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FINAL REPORT

PROJECT NO. I-82-80

**Slow-Release Water-Borne Microcapsules for
Incorporation of Pesticides into Soils.**

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C.S. Helling**

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מרכז סיגרם למדעי
הקרקע והמים

Slow-Release Water-Borne Microcapsules for
Incorporation of Pesticides into Soils

Final Report

BARD Research Project No.I-82-80

Written by

Yechezkel Mualem

June, 1985

ABSTRACT

The objectives of this study were to produce microcapsules, investigate their slow release characteristics, dispersion in water, mobility in granular soil and effectiveness in bioassay tests.

Two herbicides, atrazine and trifluralin, were selected for encapsulation. The two herbicides represent chemicals with low to very low mobility in soil, and thus have to be incorporated to soil mechanically. By encapsulation the atrazine and the trifluralin may acquire mobility via irrigation in coarse structured soils. When applied mechanically, the encapsulated pesticides are expected to be better distributed in the soil than the free chemicals and coupled by their slow release property increase their effectiveness.

Formulated pesticide had undergone a series of tests including density measurement, size distribution, stability in suspension and chemical analysis to quantify and validate their content. Slow release in mixed water and in hydrostatic conditions were observed as this property was expected to bear a significant effect on the actual pesticide active time.

Mobility of the microcapsules in saturated soil was estimated on basis of breakthrough experiments and measurement of recovery, for various lengths of soil layers.

Bioassay tests of selected formulation were carried out in growth chambers using sensitive plants. Controls and unformulated pesticides were included in each chamber for comparison. Thereby, the effect of the microencapsulation and the slow release in unsaturated soil on availability of active pesticide, was studied.

In the three years research project, microcapsules of herbicides were produced and examined by a series of preliminary physical and biological

tests. The experimental results indicate in some cases very significant improvements in efficiency achieved by the microencapsulation. It indicates that investigation of the applicability of pesticide microcapsules may have important contributions to agriculture. However, our most difficult problem was production of microcapsules with desired chemical and physical properties. Research should be continued with better products of microcapsules.

INTRODUCTION

Pesticides used on croplands are applied either directly to foliage or to the soil. The effectiveness of soil-applied pesticides depends mainly on maintaining a sufficient concentration within the proper soil zone. Because of such problems as degradation, adsorption and volatilization losses, the active concentration diminishes with time, often too quickly. As a consequence, soil application of pesticides requires relatively large dosages which might be expensive and/or have health hazards.

In some cases, the limited mobility of otherwise efficient pesticides are a very significant restrictive factor. For example, Thiabendazol Benomyl are effective compounds against vascular diseases once they are absorbed by the plant roots. However, only limited use can be made of them in the field, since their mobility in soils is very low and they are inactivated on their way from the application site to the roots. In fact, none of the existing methods of application are able to overcome this problem.

If, instead, pesticides could be applied in some manner whereby degradation and deactivation rates are restricted while mobility is controlled, some serious problems associated with their conventional use would have been reduced.

Numerous techniques for preparation of controlled release microparticles (name microencapsulations) have been described. There are two main types (I) The reservoir device where the agent is enclosed within an inert membrane. The chemicals diffuse out under concentration gradient between the solution on either side of the membrane. (II) The mono-lithic device where agent is spread as a solid throughout the spherical matrix. Extensive surveys are available (Scher, 1973; Tanquary & Lacey, 1974; Kedonius, 1980).

Incorporation of agrochemicals into soils by microcapsules dispersed in irrigated water has not been reported in scientific literature. Therefore, discussion is limited to related aspects of encapsulation and movements of particulate matter in porous media.

Biological and engineering aspects related to controlled release technology have attracted increasing attention and widely reviewed (Das, 1983). Results of bioassay tests and application of pesticide microcapsules are reviewed in the attached report by Dr. Helling.

It is conceivable that scientific and technological findings related to movement of microorganisms and colloids in porous media might be relevant (at least qualitatively) to our study. Complementary background may be found in theories on hydrodynamic chromatography (Small, 1974) and potential barrier chromatography (Ruckenstein and Prieve, 1976; Ruckenstein et al., 1977). Different types of particles have been investigated, the most common being algae, bacteria, viruses and inorganic colloids. A few studies have been published on fungi spores, nematodes, Schistosoma cercaria and synthetic particles. The majority of these studies have been laboratory experimented with sand, silica or diatomite, active carbon gravels and glass beads.

For a given system there exists a size of suspended particles for

which the mobility is a maximum (Yao et al., 1971; Folkman and Wachs, 1970). For particles larger than this size, the dominant retention mechanism is sedimentation and/or interception. For suspended particles considerably smaller than 1, the dominant mechanism is diffusion.

Microparticles must have surface properties which cause them to form a stable suspension. Flocculation decreases the mobility of microparticles in porous media drastically. The ability of suspended particles to associate into floccules, or to attach to the surface of the stationary phase can be explained by the double layer theory. Since the sign and intensity of the electrical double layer can be affected by changing the properties of the ambient solution, such changes will also affect the attachment efficiency. However, the double layer model (including van der Waal's interactions), can provide only explanation for the attachment step, and models invoking chemical bridging or specific adsorption have been proposed (O'Melia and Stumm, 1967) to account for systems which do not fit the electrical double layer model.

Particle density, temperature and suspended particles concentration may also affect the microparticles mobility in porous media. Retention by sedimentation increases with higher particle density. This effect is enhanced when the particle size is increased. Higher temperature increases the effectiveness of diffusion, flocculation and sedimentation mechanisms. However, since temperature also affects all the attachment mechanisms, its net effect on retention can neither be easily interpreted nor predicted (Hunter and Alexander, 1963).

As a first approximation, the transport efficiency may be considered independent of the applied concentration (Yao et al., 1971). Folkman and Wachs (1970) found that higher filtration was obtained with lower initial

concentration of algal suspension. Obviously the postulate made by Yao et al. refers to very stable particle suspension; the discrepancies between the above-mentioned findings may be explained by the low stability of the algal suspension.

A few investigations have been carried out on real soils by Burges, 1958; Krone et al. 1958; Drewry & Eliassen, 1968; Griffin, 1970, and Wollum & Cassel, 1978. Based on theoretical considerations, it was concluded that irregularities and rough surface geometry characterizing real soils increase the role of interception and diffusion in the translation mechanism of particulate matter. Consequently, decreasing water content results in greater retention of suspended particles.

The importance of water content was demonstrated both for nonconvective (Griffin, 1970) and for convective movement of bacteria in soils (Bitton et al., 1970). The latter investigators measured the concentration of Klebsiella aerogenes in sandy soil columns wetted by placing them in bacterial suspension. Their results show that the concentration of mobile bacteria was practically zero at water tensions higher than $1/3$ atmosphere but remained essentially unchanged through most of the wet zone, where water was held at lower tensions. Krone et al. (1958) have reported on the ability of coliform bacteria to move through partially saturated sand columns, where the equivalent diameter of the pore was 0.15 mm.

Burges (1958) measured the vertical movement of fungal spores in sandy soil columns. He found that spores with hydrophylic surfaces moved almost freely whereas spores with hydrophobic surfaces stayed in the upper soil layer and moved only slightly under the same conditions. The water content in this work was not specified. Bitton et al. (1974) also demonstrated the effect of bacterial surface on their mobility in soils.

Lahav and Tropp (1980) conducted preliminary tests on the mobility of monolithic microspheres in sandy soils. Observed breakthrough curves indicate that recovered microspheres in the effluent depend on the flow rate in the soil column.

In conclusion, it is expected that the mobility of the proposed microparticles will depend not only on the physical and chemical conditions prevailing in the soil, but also on the methods of their application. The parameters which can be manipulated in order to change the microcapsules mobility and their efficiency as agrochemical carriers in a given soil are flow rate, chemical composition of irrigation water, soil water content and the microparticles surface properties, size, release characteristics and density. The complex relationships between these parameters must be determined experimentally.

The objectives of the present research are:

- a) To produce microcapsules of pesticides having proper physical and chemical properties.
- b) To study the slow release of chemicals from the microcapsules.
- c) To study some aspects of the phenomena of the microparticle transport in granular soil.
- d) To study the efficiency of microincapsulation on weed control.

We expect to significantly improve the applicability of some pesticides, increase their biological efficiency, and reduce the long-term adverse effect associated with usage of large quantities of pesticides. This method is expected to be very useful for relatively coarse soils, and if successful, would be a significant contribution to modern agriculture, in case the method proves itself effective in the field and cuts costs for the farmer.

DESCRIPTION OF THE RESEARCH WORK

Production of the Microcapsules

Two herbicides, atrazine and trifluralin, were chosen to represent chemicals with low to very low mobility in soil. Both chemicals have very low solubility in water. By encapsulation, the herbicides could be dispersed in water and transported to the root zone by irrigation when applied in case of granular soil. The slow release from these microcapsules was expected to prolong their activity and enhance their effectiveness so that application rate can be reduced to a minimum, compared to doses of application of the commercial formulations. Thereby, we hope, encapsulation of pesticides would diminish pollution hazards as well.

Two different techniques of microencapsulation were applied: (1) Polycondensation at the interface between two phases, and (2) Phase separation. Encapsulation was done in various polymers in order to attain desired properties, namely, prolonged slow release and good dispersion in water. In some cases, double coating of the microcapsules was done to improve slow release characteristics. In order to avoid confusion, we numbered the microcapsules formulation according to their serial number and the initial letters ATRXX and TRFXX for the atrazin and trifluralin products, respectively. For more details on production of the microcapsules, see Appendix A.

Chemical Analysis and Test of the Microcapsule Physical Properties

After encapsulation, the relative content of herbicides was determined on weight basis using samples of the products. Usually, the pesticide content in the microcapsules was independently tested again by another team

member as detailed in the attached reports of A. Markus and C.S. Helling.

The microcapsule size distribution was determined under wet conditions, following production, using a sedigraph. The objective was to produce microcapsules of the size of several microns only, considerably smaller than the large pore size of the soil to which they would be applied, so that they can move readily in the upper soil layer. This objective could not be accomplished in the production process. In principle, it is possible, however, to obtain part of the small size fraction of the microcapsules by wet sieving. This has not been done because of the high cost. Figure 1 shows the size distribution of two atrazine formulations which are characteristic of other products as well. In these two cases, over 65-78% of the microcapsules are smaller than 10 and 75-86% smaller than 15 . While the size of the microcapsules has an extremely important effect on stability in suspension and mobility in soil, small size requirements may reduce the pesticide content in the microcapsules and affect their slow release potential.

The shape of the microcapsules was observed by electron microscope. Our objective was to produce microcapsules having a smooth surface and a spherical shape. We could only partially approach this goal. Figures 2 and 3 show two series of atrazine and trifluralin microcapsules, respectively. While some of the microcapsules are spherical, others have distorted shapes, with cavities, folded coating and residue fragments on their surface. These results are related to the solubility of chemical compound and the applied method of encapsulation. Yet, this property should and can be improved with better equipment and procedures.

Fig. 1. Particle size distribution of two preparation of atrazine microcapsules.

Fig. 2a. The shape of different products of atrazine microcapsules.
The pictures are taken by electron microscope.

Fig. 2b. The shape of different formulations of trifluralin microcapsules.

Fig. 3. A sample of the dispersed microcapsules in the influent and (b) enlarged section of figure 3a. The blank marks in the right bottom of the figures indicate a size of 10μ .

Dispersion and Sedimentation Rate of the Microcapsules

The properties of the microcapsules containing atrazine or trifluraline to disperse in aqueous solution and form a stable suspension were tested for various products. Often the microcapsules tend to form aggregates while in containers. Consequently, a considerable fraction either sank to the bottom or floated when immersed in water. Application of dispersant improved considerably the dispersibility of the microcapsules in aqueous solutions.

The effect of the dispersant on sedimentation rate was tested using spectrophotometer at 440 nm. Figure 4 shows a typical pattern of the microcapsule concentration in suspension as a function of time in case of effective dispersant. The higher the dispersant concentration, the suspension is more stable.

Further discussions of this subject are given in Appendix B and separate report by Y. Muallem and S. Friedman.

Slow release

Slow release tests were carried out to determine the diffusion rate of the chemicals from the microcapsules. This test was carried out only on preparations that displayed other physical properties that met basic standards, reasonable size distribution, enough pesticide content and good dispersion in aqueous solution.

