake did not relate to droplet deposit with various surfactants. However, the poor relationship between fluazifop-P uptake and efficacy with surfactants in high spray volume (Nalewaja et al. in press) and greater uptake of glyphosate in concentrated droplets confined to a small area (Cramner and Linscott 1991) indicate an importance of spray deposit. Tween® 20 gave a glyphosate deposit that was amorphous compared to a crystalline deposit for glyphosate alone (MacIsaac et al. 1991). Deposits having a grainy texture and poor contact with the leaf surface related to the antagonism of glyphosate phytotoxicity by calcium chloride (Nalewaja et al. 1992).

Linear alcohol ethoxylate surfactants differ in enhancement of glyphosate and fluazifop-P phytotoxicity (Nalewaja et al. in press). Experiments were conducted to determine spray droplet residual from glyphosate and fluazifop-P as influenced by linear alcohol ethoxylate surfactants with various alcohol carbon chain lengths and ethylene oxide percentages.

EXPERIMENTAL METHOD

Surfactants had linear alcohol moieties of C_{8-10}, C_{12-14}, and C_{16-18} with EO % of 40-46, 60-62, and 80. Spring wheat (Triticum aestivum, cv. 'Marshall') was grown in a greenhouse potting mixture contained in 0.5 L plastic pots. One week

after emergence, plants were thinned to three per pot for the scanning electron droplet residue determinations. Plants were grown under natural sunlight supplemented with metal halide lamps giving 450 uE/m^2/s photosynthetic photon flux density at plant level, for a 16 h light period. Plants were watered and fertilized for healthy growth.

Treatment was similar to that used for the earlier reported efficacy experiments (Nalewaja et al. in press) and was to 2.5-leaf wheat plants using a moving nozzle sprayer delivering 160 L/ha with a 650067 flat fan nozzle operated at 270 kPa. The spray carrier was distilled water. Glyphosate was applied at 200 g acid equivalent (a.e.)/ha of a formulation without surfactant (Honcho®[2]) and fluazifop-P at 40 g active ingredient (a.i.)/ha of the commercial liquid formulation (Fusilade 2000®[3]).

SEM photographs were taken for glyphosate applied with 1% surfactant as is used commercially with formulations without surfactant and fluazifop-P with 0.25% and compared to efficacy data previously reported (Nalewaja and Matysiak 1995). SEMs were from sprayed plants that were transferred to the North Dakota State University Electron Microscopy Center. Four to eight treatments were processed at a time, and the interval between treatment and scanning electron microscopy (SEM) examination was between 1 and 3 h.

Portions of leaves were removed from sprayed plants and mounted on aluminum stubs using double sticky carbon tape. These fresh, fully hydrated specimens were then examined and photographed using a JEOL JSM 6300 scanning electron microscope operated at accelerating voltages of 1-2KV. This technique allowed examination of spray droplet residual on the cuticle and epidermal surfaces that were unaltered by chemical fixation or dehydration. Photographs were prepared for four typical droplet residuals per treatment. Pictures presented were selected for clarity and to be representative of the group.

RESULTS AND DISCUSSION

Glyphosate spray droplet residual discernability increased as EO% and linear alcohol carbon chain length increased (Fig.1). Spray droplet residual containing C_{8-10} alcohols with 40 to 46% EO spread extensively (Fig. 1a) covering a large area that was barely visible. Glyphosate residuals had closer contact with the wheat leaf surface when with C_{8-10} (Fig. 1A-C) than C_{12-14} or C_{16-18} (Fig.1D-I), regardless of EO%. Thickness of the residue appeared to increase with EO% and alcohol carbon chain length. Residual thickness generally increased as droplet spread decreased, as would be expected for similar size droplets (Fig.1). Close contact between the deposit and the leaf surface would benefit transfer of the glyphosate to the leaf. A thick deposit would increase the amount of herbicide at a site for absorption. Short chain alcohols may have greater solubility in cuticular wax accounting for droplet spread and close contact of the deposits with the leaf

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[2]Monsanto Company, St. Louis, MO.

[3]Zeneca, Wilmington, DE.

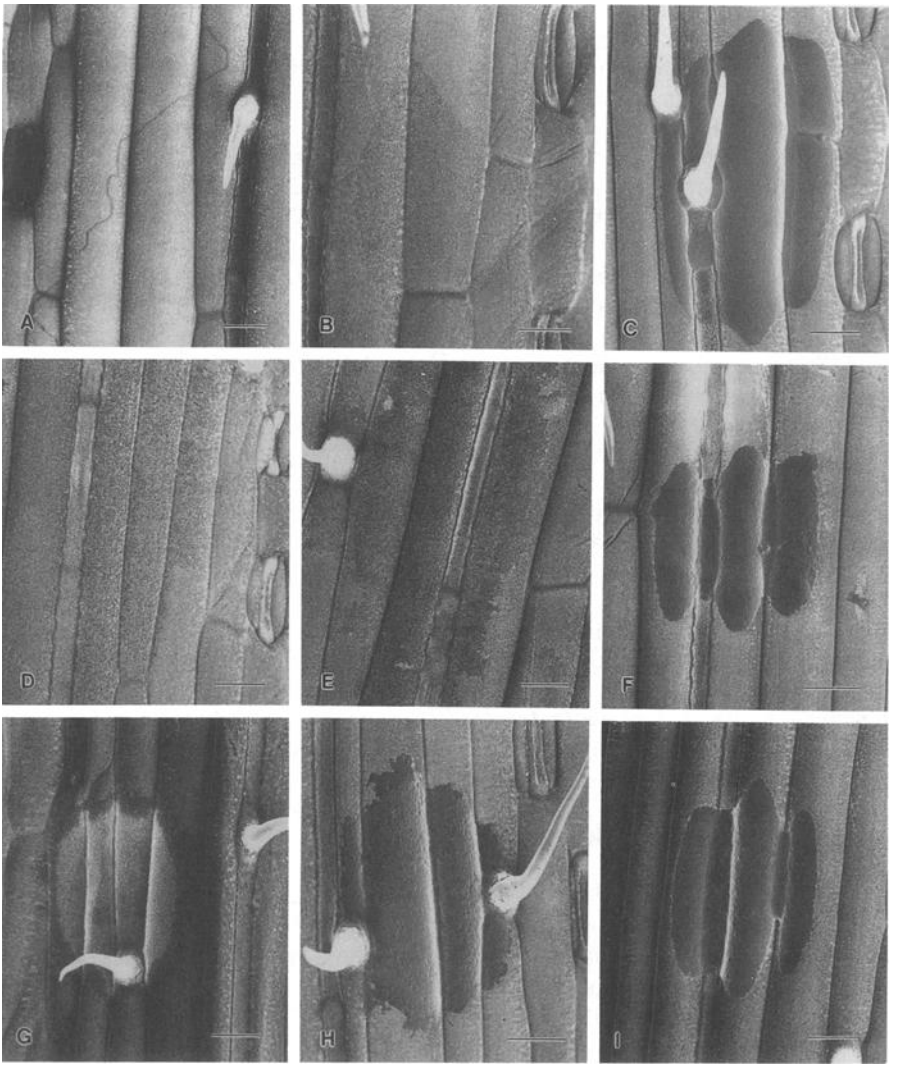

FIG. 1 -- Spray droplet residual from glyphosate at 200 g/ha on wheat leaf when applied with linear alcohol ethoxylates (LAE) having various ethoxylation (EO) A. LAE C_{8-10}, EO 40, B. EO 60, C. EO 80; D. LAE C_{12-14}, EO 40, E. EO 60, F. EO 80; and G. LAE C_{16-18}, EO 46, H. EO 62, I. EO 80. All bars = 30 μm and magnification was 300 to 400X.

surface. Close deposit contact with the leaf surface and high EO content in LAE surfactants would appear a prerequisite for cuticular hydration important to the absorption of water soluble herbicides (Stock and Holloway 1993).

Glyphosate residual deposits that were thick and had close contact with the leaf surface related to glyphosate phytotoxicity to wheat (Fig. 1, Table 1). Glyphosate phytotoxicity was the greatest when applied with LAE surfactants with alcohol having C_{8-10} and C_{12-14} with 80% EO and these surfactants applied with glyphosate left a thick deposit that had close contact with the wheat leaf surface (Fig. 1D,F,I). Glyphosate phytotoxicity was enhanced similarly by C_{16-18} alcohols regardless of EO %, but less than by C_{8-10} or C_{12-14} with 80% EO. These LAE surfactants all left thick deposits, but the more effective C_{8-10} and C_{12-14} alcohols with 80% EO appeared to give deposits with closer leaf contact (Fig. 1).

Glyphosate phytotoxicity to or absorption by various plants is greatest when applied with surfactants having high HLB values or EO% (Nalewaja and Matysiak 1995). The effectiveness of high HLB surfactants has been attributed to their hydrophilicity that served as a glyphosate solvent and hydrates the cuticle for absorption of water soluble glyphosate. These high HLB surfactants reduced droplet spread, increased drying time (Nalewaja and Matysiak 1995), and increased spray deposits thickness (Fig. 1). Thick deposits would provide a concentrated glyphosate deposit that is positive for efficacy (Cramner and Linscott 1990) and for absorption (Nalewaja and Matysiak, 1995).

SEM photographs of spray droplet deposits support the concept that an effective surfactant for glyphosate gives a thick uniform spray droplet residual

Table 1. Wheat fresh weight reduction from glyphosate at 200 g a.i./ha with LAE surfactants at 1% and fluazifop-P at 40 g a.i./ha with 0.25% LAE surfactants applied at 80 L/ha. Data published previously and included for reference with permission (Nalewaja and Matysiak 1995).

Surfactant[a]				
Carbon No.	EO %	HLB value	Glyphosate	Fluazifop-P
			% FWR[a]	
8-10	40	8	13	69
8-10	60	12	16	74
8-10	80	16	65	74
12-14	40	8	17	63
12-14	60	12	23	71
12-14	80	16	70	70
16-18	46	9	60	61
16-18	62	12.5	50	53
16-18	80	16	62	56
No surfactant			7	44
LSD 5%			7	6

[a]EO is ethylene oxide content; HLB is hydrophilic:lipophilic balance; FWR is fresh weight reduction.

having close contact with the leaf. These surfactants would need to also give spray retention and have the solubility characteristic for glyhosate absorption. Surfactants that do not cause droplet spread would provide a thick glyphosate spray deposit shown to enhance glyphosate phytotoxicity. Selection of surfactants for glyphosate that do not spread is contrary to the common belief that droplet spread is important to herbicide efficacy.

LAE surfactants had less influence on spray deposits from fluazifop-P than from glyphosate (Fig.2). The only clearly discernable deposits were with the C_{16-18} alcohols (Fig. 2G-I). The presence of the fluazifop-P and its formulants apparently greatly influenced the spray deposit. The least discernable residual for fluazifop-P occurred when applied with LAE with C_{12-14} and 40% EO (Fig. 2D), but for glyphosate when with C_{8-10} and 40% EO (Fig 1A). The most detectable deposits were with the long chain alcohols, C_{16-18}, for both herbicides. However, the crystalline cuticular wax surface was visible through the deposits for fluazifop-P with all LAE surfactants, except C_{16-18} and 80% EO. Direct comparisons of spray droplet residual for glyphosate and fluazifop-P is not possible because glyphosate was applied with 1% and fluazifop-P with 0.25% LAE surfactant. The percentages selected were to represent amounts commonly used commercially. Fluazifop-P formulation emulsifier and solvent would add to the residual, but glyphosate was formulated in water without surfactants.

Fluazifop-P is lipid soluble and formulated as an emulsifiable concentrate (Ahrens 1994). The lipid soluble fluazifop-P and its formulants apparently caused the spray deposits when applied with LAE surfactants to dissolve into the cuticular wax, except C_{16-18} containing 80% EO. LAE with C_{16-18} and 80% EO probably would be the most viscous of the LAE surfactants accounting for the large appearing residual.

Fluazifop-P phytotoxicity to wheat generally decreased as LAE alcohol carbon chain length increased. Appearance of the spray droplet residual does not easily indicate efficacy for fluazifop-P. However, the most effective LAE surfactants had C_{8-10} or C_{12-14} alcohols and 60 or 80% E0, all which left detectable residuals that appeared to blend into the epicuticular wax.

The C_{16-18} alcohol (Fig.2G-I) LAE surfactants left the most residual on the surface. The surface residual indicates that the apparently higher melting point C_{16-18} alcohol LAE possibly reduced fluazifop-P diffusion into the leaf, accounting for the reduced absorption (Nalewaja and Matysiak 1995) and phytotoxicity (Table 1). Spray retention was not a factor in fluazifop-P efficacy, which was similar with all LAE surfactants, except C_{12-14} with 40% EO which gave slightly greater spray retention than the other LAE (Nalewaja et al. in press).