Suspensions of microcapsules at various concentrations were formed and aliquots were taken at various time intervals to determine the concentration of the released chemicals. It was found that often most of the atrazine content in the microcapsules was released very fast, sometimes within minutes from immersion in water. Figure 5 illustrates this property. In other cases, the diffusion rate was faster and in some lower.

Considerably better slow release characteristics were observed for a

Fig. 4. Concentration of atrazine microcapsules in aqueous solution as a function of time for three different concentration of dispersant.

Fig. 5. Slow release characteristic of the atrazine.

trifluralin microcapsule (Figure 6).

There seems to be a clear indication between the slow release characteristics and the efficacy of the various microcapsule preparations displayed in bioassay tests. Unfortunately, the various microcapsule formulations we produced (see report by Dr. Markus) did not have the optimal properties we expected.

More detailed discussion of this aspect is given in following reports in Appendix B and separate report by Y. Muallem and S. Friedman.

Mobility in Soil

The objectives of this part were: (a) to examine some basic principles of microparticles transport in soil, and (b) to establish a standard test for the mobility of the microcapsules on a rather comparative basis.

Clean sand was packed into columns of different lengths but identical cross sections and flow column structure. The bulk density was measured to derive the porosity of the soil. Suspension of the microcapsules with different concentrations using dispersant to improve dispersion was used. The microcapsule concentration in the effluent was measured continuously spectrophotometrically. The hydraulic gradient in the flow column was observed during experiments through piezometers in the inlet and outlet chambers as well as in the soil.

Some preliminary experiments were carried out using Polystyrene Latex microspheres with a nominal diameter of about 1.1 μ .

We found that in case of very small particles that form a rather stable colloidal suspension, the flow behaved as a linear system and superposition approach is quite sound. Recovery of the microparticles that entered the soil almost unity in this case. Differences in the soil

Fig. 6. Slow release characteristic of the trifluralin.

properties were observed, as the amount of suspension that passed through the soil increased. In case of larger particles, the mobility in the soil was gradually inhibited.

Detailed description of the microparticles transport in soil study and analysis of various factors affecting flow is given in a separate report.

Bioassay tests

The efficacy of the encapsulated herbicides depend on both soil and microcapsule characteristics as well as the method of application. The objectives of this part were (a) to attain a good indication about the slow release characteristics of the microcapsules as reflected by bioassay comparative study of encapsulated versus non-encapsulated effect and (b) to test the phenomenon on different soils and plants. Therefore, in the framework of the project, this study was carried out simultaneously in the U.S.A. and Israel.

The herbicide concentration varied according to the plant test. The sensitivity of wheat in Israel and the U.S.A. was found in this study to be extremely different and required doses by order of magnitude higher in Israel to attain the same effect. In wheat and oats, the atrazine content applied to soil on a weight basis, was 0 to 500 ppm, while in the U.S. 0 to 50 ppm. Setaria was found much more sensitive and the atrazine concentration rate was below 2 ppm.

Known amounts of the atrazine preparation under study were carefully mixed with air-dry soil using three "dilution" steps in order to ensure homogeneous distribution, followed by a dropwise wetting of the mixture to the desired water content.

The wet soil was transferred into plastic containers in which and 16 seeds were sown. The amount of soil in each of the containers was 150 g.

Typically, atrazine reduces the germination and growth rates of wheat; at high concentrations it inhibits entirely the germination. This effect is observed when either commercial or pure atrazine is added to the soil. To test the efficiency of the encapsulated atrazine, the preparations were mixed with the soil, wetted and sown with wheat seeds, as explained above. It has been found that the encapsulated atrazine was also effective.

In order to determine the effect of incubation time in the soil on the atrazine effectiveness, the wet atrazine-soil mixtures were incubated for various periods of time before sowing. Typical results are shown in Figure 7 where the pre-sowing incubation time was one month. The results show that the effectiveness of the three preparations decreased as a result of the incubation. Apparently, there is a tendency of the encapsulated atrazine to retain some of its activity better than the commercial atrazine. Similar tendency was observed using Bet-Dagan soil. However, on the overall no statistically significant improvement of bioactivity was attained by the formulation of atrazine. This conclusion is well reflected by the typical results of Figs. 8 and 9 showing the fresh weight of setaria relative to control as a function of active atrazine concentration and the corresponding variation coefficient, respectively. This result is directly related to properties of the produced microcapsules: the fractured shape, relatively low content of active ingredient and the quick release as discussed above.

Bioassay tests were also carried out with microencapsulated formulation of trifluralin to examine their relative efficacy in weed control. Test plants and soils were the same as in the case of atrazine bioassay tests. At harvest, height, fresh and dry weight were measured. The soil was recropped twice after different intervals of time (up to 6

Fig. 7. Wheat seedling length (% of control) 12 days after sowing Rehovot soil.

Fig. 8. Fresh weight of setaria relative to control plant weight as a function of active atrazine concentration in soil (first harvest).

Fig. 9. Coefficient of variance for the formulated and unformulated atrazine as a function of active atrazine concentration in soil.

months from the first crop).

Comparison between formulated and unformulated trifluralin showed three groups of microcapsules: less phytotoxic, identical, and more phytotoxic by the unformulated pesticide. On the whole, however, trifluralin encapsulation seems to be a more promising technique than encapsulation of the atrazine for two reasons: (i) higher persistence in soil and of the trifluralin, and (ii) in some cases considerably higher relative phytotoxicity (Figure 10). For detailed studies, see Appendix C and attached reports by Helling and Pillai, Mualem and Friedman.

Fig.10. Growth response of oats to different application rates of analytical and encapsulated trifluralin (First crop).

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APPENDIX A - ENCAPSULATION OF ATRAZINE AND TRIFLURALIN

Written by Arie Markus

The herbicides atrazine and trifluralin were encapsulated in several types of polymers, both to make them more readily dispersable in water and to prolong their activity.

Capsules enclosed in single and in double shells were made. A second shell was made only for those atrazine capsules that showed good dispersion in water because the first shell did not adequately prevent the atrazine from dissolving in water. The primary encapsulation was done by the technique of polymerization (Kydonieus, 1980) in the interface between two phases. To make the second shell the phase separation technique was used with fatty acids and higher alcohols as the envelope materials. The phytotoxicity of the encapsulated atrazine and trifluralin was checked with plants in growth chambers. The phytotoxicity of trifluralin was stable for at least two months.

Many possible formulations for capsules were tested. This report describes the procedures for making only those formulations that gave the best results for atrazine these were ATR23, ATR 26, ATR 37, ATR 38, ATR 23+Ce^t. and ATR 23+Pal., and for trifluralin, tref 5, tref 6, tref 11, Tref 12, Tref 13, Tref 17, Tref 10,

A large variety of detergents were also tested as dispersants

The chemical equations showing the synthesis of the different envelope materials are shown below.

ATR 23

I toluene -2,4- diisocyanate (TDI)

II p-phenylenediamine

ATR 26 and Tref 12

(Secondary shells for ATR 23 and ATR 26 were made later with palmitic acid and cetyl alcohol.)

ATR 37 and Tref 18

ATR 38 and Tref 17

Tref 5

Tref 6

Tref 11

Tref 13

Pref 14

Sources of materials and analytical methods

<u>Material</u>	<u>Sources</u>
atrazine	Agan chemicals
trifluralin	Agan
TDI	Merck & Co., Inc.
toluene-2,6-diamine	Fluka
palmitic acid	Fluka
1,8-naphthylenediamine	Fluka
1,5-naphthylenediamine	Fluka
2,4-diaminophenol hydrochloride	Fluka
p-phenylenediamine	Merck
Cetyl alcohol	Merck
2-nitro-p-phenylenediamine	Merck
toluene-2,4-diamine	Aldrich Chemical Co.
2,4-diaminoanisole sulfate	Hoechst
polymethylene polyphenylisocyanate	Polyscience
DKS Depressor 801	Daiichi Kogyo Seiyaku

After encapsulation of trifluralin, the amount of fluorine was measured by combustion with oxygen and determination of with an ion-selective electrode. With encapsulated atrazine, chlorine was analysed by the Shöniger method. The shape of the synthesized capsules was determined with a scanning electron microscope (JEOLJSM 35).

EXPERIMENTAL

ATR 23

solution A	500 ml	water
	2.5 g	Tween 80

	20 g atrazine	atrazine was dissolved in the chloroform by heating 1
solution B	150 ml chloroform	
	10 g TDI	

solution C	15 g p-phenylenediamine
	HCl solution

An emulsion of solution A in B was made in a round-bottom flask and the solution C was added. After stirring for 0.5 hr, a solution of NaOH was added to the reaction mixture to bring the pH to 10. The reaction mixture was stirred overnight and filtered. The material retained on the filter was washed with a large amount of water, airdried, and passed through a mesh to obtain the fraction < 80 μ

Anal: Cl, 7.2%

ATR 26

solution A	500 ml water
	2.5 g Tween 80

solution B	20 g atrazine
	150 ml chloroform
	10 g TDI

Solution C	10 g toluene-2,6-diamine dissolved in HCl solution
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Anal: Cl, 6.4%

ATR 37

Solution A 500 ml water
 2.5 g Tween 80

solution B 20 g atrazine
 150 ml chloroform dissolved with heating
 10 g TDI

solution C 1,8-naphthylenediamine in HCl.

The reaction was carried out as for ATF 23.

Anal: Cl, 6.1%

ATR 38

solution A 500 ml water
 2.5 g Tween 80

solution B 20 g atrazine
 150 ml chloroform
 10 g TDI

Solution C 1,5-naphthylenediamine in HCl.

The reaction was carried out as for ATR 23.

Anal: Cl, 4.7 %

ATR 23 + Pal

Water (500 ml) and palmitic acid (2g) were placed in a round-bottom flask and warmed to melt the palmitic acid. ATR 23 capsules were then added. The reaction mixture was stirred for 10 minutes, cooled, and filtered. The capsules were then air dried.

Anal: Cl, 6.8%.

ATR 23 + Cet

The reaction was done in the same way as with palmitic acid but with cetyl alcohol as the second shell.

Anal: Cl, 6.8%

Tref 5

Solution A	500 ml water
	2.5 g Tween
Solution B	20 g trifluralin
	100 ml chloroform
	10 g TDI
Solution C	10 g toluene-2,4-diamine in 1M HCl

An emulsion of solution in A was made and then solution C was added. After stirring for several minutes, the pH of the solution was increased to 10 by adding a NaOH solution. The reaction mixture was stirred overnight and filtered. The material retained on the filter was washed with a large amount of water, air-dried, and passed through a mesh to obtain the fraction < 80 μ in diameter.

Anal: F, 10.1%

Other Tref formulations

All the other trifluralin formulations were made in the same way. The monomers used and the analytical results obtained are summarized in Table I.

TABLE I
Tref Formulations

Capsules	Monomer II *	Analytical results, %F
Tref 6	2-nitro-p-phenylenediamine	15.3
Tref 11	polymethylene polyphenylisocyanate	12
Tref 12	toluene-2,6-diamine	9.5
Tref 13	2,4-diaminophenylhydrochloride	8.8
Tref 14	2,4-diaminoanisole sulfate	10.3
Tref 17	1,5-naphthylenediamine	6.7
Tref 18	1,8-naphthylenediamine	6.4

* Monomer I was TDI in every case

Suspension of capsules

A large variety of detergents and mixtures of detergents were tested as dispersants for the capsules. The detergents that showed the best suspension ability were DKS80 and sodium dodecylbenzenesulfonate. Of these two, DKS801 seemed better, because less of this detergent was needed to make the suspension, and the capsules remained suspended for a longer time.

DKS801 (200 mg) was dissolved with heating in 1 liter of water. One gram of capsules was suspended in 100 ml of the solution, and the suspension was left undisturbed for 24 h. If the capsules remained in suspension for 24 h they were considered suitable for use in further experiments.

Determination of dispersion and rate of release properties of various batches of the microcapsules prepared for this project.

Prepared by Nadav Aharonson as part of the report on BARD project No. I 82/80 (1984)

The different products of microcapsules containing atrazine or trifluralin were tested with regard to their dispersion properties in aqueous solutions. The rate of release of the pesticide from the capsule was also examined. Methods were developed to determine these properties of the capsules.

Methods

Determination of the stability of microcapsules as a suspension in aqueous solutions

Microcapsules at various concentrations (20-100 ppm) were dispersed in 100 ml of distilled water together with a dispergent at a ratio of 1:5, 1:1 and 5:1 of microcapsules: despergent.

The suspension was stirred for 30 min and aliquouts of 5 ml each were withdrawn at different time intervals and measured in a spectrophotometer at 440 nm.

2. Determination of rate of release of atrazine and trifluralin from microcapsules.

Microcapsules at various concentrations were dispersed in 100 ml of distilled water together with a despergent at a ratio of 1:1. The suspension was stirred during the experiment. Aliquouts of 5 ml each were centrifuged at 7000 rpm and the upper 4 ml centrifuged solution transferred to a separatory funnel. Distilled water was added to

bring the clear aqueous phase to 10 ml and then extracted 3 times with 15 ml ethyl acetate. The ethyl acetate extracts were combined, dried over anhydrous Na_2SO_4 and evaporated to dryness on a rotary evaporator. The residues were dissolved in acetone and determined on a Varian 3700 gas chromatograph that was equipped with a glass (180 x 0.2 i.d. cm) column packed with 3% OV-17 on 80/100 mesh Gas. Chrom Q. The detector used was the thermionic specific type (TSD).

Results

1. The stability of the microcapsules in suspension.

Many of the microcapsules preparations were tested in aqueous solutions with three concentrations of the despergent. Results for the most stable preparations of atrazine are give in Figures 1-7. Atrazine microcapsules preparation No. 26 and No. 26 (new) were found most stable in solution whereas preparations 20, 33 and 36 were found much less stable than atrazine 26 and 23. Most of the preparations showed considerable differences in stability at ratio herbicide: despergent of 100:500, 100:100 and 500:100, and only atrazine 26 (new) was found to be quite stable even at the lowest concentration of the despergent.

Trifluralin preparations No. 5 and No. 10 were found to contain 41.5% and 57% of the herbicide. It was very difficult to disperse these microcapsules in aqueous solutions.

2. Release rate of atrazine from the microcapsules.

All the preparations of atrazine that were tested did not exhibit slow release properties. The content of the microcapsules was released within minutes and not hours from the beginning of the experiment.

This was shown in Figures 8-10 for a few samples chosen from the many preparations that were tested.

Preparation of treflan 5 (trifluralin microcapsules) exhibited slow release properties. The release of treflan 5 from the microcapsules in a 25 ppm aqueous suspension was: 0.31, 2.0, 2.9 and 7.2 ug treflan/ml after 15 min, 1, 24 and 72 hours from the beginning of the experiment, respectively.

The release rate of treflan 10 was 6.0, 6.0, 3.0 and 3.7 ug/ml at 15 min, 1, 24 and 72 hours, respectively.

Figure 1. The stability of Atrazine 20 (microcapsules preparation) in aqueous suspensions at three different concentrations of the despersant. The concentration of atrazine microcapsules in the suspension was 100 ppm.

Figure 2. The stability of Atrazine 23 (microcapsules preparation) in aqueous suspensions at three different concentrations of the despersant. The concentration of atrazine microcapsules in the suspension was 100 ppm.

Figure 3. The stability of Atrazine 23 "new" (microcapsules preparation) in aqueous suspensions at three different concentrations of the desfergent. The concentration of atrazine microcapsules in the suspension was 100 ppm.

Figure 4. The stability of Atrazine 26 (microcapsules preparation) in aqueous suspensions at three different concentrations of the desfergent. The concentration of atrazine microcapsules in the suspension was 100 ppm.

Figure 5. The stability of Atrazine 26 "new" (microcapsules preparation) in aqueous suspensions at three different concentrations of the despersent. The concentration of atrazine microcapsules in the suspension was 100 ppm.

Figure 6. The stability of Atrazine 33 (microcapsules preparation) in aqueous suspensions at three different concentrations of the despersent. The concentration of atrazine microcapsules in the suspension was 100 ppm.

Figure 7. The stability of Atrazine 34 (microcapsules preparation) in aqueous suspensions at three different concentrations of the deserpent. The concentration of atrazine microcapsules in the suspension was 100 ppm.

Figure 8. Rate of release of atrazine 23 cel (O), 23 pal (□) and 30 (□) from the microcapsules, in an aqueous suspension at 30 ppm concentration of the microcapsules. The ratio of microcapsules and deserpent in solution was 1:1.

Figure 9. Rate of release of atrazine 23 new (●) and 26 new (○) from the microcapsules, in an aqueous suspension at 20 and 100 ppm concentration of the microcapsules. The ratio of microcapsules and despergent in solution was 1:1.

Figure 10. Rate of release of atrazine 33 (), 34 (), and 36 () from the microcapsules, in an aqueous suspension at 100 ppm concentration of the microcapsules. The ratio of microcapsules and despergent in solution was 1:1.

APPENDIX C - BIOASSAY OF PRODUCTS

Written by N. Lahav and D. Tropp

The effectiveness of the preparations under study depends on many factors, of which mobility and slow release characteristics are the most obvious ones. In the present part, we concentrated on the slow release characteristics of these preparations in the soil, as reflected by bioassays. Focusing on these properties means that the microcapsules under study were mixed with dry soil before adding water.

Most of the preparations studied were microcapsules of atrazine in various polymeric matrices. Some of them contained trifluralin.

Rehovot sandy soil was used in most of the experiments. A few observations were carried out using Bet-Dagan clay loam soil.

Known amounts of the atrazine preparation under study, in the form of powder, were carefully mixed with the air-dry soil, using three "dilution" steps in order to mix the preparation gradually with increasing soil volumes. This technique was found to insure homogeneous distribution of the herbicide.

Atrazine concentrations varied according to the test plants. For wheat and oats, the concentrations were 0, 125, 250 and 500 ppm. In the case of setaria, the atrazine concentration range was below 2 ppm.

Wetting to the desired water content was accomplished using a 2/3 Houghland nutrient solution either by a dropwise wetting and mixing, followed by transferring the wet soil into the container, or by simply adding the calculated amount of solution into the container filled with soil.

The containers were 190 cm³ cone-shaped plastic cups with a perforated bottom. The amount of soil packed in such a container was either 150 or 200 gm.

Three plant species were tested and their sensitivity toward atrazine was compared, namely Wheat (*Triticum* sp. Sion), Oat (*Avena* sp. arod 88/6), and *Setaria verticulata*. 16 seeds were sown in each container. At the end of the experiment, the shoot fresh weight was determined gravimetrically. In several experiments, the dry matter (70°C), plant length or the fresh weight of the root were also determined. Since these three parameters gave essentially the same information, it was decided to base our study on the shoot fresh weight.

A 12-hour light period was normally employed with a constant temperature of 26°C. In several experiments room temperature was used.

The growth period was 10-14 days.

Typically atrazine reduced growth rate after germination, caused chlorosis and, at high concentrations - death.

Big differences between the three plant species under study were observed regarding their sensitivity to atrazine: At atrazine rate of 3 ppm, setaria seedlings did not grow beyond the first three or four days after germination; they became chlorotic and died. The varieties of wheat and oats tested were much less sensitive to atrazine compared with setaria. Visual damage was normally detected only above atrazine concentration of 5 ppm.

Typical results of bioassay of atrazine using wheat are shown in Figure 1, where the yield was measured by the length of the wheat plants. It is seen that at atrazine rate of 125 ppm, the effectiveness reaches its maximum (Figure 1a). The effect is pronounced also after 1 month incubation (Figure 1b).

Lower atrazine concentrations, in the range of 0 to 10 ppm, were much less effective and could hardly be detected.

Setaria was found to be very sensitive to atrazine and therefore was selected as the standard test plant.

Figure 2 shows the shoot fresh weight of setaria seedlings as a function of atrazine concentration where preparation 23* of microencapsulated atrazine was used. Commercial atrazine was significantly less effective than the encapsulated one at atrazine concentration of 1 ppm. It has been shown that preparation 23 released its content rather rapidly and thus is an effective source of atrazine. Its efficiency compared to commercial atrazine may be explained by a better mixing in the soil. Presumably, the commercial atrazine form small lumps and aggregates, and thus is not well mixed in the soil as compared with preparation 23*.

Figure 3 shows the shoot fresh weight of setaria seedlings as a function of atrazine concentration applied as preparation 38.

The above bioassays demonstrate that preparations 23* and 38, as well as others which are not shown here, can serve as atrazine source which is effectively released to the soil.

The bioassay reflects the effectiveness of the dispersion of the preparations under study in the soil volume, as well as its release characteristics. Moreover, it can be used quantitatively or semi-quantitatively to compare the effectiveness of different preparations. Together with the data on the water dispersibility and the mobility of the microcapsules in the soil profile, one can have a solid basis for both the study of the behavior of different microcapsules in the soil and the selection of the most successful ones for the next stage of the project.

O - pure atrazine
 Δ - commercial atrazine
 ▽ - encapsulated atrazine

Figure 1

Wheat seedling length (% of control) 12 days after sowing. Rehovot soil.

- a. Sowing immediately after wetting of the soil.
- b. Sowing 1 month after wetting of the soil.

ATR. 23+CET. EFFECTS OF SETARIA

Fig. 2: ATRAZINE CONCENTRATION (ppm)

ATR.38 EFFECTS OF SETARIA

Fig. 3 : ATRAZINE CONCENTRATION (ppm)

ATX EFFECTS OF SETARIA

Fig. 4: ATRAZINE CONCENTRATION (ppm)

BIOASSAY TESTS OF ATRAZINE AND TRIFLURALIN MICROCAPSULES IN A SILT LOAM SOIL

Final Report

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BIOASSAY TESTS OF ATRAZINE AND TRIFLURALIN MICROCAPSULES IN A SILT LOAM SOIL

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ABSTRACT

A bioassay protocol was developed to compare relative efficacy of new microencapsulated formulations of atrazine and trifluralin. Oats (Avena sativa 'Victory') were grown for 15 days after seeding in treated soil; at harvest, height, fresh and dry weight, and general appearance (phytotoxicity index) were measured. The soil was recropped at 2 and 6 months. Twenty-two microencapsulated atrazine, 14 microencapsulated trifluralin, and one commercial atrazine formulation (80W, a wettable powder) were compared with the technical grade herbicides and with untreated controls.

The first-harvest GR₅₀ values were estimated graphically and by the use of 3-parameter logistic equations. GR₅₀ was ca. 0.2-0.3 ppm for unformulated atrazine; most atrazine formulations were similarly phytotoxic. However, ATR-36 had a GR₅₀ value of ca. 0.6 ppm and ATR-29, ca. 0.45 ppm. Several other microencapsulated formulations which may also have diminished phytotoxicity include ATR-23*, -26, -26*, and -32. Formulations ATR-30 and -39 might be slightly more phytotoxic than unformulated atrazine.

Trifluralin was less phytotoxic to oats, and GR₅₀ values were less accurately estimated. The GR₅₀ of technical trifluralin was ca. 4-5 ppm. In

contrast to atrazine, a number of microencapsulated trifluralin treatment tended to cause enhanced efficacy: TRIF-5, -12, -13, -16, and -18. TRIF-15 seemed to be less phytotoxic than unformulated trifluralin.

Both technical and formulated atrazine had lost ca. 80% of the initial phytotoxicity (estimated by dry weight-based GR₅₀) after 2 months. Formulations ATR-23*, -26*, -31, -32, and -36 were notably less active than unformulated atrazine, generally confirming trends observed in the first harvest. By 6 months, formulations possibly exhibiting phytotoxicity included ATR-23-Cet, -23-Pal, -29, -30, -31, -32, -34, -37, -38, and -39. Four of these (ATR-23-Cet, -23-Pal, -38, and -39) seemed to have greater residual activity at 6 months that did atrazine or atrazine-80W.

Trifluralin was much more persistent in soil than was atrazine. At the 2-month cropping, only microencapsulations TRIF-1, -5, -16, -17, and -18 seemed to have lost bioactivity. Formulations TRIF-13, -12, -6, -14, -9, and -11 (in decreasing order of phytotoxicity) seem to be active at initial rates 2-3X lower than unformulated trifluralin. The 6-month data indicated continued measurable residues in all formulations except TRIF-1. TRIF-15 was more toxic at 6 months than when initially applied, suggesting very slow release of herbicide.