LAE surfactants all greatly changed the appearance of the glyphosate spray deposit (Fig. 1A-I) compared to that of glyphosate applied alone (Fig 3A). The light area may represent cuticular disruption from the initial droplet and the dark area the glyphosate deposit. Glyphosate is highly water soluble and would probably remain in solution as the droplet dried and would only precipitate when the droplet became small and concentrated. The dark area has a broken edge over the anticlinal cell wall area indicating poor contact with the epicuticular surface which would account for the poor efficacy of glyphosate applied without LAE surfactant

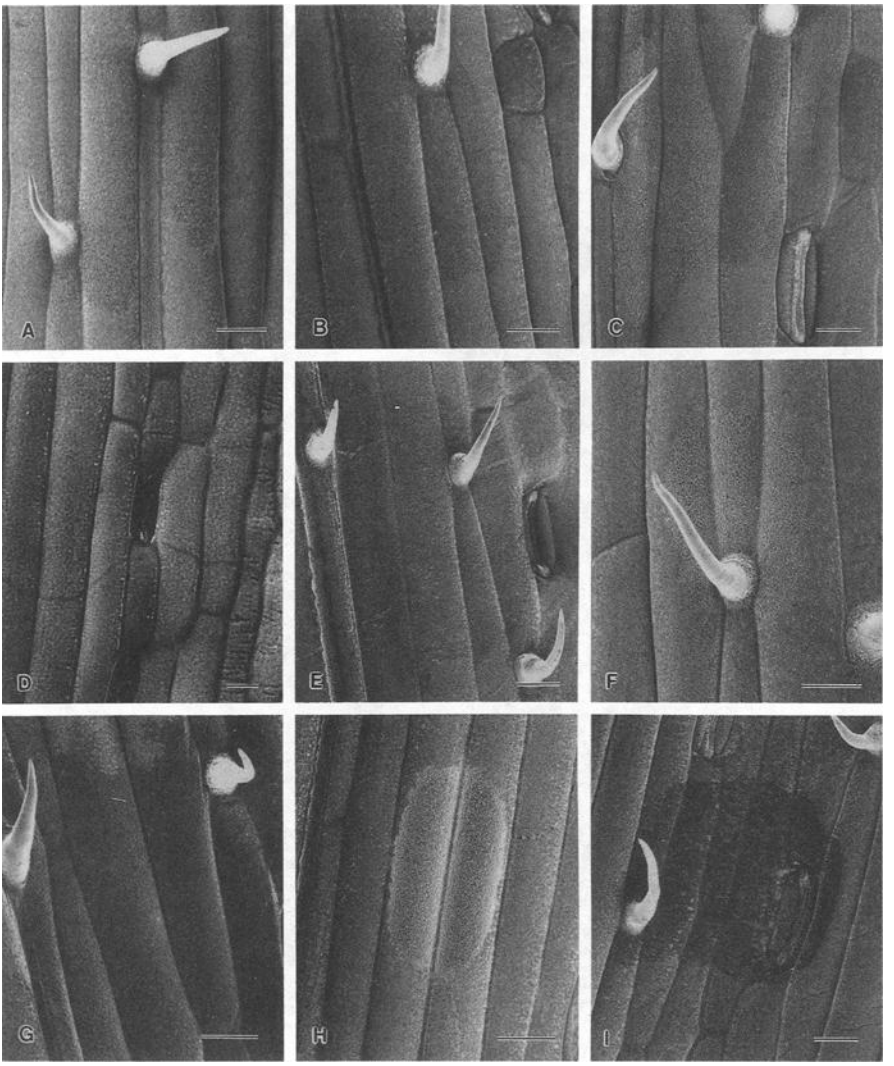

FIG. 2 -- Spray droplet residual from fluazifop-P at 40 g/ha on wheat leaf when applied with linear alcohol ethoxylates (LAE) having various ethoxylation (EO) A. LAE C_{8-10}, EO 40, B. EO 60, C. EO 80; D. LAE C_{12-14}, EO 40, E. EO 60, F. EO 80; and G. LAE C_{16-18}, EO 46, H. EO 62, I. EO 80. All bars = 30 μm and magnification was 230 to 450X.

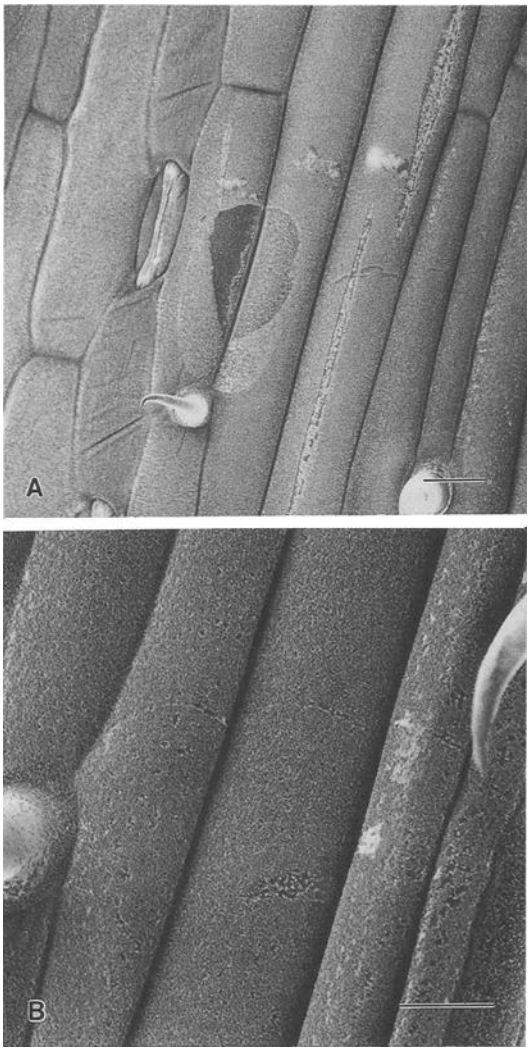

FIG. 3 -- Spray droplet deposit on wheat leaves from A. glyphosate at 200 g/ha and B. from fluazifop-P at 40 g/a, applied without LAE surfactant.

(Table 1). LAE surfactants all enhanced fluazifip-P efficacy (Table 1). The less discernable fluazifop-P deposit when without LAE and the lack of efficacy indicates possible adsorption in the cuticle. LAE (Fig. 1B) surfactants applied with fluazifop-P may serve as a co-solvent for absorption.

CONCLUSION

Herbicide spray droplet deposit characteristics help explain the surfactant enhancement of herbicide phytotoxicity. Surfactants that appear to dissolve into cuticular wax indicate effectiveness with lipophilic fluazifop-P, while those that left a large deposit on the surface but had excellent contact with the wax were effective with water soluble glyphosate.

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Zenon Woznica,[1] John D. Nalewaja,[2] and Edward F. Szelezniak[3]

MON 37532 PHYTOTOXICITY IS AFFECTED BY SURFACTANT AND AMMONIUM NITRATE

REFERENCE: Woznica, Z., Nalewaja, J. D., and Szelezniak, E. F., ''**MON 37532 Phytotoxicity is Affected by Surfactant and Ammonium Nitrate,**'' Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: Herbicides often are applied with surfactants and with the addition of 2 to 4% (v/v) 28% nitrogen liquid fertilizer (ammonium nitrate + urea). Greenhouse experiments were conducted to determine the influence of surfactant hydrophilic:lipophilic balance (HLB) on efficacy of MON 37532, a new herbicide by Monsanto, applied with surfactant alone and with ammonium nitrate. Linear alcohol ethoxylate (LAE) surfactants with C_{8-10} or C_{12-14} alcohol were or tended to be more effective with 12 than 8 or 16 HLB. HLB did not influence C_{16-18} LAE surfactant enhancement of MON 37532 phytotoxicity to oats. However, all LAE were similar in enhancing phytotoxicity to green foxtail, except C_{12-14} with 8 HLB was less effective. The presence of ammonium nitrate increased or decreased LAE efficacy depending on the specific LAE and varied with species. Ethoxylated alkylphenols, Triton® X, and secondary alcohol ethoxylate, Tergitol® 15-S surfactants generally increased in efficacy as HLB increased. Ammonium nitrate did not enhance efficacy of Tergitol® 15-S or Igepal CO, but with Triton® X often enhanced MON 37532 phytotoxicity to green foxtail. MON 37532 phytotoxicity enhancement from ammonium nitrate was inpart from increase spray retention, and did not appear to relate to droplet deposit.

KEYWORDS: surfactant, HLB, ammonium nitrate, green foxtail, Japanese brome, cheat, oat.

Adjuvants are commonly added to postemergence applied herbicide spray solutions to enhance phytotoxicity. The HLB value and chemical composition of the surfactant component in an adjuvant, as well as salts in the spray solution and the plant species to which the spray is applied all interact in adjuvant efficacy with a herbicide (Manthey et al. 1995a, Manthey et al. 1995b).

MON 37532 is a new sulfonylurea herbicide for postemergence control of several grass weed species in small grains (Monsanto Company, St. Louis, MO). Surfactants with HLB values of 12 to 17 and those leaving a gel-like deposit were most effective with the sulfonylurea

[1]Associate Professor, Agronomy Dep., Agricultural University, 60-627 Poznan, Poland

[2]Professor, Plant Sciences, North Dakota State University, Fargo, ND 58105-5051

[3]Research Scientist, Institute of Plant and Soil Science, Pulawy, Poland

herbicide rimsulfuron for giant foxtail (Setaria faberii) and velvetleaf (Abutilon theophrasti) control (Green and Green 1993). The HLB for optimum octoxynol enhancement was about 15 for rimsulfuron and 16 for nicosulfuron and primisulfuron of green foxtail control (Manthey et al 1995a, 1995b). The optimum HLB for these three sulfonylurea herbicides does not relate to their water solubility at pH 7. Primisulfuron and nicosulfuron responded to the same HLB value, but primisulfuron is less water soluble (243 mg/L) than nicosulfuron (12,200 mg/L, pH 6.85) (Green and Green 1993).

The species to which the herbicide is applied also is important to the optimum surfactant HLB value. The optimum HLB for octoxynol and nonoxynol with primisulfuron was <13 for sunflower (Helianthus annuus) and >17 for kochia (Kochia scoparia) (Manthey et al. 1995a). Difference in species response to surfactants probably is from cuticular difference affect spray droplet retention and/or surfactant penetration.

MON 37532 controls grass weeds in wheat. The grasses controlled by MON 37532 differ anatomically, green foxtail (Setaria viridis) and wild oat (Avena fatua) are non-pubescent while downy brome (Bromus tectorum), Japanese brome (Bromus japonicus), and cheat (Bromus secalinus) are very pubescent on both the leaves and stems. The first function of a surfactant important to herbicide efficacy is to promote spray droplet retention on the plant and second, to aid in herbicide penetration of the epicuticular leaf surface. Spray retention by grasses is difficult because of vertically oriented leaves and most grasses have a water repelling crystalline surface. The optimum surfactant HLB value for MON 37532 could differ for the species being controlled.

Surfactants are often applied with an ammonium salt. Ammonium sulfate and ammonium nitrate have enhanced glyphosate (Nalewaja and Matysiak 1991), and sethoxydim (Nalewaja et al. 1989) phytotoxicity by overcoming antagonistic cations in the spray carrier or by directly enhancing glyphosate phytotoxicity (Nalewaja and Matysiak 1992). Surfactant effectiveness with nicosulfuron differed depending on the specific ammonium salt in the spray (Nalewaja et al. 1995). A 28% nitrogen solution of approximately 50:50% ammonium nitrate:urea is commonly used commercially as an adjuvant. The most important component appears to be the ammonium nitrate. Ammonium ions have increased the uptake of imazethapyr by cells (Gronwald et al. 1993) may account for the enhanced herbicide efficacy. However, ammonium salts did not all enhance nicosulfuron and certain salts were antagonistic with specific surfactants (Nalewaja et al. 1995).

Experiments were conducted to determine MON 37532 phytotoxicity to green foxtail, Japanese brome, cheat, or tame oats (Avena sativa cv. Valley) as influenced by HLB value of surfactants and applied with and without ammonium nitrate. Ammonium nitrate was selected because it is apparently the major active component of 28% nitrogen fertilize commonly used as a spray adjuvant.

EXPERIMENTAL METHOD

General Procedure

Green foxtail, Japanese brome, cheat, and oat were seeded in a commercial peat based greenhouse soil contained in 3 by 20 cm plant growth cones. Plants were thinned to four per cone within 1 wk after emergence and watered and fertilized for healthy growth. Natural day length was supplemented for a 16-h photoperiod with metal-halide lamps with a plant level intensity of 450 $uE \cdot m^{-2} \cdot s^{-1}$. The greenhouse was maintained at 20 C at night and 25 C during the day with a 5 C variation. Treatments were applied to 2-to 3-leaf plants using a moving nozzle pot sprayer that delivered 160 L/ha through a flat fan 8001 nozzle. The soil was covered with vermiculite before treatment. The

vermiculite was removed after treatment to reduce possible herbicide absorption from the soil.

Surfactants with various HLB values evaluated for efficacy with MON 37532 were: linear alcohol ethoxylates (LAE) on green foxtail, Japanese brome, and oats; secondary alcohol ethoxylate (Tergitol® 15-S[4]), octylphenol ethoxylate (Triton® X[4]), and ethoxylated nonylphenol ethoxylate (Igepal CO) on green foxtail and cheat. Surfactants were applied at 0.25% (v/v) of spray for liquids and 0.25% (w/v) for solids. Surfactants also were applied with ammonium nitrate at 0.25% (w/v). Ammonium nitrate was analytical grade and the spray carrier was distilled water.

MON 37532 was applied at 5 g active ingredient (ai) to Japanese brome, 7.5 to 15 g/ha to green foxtail, cheat and oat. Shoot fresh weight was determined 14 to 21 d after treatment. Data were converted to percent shoot fresh weight reduction compared to untreated plants. Experiments were conducted in a randomized complete block design. Each treatment was replicated four times and each experiment was repeated. Means were separated using Fisher Protected LSD Test at 5% probability.

Spray Retention Experiments

MON 37532 spray retained by green foxtail and cheat was determined for Triton® X-45, X-102, and X-405; Tergitol® 15-S-5, 15-S-9, and 15-S-40; Igepal CO-530, CO-710, and CO-977 surfactants at 0.25% (v/v) with or without 0.25% (w/v) of ammonium nitrate. The amount of spray retained was determined by including Chicago Blue Sky dye[5] at 7.5 g/L in the spray solution. Plants were excised at soil level after the spray droplets dried, placed in a test tube containing 15 ml of distilled water and 0.1% polyoxyethylene sorbitan monolaurate (Tween® 20[6]) and 0.01% commercial antifoam[7] and shaken for 20 s. The amount of spray retained was determined from standard curve prepared with various dye concentrations.

Scanning Electron Microscopic Examination of Spray Drop Residues

Scanning electron microscopic (SEM) photographs were taken for MON 37532 applied with 0.25% (v/v) Triton® X-45 and X-405 with or without ammonium nitrate at 0.25% (w/v). SEM were from sprayed plants that were transferred to the North Dakota State University Electron Microscopy Center. All treatments were processed at a time and the interval between treatments and SEM examination was between 1 and 3 h. Portions of leaves were removed from sprayed plants and mounted on aluminum stubs using double sticky carbon tape. These fresh, fully hydrated specimens were then examined and photographed using a JEOL JSM 6300 scanning electron microscope operated at accelerating voltages of 1-2KV. This technique allowed examination of spray droplet residual on the cuticle and epidermal surfaces that were unaltered by chemical fixation or dehydration. Photographs were prepared for two typical droplet residuals per treatment. Pictures presented were selected for clarity and to be representative of the group.