INTRODUCTION

Formulations of pesticides are, in simplest terms, mixtures of the pesticide and other substances that facilitate field application of the pure biocide. In some cases, the pesticide molecule itself is chemically modified, e.g., by formation of esters or salts. Controlled release formulations are designed to release gradually the active ingredient into the environment. This is achieved by dissolving, encapsulating, or dispersing the agrichemical in a polymer (so

loss occurs by diffusion) or by chemical bonding the agrichemical to the polymer via a hydrolyzable linkage (so release follows a degradative reaction).

The relatively new controlled release formulations of pesticides (Scher, 1977a) offer the potential advantage of extending the persistence of the bio-cide. The dose rate of pesticide may thereby be reduced, soil incorporation may be delayed, or pest control might be achieved with single as opposed to multiple applications. Microencapsulated pesticides, a type of controlled release formulation, are 1-200 μm particles composed of a solid or liquid core (containing the pesticide) surrounded by a wall (Scher, 1977b). Microencapsulation has extended the persistence of such herbicides as butylate (Nash, 1983), EPTC (Schreiber et al., 1978), and trifluralin (Coffman and Gentner, 1980; White and Schreiber, 1984), and has reduced the volatilization of chlorpropham (Turner et al., 1978). Slower release from microcapsules as compared to standard formulations tended to reduce leaching of dichlobenil (Connick et al., 1984), carbofuran (Pascucci and Kirda, 1985), and picloram (Bauer, 1980) in laboratory tests.

The principal objective of the overall BARD project was to develop micro-encapsulated pesticides that could be physically transported by water into the soil profile. Transfer of this technology to practical field use necessitates that such formulations remain biologically active. It was the objective, in this part of the project, to evaluate biological activity of microencapsulated pesticides that had earlier shown promise in soil leaching test.

Two herbicides were selected for formulation, atrazine and trifluralin. These were chosen because of their (a) widespread use; (b) amenity to biological and chemical assay; and (c) leaching characteristics. According to the classification of Helling (1971), atrazine is moderately mobile and trifluralin, immobile, in the standard Hagerstown silty clay loam. Thus, although atrazine

will leach to some extent, trifluralin should not; this has been confirmed by abundant laboratory and field observations. Microencapsulation (if project goals are achieved) then has the potential, particularly with trifluralin, of allowing deeper placement of the herbicide without mechanical incorporation. This is advantageous since best weed control with trifluralin is where the herbicide is incorporated uniformly to a depth of 2.5-5 cm rather than 10 cm or when left at the surface (Khalifa et al., 1983; Talbert and Frans, 1968).

Although mobile microcapsules would likely be most effective in irrigated sandy soils, these bioassay tests were conducted with a silt loam soil. It was chosen as a typical medium textured soil characteristic of much of eastern United States. Phytotoxicity will be less than in coarser soils, because of herbicide adsorption, and thus the silt loam constitutes a more rigorous testing medium for our initial studies of efficacy.

Bioassay methods for herbicides were reviewed by Santelmann (1977), and the general method selected for the BARD project was typical of a small pot bioassay approach. Oats was chosen for bioassaying both herbicides. It has been widely used with atrazine, to which it is very sensitive. It is less sensitive to trifluralin, but we believed the advantages of conducting all tests identically outweighed alternative procedures. A 15-day plant-to-harvest cycle was sufficient time for complete kill of plants treated at the highest rates of atrazine.

MATERIALS AND METHODS

Formulation Analyses. All formulations were extracted and analyzed by gas chromatography (GC) to determine their percentage of active ingredient (AI). A typical early procedure for trifluralin was to shake ca. 10 mg of formulation

with 10 ml EtOAc for up to 170 h, at 28°C. Samples were withdrawn for analysis at 16, 83, and 170 h; 1 ml of suspension was centrifuged at 1500 rpm for 1 h and 0.1 ml of the supernate was diluted to 10 ml with EtOAc for GC analysis. Later, a 10-mg sample/8-ml EtOAc suspension was shaken for 40 h; centrifuged 1 h at 3500 rpm; and 0.1 ml diluted to 10 ml for final analysis. The solvent used to extract atrazine was methanol.

All analyses used a Hewlett-Packard 5880A gas chromatograph with automatic sample injection. For atrazine, typical run conditions were: temperatures---injector (200°), oven (180°), nitrogen-phosphorus (NP) detector (250°C); flow rates (ml min⁻¹)---carrier (He, 28.4), detector (H₂, 3.6, and air, 101); column---2-mm (i.d.) X 1.7-m glass, packed with 3% SP-2100 on 80/100 Chromosorb WHP.

Trifluralin was analyzed by a ⁶³Ni electron capture (EC) detector after separation in a 0.25-mm (i.d.) X 30-m fused silica capillary column that contained DB-1, a bonded phase silicone (film thickness, 0.25 µm). The carrier gas was hydrogen (1.7 ml min⁻¹); the makeup gas was argon:methane [95:5 (v/v)] (85 ml min⁻¹). Split vent and septum purge flow rates were, respectively, 30 and 10 ml min⁻¹. Injector and detector temperatures were 200° and 250°C, respectively. Splitless injection and temperature programming were used as follows: initial oven temperature = 55° for 0.6 min; inlet purge valve off; temperature increased at 10° min⁻¹ to 180°, then held 10 min. Integration for both herbicides was by peak area.

Some analyses of atrazine were also made by using the capillary EC system, using conditions as described for trifluralin. Similarly, trifluralin was sometimes analyzed by packed column (NP detector) at 190°.

Bioassay. Duffield silt loam (Ultic Hapludalfs subgroup) was collected from a grassy field plot in Frederick County, Maryland in October 1982 and again in May 1983. The Duffield series consists of well-drained soils developed from impure limestone. The soil was sieved to pass 2 mm and stored moist at ambient temperature prior to use. Soil organic matter content was 2.5%; pH = 6.3.

The bioassay was conducted with ca. 190 g (o.d. equiv.) of soil per 250-ml white styrofoam cup, which served as pots. Treatment rates varied according to the % AI in each formulation, but were within the range 0.05-50 ppm as the formulation. Treatment was by serial dilution, i.e., after thoroughly mixing the highest rate, portions were withdrawn and blended with untreated soil to achieve lower rates. Untreated soil and unformulated (analytical) atrazine or trifluralin were included for comparison. Initially, six replications of the six rates were used; later, four reps of treatments and eight of controls were used.

Each pot was planted with 12-15 seeds of oats (Avena sativa 'Victory'), moistened, and maintained in growth chambers at 25° with 12-h daylength. Light intensity was ca. $2.3-3.2 \times 10^4$ lux, depending on chamber position, at the beginning of these experiments. Pots were positioned as randomized blocks, with four replications. Each pot was thinned to 8 plants at Day 7, watered with Hoagland's nutrient solution on Day 8, and harvested on Day 15. A typical experiment consisted of complete sets of control, analytical herbicide, and three formulations.

At harvest, a phytotoxicity index rating (0 = complete kill, 10 = no effect) preceded measurement of height, fresh weight, and oven-dry weight.

Following the first harvest (15-day), pots were stored for 2 months at ambient temperature; the soil was then remixed and recropped for 15 days. This process was again repeated with remixing and cropping at ca. 6 months (3rd harvest cycle).

Volatilization. To assess possible loss of pesticides by volatilization, the following experiment was done. Gray polyurethane foam plugs, 22 mm Ø X 38 mm long, were Soxhlet-extracted sequentially with benzene:methanol (1:1, v/v) methanol, and hexane. The plugs were laterally sectioned in thirds; the lower two sections were inserted into 30-ml glass columns [22 mm (i.d.) X 112 mm]. These were just above a 1-g layer of pesticide or formulation, separated from the foam plugs by discs of glass fiber filter paper and supported by a sintered frit. Nitrogen gas at 10 ml min⁻¹ passed through a water trap, then through the pesticide and foam plug. The apparatus was maintained in an incubator at 30°. After 24 h, the plugs were removed and extracted with hexane; these extracts were analyzed by GC as described earlier.

Atrazine Extraction from Aged Soil. Soil treated with ATR-1 formulation was analyzed for atrazine residues following the final, 6-month harvest. The initial treatment was 5 ppm formulation. Moist soil subsamples (25 g of each) were Soxhlet-extracted for 24 h with EtOAc, methanol, or 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113; spectrophotometric grade, 99+%). Extracts were evaporated under vacuum, dissolved in hexane, dried with anhydrous Na₂SO₄, diluted to 5 ml, and analyzed by GC using the NP detector as described for formulation analysis. A control soil was extracted with EtOAc only.

RESULTS

Formulation Analyses. Representative gas chromatograms are shown for trifluralin (ECD; Fig. 1), atrazine (NPD; Fig. 2), and TRIF-14 (NPD; Fig. 3). The limit of detection for both herbicides in the NP system was ca. 50 ppb solution concentration, or ca. 50 pg injected. Trifluralin was ca. 40-45X more sensitive to EC detection than was atrazine, and its limit of detection was ca. 5 ppb.

【hp】 5880A SAMPLER INJECTION @ 13:00 APR 13, 1983

SAMPLE # : ID CODE :
3 TRIF 0.05 PPM

AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASLINE	AREA %
0.00						
0.00						
0.00						
0.00						
11.00						
11.91	54.52	VV	0.075	11.34	134.43	3.152
13.39	1675.21	BB	-----	748.05	135.14	96.848
14.00						

BASLINE @ START RUN = 133.23

THRESHOLD @ START RUN = 4

PEAK WIDTH @ START RUN = 0.04

RP: REJECT -> 100000

RP: REJECT -> 0

RP: REJECT -> 100000

TOTAL AREA = 1729.74

MULTIPLIER = 1

Fig. 1 Gas chromatogram of trifluralin, using electron-capture detector.
Herbicide retention time is 13.39 min.

HP 5980A SAMPLER INJECTION @ 19:27 FEB 1, 1983

SAMPLE # : ID CODE :

6 ATR 1 PPM

AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASELINE	AREA %
0.00						
0.00						
0.00						
0.63	154.08	BB	*-----	4.61	16.73	21.428
2.03	565.00	BH	0.11 *	79.21	20.94	78.572
3.00						

RP: REJECT → 1000

TOTAL AREA = 719.08

MULTIPLIER = 1

Fig. 2 Gas chromatogram of atrazine (in EtOAc), using N-P detector. Oven temperature = 190°C. Herbicide retention time is 2.03 min.

【hp】 5880A SAMPLER INJECTION @ 08:44 APR 7, 1983

SAMPLE # : ID CODE :
88 TRI-14, 170H

AREA %

RT	AREA	TYPE	WIDTH	HEIGHT	BASLINE	AREA %
0.00						
0.00						
0.00						
1.11	43.48	BB	0.057	11.85	19.18	22.912
1.51	146.28	BB	0.072	31.87	19.32	77.088
TOTAL AREA = 189.76						
MULTIPLIER = 1						

Fig. 3 Trifluralin analysis, by N-P gas chromatography, of microencapsulated formulation TRIF-14. Oven temp. = 190°C. Column = 2-mm X 1.7-m glass with 3% SP-2100 on 80/100 Chromosorb WHP. Sample was initially shaken for 170 h with EtOAc. Trifluralin retention time = 1.51 min. Peak at 1.11 min. is internal standard, 2,6-dinitro-4-trifluoromethyl-N-methyl-aniline.

Although most earlier analyses included an internal standard (see Fig. 3), quantitation was later based solely on herbicide's peak area.

Results of the formulation analyses are in Tables 1 and 2. Since results by EC and NP methods were very similar, many estimates of % AI are averages of both methods. There was no consistent effect of solvent extraction time on herbicide content (16-170 h), indicating rapid equilibration. For some later tests, a single period of 48 h was therefore used. Atrazine and trifluralin analyses performed originally in Israel were based on total Cl or F and were usually somewhat higher than these more selective GC analyses. Atrazine concentration in microcapsules ranged from 0.3% (ATR-26) to 62% (ATR-23*). It is reassuring that atrazine-80W, nominally a 80% wettable powder formulation, did contain 79.2% atrazine. Trifluralin microcapsules contained 36% (TRIF-1) to 84% (TRIF-15) herbicide.

Bioassay. Two parameters measured at harvest are reported for atrazine (Table 1) and trifluralin (Table 2) treatments. Although nearly all experiments contained unformulated herbicide, only a few such examples are shown. Of 22 microencapsulated atrazine formulations, plus atrazine-80W, only ATR-26 was not present at a rate sufficient to give at least 50% growth reduction relative to control plants (GR₅₀). Except for slight phytotoxicity at the highest rate (0.165 ppm AI), the ATR-26 treatment was ineffective and could constitute a formulation blank.

Typical atrazine symptoms ranged from slight tip burn on leaves to chlorosis, stunting, and complete kill as herbicide levels increased. Since germination was not inhibited, some growth was always seen; thus, reduction in growth relative to control plants rarely exceeded 85%.

Table 1. Bioassay of atrazine microencapsulated formulations.

Formulation	Composition (AI), %	Rate, ppm		Phytotoxicity rating			Reduction in dry weight, % of control		
		Formula-	AI	1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
Atrazine (analytical, Agam Chem.)	100	-	0.05	10.0	10.0	9.5	13.1	3.3	(1.7)
	"	-	0.1	10.0	10.0	9.5	27.7	(2.9)	(4.3)
	"	-	0.5	4.1	10.0	9.5	78.5	7.4	5.8
	"	-	1.0	0.9	6.4	9.5	84.8	52.0	(5.7)
	"	-	2.5	0.0	1.6	9.8	88.0	74.1	(7.1)
	"	-	5.0	0.0	1.0	8.4	88.6	75.2	31.5
Atrazine 80W	79.2	0.05	0.04	10.0	10.0	10.0	3.4	21.4	6.4
	"	0.1	0.08	9.9	10.0	10.0	6.6	0.2	3.4
	"	0.5	0.4	3.5	10.0	10.0	59.5	0.0	(2.8)
	"	1.0	0.8	1.6	7.0	10.0	72.1	39.0	(3.9)
	"	2.5	2.0	0.4	1.0	9.8	75.4	67.0	(2.2)
	"	5.0	4.0	0.2	0.6	8.7	74.9	70.0	27.7
ATR 1	59.2	0.05	0.03	10.0	10.0	10.0	9.9	(5.2)	(5.0)
	"	0.1	0.06	10.0	10.0	10.0	12.1	(7.5)	(17.6)
	"	0.5	0.30	6.6	10.0	10.0	60.8	(8.5)	(14.2)
	"	1.0	0.59	2.0	10.0	10.0	74.0	1.1	2.9
	"	2.5	1.5	0.8	2.8	10.0	77.0	61.4	(22.6)
	"	5.0	3.0	0.3	1.2	9.8	77.0	66.4	4.6
ATR 2	21.1	0.05	0.01	10.0	10.0	10.0	12.2	(1.9)	(20.5)
	"	0.1	0.02	10.0	10.0	10.0	10.0	(0.9)	(26.0)
	"	0.5	0.11	9.6	10.0	10.0	19.0	(6.8)	(11.7)
	"	1.0	0.21	8.3	10.0	10.0	44.3	(16.7)	(20.0)
	"	2.5	0.53	2.6	9.1	10.0	71.8	11.9	(8.4)
	"	5.0	1.05	0.8	4.4	10.0	74.3	51.7	(21.3)

Table 1 (Cont.).

Formulation	Composi- tion (AI), %	Rate, ppm Formula- tion	AI	Phytotoxicity rating			Reduction in dry weight, % of control		
				1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
ATR 4	34.2	0.05	0.018	10.0	10.0	10.0	5.6	(0.4)	14.1
"	"	0.1	0.034	10.0	10.0	10.0	10.0	(0.7)	7.2
"	"	0.5	0.17	9.5	10.0	10.0	34.3	(11.4)	4.3
"	"	1.0	0.34	4.0	10.0	10.0	66.0	(2.5)	0.9
"	"	2.5	0.85	1.4	7.9	10.0	71.1	41.1	(3.2)
"	"	5.0	1.7	0.4	2.0	10.0	75.6	66.2	4.3
ATR 5	39.3	0.05	0.02	10.0	10.0	10.0	15.2	(6.1)	0.0
"	"	0.1	0.04	10.0	10.0	10.0	17.4	(2.6)	5.9
"	"	0.5	0.2	8.9	10.0	10.0	41.9	(12.0)	19.7
"	"	1.0	0.4	3.3	10.0	10.0	59.3	(16.3)	10.0
"	"	2.5	1.0	1.0	6.3	10.0	72.9	47.3	0.9
"	"	5.0	2.0	0.1	1.9	10.0	78.4	62.7	(6.1)
ATR 23	2.6	0.5	0.013	10.0	10.0	10.0	(4.4)	(2.0)	(2.7)
"	"	1.0	0.026	10.0	10.0	10.0	(3.5)	4.4	(2.8)
"	"	5.0	0.13	10.0	10.0	10.0	3.8	(0.3)	6.6
"	"	10.0	0.26	6.5	10.0	10.0	48.6	2.8	(7.0)
"	"	25.0	0.65	0.8	9.8	10.0	76.1	23.0	(2.7)
"	"	50.0	1.30	0.2	4.0	10.0	77.0	68.2	6.7
ATR 23*	62.3	0.1	0.06	10.0	10.0	10.0	(33.2)	(2.5)	20.3
"	"	0.2	0.12	10.0	10.0	10.0	(7.9)	(12.7)	5.2
"	"	1.0	0.62	3.5	10.0	10.0	53.3	(31.8)	(1.0)
"	"	2.0	1.24	0.8	10.0	10.0	65.1	(34.4)	(2.4)
"	"	5.0	3.1	0.1	8.7	10.0	69.8	28.3	0.6
"	"	10.0	6.2	0.1	3.8	10.0	68.2	61.2	(1.6)

Table 1 (Cont.).

Formulation	Composi- tion (AI), %	Rate, ppm		Phytotoxicity rating			Reduction in dry weight, % of control		
		Formula- tion	AI	1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
ATR 23-Cet	29.1	0.1	0.029	10.0	10.0	10.0	23.0	(3.7)	6.0
	"	0.2	0.058	10.0	10.0	10.0	21.3	(12.5)	(11.9)
	"	1.0	0.29	5.1	10.0	10.0	69.6	(11.2)	(10.4)
	"	2.0	0.58	0.6	10.0	10.0	79.1	(14.4)	(7.1)
	"	5.0	1.46	0.0	2.9	10.0	85.0	54.6	11.3
	"	10.0	2.9	0.0	0.9	5.6	83.5	68.4	47.2
ATR 23-Pal	27.7	0.1	0.028	10.0	10.0	10.0	15.7	(10.6)	8.6
	"	0.2	0.055	10.0	10.0	10.0	12.2	12.3	(1.5)
	"	1.0	0.28	4.3	10.0	10.0	68.9	(7.4)	(6.3)
	"	2.0	0.55	0.6	10.0	10.0	81.4	(6.0)	(16.3)
	"	5.0	1.39	0.0	2.1	10.0	85.2	63.3	4.1
	"	10.0	2.77	0.0	0.4	4.5	84.7	69.7	47.5
ATR 26	0.33	0.5	0.0017	10.0	10.0	10.0	(1.0)	(3.1)	(0.7)
	"	1.0	0.0033	10.0	10.0	10.0	(3.2)	(8.5)	(0.9)
	"	5.0	0.0165	10.0	10.0	10.0	(7.4)	(2.0)	(5.5)
	"	10.0	0.033	10.0	10.0	10.0	1.1	1.0	(1.2)
	"	25.0	0.083	10.0	10.0	10.0	(2.0)	(6.8)	(1.7)
	"	50.0	0.165	9.5	10.0	10.0	9.8	5.6	(3.3)
ATR 26*	42.4	0.1	0.04	10.0	10.0	10.0	(13.2)	(2.8)	17.3
	"	0.2	0.08	10.0	10.0	10.0	(5.4)	(0.5)	(4.8)
	"	1.0	0.4	4.8	10.0	10.0	52.4	(22.3)	18.1
	"	2.0	0.8	1.3	10.0	10.0	64.8	(28.0)	21.4
	"	5.0	2.1	0.1	8.5	10.0	68.6	22.1	(10.5)
	"	10.0	4.2	0.0	4.0	10.0	71.2	56.2	2.0

Table 1 (Cont.).

Formulation	Composi- tion (AI), %	Rate, ppm		Phytotoxicity rating			Reduction in dry weight, % of control		
		Formula- tion	AI	1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
ATR 28	4.2	0.5	0.021	10.0	10.0	10.0	(10.7)	7.2	1.9
	"	1.0	0.042	10.0	10.0	10.0	4.5	(3.3)	1.1
	"	5.0	0.21	7.4	10.0	10.0	32.5	(3.6)	(5.3)
	"	10.0	0.42	2.6	10.0	10.0	70.3	0.0	(6.6)
	"	25.0	1.05	0.5	6.3	10.0	77.1	51.7	(1.6)
	"	50.0	2.1	0.3	3.1	10.0	78.2	71.3	4.1
ATR 29	6.1	0.5	0.031	10.0	10.0	10.0	(0.8)	0.0	5.7
	"	1.0	0.061	10.0	10.0	10.0	(1.1)	4.9	1.8
	"	5.0	0.305	8.6	10.0	10.0	23.4	4.2	3.4
	"	10.0	0.61	3.9	10.0	10.0	65.6	10.6	(3.1)
	"	25.0	1.53	0.8	5.0	10.0	74.1	63.2	3.3
	"	50.0	3.05	0.3	0.7	9.5	76.2	75.8	20.3
ATR 30	4.3	0.5	0.013	9.5	10.0	10.0	(6.1)	(2.4)	11.4
	"	1.0	0.026	9.5	10.0	10.0	4.2	(10.2)	14.7
	"	5.0	0.13	5.2	10.0	10.0	56.0	(17.7)	21.6
	"	10.0	0.55	3.0	10.0	10.0	66.0	(17.6)	9.1
	"	25.0	1.39	1.0	8.3	10.0	70.8	(19.4)	8.7
	"	50.0	2.77	0.6	4.0	10.0	72.7	59.3	27.0
ATR 31	3.7	0.5	0.018	9.5	10.0	10.0	(5.4)	0.0	8.2
	"	1.0	0.037	9.5	10.0	10.0	0.6	(5.4)	11.6
	"	5.0	0.18	9.0	10.0	10.0	21.4	(3.0)	16.7
	"	10.0	0.37	5.5	10.0	10.0	63.3	(27.2)	17.3
	"	25.0	0.93	2.5	9.9	10.0	71.3	(8.3)	4.3
	"	50.0	1.85	1.1	4.5	10.0	71.2	45.4	21.9

Table 1 (Cont.).

Formulation	Composi- tion (AI), %	Rate, ppm		Phytotoxicity rating			Reduction in dry weight, % of control		
		Formula- tion	AI	1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
ATR 32	3.6	0.5	0.018	9.5	10.0	10.0	(6.5)	(6.0)	(0.8)
	"	1.0	0.036	9.5	10.0	10.0	(0.8)	(11.0)	14.1
	"	5.0	0.18	9.0	10.0	10.0	32.6	(13.4)	7.0
	"	10.0	0.36	5.0	10.0	10.0	39.4	(19.2)	(5.1)
	"	25.0	0.92	2.4	10.0	10.0	67.6	(7.8)	0.9
	"	50.0	1.8	0.5	6.1	10.0	73.0	37.4	28.4
ATR 33	37.5	0.1	0.038	10.0	10.0	10.0	5.6	9.7	8.1
	"	0.2	0.075	10.0	10.0	10.0	(6.5)	1.8	25.4
	"	1.0	0.38	4.4	10.0	10.0	64.3	3.3	21.9
	"	2.0	0.75	0.6	10.0	10.0	74.9	0.7	20.6
	"	5.0	1.88	0.0	6.8	10.0	83.2	44.0	11.8
	"	10.0	3.8	0.0	0.8	9.8	84.0	69.1	11.6
ATR 34	37.2	0.1	0.037	10.0	10.0	10.0	6.0	9.6	32.7
	"	0.2	0.074	10.0	10.0	10.0	2.2	12.2	27.3
	"	1.0	0.37	4.8	10.0	10.0	66.5	1.7	16.4
	"	2.0	0.74	1.1	10.0	10.0	77.1	3.4	13.5
	"	5.0	1.86	0.0	5.0	10.0	82.2	57.6	6.4
	"	10.0	3.7	0.0	0.6	10.0	85.6	71.5	33.0
ATR 35	3.2	0.5	0.016	10.0	10.0	10.0	(3.8)	11.0	35.7
	"	1.0	0.032	10.0	10.0	10.0	(6.5)	16.3	20.9
	"	5.0	0.16	9.5	10.0	10.0	27.6	8.1	41.1
	"	10.0	0.32	4.5	10.0	10.0	66.1	10.7	11.8
	"	25.0	0.80	0.5	10.0	10.0	81.7	(9.1)	22.4
	"	50.0	1.60	0.0	5.3	10.0	82.8	45.6	5.3

Table 1 (Cont.).