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[4]Union Carbide Corporation, Danbury, CT

[5]Sigma Chemical Company, St. Louis, MO

[6]ICI Surfactants, Wilmington, DE

[7]Foambuster, Ostlund Chemical, Fargo, ND

RESULTS AND DISCUSSION

Triton® X, Tergitol® 15-S, and Igepal CO surfactants generally enhanced MON 37532 phytotoxicity to green foxtail more when having a high than low HLB value (Table 1). However, Igepal CO efficacy generally increased through HLB 17.2, Igepal CO-887, and than decreased with HLB 18.2, Igepal CO-977, with or without ammonium nitrate. Triton® X and Tergitol® 15-S continued to increase in effectiveness through HLB 17.9 and 18.0, respectively, the highest values used in the experiments.

Table 1 -- Green foxtail percent fresh weight reduction (% FWR) from MON 37532 at 15 g/ha as influenced by surfactants alone or with ammonium nitrate

Surfactant	HLB	Ammonium nitrate	
		None	0.25%
		% FWR	
Octyphenol ethoxylate			
None	--	29	29
Triton® X-45	10.4	39	39
Triton® X-100	13.5	42	51
Triton® X-102	14.6	43	60
Triton® X-114	12.4	42	48
Triton® X-165	15.8	46	68
Triton® X-305	17.3	64	72
Triton® X-405	17.9	66	76
LSD 5%		5	
Secondary alcohol ethoxylate			
None	--	27	32
Tergitol® 15-S-5	10.5	27	35
Tergitol® 15-S-7	12.1	31	34
Tergitol® 15-S-9	13.3	27	28
Tergitol® 15-S-15	15.4	32	35
Tergitol® 15-S-20	16.3	44	40
Tergitol® 15-S-30	16.3	50	51
Tergitol® 15-S-40	18.0	63	53
LSD 5%		10	
Nonylphenol ethoxylate			
None	--	25	24
Igepal CO-430	8.8	40	41
Igepal CO-530	10.8	40	40
Igepal CO-610	12.2	36	36
Igepal CO-630	13.0	46	45
Igepal CO-710	13.6	46	54
Igepal CO-720	14.2	48	54
Igepal CO-730	15.0	51	62
Igepal CO-887	17.2	72	69
Igepal CO-977	18.2	58	41
LSD 5%		15	

LAE surfactants were all similar in enhancement of MON 37532 phytotoxicity to green foxtail, except C_{12-14} linear alcohol with 8 HLB was less effective than the other LAE with or without ammonium nitrate (Table 2). High HLB of Igepal CO, Triton® X and Tergitol® 15-S surfactant enhancement of MON 37532 for green foxtail was similar to other sulfonylurea herbicides (Green and Green 1993) and glyphosate (Nalewaja et al. 1995). However, MON 37532 phytotoxicity to green foxtail was not influenced by LAE HLB or alcohol carbon chain length, except for reduced phytotoxicity with LAE C_{12-14} with 8 HLB. MON 37532 differed from nicosulfuron in response to LAE surfactants as high HLB and alcohol chain length increased phytotoxicity to large crabgrass (Digitaria sanguinalis) (unpublished data). These results suggest that surfactant and herbicide chemistry are more important than HLB in determining surfactant efficacy with some herbicides.

LAE surfactants differed more in influencing MON 37532 phytotoxicity to oats than green foxtail (Table 2). LAE C_{8-10} with 12 HLB greatly enhanced phytotoxicity to oats and all C_{16-18} LAE surfactants were more effective than C_{12-14} surfactants. HLB was important to MON 37532 efficacy for oats when LAE contained short (C_{8-10}) alcohol carbon chains, of minor importance with the intermediate (C_{12-14}) alcohol, and not important to the long chain (C_{16-18}) alcohols. In the greenhouse, leaves of oats were more erect than those of green foxtail at treatment and the enhancement from LAE C_{8-10} with 12 HLB might be from greater spray retention. Glyphosate spray retained by wheat was greatest when applied with LAE C_{8-10} with 12 HLB and two to three times greater than when applied with the longer chain alcohols (Nalewaja et al. 1993).

TABLE 2 -- Japanese brome, green foxtail and oat fresh weight reduction (% FWR) from MON 37532 as influenced by linear alcohol ethoxylates (LAE) alone or with ammonium nitrate[a]

		Japanese brome		Green foxtail		Oat	
		Ammonium nitrate, (w/v)					
LAE	HLB	None	0.25%	None	0.25%	None	0.25%
		% FWR					
None	--	--	--	17	4	15	6
810-40	8	11	28	42	37	22	39
810-60	12	24	49	44	44	68	40
810-80	16	18	44	38	33	44	34
1214-40	8	36	45	22	27	31	27
1214-60	12	48	54	43	36	38	50
1214-80	16	48	63	42	36	27	38
1618-46	9	69	48	44	42	44	34
1618-62	12.6	45	39	42	34	46	31
1618-80	16	32	39	41	36	48	49
LSD 5%		9		6		9	

[a]MON 37532 rate 7.5 g/ha, Japanese brome; 20 g/ha, green foxtail; 10 g/ha, tame oat.

Ammonium nitrate enhanced MON 37532 phytotoxicity to green foxtail when with most Triton® X surfactants, but had no effect on phytotoxicity when with Igepal CO or Tergitol® 15-S surfactants, except 15-S-40 (Table 1). Ammonium nitrate had no effect on MON 37532 phytotoxicity when applied with LAE having 6 to 8 or higher carbon number with 12 to 16 HLB. However, ammonium nitrate was or tended to be antagonistic to MON 37532 phytotoxicity to green foxtail when applied with LAE C_{12-14} or C_{16-18} with 12 to 16 HLB. The general antagonism from ammonium nitrate with high HLB and long carbon chain alcohols indicates that physical form of the deposit may be important. These LAE alcohols are generally solids or gels and when with ammonium nitrate may form a solid deposit that prevents herbicide absorption. The data indicate that the benefit from ammonium nitrate as an adjuvant is highly dependent on the associated surfactant. The response might have differed had another nitrogen compound been used as occurred for nicosulfuron applied with surfactants and various salts (Nalewaja et al. 1995). Ammonium nitrate was selected because it is about 50% of 28% liquid nitrogen fertilizer commonly used as an adjuvant with many herbicides.

Ammonium nitrate both enhanced and antagonized MON 37532 phytotoxicity to oats and Japanese brome depending on the presence of specific LAE surfactants (Table 2). The greater response to ammonium nitrate for oats and Japanese brome than green foxtail further indicates the importance of species in adjuvant efficacy. Similar differences have occurred with broadleaf plants where fertilizer 10-34-0 enhanced phytotoxicity of acifluorfen + bentazon to velvetleaf, but not to soybean (Smith et al. 1995).

The specific negative and limited positive responses to ammonium nitrate suggests an interaction with the surfactant, possibly relating to spray droplet deposit. Ammonium nitrate should not have antagonized phytotoxicity if its function was enhancement of herbicide absorption through the cell membrane. The response to nitrogen fertilizer might not always positive as certain surfactants could prevent the ammonium ion from reaching the cell membrane. Ammonium nitrate may physically trap MON 37532 away from the surfactant or plant surface causing antagonism, as reported for glyphosate (Nalewaja et al. 1992).

MON 37532 phytotoxicity to Japanese brome tended to be or was enhanced by the inclusion of ammonium nitrate with the lower molecular weight LAE, C_{8-10} and C_{12-14} (Table 2). The enhancement of MON 37532 applied with surfactants in the presence of ammonium nitrate to Japanese brome but not to green foxtail could relate to pubescence and leaf angle. Japanese brome is pubescent and has erect leaves while green foxtail is not pubescent and has horizontal leaves.

An experiment was conducted using cheat, similar leaf characteristics to Japanese brome, and green foxtail to determine retention of MON 37532 spray as effected by surfactants and ammonium nitrate. Spray retained (Table 3) did not explain differences in phytotoxicity from surfactants or ammonium nitrate to either cheat (Table 3) or green foxtail (Table 1). Ammonium nitrate only enhanced spray retained by cheat when applied with Triton® X-45 or X-102, but MON 37532 phytotoxicity to cheat was reduced or not affected. Spray retained tended to be increased by ammonium nitrate applied with Tergitol 15-S-5 which related to increased MON 37532 phytotoxicity to cheat. Cheat retained more spray when with the higher HLB Triton® and Tergitol surfactants which related to increased MON 37532 phytotoxicity (Table 3). However with Igepal CO surfactants, the spray retained also increased with higher HLB, but MON 37532 phytotoxicity to cheat decreased when with the highest HLB, Igepal CO-977.

Ammonium nitrate enhanced spray retention by green foxtail when applied with all surfactants, except Igepal CO-710 (Table 3). However, ammonium nitrate only enhanced MON 37532 phytotoxicity to green foxtail

Table 3 -- Percent spray retained (%SR) by green foxtail and cheat and cheat fresh weight reduction (% FWR) from MON 37532[a] as influenced by surfactants alone or with ammonium nitrate

		Green foxtail		Cheat			
		Ammonium nitrate, (% w/v)					
Surfactant	HLB	None	0.25%	None	0.25%	None	0.25%
		% SR[b]				% FWR	
None	--	100	96	100	84	23	23
Triton® X-45	10.4	137	181	96	127	61	48
Triton® X-102	14.6	227	267	129	146	76	71
Triton® X-405	17.9	163	180	143	148	71	61
Tergitol® 15-S-5	10.5	151	197	121	133	56	62
Tergitol® 15-S-9	13.3	256	227	157	156	86	68
Tergitol® 15-S-40	18.0	169	242	152	146	81	73
Igepal CO-530	10.8	212	251	130	123	69	65
Igepal CO-710	13.6	264	254	147	146	83	85
Igepal CO-977	18.2	201	225	147	142	67	63
LSD 5%		15		15		5	

[a]MON 37532 rate 15 g/ha, green foxtail; 7.5 g/ha, cheat.
[b]Spray retained as a percentage of treatment with no surfactant and ammonium nitrate.

when with Trition X-102, X-405, and Igepal CO-977. These data indicate that adjuvants differ in enhancement of MON 37532 phytotoxicity and that the benefit from the inclusion of ammonium nitrate in the spray is dependent upon the specific surfactant and species being controlled. The potential benefit from the use of ammonium with MON 37532 may be from increased spray retention, but the apparent affect upon absorption often exceeds the benefit of increased spray retention.

Spray droplet deposit characteristics for MON 37532 applied alone or with Trition® X-45 and X-405 and ammonium nitrate on cheat generally related to the concept that a uniform deposit with close epicuticular contact is positive to herbicide phytotoxicity (Fig. 1). MON 37532 applied with Triton® X-405 without ammonium nitrate left a uniform deposit that appeared to blend closely with or into the cheat cuticular surface, but when with ammonium nitrate, the deposit appeared crusty and above the cuticular surface, probably reducing absorption and accounting for the reduced phytotoxicity from ammonium nitrate (Table 3). The reduced phytotoxicity from ammonium nitrate when with Triton X-45 was not easily detected from the small droplets present.

Spray deposit characteristics varied widely on green foxtail for MON 37532 applied with Triton® X surfactant and ammonium nitrate (Fig. 2) but deposit contact with the leaf did not easily relate to efficacy (Table 1). Ammonium nitrate applied with Triton® X-45 did not enhance MON 37532 phytotoxicity to green foxtail, even though deposits had a close contact (Fig. 2D) with the leaf surface and spray retention was

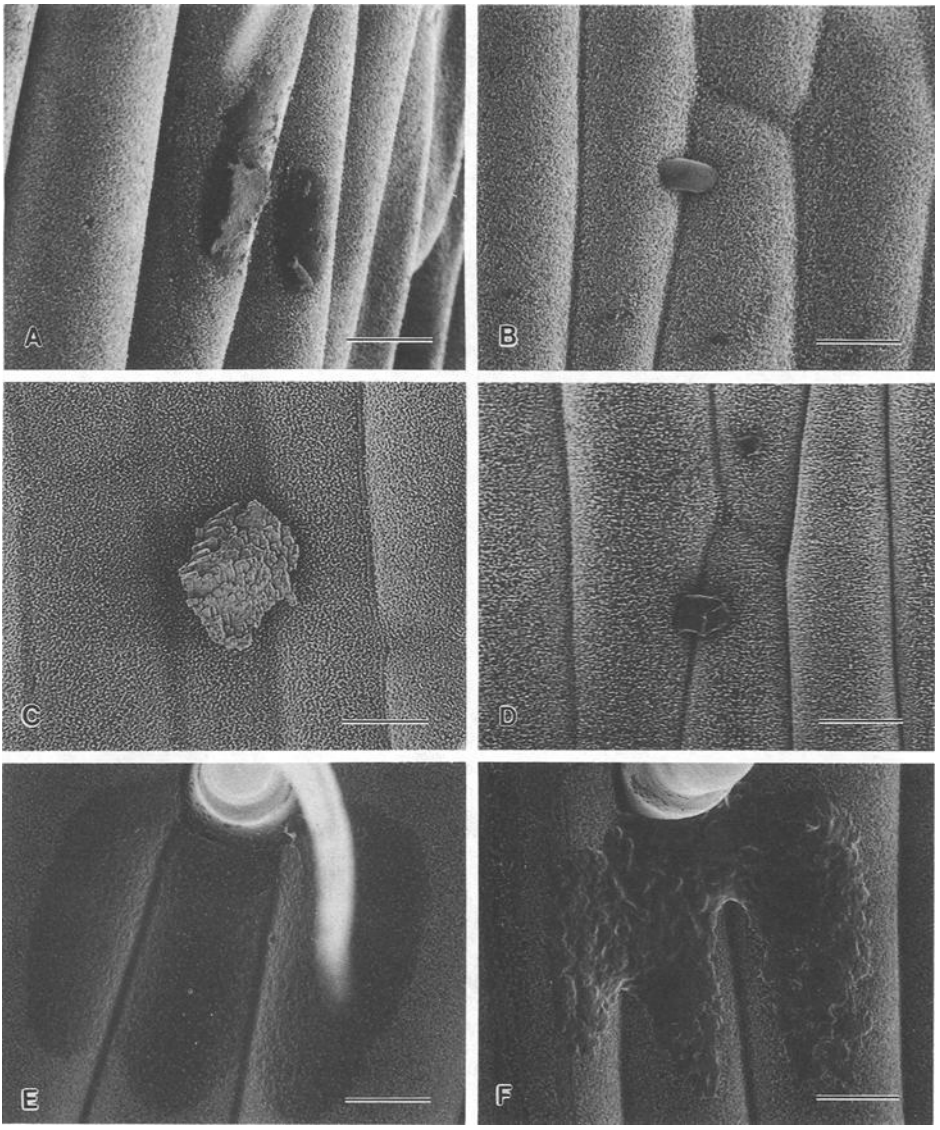

FIG. 1 -- Spray droplet residual on cheat leaf of MON 37532 at 7.5 g/ha applied A. alone, B. with ammonium nitrate (AMN), C. Triton® X-45, and D. Triton® X-45 + AMN, E. Triton® X-405, and F. Triton® X-405 + AMN. Bars represent 20 μm.