Formulation	Composi- tion (AI), %	Rate, ppm Formula- tion	AI	Phytotoxicity rating			Reduction in dry weight, % of control		
				1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
ATR 36	2.6	0.5	0.013	10.0	10.0	10.0	(28.1)	(6.0)	(2.6)
	"	1.0	0.026	10.0	10.0	10.0	(6.7)	(1.3)	(2.0)
	"	5.0	0.13	10.0	10.0	10.0	(34.6)	(2.5)	15.1
	"	10.0	0.26	10.0	10.0	10.0	(8.8)	(10.6)	9.1
	"	25.0	0.65	3.8	10.0	9.9	51.6	(10.3)	13.5
	"	50.0	1.30	0.5	9.9	10.0	60.3	(15.8)	13.4
ATR 37	19.2	0.1	0.019	10.0	10.0	10.0	7.0	(10.2)	15.5
	"	0.2	0.038	10.0	10.0	10.0	5.9	1.8	21.2
	"	1.0	0.19	8.5	10.0	10.0	38.0	0.7	13.7
	"	2.0	0.38	2.7	10.0	10.0	74.1	(13.9)	(5.7)
	"	5.0	0.96	0.1	4.7	9.0	81.8	47.8	18.2
	"	10.0	1.92	0.0	2.9	3.5	84.4	59.8	59.6
ATR 38	23.8	0.1	0.024	10.0	10.0	10.0	(1.1)	(1.3)	11.1
	"	0.2	0.048	10.0	10.0	10.0	8.6	6.8	(11.3)
	"	1.0	0.24	8.9	10.0	10.0	30.3	2.7	7.9
	"	2.0	0.48	3.4	9.4	10.0	74.0	2.1	(0.3)
	"	5.0	1.19	0.1	5.0	5.0	80.4	48.0	46.7
	"	10.0	2.38	0.0	2.4	2.1	83.3	58.3	58.2
ATR 39	27.7	0.1	0.028	10.0	10.0	10.0	9.3	5.3	(3.5)
	"	0.2	0.055	10.0	10.0	10.0	10.4	(8.7)	2.5
	"	1.0	0.28	4.9	10.0	10.0	71.5	(17.3)	(3.1)
	"	2.0	0.55	1.0	10.0	10.0	79.7	(11.1)	6.2
	"	5.0	1.39	0.0	3.0	10.0	84.7	52.8	(5.9)
	"	10.0	2.8	0.0	0.8	9.3	84.7	64.4	30.1

Table 2. Bioassay of trifluralin microencapsulated formulations.

Formulation	Composition (AI), %	Rate, ppm		Phytotoxicity rating			Reduction in dry weight, % of control		
		Formula-	AI	1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
Trifluralin (analytical)	99.5+	-	0.05	10.0	10.0	10.0	15.3	2.0	5.3
	"	-	0.10	10.0	10.0	10.0	15.0	3.6	4.5
	"	-	0.50	10.0	10.0	10.0	11.5	(4.4)	8.1
	"	-	1.00	9.7	9.7	10.0	8.4	8.0	0.0
	"	-	2.5	9.8	9.8	10.0	10.2	25.3	7.5
	"	-	5.0	9.8	7.8	10.0	6.0	33.0	9.5
Trifluralin (analytical)	99.5+	-	0.25	10.0	10.0	10.0	4.0	(0.9)	(9.5)
	"	-	0.50	9.9	10.0	10.0	6.2	(4.7)	5.3
	"	-	2.5	9.5	8.7	10.0	(8.0)	23.8	(2.1)
	"	-	5.0	9.1	5.8	6.4	(9.0)	45.4	39.1
	"	-	12.5	6.6	8.4	4.1	7.0	28.9	57.7
	"	-	25.0	2.8	3.0	2.9	36.7	63.9	61.0
TRIF 1	35.6	0.05	0.018	10.0	10.0	10.0	3.4	(1.3)	(6.6)
	"	0.1	0.036	10.0	10.0	10.0	12.0	1.3	(9.5)
	"	0.5	0.18	10.0	10.0	10.0	(4.0)	(10.0)	(14.1)
	"	1.0	0.36	9.8	10.0	10.0	(12.0)	(7.3)	(10.0)
	"	2.5	0.89	9.2	10.0	10.0	(20.0)	(12.2)	(18.0)
	"	5.0	1.78	8.4	9.8	10.0	3.8	(22.7)	(28.3)
TRIF 5	36.7	0.5	0.18	9.8	10.0	10.0	(7.0)	(1.2)	1.1
	"	1.0	0.37	9.1	10.0	10.0	(9.0)	9.3	(9.0)
	"	5.0	1.84	3.9	9.5	10.0	50.0	13.9	(4.4)
	"	10.0	3.7	3.8	8.4	7.6	39.0	22.3	28.4
	"	25.0	9.2	2.2	6.5	3.6	71.0	35.9	58.6
	"	50.0	18.4	0.3	2.8	2.0	76.0	73.7	66.0

Table 2 (Cont.).

Formulation	Composi- tion (AI), %	Rate, ppm		Phytotoxicity rating			Reduction in dry weight, % of control		
		Formula- tion	AI	1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
TRIF 6	44.0	0.5	0.22	10.0	10.0	10.0	5.4	2.7	14.9
"	"	1.0	0.44	9.8	10.0	10.0	(2.3)	(5.3)	0.3
"	"	5.0	2.2	3.4	7.3	10.0	17.7	40.0	11.9
"	"	10.0	4.4	3.0	4.2	5.9	34.1	52.8	37.5
"	"	25.0	11.0	1.3	3.5	3.4	63.7	58.8	53.4
"	"	50.0	22.0	1.3	1.0	0.6	65.0	84.1	63.4
TRIF 8	40.3	0.5	0.20	10.0	10.0	10.0	(1.6)	2.0	9.4
"	"	1.0	0.40	9.8	10.0	10.0	(5.4)	(2.0)	5.7
"	"	5.0	2.0	6.3	8.0	10.0	23.7	15.3	13.6
"	"	10.0	4.0	4.3	6.0	7.0	45.7	43.2	32.7
"	"	25.0	10.1	2.9	2.5	2.2	52.0	68.2	53.7
"	"	50.0	20.2	1.8	2.0	0.5	67.8	77.5	70.4
TRIF 9	37.0	0.5	0.18	10.0	10.0	10.0	(20.0)	(16.3)	(15.5)
"	"	1.0	0.37	10.0	10.0	10.0	(21.0)	(26.3)	(16.7)
"	"	5.0	1.85	8.4	9.3	10.0	4.2	6.6	6.6
"	"	10.0	3.7	5.4	5.0	8.2	18.6	39.8	20.5
"	"	25.0	9.3	2.1	2.4	4.8	48.4	63.7	47.0
"	"	50.0	18.5	1.0	0.5	4.0	57.6	81.4	63.2
TRIF 10	51.3	0.5	0.26	10.0	10.0	10.0	7.5	1.0	(0.3)
"	"	1.0	0.51	9.8	10.0	10.0	4.5	(9.8)	0.7
"	"	5.0	2.6	5.0	7.8	9.5	21.8	30.5	18.3
"	"	10.0	5.1	3.6	4.7	8.6	45.3	42.9	37.6
"	"	25.0	12.8	2.1	1.5	3.8	47.7	68.8	54.8
"	"	50.0	25.7	1.0	0.6	2.0	60.5	57.8	58.5

Table 2 (Cont.).

Formulation	Composition (AI), %	Rate, ppm		Phytotoxicity rating			Reduction in dry weight, % of control		
		Formula-	AI	1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
TRIF 11	60.8	0.5	0.30	10.0	10.0	10.0	1.0	(8.6)	(0.2)
	"	1.0	0.61	9.8	10.0	10.0	7.5	(15.3)	(3.8)
	"	5.0	3.0	5.4	6.5	9.9	17.6	22.1	12.1
	"	10.0	6.1	2.4	3.0	8.6	50.0	46.1	29.3
	"	25.0	15.2	1.8	0.9	5.6	53.6	74.1	45.2
TRIF 12	"	50.0	30.4	0.6	0.6	2.6	70.9	87.1	69.2
	47.3	0.5	0.24	10.0	10.0	10.0	(9.8)	(7.6)	4.0
	"	1.0	0.47	10.0	10.0	10.0	(17.2)	(6.5)	12.3
	"	5.0	2.4	6.8	8.4	8.8	44.5	29.2	32.0
	"	10.0	4.7	5.8	4.1	8.6	52.8	56.9	48.3
TRIF 13	"	25.0	11.8	2.8	2.0	3.6	62.1	67.7	66.5
	"	50.0	23.7	2.0	0.6	1.9	56.1	83.5	73.8
	39.2	0.5	0.20	10.0	10.0	10.0	(6.6)	(1.4)	4.9
	"	1.0	0.39	10.0	10.0	10.0	5.3	(3.5)	17.4
	"	5.0	1.96	6.0	8.1	9.2	45.0	33.1	28.1
TRIF 14	"	10.0	3.9	5.8	5.0	5.5	43.7	54.8	58.7
	"	25.0	9.8	2.5	2.7	4.1	67.7	64.4	60.6
	"	50.0	19.6	1.6	0.1	2.2	74.7	82.2	70.4
	46.4	0.5	0.23	10.0	10.0	10.0	2.0	(0.6)	12.3
	"	1.0	0.46	10.0	10.0	10.0	(1.8)	2.2	11.9
	"	5.0	2.3	8.8	5.8	9.2	24.2	41.1	29.7
	"	10.0	4.6	5.0	6.0	6.9	56.1	50.8	55.7
	"	25.0	11.6	2.4	2.0	5.2	67.2	73.4	56.2
	"	50.0	23.2	0.8	0.1	1.9	82.1	80.0	68.9

Table 2 (Cont.).

Formulation	Composition (AI), %	Rate, ppm		Phytotoxicity rating			Reduction in dry weight, % of control		
		Formula- tion	AI	1st crop	2nd crop	3rd crop	1st crop	2nd crop	3rd crop
TRIF 15	85.6	0.25	0.21	10.0	10.0	10.0	2.8	(4.1)	(3.8)
	"	0.5	0.43	10.0	10.0	10.0	(5.2)	3.3	2.4
	"	2.5	2.1	10.0	8.5	7.0	(7.6)	8.9	36.6
	"	5.0	4.3	10.0	8.5	5.3	10.1	18.0	52.7
	"	12.5	10.7	7.9	6.2	4.1	31.7	29.9	62.5
	"	25.0	21.4	5.5	4.2	3.1	54.3	53.6	71.3
TRIF 16	49.5	0.5	0.25	10.0	10.0	10.0	2.3	3.9	(15.2)
	"	1.0	0.50	10.0	10.0	10.0	1.8	(15.0)	(21.4)
	"	5.0	2.5	7.4	8.6	9.0	43.3	8.6	6.3
	"	10.0	5.0	5.8	8.0	7.0	56.1	20.8	27.8
	"	25.0	12.4	3.0	6.5	5.6	59.3	28.6	40.6
	"	50.0	24.8	2.6	4.1	4.0	64.4	41.3	49.2
TRIF 17	42.4	0.5	0.21	10.0	10.0	10.0	0.5	(5.3)	(5.6)
	"	1.0	0.42	10.0	10.0	10.0	0.5	(11.0)	(16.1)
	"	5.0	2.1	9.0	9.6	7.6	28.8	1.0	24.5
	"	10.0	4.2	5.9	7.5	5.8	46.8	25.9	40.5
	"	25.0	10.6	4.7	6.5	5.0	49.7	38.9	46.6
	"	50.0	21.2	2.0	4.4	2.1	70.8	50.6	76.2
TRIF 18	43.3	0.5	0.21	10.0	10.0	10.0	9.5	(10.1)	(4.0)
	"	1.0	0.43	10.0	10.0	10.0	4.7	(0.1)	(4.3)
	"	5.0	2.2	9.1	9.4	8.9	37.6	17.4	19.8
	"	10.0	4.3	4.4	7.0	5.6	57.9	27.7	47.9
	"	25.0	10.8	4.4	4.1	4.4	56.5	48.7	56.5
	"	50.0	21.7	1.1	3.8	2.6	74.2	54.4	70.9

Height measurements are not shown in Tables 1 and 2. These were somewhat more variable and less responsive than weight to herbicide rate changes. Fresh weight differences were apparent, e.g., between severely affected plants and those which had already died. These data are used later, but for brevity, are not included here. Fresh weight may be more sensitive to soil moisture status immediately before and during harvest than oven-dry weight would be.