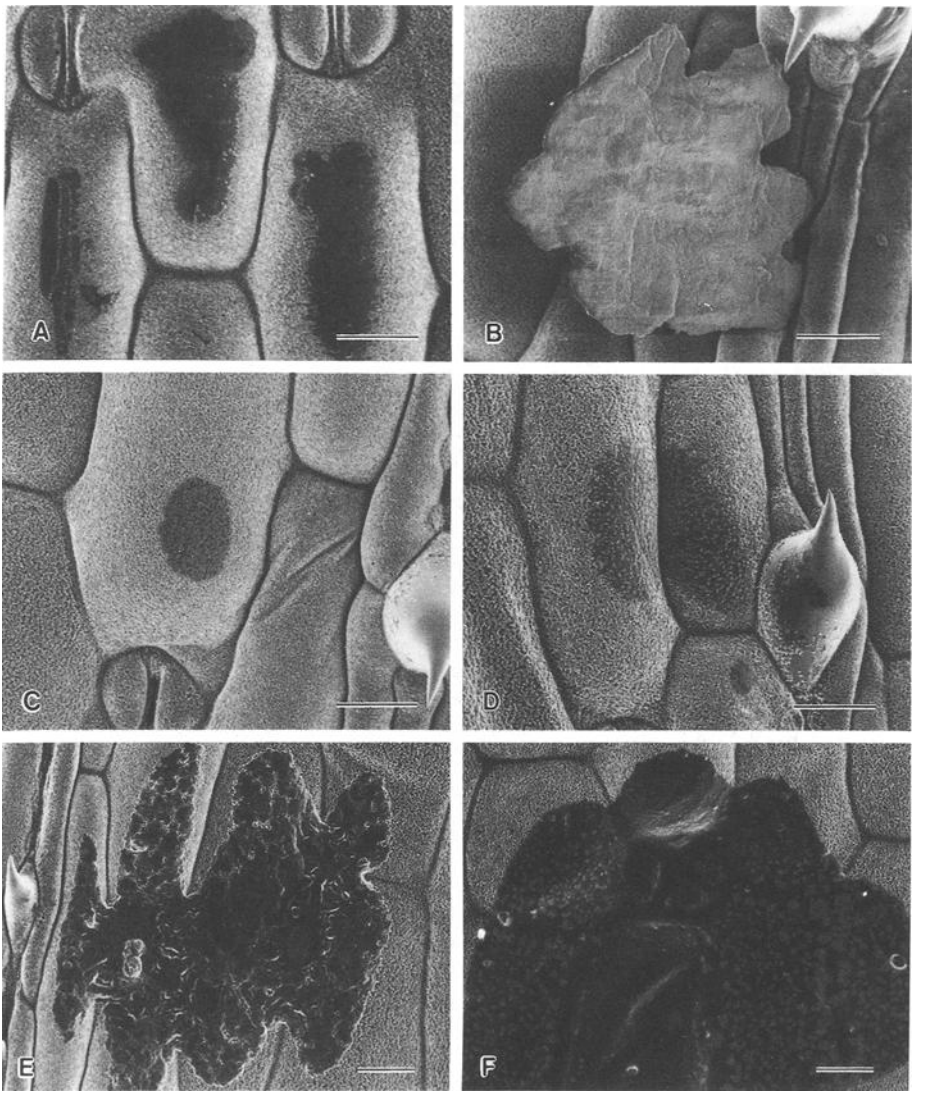

FIG. 2 -- Spray droplet residual on green foxtail leaf of MON 37532 at 15 g/ha applied A. alone, B. with ammonium nitrate (AMN), C. Triton® X-45, D. Triton® X-45 + AMN, E. Triton® X-405, and F. Triton® X-405 + AMN. Bars represent 20 μm.

increased (Table 3). Further, ammonium nitrate with Triton® X-405 enhanced MON 37532 phytotoxicity, but the spray deposit had less contact with the leaf surface (Fig. 2E) than when without ammonium nitrate (Fig. 2F). Droplet residual from Triton® X-405 applied with ammonium nitrate did not appear to have as close contact over the anticlinal cell wall areas as when without ammonium nitrate. However, contact appeared better than on cheat (Fig. 1F) where ammonium nitrate was antagonistic to MON 37532 phytotoxicity (Table 3). MON 37532 with Triton® X-405 appeared to blend into the cuticular surface of cheat more than green foxtail (Fig. 1E, 2E) indicating a greater solubility in the cheat epicuticular wax.

An adjuvants influence on the physical appearance of spray deposits inpart explains the reason for efficacy. Deposits having close contact with the leaf surface alone does not assure efficacy as deposits with similar contact differed in efficacy. The deposits internal component solubilities with the leaf wax and the specific herbicide are apparently also important to adjuvant enhancement of MON 37532 phytotoxicity.

CONCLUSION

MON 37532 phytotoxicity enhancement from surfactants differed greatly depending on surfactant type and HLB. Ammonium nitrate enhanced, had no effect, or antagonized MON 37532 phytotoxicity differently depending on surfactant and species. Ammonium nitrate often increases MON 37532 spray retention, but does not always enhance phytotoxicity because of reduced spray droplet residual contact with the leaf.

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Frank A. Manthey,[1] Lynn S. Dahleen,[2] John D. Nalewaja,[1] and Janet D. Davidson[1]

MEASURING PROTON EXTRUSION FROM CELL MEMBRANES OF BARLEY CALLI TO EVALUATE SURFACTANT PHYTOTOXICITY

REFERENCE: Manthey, F. A., Dahleen, L. S., Nalewaja, J. D., and Davidson, J. D., **''Measuring Proton Extrusion from Cell Membranes of Barley Calli to Evaluate Surfactant Phytotoxicity,''** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: A technique that measured extrusion of protons by barley (*Hordeum vulgare*) calli into the incubation solution was developed to detect surfactant phytotoxicity. Parameters for proton extrusion by barley calli were: 0.1 mg/L 2,4-D [(2,4-dichlorophenoxy)acetic acid] in 10% (v/v) L1 medium using 125 mg barley calli in 3 ml incubation solution with continuous aeration. If foaming occurred, the continuous aeration could be replaced by rotary shaking at 100 rpm. Proton extrusion was similar for the three barley genotypes evaluated. Proton extrusion from barley calli and electrolyte leakage from potato (*Solanum tuberosum*) discs were compared for their ability to detect surfactant phytotoxicity. Anionic, cationic, and nonionic surfactants were evaluated for phytotoxicity. Phytotoxicity was detected at equal or lower surfactant concentrations when tested by proton extrusion (pH change) from barley calli than by electrolyte leakage (electroconductivity) from potato tubers. Surfactant solutions with high or low pH interfered with the proton extrusion method and made direct comparison difficult, while surfactant solutions with high electroconductivity reduced the sensitivity of the electroconductivity method. Thus, the proton extrusion and electrolyte leakage methods complimented each other and provided more information about surfactant phytotoxicity than either method alone.

KEYWORDS: Anionic, cationic, nonionic, surfactants, electroconductivity, cell membrane permeability.

Surfactants may be biologically active and thus influence pesticide efficacy. Surfactants can readily penetrate leaf cuticle and be absorbed into the underlying cells where they can affect cellular processes (Silcox and Holloway 1989; Parr 1982). Altered cell membrane permeability is a common effect of surfactants (Helenius and Simons 1975; Manthey et al. 1996; Parr 1982). Surfactants may facilitate movement of certain herbicides into the cell by increasing the permeability of the membrane (St. John et al. 1974; Watson et al. 1980).

[1]Research Scientist, Professor, and Agricultural Research Technician, respectively, Department of Plant Sciences, North Dakota State University, Fargo, ND.

[2]Research Geneticist, USDA/ARS, Fargo, ND.

However, altering membrane integrity often results in cellular injury. Cellular injury by surfactants can affect pesticide efficacy by restricting foliar absorption into and translocation out of the underlying cells (St. John et al. 1974).

A quick, simple technique is needed that can detect small changes in cell membrane permeability. Changes in cell membrane permeability often are detected by measuring electrolyte leakage from potato tubers or treated leaves (Manthey et al. 1996; Vanstone and Stobbe 1977). Electrolyte leakage is determined by measuring the change in electroconductivity of an incubation solution. A disadvantage of this technique is that some incubation solutions inherently contain high levels of electrolytes, making it difficult to detect small increases in cell membrane permeability. Ethylene evolution has been used to detect cellular injury of leaves treated with surfactants (Knoche et al. 1992; Matsui et al. 1992). However, the ethylene assay is a more complex procedure and requires sophisticated equipment compared to the electroconductivity assay.

The proton extrusion technique described in this paper provides an alternative to the electroconductivity technique. Normal functioning cell membranes extrude protons in an effort to maintain cytoplasmic pH near 7.0. Protons accumulating on the outside of the membrane are unable to return to the inside except through specific channels or sites. The buildup of protons decreases extracellular pH, which can be measured using a pH electrode (Reuveni et al. 1987; Shimabukuro et al. 1982). Surfactants that alter cell membrane permeability allow protons to return inside the cell and prevent the buildup of extracellular protons, preventing a normal decrease in extracellular pH.

Experiments were conducted to determine optimal conditions for measuring proton extrusion from barley calli. Factors evaluated were: liquid medium and 2,4-D concentration, genotype response, and ratio of callus weight to incubation solution volume. Electrolyte leakage from potato discs and proton extrusion from barley calli were compared to determine their ability to detect surfactant-enhanced cell membrane permeability.

EXPERIMENTAL METHOD

Plant material

Barley used to initiate calli was grown in the greenhouse at 20 to 24°C during the day and 13 to 17°C at night. A daylength of 16 h was maintained by supplemental lighting with mercury halide lamps.

The procedure reported by Dahleen (1995) was used for embryo culture initiation. Culture medium was MS (Murashige and Skoog, 1962) with copper sulfate increased to 50 uM, supplemented with 4.5 mg/L 2,4-D, 30 g/L maltose, 0.25 g/L myo-inositol, 1 g/L casein hydrolysate, and solidified with 3.5 g/L gellan gum. The culture medium was sterilized by autoclaving. Every 4 wk, actively growing white or yellow colored calli were transferred to fresh medium. Callus morphology was similar for all cultures used.

Proton extrusion method

Proton extrusion was measured by placing barley calli in a 22 by 195 mm glass test tube containing 3 mL of incubation solution [L1 liquid medium (Lazzeri et al, 1991) with 50 g/L maltose]. The L1 medium was sterilized by filtration. The solution pH was monitored continuously for 2 h using a pH meter connected to a strip-chart recorder set at 6 cm/h. Two treatments were run simultaneously by using two pH meters. The system equilibration time ranged from 10 to 30 min. The change of pH from 30 min to 120 min was used to determine calli response to treatment.

Optimizing proton extrusion

Experiments were conducted to determine the conditions required to maximize proton extrusion. The experimental design for these experiments was a randomized complete block with eight replicates. The same pH meter was used for all treatments within a replicate. Means were separated using Fisher's Protected LSD at the 0.05 probability level.

2,4-D concentration--'Morex' barley calli, 500 mg, were incubated with continuous aeration in 3 ml of 50% L1 medium containing 0, 0.01, 0.1, 1, and 10 mg/L 2,4-D.

L1 medium concentration--Morex barley calli, 500 mg, were incubated with continuous aeration in 3 mL of solution containing 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% (v/v) L1 medium and 0.1 mg/L 2,4-D. L1 medium was diluted with distilled water.

Callus weight/solution volume--Morex barley calli were incubated with continuous aeration in 3 mL of 10% (v/v) L1 medium containing 0.1 mg/L 2,4-D. Callus weights tested were 62, 125, 250, 500, and 750 mg.

Barley genotype--Calli from 'Harrington', 'Hector', and Morex barley, 125 mg, were incubated with continuous aeration in 3 mL of 10% (v/v) L1 medium containing 0.1 mg/L 2,4-D.

Solution aeration and agitation--Hector barley calli, 125 mg, were incubated in 3 mL of 10% (v/v) L1 medium containing 0.1 mg/L 2,4-D. Solution agitation treatments were continuous aeration; 10 min aeration; no aeration; and continuous shaking on a rotary shaker at 100 rpm.

Electroconductivity method

Eight mm diameter cylinders were removed from Russet potatoes using a cork borer. The excised cylinders were sectioned transversely into 2 mm thick discs. Potato discs were rinsed 12 h in tap water to remove electrolytes from cells damaged during sectioning.

Fifteen discs were incubated in 10 mL incubation solution for 2 h at room temperature. The incubation solution contained 10% (v/v) L1 medium and 0.1 mg/L 2,4-D. After 2 h, electroconductivity of the incubation solution was measured with a conductivity bridge using a conductivity cell (k=1.0). Data were corrected for electroconductivity innate to the incubation solution.

Method comparison

Proton extrusion and electoconductivity methods were compared using the surfactants presented in Table 1. Each surfactant was considered a separate experiment. Surfactants were evaluated at 0.001, 0.01, 0.1 and 1% active ingredient (v/v) in 10% (v/v) L1 medium containing 0.1 mg/L 2,4-D. Barley calli and potato discs were first incubated 2 h in L1 medium containing surfactant. The barley calli and potato discs were then rinsed 2 min in running tap water and reincubated 2 h in L1 medium without surfactant. Barley callus solution pH and potato disc solution electroconductivity were determined after both 2 h incubations using the procedures described above. The experimental design for both methods was a randomized complete block with six replicates. Means were separated using Fisher's Protected LSD at the 0.05 probability level.

TABLE 1 -- Ionic class, trade name, and general chemistry of surfactants.

Ionic class	Trade name[a]	General chemistry
Nonionic	Triton® X-100	Octylphenol ethoxylate
Nonionic	Triton® X-405	Octylphenol ethoxylate
Cationic	Arquad® 2C-75	Dicoco dimethyl ammonium chloride
Cationic	MON 0818	Polyethoxylated tallow amine
Anionic	Gafac® RS-710	Free acid of complex organic phosphate ester
Anionic	Steol® CS-130	Sodium laureth sulfate

[a]Triton® from Union Carbide Chemicals and Plastics Co., Danbury, CT; Arquad® from Akzo Chemicals Inc., Chicago, IL; MON from Monsanto Co., St. Louis, MO; Gafac® from Rhone-Poulenc/GAF, Princeton, NJ.; and Steol® from Stepan Co., Maywood, NJ.

RESULTS AND DISCUSSION

Optimizing proton extrusion

The cell membrane proton pump is stimulated by an auxin plant hormone, indole-acetic acid (Rayle 1973). 2,4-D is a synthetic auxin that mimics indole-acetic acid. Barley calli proton extrusion as measured by a decrease in solution pH and an increase in proton concentration (acidification) was greatest when the L1 medium contained 0.1 mg/L 2,4-D; fewer protons were pumped out of the cells at lower or higher 2,4-D concentrations (Table 2). These results indicate that the 2,4-D concentration was important in optimizing proton efflux which is controlled in part by the proton pump.

TABLE 2 -- The effect of 2,4-D concentration in 50% (v/v) L1 medium on 2,4-D-induced acidification by barley calli.

2,4-D concentration	Initial pH	Net change pH	Net change Proton concentration
mg/L			10^{-6} M
0	5.86	-0.42	2.25
0.01	5.86	-0.43	2.34
0.1	5.85	-0.58	3.96
1	5.80	-0.40	2.39
10	5.70	-0.36	2.57
distilled water	6.87	-0.05	0.02
LSD (0.05)		0.06	0.66

Net change in the incubation solution pH by barley calli was greatest when the incubation solution contained 10% (v/v) L1 medium; intermediate with 30 to 50% L1 medium; and least with 0 or 60 to 100% L1 medium (Table 3). The net change in proton concentration was greatest when the incubation solution contained 10% (v/v) L1 medium; intermediate with 20 to 100% L1 medium; and least with 0% L1 medium (100% distilled water). The apparent inconsistency between the net change in pH and net change in proton concentration is due to the geometric relationship between pH and proton concentration. pH is the negative log of the proton concentration. A 0.5 unit change in pH from 7.0 to 6.5 requires 2.16×10^{-7} M protons while a pH change from 6.5 to 6.0 requires 6.84×10^{-7} M protons. Therefore, more protons are required for a unit change

in pH as the solution pH decreases. The initial pH of the incubation solution ranged from 6.45 with no L1 medium to 5.38 with 100% L1 medium. A similar increase in protons in the incubation solution would cause a greater change in pH with the high pH solution than with a low pH solution. Net change in pH and net change in proton concentration would be equivalent measurements of proton extrusion as long as the initial pH was the same. The similarity between both measurements can be seen in Table 4.

Table 3 -- Effect of concentration of L1 medium containing 0.1 mg/L 2,4-D on acidification by barley calli.

L1 medium concentration	Initial pH	Net change	
		pH	Proton concentration
%, v/v			10^{-6} M
0	6.45	-0.21	0.22
10	6.39	-1.22	6.35
20	6.32	-0.94	3.69
30	6.12	-0.69	2.96
40	5.95	-0.61	3.45
50	5.81	-0.54	3.82
60	5.69	-0.43	3.45
70	5.60	-0.39	3.66
80	5.53	-0.42	4.81
90	5.46	-0.33	3.94
100	5.38	-0.22	2.75
LSD (0.05)	0.11	0.31	1.46

Callus weight of 125 mg/3 mL of incubation solution gave the greatest acidification, 62 and 250 mg/3 mL gave intermediate acidification, and 500 and 750 mg/3 mL gave the least acidification (Table 4). The low acidification from the high callus weights was not expected and may be from physical damage to the calli by the pH electrode. There was little room for the pH electrode in the test tube containing 750 mg calli.

TABLE 4 -- The effect of barley calli weight/3 mL incubation solution on acidification[a].

Calli weight	Net change	
	pH	Proton concentration
mg/3mL		10^{-6} M
62	-0.88	3.15
125	-1.07	5.14
250	-0.92	3.50
500	-0.65	1.66
750	-0.50	1.03
LSD (0.05)	0.15	0.28

[a]Initial pH of incubation solution was 6.32.

Proton extrusion was similar for calli from Harrington, Hector, and Morex barley. The initial incubation solution pH, 6.34, was reduced 0.74 pH units by Harrington, 0.81 pH units by Hector, and 0.69 pH units by Morex calli (LSD 5%=NS). The net change in proton concentration was

similar with all three barley genotypes (data not presented). Thus, the proton extrusion method performed adequately, regardless of barley genotype used to initiate calli.

When the incubation solution with continuous aeration contained certain surfactants, i.e., Triton® X-100, the foam produced by continuous aeration would lift pieces of calli out of solution. Aeration and agitation treatments with initial incubation solution pH 6.17 indicated that acidification was least with no aeration or agitation, -0.39 pH units; intermediate with shaking at 100 rpm, -0.72 pH units; and greatest with continuous aeration, -0.98 pH units (LSD 5%=0.24). Shaking on a rotary shaker at 100 rpm did not cause foaming in the incubation solution and barley calli reduced pH sufficiently. Thus, continuous aeration could be replaced with rotary shaking at 100 rpm when working with surfactants that foam.

These results indicate that the best conditions for proton extrusion from barley calli were 0.1 mg/L 2,4-D in 10% L1 medium using 125 mg calli in 3 ml incubation solution with continuous aeration. Further, barley genotype selection was not important in optimizing the proton extrusion method. If foaming occurred, the continuous aeration could be replaced by rotary shaking at 100 rpm. Change in pH is a satisfactory measurement of proton concentration if the initial pH of the incubation solution is similar for all treatments. However, if the initial pH differs among treatments, then the change in the proton concentration should be calculated to prevent erroneous conclusions due to the geometric relationship between pH and proton concentration.

Method comparison

Nonionic, cationic, and anionic surfactants are used in pesticide formulations. Six surfactants (two anionic, two cationic, and two nonionic) were selected to compare the effectiveness of barley callus proton extrusion and potato electroconductivity methods (Table 1). The nonionic surfactants, Triton® X-100 and Triton® X-405, were selected based on their phytotoxicity. In general, Triton® X-100 is phytotoxic and Triton® X-405 is not phytotoxic (Lownds and Bukovac 1988; Manthey et al. 1996). The cationic surfactant MON 0818 was selected based on its use in the glyphosate formulation for Roundup®. The remaining cationic surfactant, Arquad® 2C-75, and the anionic surfactants, Gafac® RS-710 and Steol® CS-130, were selected randomly.

Electroconductivity of the calli incubation solution was measured (data not presented). However, electrolyte leakage from 125 mg calli in 3 mL solution was too small relative to the inherent electroconductivity of the incubation solution to adequately detect surfactant induced change in cell membrane permeability.

Triton® X-100 at 0.001% (v/v) reduced acidification by barley calli of the incubation solution and stopped acidification at 0.1 and 1% (Table 5). The rise in pH at 0.1 and 1% indicates inhibition of the cell membrane proton pump and proton movement back into the cell to achieve equilibrium between protons inside and outside the cell. Calli injured by Triton® X-100 did not resume proton pump acidification when rinsed and incubated in solution without surfactant.

Triton® X-100 at 0.001 or 0.01% did not enhance electrolyte leakage from potato discs but caused leakage at 0.1 and 1% (Table 5). Enhanced electrolyte leakage from potato discs incubated with 0.1 or 1% Triton® X-100 continued after rinsing with distilled water and incubating in solution without surfactant. Even though Triton® X-100 at 0.001% (v/v) did not affect cell membrane permeability as measured by electrolyte leakage, it did reduce acidification of the incubation solution. Thus, the proton extrusion method was more sensitive in detecting cell membrane injury from Triton® X-100 than was the electrolyte leakage method.

TABLE 5 -- 2,4-D-induced acidification by barley calli and electrolyte leakage from potato discs as influenced by the concentration of nonionic surfactants, Triton® X-100 and Triton® X-405.

			Barley calli						
			Net pH change		Net proton concentration		Potato discs		
Surfactant	Concentration	Initial pH	2 h	2 HAR[a]	2 h	2 HAR	Initial EC[a]	Net change 2 h	2 HAR
	% v/v				— 10^{-7} M —		— micromhos —		
Triton® X-100									
	0	6.8	-0.70	-0.66	6.36	5.67	388	69	108
	0.001	6.8	-0.33	-0.42	1.81	2.59	399	65	112
	0.01	6.8	-0.08	-0.19	0.33	0.87	416	52	117
	0.1	6.9	0.13	-0.01	-0.33	0.04	403	184	194
	1	6.9	0.09	0.04	-0.24	-0.13	407	321	361
LSD (0.05)			0.24	0.26	2.13	2.50		33	20
Triton® X-405									
	0	6.8	-0.77	-0.73	7.75	6.93	417	49	99
	0.001	6.8	-0.69	-0.65	6.18	5.50	413	44	108
	0.01	6.8	-0.73	-0.69	6.93	6.18	416	44	97
	0.1	6.9	-0.58	-0.69	3.53	6.18	420	44	109
	1	6.9	-0.50	-0.61	2.72	4.88	400	44	102
LSD (0.05)			NS	NS	NS	NS		NS	NS

[a]HAR is hours after rinse; and EC is electroconductivity.

Acidification of the incubation solution by barley calli and electrolyte leakage from potato discs were not affected by Triton® X-405 (Table 5). Thus, Triton® X-405 was less phytotoxic than Triton® X-100. These results are in agreement with intact plant response to foliar application of Triton® X-100 and Triton® X-405 (Lownds and Bukovac 1988; Manthey et al. 1996).

Phytotoxicity apparently occurs from the solubilization of plant membranes by the surfactant. Helenius and Simons (1975) reported that maximum solubilization of plant membranes occurred with surfactants with HLB values in the range of 12.5 to 14.5. Triton® X-100 (HLB=13.5) mimics cell membrane lipids and affects membranes at a concentration of 0.01% or less (Caux and Weinberger 1993). Large hydrophilic surfactant molecules (such as Triton® X-405, HLB=17.9) may be too bulky and lack sufficient lipophilicity to interact strongly with the cell membrane.

The cationic surfactant Arquad® 2C-75 at 0.01% reduced barley calli acidification but did not enhance electrolyte leakage from potato discs (Table 6). Acidification by barley calli was completely inhibited and electrolyte leakage was enhanced by Arquad® 2C-75 at 0.1 and 1%. The rise in pH and corresponding decrease in proton concentration at 0.1 and 1% Arquad 2C-75 indicates the inhibition of the cell membrane proton pump and the movement of protons back into the cell. Acidification was partially restored after rinsing the barley calli incubated with Arquad® 2C-75 at 0.01%, but not at 0.1 or 1%. Similarly, electrolyte leakage from potato discs incubated with Arquad® 2C-75 at 0.1 or 1% continued after rinsing and placement in solution without Arquad® 2C-75. The proton extrusion method detected phytotoxicity from Arquad® 2C-75 at 0.01% or greater while electroconductivity detected injury at 0.1% or greater. Calli injured by Arquad® 2C-75 at 0.1 or 1% did not resume proton pump acidification and electrolyte leakage continued after being rinsed and incubated in fresh solution.

TABLE 6 -- 2,4-D-induced acidification by barley calli and electrolyte leakage from potato discs as influenced by the concentration of cationic surfactants, Arquad® 2C-75 and MON 0818.

		Barley calli				Potato discs		
Surfac-	Concen-	Initial	Net pH change		Net proton concentration		Initial	Net change
tant	tration	pH	2 h	2 HAR[a]	2 h	2 HAR	EC[a]	2 h 2 HAR
	% v/v				— 10^{-7} M —		— micromhos —	
Arquad® 2C-75								
	0	6.7	-0.46	-0.53	3.75	4.76	406	58 113
	0.001	6.8	-0.54	-0.43	3.92	3.37	402	52 119
	0.01	6.8	-0.23	-0.35	1.11	2.47	404	60 114
	0.1	6.7	0.04	-0.09	-0.13	0.45	418	129 154
	1	6.0	0.20	0.00	-3.69	0.00	496	413 275
LSD (0.05)			0.14	0.24	0.95	1.86		14 12
MON 0818								
	0	6.7	-0.63	-0.64	6.51	6.71	409	45 98
	0.001	6.8	-0.63	-0.64	5.18	6.71	400	46 95
	0.01	7.1	-0.38	-0.49	0.93	4.17	406	101 141
	0.1	7.4	-0.19	-0.17	0.22	0.95	420	313 303
	1	8.4	-0.86	-0.18	0.19	1.02	431	581 441
LSD (0.05)			0.20	0.30	1.83	3.38		28 16

[a]HAR is hours after rinse; and EC is electroconductivity.

Based on the net change in pH, the cationic surfactant MON 0818 at 0.01 and 0.1% (v/v) reduced proton extrusion, but at 1% appeared to enhance proton extrusion (Table 6). However, the net proton concentration indicated a reduction in proton extrusion at 0.01, 0.1 and 1% (v/v) MON 0818. The different results between net change in pH and net proton concentration is do to their geometric relationship. MON 0818 increased the initial pH of the incubation solution from 6.7 with no MON 0818 to 8.4 with 1% (v/v) MON 0818. More protons are needed to cause a unit change in pH at low than at high pH. Thus at pH 8.4, 0.19 X 10^{-7} M protons reduced pH 0.86 units while at pH 7.4 0.22 X 10^{-7} M protons reduced pH only 0.19 units.