The loss of phytotoxicity with time is apparent on inspection of 2nd and 3rd cropping results. Data in parentheses (Tables 1 and 2) show average growth enhancement relative to controls, i.e., an indication of variability. The conditions of storage between croppings were such that the soil (initially moist and covered) gradually dried out. Thus, microbial degradation should become minimal, somewhat prolonging persistence. At the highest rates selected for these microencapsulated atrazine formulations, none gave unambiguous evidence of remaining toxic after 6 months. Atrazine and atrazine-80W, originally applied at slightly higher rates than the microencapsulated atrazines, do show toxicity.

Trifluralin was much less toxic to oats than was atrazine. Phytotoxicity was indicated by slightly shorter, darker green plants progressing (at the highest rates) to severely stunted plants or even germination inhibition. In contrast to atrazine, differences among plants within a pot were greater in trifluralin-treated soil (at high rates). Root growth at these rates was confined to the upper 1-2 cm zone. During the 2nd and 3rd cropping cycles, soil in the higher-rate trifluralin (analytical and formulated) pots was noticeably lacking good granular structure; lack of proper aeration then may have contributed to poor growth.

Trifluralin persistence seemed enhanced by encapsulation. For example, only formulations TRIF-5, -16, -17, and -18 seemed to have reduced bioactivity

by the second harvest (TRIF-1 caused no phytotoxicity during the 2nd or 3rd cropping), although only TRIF-16 showed this reduction at 6 months. TRIF-15 may be slightly more active than unformulated trifluralin, after 6 months, but all other formulations tested were of comparable activity to the parent.

Statistical analyses of the atrazine and trifluralin results began with an analysis of variance for ATR-1, -2, -4, and -5 treatments. Replication effects were nonsignificant (Tables 3 and 4), indicating that the position of pots within the growth chamber did not affect results. As expected, treatment rates were linearly related to growth, whether measured by height or weight. The effect was less significant by the 2nd cropping (Table 4). The highest F values occurred when fresh weight was measured in the 1st harvest (Table 3); by the 2nd harvest, both fresh and dry weight responses were equally significant. Parallelism represents the tendency for the decline in growth to occur at the same rate for all treatments with incremental additions of herbicide (AI basis). Fresh weight changes were not uniformly parallel, however (Tables 3 and 4). Significant deviations from a linear decline in growth were found; by the 2nd harvest, all four treatments had a strong quadratic component (Table 4), which apparently reflected some growth stimulation at the lowest rates. Reexamination of 2nd cycle growth suggests that this may have occurred for other atrazine formulations as well (Table 1).

The most convenient comparison of formulations was by 1st-harvest GR₅₀ values. These were first computed by plotting growth curves (Figs. 4-5), then visually estimating the GR₅₀. Such data are summarized in Table 5. Curve-fitting the atrazine results (Fig. 4) was relatively easy; trifluralin data (Fig. 5), however, seemed to indicate growth stimulation, at low herbicide levels, and an unexpected deviation at high rates (see especially TRIF-16, -17,

Table 4. Analysis of variance for bioassay of treatments ATR-1, -2, -4, and -5; second cropping.

Source	df	Height			Fresh weight			Dry weight		
		Mean square	F-ratio	Signif.	Mean square	F-ratio	Signif.	Mean square	F-ratio	Signif.
Growth	1	250.91	46.67	<.002	3.47	0.64	NS	2.56	77.00	<.001
Rep (Growth)	4	5.38	1.57	NS	5.44	0.73	NS	0.03	0.36	NS
Treatment	24	57.93	--	--	267.23	--	--	2.70	--	--
Linear trends										
ATR-1	1	137.41	40.06	<.01	1224.11	163.06	<.001	15.80	171.54	<.001
ATR-2	1	90.22	26.31	<.01	195.30	26.02	<.001	3.11	33.77	<.001
ATR-4	1	312.62	91.15	<.01	729.82	97.22	<.001	8.20	89.08	<.001
ATR-5	1	270.17	78.77	<.01	813.81	108.41	<.001	9.39	102.00	<.001
Control vs. treatments	1	3.21	0.94	NS	70.94	9.45	<.001	0.77	8.34	<.001
Deviations	12	33.61	9.80	<.01	20.47	2.73	<.01	1.72	18.71	<.001
ATR-1 Quadratic	1				595.80					
ATR-2 Quadratic	1				498.27					
ATR-4 Quadratic	1				541.43					
ATR-5 Quadratic	1				801.70					
Parallelism	3	58.16	3.79		232.23		<.001	0.01	0.10	NS
Error	120	3.43			7.51			0.092		

¹Deviations df = 16 for Height and Dry weight parameters.

Fig. 4A Growth response of oats (dry weight) to unfurulated atrazine treatments, at first harvest.

Fig. 4B Growth response of oats (dry weight) to formulated atrazine treatments, at first harvest.

Fig. 4C Growth response of oats (dry weight) to formulated atrazine treatments, at first harvest.

Fig. 5 Growth response of oats (by weight) to trifluralin treatments, at first harvest.

Table 5. Relative phytotoxicity of atrazine and trifluralin microencapsulated formulations, as estimated graphically.

Formulation	GR ₅₀ , ¹ ppm AI
Atrazine, anal.	0.17
Atrazine, anal. [Expt. 4]	0.42
Atrazine, anal. [Expt. 11]	0.32
Atrazine 80W	0.30
ATR 1	0.23
ATR 2	0.24
ATR 4	0.23
ATR 5	0.27
ATR 23	0.26
ATR 23*	0.56
ATR 23-Cet	0.15
ATR 23-Pal	0.17
ATR 26	0.42
ATR 26*	0.40
ATR 28	0.23
ATR 29	0.43
ATR 30	0.11
ATR 31	0.30
ATR 32	0.40
ATR 33	0.27
ATR 34	0.25
ATR 35	0.24
ATR 36	0.63
ATR 37	0.24
ATR 38	0.31
ATR 39	0.19
Trifluralin, anal. [Expt.]	342
TRIF 1	52
TRIF 5	2.8
TRIF 6	5.5
TRIF 8	5.4
TRIF 9	9.7
TRIF 10	9.4
TRIF 11	7.1
TRIF 12	3.7
TRIF 13	3.0
TRIF 14	4.1
TRIF 15	18.2
TRIF 16	3.1
TRIF 17	10.0
TRIF 18	2.5

¹Rate of soil-incorporated herbicide (AI basis) which gave 50% growth reduction (GR₅₀), on a dry weight basis, of oats (*Avena sativa* 'Victory'), relative to untreated control plants. Planting was immediately after soil treatment and harvest was 15 days later.

²Value estimated by extrapolation.

and -18). Based on graphical analysis (Table 5), atrazine and its formulations had GR₅₀ values of ca. 0.2-0.4 ppm AI. One microencapsulated atrazine (ATR-30) seemed much more toxic (GR₅₀ = 0.11) than unformulated atrazine, whereas several (ATR-23*, -26, -26*, -29, -32, and -36) were less toxic.

Trifluralin GR₅₀'s (Table 5) were ca. 3-10 ppm. The unformulated trifluralin value (34 ppm) was based on extrapolation; tests with a higher dose range demonstrated that the actual GR₅₀ is ca. 5 ppm. Formulations which on 1st cropping seemed more toxic than trifluralin alone include TRIF-18, -5, -13, -16, -12, and -14. Least effective formulations were TRIF-15, -17, -9, and -10.

The GR₅₀ was also estimated mathematically by a nonlinear three-parameter logistic equation:

$$y = \frac{b_0}{1 + (x/c)^d} \quad [1]$$

where y = plant growth (fresh or dry weight, mg), b_0 = weight of control plants, x = herbicide application rate (ppm as AI), c = herbicide rate which reduces weight by 50%, i.e., the GR₅₀ value; and d = slope. This equation is suited for describing a sigmoidal curve such as that below:

The results of this approach, for atrazine, are summarized in Table 6. Several GR₅₀ values must be discarded, e.g., 0.60 ppm for two analytical atrazine treatments. These data were better represented by a four-parameter

Table 6. Relative phytotoxicity of atrazine microencapsulated formulations as estimated by nonlinear 3-parameter logistic equations.

Formulation	Expt. no.	Fresh weight ¹			Dry weight		
		b ₀	c	d	b ₀	c	d
Atrazine, anal.	3	28.01	0.30	3.16	4.82	0.17	0.78
" "	4	21.53	0.40	1.14	3.00	0.60	1.00
" "	5	29.21	0.35	1.86	7.28	0.13	0.93
" "	6	22.34	0.36	1.61	3.77	0.16	0.66
" "	10	34.29	0.24	1.92	4.81	0.24	1.17
" "	11	34.60	0.38	3.77	4.00	0.60	1.38
Atrazine 80W	2	21.91	0.25	2.08	3.47	0.18	0.67
ATR 1	1	19.33	0.30	1.97	4.11	0.19	0.75
ATR 2	1	19.09	0.36	2.73	3.04	0.30	1.13
ATR 4	1	19.47	0.31	2.99	3.41	0.21	0.78
ATR 5	1	18.31	0.31	3.93	3.14	0.26	0.73
ATR 23	3	28.75	0.27	3.65	3.56	0.31	1.49
ATR 23*	6	25.76	0.36	1.62	4.00	0.30	1.00
ATR 23-Cet	10	29.45	0.20	2.41	4.00	0.30	1.10
ATR 23-Pal	10	29.68	0.23	3.12	5.62	0.14	0.98
ATR 26	3	20.92	1.50	2.50	3.30	1.50	2.50
ATR 26*	6	21.59	0.31	1.88	3.58	0.17	0.62
ATR 28	3	28.81	0.27	3.62	4.43	0.15	0.79
ATR 29	3	28.32	0.47	3.89	3.68	0.46	0.98
ATR 30	4	23.10	0.19	0.96	3.00	0.30	1.00
ATR 31	4	20.73	0.35	1.39	3.40	0.24	0.78
ATR 32	4	21.94	0.37	1.59	3.90	0.14	0.64
ATR 33	5	28.41	0.35	3.27	5.10	0.25	1.12
ATR 34	5	31.97	0.23	1.81	6.74	0.11	0.85
ATR 35	5	28.38	0.28	3.73	4.87	0.22	1.29
ATR 36	6	24.28	0.57	3.68	3.02	0.64	1.85
ATR 37	11	34.03	0.26	3.83	4.58	0.23	1.19
ATR 38	11	33.06	0.37	4.23	4.76	0.30	1.15
ATR 39	10	32.00	0.23	2.71	6.40	0.11	0.92

¹Equation used is $y = b_0/[1 + (x/c)^d]$, where y = plant growth (weight of tops, mg); b_0 = weight of control (untreated) plants; x = herbicide application rate (AI, ppm); c = herbicide rate which reduces weight by 50%, i.e., the GR₅₀ value; and d = slope. Results are based on the growth of oats (*Avena sativa* 'Victory') planted immediately after soil treatment and harvested 15 days later.

logistic equation,

$$y = a + \frac{b_0}{1 + (x/c)^d} \quad [2]$$

which yielded GR₅₀ estimates of 0.30 ppm. Equation [1] is actually a special case of Equation [2] in which $a = 0$. The values of 1.50 for ATR-26 were not used since they are based on extrapolation.

For comparison, the three GR₅₀ estimates for unformulated atrazine averaged 0.25 ± 0.12 , and 0.31 ± 0.09 ppm, by the methods above; together, the formulation estimates averaged 0.29 ± 0.11 ppm, or the same as the overall nonformulated atrazine GR₅₀, 0.27 ± 0.09 .