Barley calli injured by MON 0818 resumed proton extrusion when rinsed and incubated in solution without surfactant. Proton extrusion was not restored after rinsing calli injured by MON 0818 at 0.1 or 1% (v/v).

Electrolyte leakage from potato discs increased as MON 0818 increased from 0.01 to 1% (v/v) (Table 6). Enhanced electrolyte leakage continued after potato discs were rinsed with distilled water.

Proton extrusion and electroconductivity methods provided similar results, with injury being detected at 0.01% (v/v) and increasing with increased concentration of MON 0818. The exception was the 'false' reading for the first incubation due to the high pH of 1% (v/v) MON 0818 using the proton extrusion method. Cellular injury from 1% (v/v) MON 0818 was apparent from the net proton concentration after the first incubation and from the net pH change and net proton concentration following rinsing the calli in the second incubation.

The anionic surfactant Gafac® RS-710 at 0.001 (v/v) reduced and at 0.01, 0.1 and 1% completely inhibited barley calli acidification of the incubation solution (Table 7). Gafac® RS-710 is a free acid of a complex organic phosphate ester. Gafac® RS-710 reduced incubation solution pH from 6.9 without Gafac® RS-710 to 3.5 with 0.1% (v/v) and 2.8 with 1% Gafac® RS-710. The low pH of the initial incubation solution containing 0.1 and 1% (v/v) Gafac® RS-710 probably inhibited

proton extrusion as the proton gradient across the cell membrane would strongly favor proton movement into the cell. This is reflected by the large decrease in proton concentration with Gafac® RS-710 at 0.1 and 1% (v/v). Proton extrusion was restored after rinsing the barley calli incubated with Gafac® RS-710 at 0.001% (v/v) and partially restored with calli incubated with Gafac® RS-710 at 0.01%. The large increase in proton concentration after rinsing the barley calli treated with 1% (v/v) Gafac® RS-710 probably is due to surfactant moving out of the calli. This would indicate that the 2 min rinse was not long enough to remove Gafac® RS-710 from the calli.

TABLE 7 -- 2,4-D-induced acidification by barley calli and electrolyte leakage from potato discs as influenced by the concentration of anionic surfactants, Gafac® RS-710 and Steol® CS-130.

		Barley calli				Potato discs		
			Net pH change		Net proton concentration			
Surfactant	Concentration	Initial pH	2 h	2 HAR[a]	2 h	2 HAR	Initial EC[a]	Net change 2 h	Net change 2 HAR
	% v/v				— 10^{-7} M —		— micromhos —		
Gafac® RS-710									
	0	6.9	-0.55	-0.57	6.40	6.82	363	58	130
	0.001	6.9	-0.30	-0.49	3.15	5.25	403	22	124
	0.01	6.5	0.01	-0.31	-0.14	2.62	418	24	107
	0.1	3.5	1.41	-0.13	-4815.00	0.88	503	-5	186
	1	2.8	0.12	-1.29	-7600.00	46.49	1692	-668	198
LSD (0.05)			0.16	0.23	10.46	6.55		17	35
Steol® CS-130									
	0	6.8	-0.46	-0.48	2.99	3.21	431	50	132
	0.001	6.9	-0.50	-0.42	2.72	2.58	407	56	126
	0.01	7.0	-0.28	-0.31	0.91	1.66	421	49	121
	0.1	7.1	-0.27	-0.23	0.69	1.11	500	148	225
	1	7.8	-0.04	-0.21	0.02	0.99	1128	385	241
LSD (0.05)			0.20	0.20	1.58	0.55		24	9

[a]HAR is hours after rinsing; and EC is electroconductivity.

Electroconductivity of the initial incubation solution increased from 363 to 1692 micromhos with increased concentration of Gafac® RS-710 of 0 to 1% (v/v) (Table 7). Conversely, the electroconductivity of the incubation solution after 2 h incubation decreased with increased concentration of Gafac® RS-710. The decrease in electroconductivity indicates that Gafac® RS-710 was absorbed into the barley callus cells. This was most pronounced with 1% (v/v) Gafac® RS-710.

After potato discs were rinsed, electrolytes leaked most from cells of potatoes previously treated with Gafac® RS-710 at 0.1 and 1% (Table 7). This leakage may be due to a change in cell membrane permeability or to excess electrolytes (Gafac® RS-710) moving out of the cells in order to reach equilibrium with the surrounding incubation solution. Movement of Gafac® RS-710 out of the cell would decrease the pH of the incubation solution. This was evident with barley calli incubated with 1% (v/v) Gafac® RS-710.

Steol® CS-130 reduced barley calli acidification of the incubation solution as concentration increased from 0.01 to 1% (v/v) (Table 7). Steol® CS-130 at 1% (v/v) inhibited acidification even though the initial incubation solution pH was 7.8. The high pH of the incubation

solution should have created a favorable gradient for the movement of protons from inside to outside the cell. Barley calli acidification was partially restored after the Steol® CS-130 was washed from the calli.

Unlike Gafac® RS-710, Steol® CS-130 at 0.1 and 1% (v/v) increased electrolyte leakage from potato discs in spite of the increased electroconductivity of the initial solution with increased concentration of Steol® CS-130 (Table 7). After potato discs were rinsed, electrolytes continued to leak from potato discs previously treated with Steol® CS-130 at 0.1 and 1% (v/v).

In general, surfactant injury was similar whether measured by proton extrusion by barley cells or by electrolyte leakage from potato discs. The proton extrusion method using barley calli generally detected surfactant injury at a lower concentration than the electroconductivity method using potato discs. For example, acidification was reduced by Triton® X-100 at 0.001% (v/v) while enhanced electrolyte leakage was detected at 0.1% (Table 5). Similarly, injury to proton extrusion, solution acidification, from Arquad® 2C-75 occurred at 0.01% (v/v), while enhanced electrolyte leakage was detected from Arquad® at 0.1% (Table 6). Recovery from apparent surfactant injury was detected for Arquad® 2C-75 and MON 0818 at 0.01% (v/v) (Table 6) at 0.01 to 1% using the proton extrusion method but not detected with the electroconductivity method. The greater sensitivity of the proton extrusion method and its ability to detect injury that is reversible may help further differentiate between surfactants that cause reversible and irreversible injury.

Proton extrusion and electroconductivity methods have limitations. Initial surfactant solutions that have high or low pH cause problems with interpreting results from the proton extrusion method, but pose no problem for the electroconductivity method. Electroconductivity data is confounded by initial surfactant solutions that are high in electrolytes. The electrolytes may diffuse into the cell during incubation and then diffuse out of the cell after washing. One method offsets the limitations of the other method and using both methods allows for the verification of injury.

CONCLUSION

The best conditions for proton extrusion with barley calli were 0.1 mg/L 2,4-D in 10% L1 medium using 125 mg calli in 3 ml incubation solution with continuous aeration. If foaming occurs, the continuous aeration can be replaced by rotary shaking at 100 rpm.

These data indicate that the proton extrusion method described in this paper can be used to detect adverse interaction between surfactants (anionic, cationic, and nonionic) and the cell membrane. Further, the proton extrusion method using barley calli detected cell membrane injury at equal or lower surfactant concentrations than did the electrolyte leakage method using potato discs. The apparent difference in sensitivity between the two methods may relate to the inherent physiological differences between barley calli and potato discs and not necessarily between the methodologies.

Caution must be used in interpreting results from the proton extrusion method if the surfactants alter solution pH. Change in pH is a satisfactory measurement of proton concentration if the initial pH of the incubation solution is similar for all treatments. However, if the initial pH differs among treatments, then the change in the proton concentration should be calculated to prevent erroneous conclusions due to the geometric relationship between pH and proton concentration. The proton extrusion and electrolyte leakage methods complimented each other and provided more information about surfactant phytotoxicity than either method alone.

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Royal G. Fader[1] and Martin J. Bukovac[2]

TRITON X-45: A UNIQUE EFFECT ON GROWTH REGULATOR SORPTION BY AND PENETRATION OF ISOLATED PLANT CUTICLES.

REFERENCE: Fader, R. G. and Bukovac, M. J. **"Triton X-45: A Unique Effect on Growth Regulator Sorption by and Penetration of Isolated Plant Cuticles,"** Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328, G. Robert Goss, Michael J. Hopkinson, and Herbert M. Collins, Eds., American Society for Testing and Materials, 1997.

ABSTRACT: A unique effect of the polyethoxylated (average 5 ethylene oxide units, EO 5) octylphenol surfactant, Triton X-45 (TX-45), on the sorption and penetration of 2-(1-naphthyl) [1-^{14}C] acetic acid (NAA) by and through enzymatically isolated tomato fruit cuticular membranes (CM) is described. TX-45 induced an unusually marked increase in cuticular sorption (55% greater than control) of NAA relative to Triton X surfactants of lower and higher EO content. This marked enhancement of NAA sorption was associated with epicuticular and cuticular waxes, for on removal of waxes, the TX-45 - mediated increase in sorption was lost. Surprisingly, a different lot of TX-45 failed to produce similar results. Evaluation of eight lots of TX-45 (0.1% w/v) revealed that three produced no enhancement while five increased NAA sorption. A TX-45 lot that enhanced NAA sorption also increased NAA transcuticular penetration of isolated tomato fruit CM in a finite-dose diffusion system. Again, the CM waxes played a critical role. Based on capillary gas-liquid chromatography, no obvious differences were found in the ethoxymer profile of selected nonenhancing and enhancing TX-45 surfactants that could be related to performance.

KEYWORDS: cuticular penetration, epicuticular waxes, cuticular waxes, naphthylacetic acid, sorption, finite-dose, surfactants

Foliar application of agrochemicals in aqueous sprays is common practice in crop production. Aerial plant organs are covered with a noncellular, lipoidal membrane, the cuticle, which is considered to be the prime barrier to penetration (Norris and Bukovac, 1968; Martin and Juniper, 1970; Bukovac et al., 1981). The cuticular surface is usually covered with waxes, which are difficult to wet and can lead to low spray droplet retention and poor coverage (Eglinton and Hamilton, 1967; Johnstone, 1973; Bukovac, 1976;

[1]Research Assistant, Department of Horticulture, Michigan State University, East Lansing, MI 48824.
[2]Professor, Department of Horticulture, Michigan State University, East Lansing, MI 48824.

Price, 1982; Baker, 1982). For maximum biological activity of systemic compounds (e.g. plant growth regulators, herbicides, etc.), the active ingredient must be retained, penetrate the cuticle and be translocated to a site of action.

Surfactants are regularly used in formulation and spray application of agrochemicals to not only improve spray solution characteristics (e.g. solubility, emulsification, surface tension, etc.), but also to increase the interaction of the spray and active ingredient with the cuticular membrane, namely wetting, retention, coverage and penetration (Behrens, 1964; Ford et al., 1965; Parr and Norman, 1965; Foy and Smith, 1969). It is well established that surfactant chemistry plays an important role in spray performance (Temple and Hilton, 1963; Smith and Foy, 1967; McWhorter, 1982; Lownds et al., 1987; Baker and Hunt, 1988; Hamburg and McCall, 1988; Silcox and Holloway, 1989). Even within a chemical surfactant class, the number of ethoxy groups may affect the interaction of the spray and the active ingredient with the cuticular membrane (Shafer and Bukovac, 1987; Stevens and Bukovac, 1987a,b; Shafer et al., 1989). We have previously shown that within the Triton X surfactant series, Triton X-45 markedly enhances sorption, an early event in the penetration process, of naphthylacetic acid (NAA) relative to oligomers with longer or shorter ethoxyethylene chains (Shafer et al., 1989; Bukovac et al., 1990; Shafer and Bukovac, 1991). Further, this enhancement appears to be related to an interaction with the cuticular waxes. In this report we characterize the nature of the Triton X-45 response on cuticular sorption and penetration of NAA and show that this effect may vary among surfactant lots.

MATERIALS AND METHODS

Isolation of tomato fruit cuticular membranes

Disks (17.5 mm in diameter) of epidermal tissue were excised by corkbore from regions free of visual defects of field grown mature tomato fruit (*Lycopersicon esculentum* Mill. cv. Sprinter, PikRed, and Sun Rise). The excised disks were incubated at room temperature in a buffered solution (50 mM sodium citrate, pH 4.0) containing 4,000 units/liter cellulase (Sigma Chemical, St. Louis, MO), 20,000 units/liter fungal pectinase (ICN Pharmaceuticals, Costa Mesa, CA), and 1 mM NaN_3 to prevent microbial growth (Orgell, 1955; Yamada et al., 1964). Enzyme solution was changed every 3 to 4 days, for at least 4 cycles or until the CM separated from the underlying tissue. The CM were extensively rinsed and any remaining cellular debris was removed under deionized (DI) water. CM minus the epicuticular waxes were prepared by briefly dipping (4 x 1 s) whole tomato fruit in chloroform prior to excising the CM disks. Cuticular waxes were removed from isolated CM by batch extraction at 50°C with chloroform:methanol (1:1 v/v) over 72 h with a minimum of 10 solution changes. CM treated in this manner were designated as dewaxed cuticular membranes (DCM). The CM and DCM were air dried flat on filter paper, and stored at room temperature. Isolated tomato fruit cuticular waxes, for use as a sorbent, were extracted by momentarily dipping (4 x 5 s) whole fruit in chloroform. The extract was filtered, dried over anhydrous Na_2SO_4, and reduced in volume with a rotary evaporator at 50°C.

Chemicals

NAA treatment solutions, 7.9 x 10^4 disintegration per min (dpm)/mL of 2-(1-naphthyl)[1-^{14}C] acetic acid (specific activity- 2.3 GBq·mmol^{-1}, 97% radiochemical purity, Amersham Corp., Arlington Heights, IL) were prepared with a sodium citrate buffer (20 mM, pH 3.2, and 1 mM sodium azide to prevent microbial growth). Surfactants (Triton X series, Rohm and Haas, Philadelphia, PA) were commercial preparations (no further purification prior to use) of α-[4-1,1,3,3-tetramethylbutyl)-phenyl-ω-hydroxypoly (oxy-1,2-ethanediyl) with an average of 3, 5, 7.5, 9.5, 12.5, 16 oxyethylene groups (Triton X-35, 45, 114, 100, 102, 165, respectively). Samples from random Triton X-45 (EO 5) lots were procured from the manufacturer and colleague holdings and coded by letter and a + (enhanced sorption) or - (no effect or depressed sorption) to simplify presentation (see Table 3). All surfactant concentrations were based on weight/volume (w/v), prepared with sodium citrate buffer.

Measurement of Sorption

Sorption for CM/buffer and DCM/buffer systems was measured utilizing the procedure of Riederer and Schönherr (1984). Randomly selected CM discs were cut into 1 mm wide strips and a 5 mg subsample was placed in a 15 x 45 mm (1 dram) glass vial with a Teflon lined cap. Aliquots (1.5 mL) of the buffered treatment solutions, containing ^{14}C-labeled NAA (79,000 dpm/mL), were pipetted into each vial. Vials without cuticle strips served as treatment blanks to correct for any sorption of NAA to the glass and Teflon cap. Vials were submerged in a water bath (25 ± 0.5 °C) and shaken horizontally. The sorbate was sampled at designated times (48, 96, 192, 720 h; equilibrium by 192 h) by transferring a 100 μL aliquot to a 20 mL scintillation vial and adding 10 mL of scintillation cocktail, either Brays (1,4-dioxane, containing 100 g naphthalene and 5 g PPO per liter) or Safety Solve (Research Products International, Mt. Prospect, IL). Radioactivity was determined by liquid scintillation spectrometry. Because the level of quenching was constant throughout an experiment, all calculations were performed using dpm. The amount of NAA sorbed was calculated by difference (Kipling, 1965): blank value minus sorbate value = amount sorbed by the cuticle phase. When desired, an apparent partition coefficient for a specific buffer pH (K^{pH}) was calculated by the equation:

$$K^{pH} = \frac{\text{dpm in cuticle phase } [\text{Bq} \cdot \text{kg}^{-1}]}{\text{dpm in supernatant phase } [\text{Bq} \cdot \text{kg}^{-1}]}$$

NAA sorption by various waxes

NAA sorption by various waxes was investigated by using isolated tomato fruit cuticular wax, filtered beeswax or commercial paraffin as sorbents. Two mg of wax, dissolved in chloroform, was plated onto the bottom of glass vials (15 x 45 mm) and allowed to thoroughly air dry overnight. Sorbate solutions were added and vials incubated

vertically in a waterbath (25°C) with gentle agitation. Care was taken not to dislodge the wax while sampling.

Surfactant pretreatment of CM

Enhanced sorption of NAA by an enhancing lot of TX-45 may be related to formation of aggregates or hemimicelles with the waxes of the CM. The NAA in the sorption solution then becomes solubilized in these aggregates and this component is measured as sorbed by the CM in our system. This possibility was evaluated by pretreating tomato fruit CM with either 1.5 mL buffer, an enhancing (TX-45I+) or nonenhancing (TX-45E−) surfactant lot (0.1%) for 24 h at 25°C with agitation. The pretreatment solution was then aspirated and NAA sorption was determined from buffer containing an enhancing or nonenhancing lot of TX-45 (see Table 4 for treatment combinations). Control vials containing CM were pretreated with buffer.

Finite-dose penetration system

A droplet/cuticle/aqueous receiver diffusion system was used for examining the effect of selected TX-45 lots on NAA penetration (diffusion) through the isolated tomato fruit CM. The apparatus and methodology have been described by Bukovac and Petracek (1993). Briefly, tomato fruit cuticle discs were mounted between two donut shaped plexiglass holders. The cuticle holder was mounted between two glass half U-cells with vacuum grease and a spring clamp. Buffer was added to each side-arm to hydrate the CM and an additional quantity (~3 mL) was added to one of the side-arms to establish hydrostatic pressure to test for leakage for a minimum of 24 h. The holders, with the CM outer morphological surface oriented to the ambient air, were attached with vacuum grease and joint clamps to Pyrex receiver cells with a 3 mL solution volume (pH 3.2 HCl adjusted DI water) equipped with a spin fin and a sampling side arm. Diffusion units were arranged on a multi-position magnetic stirring base held at 23 ± 2°C. Relative humidity ranged from 12% to 79%. NAA treatment solutions were prepared in DI water adjusted to pH 3.2 with HCl and labeled with 2.6 x 10^4 dpm/μL radiolabeled NAA. Transcuticular penetration was initiated by applying one 3 μL droplet with a microsyringe onto the cuticle. Droplets dried in ~25 to 35 min. The receiver solution was sampled (0.5 mL) via the sampling arm at 1, 2, 4, 6, 8, 10, 12, 24, and subsequently at 24 h intervals for 120 h. Later sampling was at 2 to 4 day intervals. Sample volume was replaced with pH 3.2 adjusted DI water. Radioactivity was determined as previously described and used to calculated the fraction (%) of the radioactivity applied that penetrated through the cuticle. The initial penetration rates were determined by regression of five data points during the near linear phase (4 to 12 h) of penetration.

Capillary gas-liquid chromatography

Surfactant ethoxymer distribution was examined on selected Triton X-45 lots by capillary GLC using a Varian 3700 Gas Chromotograph, equipped with a J&W (J&W Scientific, Fulsome, CA) DB-1 30 m x 0.331 mm fused silica column (25 μm film

thickness). Surfactants were diluted to 1 mg/mL with HPLC grade chloroform, and octanol (1.0 mg/mL; Gold Label, Aldrich Chemical Co., Milwaukee, WI) was included as an internal standard. The injection volume was 2 μL. Helium carrier flow rate was 1.5 mL/min. Injector and FID detector temperatures were 230° and 330°C, respectively, and the column oven temperature was programmed for a 6°C/min rise from 50-320°C with a 5 min hold at 320°C.

Surface tension

Equilibrium surface tension was measured on selected Triton X-45 surfactant lots with a Fisher surface tensiometer (Model 20; Fisher Scientific, Pittsburgh, PA) using the du Noüy ring method. Measurements were made on 20 mL of surfactant solution, 0.1% w/v in DI water, in acid-washed glass petri dishes (48 mm internal diameter) prerinsed with the appropriate surfactant lot. Duplicate measurements were made on each surfactant lot and replicated five times.

Statistics

All studies were performed with a minimum of five replications. Sorption and penetration parameters (partition coefficient, amount sorbed), simple statistics (means and standard error), and linear regressions were calculated using Lotus 1-2-3 (Lotus Development Corporation, Cambridge, MA) or Excel (Microsoft, Bellingham, WA).

RESULTS AND DISCUSSION

EO Effect

A marked increase in NAA sorption by tomato fruit CM was observed with TX-45 (TX-45B+, Table 3) and TX-114 relative to Triton surfactants with a lower or higher EO content at 0.1% (Fig. 1). The apparent partition coefficient for TX-45 (5 EO) and TX-114 (7.5 EO) was significantly higher than for Tritons X-35, X-100, X-102 and X-165 having 3, 9.5, 12.5 and 16 EO groups respectively, and about 50% greater than for the buffer control. NAA sorption was depressed by about 20% by Tritons X-35, X-100, X-102 and X-165 relative to the buffer control. This suppression is related to micelle solubilization of NAA since all surfactants were present at a concentration above their critical micelle concentration (cmc) (Shafer and Bukovac, 1988; Heredia and Bukovac, 1992). Although TX-45 and TX-114 were also present at a concentration (0.1% w/v) above their cmc (0.005 and 0.009% by wt, respectively), they unexpectedly increased sorption in our system, and appeared to have a uniquely different effect.

The ability to enhance sorption, and perhaps penetration (since sorption is the initial event in CM penetration), of foliar applied chemicals would be significant in increasing the efficiency of pesticide application. We further explored this phenomenon, focusing on Triton X-45 because we observed a different response with another Triton X-45 lot and wanted to avoid working near the cloud point (22°C) of Triton X-114. NAA was chosen as a model compound typical of weak organic acid growth regulators

and herbicides.

One may visualize that the increase in NAA sorption may be related to 1) sorption to newly created sites or to increased penetration into previously unaccessible regions of the CM if the surfactant induced swelling of the polymeric matrix of the CM or 2) solubilization of NAA by surfactant aggregates (hemimicelles) formed in association with the cuticular surface (Levitz and Van Damme, 1986).

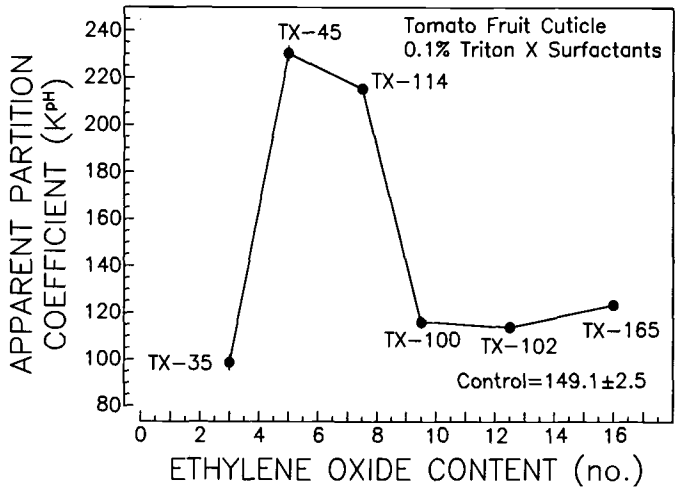

FIG. 1--Effect of ethylene oxide chain length of Triton X surfactants on NAA partitioning into enzymatically isolated tomato fruit cuticle.

Role of Waxes Associated With the Cuticle

Preliminary studies established that waxes associated with the CM played an important role in the enhancement of NAA sorption by TX-45 (Bukovac et al., 1990). We now extend these studies to show that TX-45 enhanced NAA sorption in the presence of the entire CM wax complement (i.e., epicuticular and cuticular waxes) and in the presence of the cuticular waxes after selective removal of the epicuticular wax (Table 1). TX-45 increased NAA sorption by the CM by about 18% over the control and about 49% over TX-100 at 0.1% w/v. Similar results were obtained after removal of only the epicuticular wax (surface wax). However, on removal of both the epicuticular and cuticular (embedded) waxes, the TX-45 effect was lost, NAA sorption being about 13% less than the control and equal to TX-100 (Table 1). Interestingly, NAA sorption in the presence of TX-45 was equivalent to that achieved by dewaxing the cuticle, 66.6 versus 65.4 μmole/kg. Further, NAA sorption by DCM in the presence of TX-45 was 17% less than by CM while increases of 16 and 27% were obtained for buffer only and TX-100, respectively. In DCM, both TX-45 and TX-100 resulted in less NAA sorption than the control, again the response being related to micelle solubilization of NAA with a

TABLE 1--Effect of Tritons X-45 and X-100 on NAA sorption (μmole/kg CM after 192 h) by isolated tomato cuticular membranes (CM) with and without waxes.

Buffer + surfactant (0.1% w/v)	Cuticular membrane		
	With waxes[1] (CM)	Minus epicuticular wax[2]	Minus epicuticular and cuticular waxes (DCM)[3]
Buffer only	56.6b[4]	58.0b	65.4a
Triton X-45	66.6a	67.1a	56.9b
Triton X-100	44.7c	46.5c	56.6b

[1]CM as isolated.
[2]Epicuticular wax removed from fruit by four successive 1 s dips in chloroform before isolation of CM.
[3]Cuticular and epicuticular waxes removed (DCM) by batch extraction with 1:1 methanol:chloroform; 50°C, ten changes over 3 days.
[4]Means within a column with the same letter are not significantly different, DMRT, P=0.05.

corresponding reduction in driving force for sorption (Shafer and Bukovac, 1989; Heredia and Bukovac, 1990).

Further information for the role of waxes in the TX-45/NAA sorption effect was obtained by using isolated epicuticular wax as the sorbent. Triton X-45 increased NAA sorption by waxes isolated from tomato fruit CM by about 8-fold over the control (Table 2). A similar trend was observed when beeswax was used as the sorbent (Table 2). Results on sorption by paraffin were not significant, the values being low and variable.

TABLE 2--Effect of Triton TX-45 surfactant on NAA sorption (μmole/kg wax after 192 h) by waxes from different sources used as the sorbent.

Buffer + surfactant (0.1% w/v)	Wax source[1]		
	Tomato fruit	Beeswax	Paraffin
Buffer only	15.8b[2]	6.7b	0.6a
Triton X-45	121.3a	24.2a	2.9a

[1]2 mg/vial.
[2]Means within a column with the same letter are not significantly different, DMRT, P=0.05.

These data demonstrate that the waxes associated with the CM mediate the enhancement of NAA sorption induced by TX-45. Also, the increased sorption is not the result of TX-45 effects on the cuticle's base polymer, since no enhancement was

observed after dewaxing the cuticle and a similar increase in NAA sorption was observed with waxes isolated from tomato fruit and with unrelated beeswax.

Differences Among Surfactant Lots

When a replacement lot of TX-45 (#1013, denoted as lot E−, Table 3) was used, the marked enhancement of NAA sorption was not observed. This led to an evaluation of several TX-45 lots obtained from the manufacturer (Rohm and Haas) and university and industry colleagues. The evaluation of eight different lots (coded B through I) on NAA sorption by tomato fruit CM after 192 h showed that five enhanced NAA sorption by 8 to 25%, one had no effect and two depressed sorption by 5 to 9% (Table 3). Thus, those lots which increased sorption over the control were designated plus (i.e., B+, C+, D+, G+, I+) while those that did not enhance sorption were designated minus (i.e., E−, F−, H−).

Based on a long-term time course, NAA sorption reached equilibrium quickly (48 to 96 h) in the presence of TX-45 lots that enhanced sorption (data not presented). NAA sorption equilibrium was reached in buffer alone within 96 h. NAA sorption in the presence of TX-45 lots that did not enhance sorption, was consistently less than the NAA control until about 288 h; thereafter they did not differ significantly from the control. Lot G+ approached equilibrium by 192 h (8% greater than control) and increased an additional 4% over the next 528 h.

TABLE 3--A comparison of the effect of various lots of Triton X-45 at 0.1% w/v on NAA sorption by isolated tomato fruit cuticular membranes.

Triton X-45 lot number-code	NAA sorption[1] (μmole/kg CM)	Percent of control	Surface tension ($mN \cdot m^{-1}$)
Control	58.7c[2]	100	71.2±0.04
8160-B+	71.9a	122	28.6±0.03
9422-C+	73.2a	125	28.8±0.03
9500-D+	73.5a	125	28.8±0.02
1013-E−	55.9d	95	28.6±0.07
3914-F−	59.0c	101	28.7±0.03
6687-G+[3]	63.4b	108	28.8±0.03
6203-H−	53.5e	91	28.9±0.03
7501-I+	72.8a	124	28.8±0.05

[1]Sorption after 192 h.

[2]Means within a column with the same letter are not significantly different, DMRT, P=0.05.

[3]Sorption on long term time-course equaled control.

Concentration Response

Comparison of the concentration response of an enhancing (TX-45B+) and nonenhancing (TX-45E-) lot on NAA sorption gave similar curves, except the TX-45E- curve was displaced to the left, reflecting lower sorption at concentrations of 0.1% to 1% (Fig. 2). There were no differences between the two lots at concentrations below 0.075%. The sorption optimum for TX-45B+ appeared between 0.075% and 0.1% with a 26% increase above the control, while the sorption optimum for the nonenhancer was 0.05% with a 15.8% increase over the control. Both lots showed a precipitous decrease in sorption between 0.1% and 1.0% as has been previously found for other Triton X surfactants at concentrations above the cmc (Shafer and Bukovac, 1987).

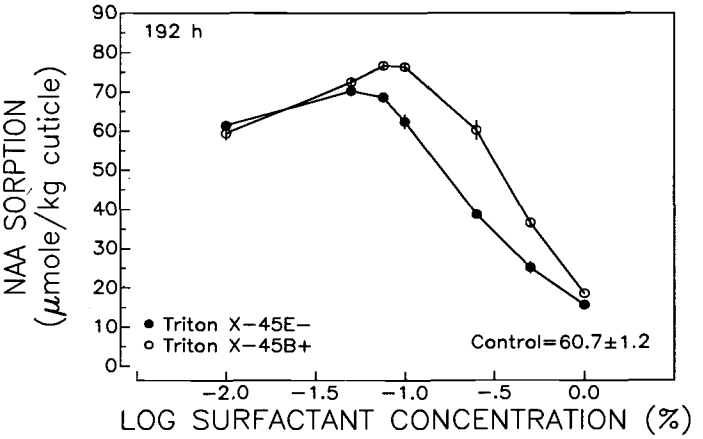

FIG. 2--Concentration response of a nonenhancing (TX-45E-) and enhancing (TX-45B+) lot of Triton X-45 on NAA sorption by enzymatically isolated tomato fruit cuticle.

Effect of Pretreatment

Pretreatment with a nonenhancing lot (TX-45E-) had no effect on NAA sorption from buffer or from buffer containing an enhancing TX-45I+ lot (Table 4). Pretreatment of CM with the nonenhancing lot slightly, but significantly, decreased (61.0 versus 58.1 μmole/kg) sorption of NAA from sorbate solution containing the nonenhancing lot and dramatically depressed the effectiveness of pretreatment with the enhancing lot (75.2 versus 59.8 μmole/kg). Pretreatment with the enhancing lot increased NAA sorption from buffer (67.6 versus 75.2 μmole/kg) and further increased NAA sorption when the sorbate solution contained the enhancing lot (80.7 versus 85.2 μmole/kg), but had no effect on NAA sorption when the nonenhancing TX-45E- was present (80.7 versus 78.9 μmole/kg). There was a significant pretreatment x sorption interaction, i.e., sorption of

NAA was affected by pretreatment (Table 4). Thus, these data show that pretreatment with the enhancing lot conditions the CM, perhaps by forming stable surfactant

TABLE 4--Effect of pretreatment of isolated tomato fruit CM with Triton X-45 nonenhancing (E-) and enhancing (I+) lots on NAA sorption.

Sorption solution[1]	Pretreatment solution[1]		
	Buffer	Triton X-45E-	Triton X-45I+
		(μmole/kg CM)	
Buffer	67.6d[2]	67.9d	75.2c
Triton X-45E-	61.0e	58.1f	59.8ef
Triton X-45I+	80.7b	78.9b	85.2a

[1]20 mM sodium citrate buffer, pH 3.2; surfactants at 0.1% w/v; pretreatment for 24 h, sorption for 192 h.
[2]Means followed by the same letters are not significantly different by DMRT at P=0.05. Pretreatment x sorption interaction significant at > P=0.001.

aggregates or hemimicelles, that increase subsequent sorption of NAA from buffer or sorbate solution containing the enhancing lot (Table 4). While pretreatment of CM with a nonenhancing lot does not prevent the effect of an enhancer, the presence of the nonenhancer in the sorbate solution severely depresses NAA sorption by CM pretreated with the enhancing lot (Table 4).

Effect on Penetration

NAA penetration through isolated tomato fruit CM was measured using a finite-dose diffusion system where the NAA was applied to the cuticle as a droplet simulating a foliar spray (Fig. 3A). Both TX-45I+ and nonenhancing TX-45E- surfactants increased the initial rate of penetration by 165% and 79%, respectively. Both surfactant lots also increased the maximum amount that penetrated over the control. However, the effect of the enhancing lot was significantly greater than the nonenhancing lot. Maximum NAA penetration after 312 h with the enhancing lot was significantly greater than with the nonenhancing lot and control, while there were no differences between the nonenhancing lot and control (Fig. 3A). There was no significant effect of either TX-45 surfactant lots on NAA penetration through dewaxed cuticles (Fig. 3B). NAA penetration was greater for all treatments through the DCM than CM, reflecting the removal of the wax barrier.

It is significant that a Triton X-45 lot that enhanced NAA sorption (Fig. 1) also increased NAA penetration through isolated tomato fruit cuticles (Fig. 3). In this study, TX-45 enhancing lots increased sorption (TX-45B+, Fig. 1, Table 3) and also penetration (TX-45I+) through the CM, but did not significantly enhance penetration through cuticles

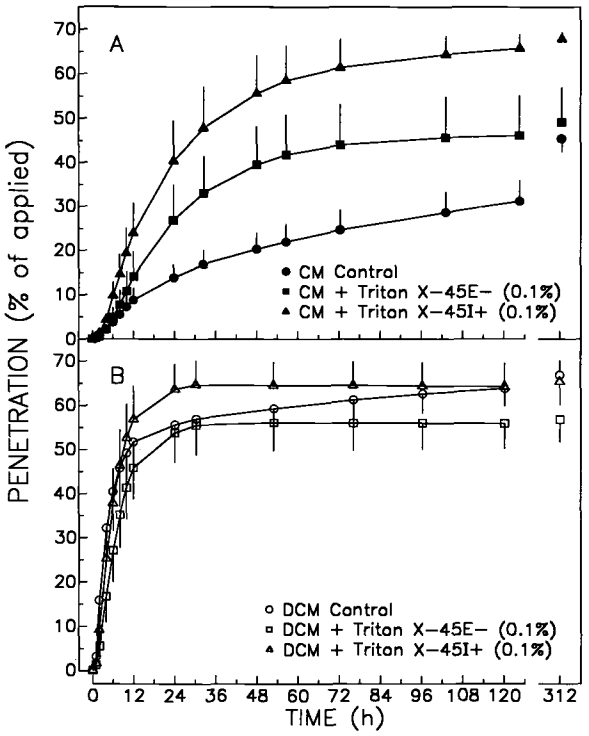

FIG. 3--Comparison of the effect of two lots of TX-45, one which enhances sorption (I+) and one which does not (E-), on penetration of NAA through isolated tomato fruit cuticle (CM) and dewaxed CM (DCM).

from which waxes were removed (Fig. 3B). Some surfactants can increase penetration through the cuticle by increasing NAA partitioning into the CM (solubility in CM) or by increasing diffusion across the CM (mobility in CM). Triton X surfactants with EO groups in the range of 5 to 10 have been shown to increase NAA diffusion coefficients (Knoche and Bukovac, 1993). The basis for the response in this study appears to be primarily by increasing sorption (Fig. 1). If surfactant complexes (aggregates, hemimicelles) are formed in conjunction with the cuticular waxes, they may solubilize NAA resulting in a localized high concentration of NAA on the surface, thus, increasing the driving force for penetration. Alternatively, the complexes may solubilize some cuticular waxes by forming mixed hemimicelles, thus reducing the effectiveness (resistance) of the wax barrier. The failure for the enhancing lot (TX-45I+) to increase NAA penetration through dewaxed cuticles (Fig. 3B) may be the result of the failure to form surfactant aggregates on the DCM, since the waxes were not present and waxes were found to be essential for enhancement of sorption (Table 1).

Observations on Enhancing and Nonenhancing Surfactant Lots

Identification of the factor(s) responsible for increased cuticular sorption and penetration would be important not only for a better understanding of the mechanisms involved, but may provide a basis for increasing efficiency of foliar application of pesticides. A GLC comparison of the eight TX-45 lots (Table 3) revealed no apparent qualitative differences that could be associated with the enhancing or nonenhancing lots. GLC traces of two strong enhancers (TX-45I+ and B+) and two without effect (TX-45E- and H-) were very similar (Fig. 4), particularly lot TX-45I+ and TX-45H- representing the extremes in their effect on sorption, namely 24% enhancement with TX-45I+ and 9% depression with TX-45H- (Table 3). None of the peaks examined are associated exclusively with the enhancing surfactants TX-45B+ or TX-45I+ (Table 5). Either a peak in one enhancer does not appear or has a much lower magnitude than in the other enhancer (peaks 2, 4, and 9), or the magnitude of a peak for one or both of the enhancers also appears in one of the non-enhancers (peaks 3, 5, 6, 7, and 8). This suggests that the enhancing effect may be due to a minor component, not obvious in these profiles. All TX-45 lots were equally effective in reducing surface tension at 0.1% w/v (Table 3).

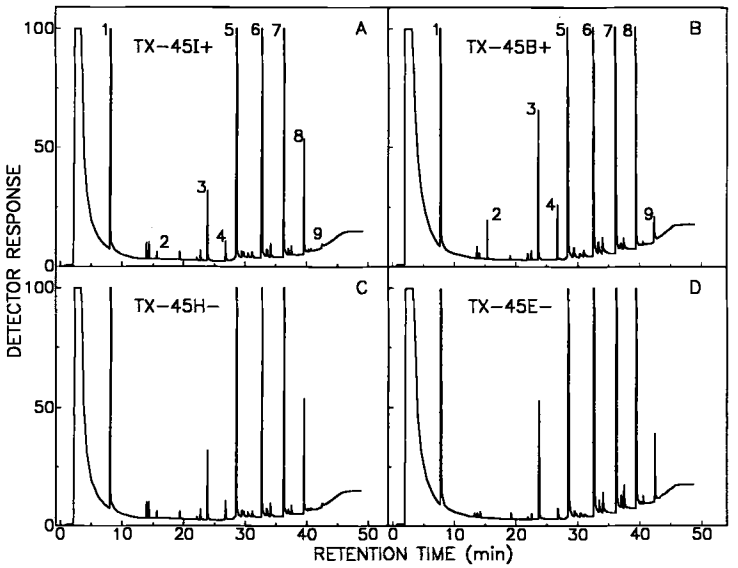

FIG. 4--Capillary gas-liquid chromotographic traces (2μg in 2μL injection volume) of four lots (I+, B+, H-, E-) of Triton X-45 surfactants with octanol (peak 1) as internal standard. Two lots, TX-45I+ (A) and TX-45B+ (B), increased NAA sorption by 22% to 24% while TX-45H- (C) and TX-45E- (D) decreased sorption by 9% and 5%, respectively.

There are some observations on the appearance of the surfactant solutions that may be of interest. There were distinct differences in the opaqueness of 0.1% solutions of the E–, B+ and I+ lots, with B+ and I+ solutions being more opaque. When allowed to stand undisturbed for a few days, the E– solution becomes clear with a distinct opaque phase covering the vessel bottom, while the B+ or I+ solutions contain scattered clear globules. The upper phases of clarified B+ and E– solutions continue to enhance and inhibit NAA sorption, respectively, but at a lower level than a corresponding unclarified solution.

TABLE 5--A comparison of selected capillary gas-liquid chromatographic elution peaks from four lots of Triton X-45 surfactant.

Peak no.[1]	Retention time (min)	Percent of internal standard			
		TX-45I+	TX-45B+	TX-45H–	TX-45E–
1[2]	7.36	100.00	100.00	100.00	100.00
2	15.20	0.01	0.69	0.13	0.15
3	23.57	2.85	2.67	1.56	2.35
4	26.59	0.35	1.35	0.50	0.33
5	28.35	17.82	20.97	12.33	20.78
6	32.54	20.65	29.92	12.54	31.80
7	36.20	12.09	22.52	11.55	27.64
8	39.51	2.76	7.81	2.54	11.54
9	42.64	0.08	0.41	0.30	1.98

[1]See Fig. 4.
[2]Internal standard.

CONCLUSIONS

We found that some production lots of Triton X-45 (5 EO) markedly increased sorption by and penetration through isolated tomato fruit cuticles relative to Triton X surfactants with shorter (3 EO) or longer (7.5 to 16 EO) oxyethylene chains. Of eight lots examined, five increased NAA sorption by 8% to 25% and three decreased (5% to 9%) or had no effect. A surfactant lot (I+) that increased NAA sorption also increased (64% after 192 h) penetration across isolated tomato fruit cuticles. Enhancement of both sorption and penetration occurred only in the presence of cuticular waxes, suggesting that these surfactant lots dramatically reduced the effectiveness of the wax barrier to sorption and penetration. The factor(s) responsible for this response is not known, but it does not appear to be related to differences in oxyethylene distribution or surface tension properties of the surfactant lots.

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