One can thus generalize that microencapsulated atrazine had the same phytotoxicity as unformulated atrazine, indicating that it was readily released during a 15-day period. Such a generalization does not address individual differences, however.

A better basis for comparing means within an experiment is the Z test statistic (Kleinbaum and Kupper, 1978) defined as

$$Z = \frac{c_{\text{trt } 1} - c_{\text{trt } 2}}{\sqrt{(SE_{\text{trt } 1})^2 + (SE_{\text{trt } 2})^2}} \quad [3]$$

where c was defined in Equation 1 and $SE_{\text{trt } 1}$ and $SE_{\text{trt } 2}$ are the associated standard errors for the c estimates of treatments 1 and 2. If $|Z| > 1.96$, the difference in c_1 and c_2 are significant at the 5% level.

Most comparisons were made between a formulation and the analytical atrazine bioassayed during the same experiment, using "dry weight c " as the basis for comparison. On this basis, atrazine (Expt. 6) was significantly more toxic than ATR-36 [$Z = 2.76$]. Similarly, ATR-23-Pal ($c = 0.14$ ppm) was more toxic than ATR-23 ($c = 0.31$ ppm) [$Z = 2.13$]; ATR-28 ($c = 0.15$ ppm), more than

ATR-29 ($c = 0.46$ ppm) [$Z = 2.46$]; and ATR-34 ($c = 0.11$ ppm), more than ATR-35 ($c = 0.22$ ppm) [$Z = 2.57$]. Graphical analysis suggests that ATR-34 and -35 do not differ; with this exception, the differences seem to be confirmed by the other two estimates of GR₅₀. In most cases, Z fails to confirm differences among treatments. However, there seem to be some cases in which c inaccurately predicts GR₅₀, i.e., based on graphical interpretation (Fig. 4, Table 5). An example is ATR-30, for which $c = 0.30$ ppm seems clearly too large.

Trifluralin phytotoxicity is compared, via c values, to that of microencapsulated formulations in Table 7. Values from Expt. 2 should be disregarded since the GR₅₀ represents an estimate extrapolated well beyond the highest applied herbicide dose. Average values of c , whether computed by fresh or dry weight, are somewhat smaller for formulated trifluralin. If the TRIF-15 fresh weight value (12.32), which is unusually large, is excluded, the average formulation c from fresh weight data becomes 2.5 ppm, well below the 5.2 ppm from analytical trifluralin.

Bioactivity differences among specific formulations seem more apparent for microencapsulations of trifluralin than atrazine. Interpretation of Table 7 data is greatly complicated by high variance in the estimates of c . This is partly due to inherent variability among replicates, but much of the error can probably be ascribed to inaccurate mathematical modeling (Table 7). The trifluralin curves (Fig. 5) had apparent growth stimulation at low rates and a response anomaly at high rates. Neither would properly modeled by Equation 1. When the Z test was used to compare analytical trifluralin with formulations run during the same experiment (Expts. 7, 9, and 12), only TRIF-6 (7.98 ppm) was significantly different from trifluralin (2.91 ppm) [$Z = 2.02$].

In general terms, the estimated GR₅₀ values of Tables 5 and 7 suggest the following order of phytotoxicity.

Table 7. Relative phytotoxicity of trifluralin microencapsulated formulations as estimated by nonlinear 3-parameter logistic equations.

Formulation	Expt. no.	Fresh weight ¹			Dry weight		
		b ₀	c	d	b ₀	c	d
Trifluralin, anal.	2	36.12	0.95	0.06	5.77	7.18	-0.16
"	7	26.13	5.33	0.39	5.93	2.91	0.09
"	9	34.08	4.58	0.44	4.71	5.18	0.44
"	12	32.59	5.59	0.44	4.23	4.84	0.40
TRIF 1							
TRIF 5	7	28.77	0.90	0.72	4.76	1.05	0.62
TRIF 6	7	22.27	4.81	0.90	3.27	7.98	0.96
TRIF 8	7	30.48	1.75	0.57	4.04	3.60	0.56
TRIF 9	8	32.15	2.87	0.81	3.73	7.86	0.90
TRIF 10	8	22.35	5.61	0.70	4.22	2.59	0.35
TRIF 11	8	23.11	4.57	0.75	3.87	3.49	0.39
TRIF 12	9	41.55	1.13	0.69	5.68	1.01	0.44
TRIF 13	9	23.12	1.50	1.00	6.57	0.65	0.49
TRIF 14	9	28.99	3.48	1.05	4.23	4.49	1.05
TRIF 15	12	27.66	12.32	1.45	3.74	4.75	0.50
TRIF 16	12	29.51	0.95	0.45	4.90	0.87	0.42
TRIF 17	12	35.56	1.34	0.57	3.89	3.66	0.60
TRIF 18	12	30.78	1.53	0.68	3.83	2.24	0.58

¹Equation used is $y = b_0/[1 + (x/c)^d]$, where y = plant growth (weight of tops, mg); b_0 = weight of control (untreated) plants; x = herbicide application rate (a.i., ppm); c = herbicide rate which reduces weight by 50%, i.e., the GR₅₀ value; and d = slope. Results are based on the growth of oats (*Avena sativa* 'Victory') planted immediately after soil treatment and harvested 15 days later.

More toxic than trifluralin (lower GR₅₀):

TRIF-16, -5, -12, -13, -18

Similar toxicity to trifluralin:

TRIF-17, -8, -14, -11, -6, -9, -10

Less toxic than trifluralin (higher GR₅₀):

TRIF-15

However, by the third harvest (Table 2) there was evidence that TRIF-16 had lost much of its toxicity, whereas TRIF-15 was now the most active formulation. This suggests that herbicide release from formulations such as TRIF-16 was fairly rapid, whereas that from TRIF-15 was much slower, thereby prolonging trifluralin's persistence in soil.

Volatilization. The vapor pressures of atrazine and trifluralin are 0.187 mPa (30°C) and 26.5 mPa (29.5°), respectively (Plimmer, 1976). That of trifluralin is sufficiently high so that major vapor phase loss can occur unless the herbicide is promptly incorporated into soil during or after application (Helling, 1976). The purpose of this experiment was to develop a method for estimating such loss from microcapsules. Only a few samples were tested by this procedure because of primary commitments to the bioassay study.

Vapor density, d_0 , was calculated as

$$d_0 = m = \frac{PM}{RT} \quad [4]$$

where d_0 is the mass per unit volume (g l^{-1}), m = mass of chemical (g; assumes volume = 1 l), P = equilibrium vapor pressure (atm), M = molecular weight (g mol^{-1}), T = temperature ($^{\circ}\text{K}$), and R = gas constant ($0.082 \text{ l atm mol}^{-1} ^{\circ}\text{K}^{-1}$). At 30°C, calculated $d_0(\text{atrazine}) = 0.016 \mu\text{g l}^{-1}$ and $d_0(\text{trifluralin}) = 3.61 \mu\text{g l}^{-1}$. The estimated maximum loss at 30°, assuming instantaneous equilibration from a nonlimiting herbicide source, was 0.23 μg and 52 μg for atrazine and trifluralin

in a system flushed by 10 ml min^{-1} air for a 24-h period. Thus, 1 g of pesticide was considered to be a nonlimiting source for this experiment. In our apparatus, the 10 ml min^{-1} air flow represented a 1.91 m h^{-1} velocity---very slow flow.

The results of a 24-h run are shown in Table 8. Virtually all trifluralin was trapped in the lower plug section (ca. 7 mm high) and the blank indicated no interfering GC peaks. The total loss of trifluralin was 3-4X higher than calculated, yielding an apparent d_0 of $10\text{-}13 \text{ } \mu\text{g l}^{-1}$. This may indicate some form of direct contamination of the foam plugs.

Table 8. Volatilization of trifluralin and five atrazine formulations in 24 h at 30°C .

Sample	Apparent quantity of herbicide trapped, μg		
	Lower plug	Upper plug	Total
----- Trifluralin -----			
Blank	0.02	0.01	0.03
Trifluralin, anal.	148.5	0.4	148.9
TRIF-1	190.0	2.4	192.4
----- Atrazine -----			
Blank	1.18	0.57	1.75
ATR-1	2.21	1.25	3.76
ATR-2	0.63	0.26	0.89
ATR-4	0.65	0.13	0.78
ATR-5	1.84	0.35	2.19
Atrazine-80W	4.32	0.43	4.75

Atrazine analysis was complicated by major interference, perhaps due to impurities trapped from the air stream. In all cases, the apparent atrazine peak was larger in the lower plug. Only with atrazine-80W and ATR-1 was this

peak substantially above the blank background. The highest residue, 4.75 μg , if corrected for the blank, becomes 3 μg which is much higher than the predicted loss.

This general approach to evaluating volatilization is likely to be successful only if (a) modifications are introduced to preclude any direct contamination of the foam plugs by the sample, and (b) the gas stream is purified further so that blanks (especially for atrazine analysis) are clean.

Atrazine Extraction from Aged Soil. Following the final, 6-month harvest of the first microencapsulated formulation tested (ATR-1), the soil was chemically extracted to determine whether parent atrazine remained. In a preliminary test, duplicate 25-g samples of moist soil were shaken overnight with 25 ml of methanol or chloroform. Samples of the control soil from this bioassay experiment were either extracted directly (as blanks) or spiked with atrazine to check recovery. Both solvents yielded only 40% recovery of the standard. The soil which was originally treated at 5 ppm (3 ppm AI) had apparent atrazine residues of 0.16 ppm (methanol extraction) or 0.06 ppm (chloroform extraction).

Extraction was repeated by using the Soxhlet technique with two other 5 ppm replications of ATR-1, as described in the Materials and Methods. Atrazine was readily detected at levels higher than analyzed in the less effective shaking procedure (Table 9). Ethyl acetate was somewhat more efficient than methanol or Freon 113 as the extraction solvent.

Bioassay measurements after the 3rd cropping (Table 3) had given no evidence of remaining herbicide. The residues found by chemical analysis, ca. 0.6-0.9 ppm, are higher than the estimated GR50 of atrazine. Thus, the atrazine which is present must be in an inaccessible form, either within the microcapsule or, more likely, partially bound to soil. This observation warrants further study.

Table 9. Atrazine residue, as determined by three extraction solvents, in ATR-1 treated (3 ppm AI) soil following the 6-month harvest.

Soil sample	Atrazine concentration, ppm		
	EtOAc	MeOH	Freon 113
Control	0	-----	-----
ATR-1, Rep. 1	0.91 $\pm$ 0.02	0.73 $\pm$ 0.01	0.63 $\pm$ 0.01
ATR-1, Rep. 5	0.68 $\pm$ 0.00	0.62 $\pm$ 0.00	0.41 $\pm$ 0.01
	$\bar{x}$ = 0.80	0.68	0.52

SUMMARY

The principal objective of this phase of the BARD project was to test microencapsulated herbicide formulations with respect to their phytotoxicity. All 22 atrazine and 14 trifluralin formulations sent from Israel were tested, usually in direct comparison with both untreated controls and unformulated parent herbicide.

The 15-day oat bioassay, conducted in growth chambers, demonstrated that all atrazine formulations, except perhaps ATR-36 and -29, were approximately as phytotoxic as unformulated atrazine. This seems to indicate fairly rapid loss of herbicide from the capsule. Thus, encapsulation has not diminished the efficacy of this soil-applied pesticide, as measured during the first 15 days. Bioactivity over longer periods, 2 and 6 months, may be less than for unformulated atrazine. However, chemically extractable atrazine (in the only treatment tested, ATR-1) after 6 months exceeded by 2-3X the concentration earlier shown to cause 50% growth reduction. Thus, the later bioassays may not be accurately representing remaining herbicide in the soil---perhaps because of bound residue formation.

Trifluralin evaluation is hampered in that the bioassay is less sensitive (necessitating rather high application rates) than desirable. Nevertheless, the tests showed both analytical and formulated trifluralin to be much more persistent than atrazine. Furthermore, microencapsulation seemed to prolong bioactivity in soil. Certain formulations may be up to twice as active as parent trifluralin, though interpretation requires caution because of high data variability.

Further research should focus on only a few very promising formulations. Presumably, the criteria would be (a) leaching characteristics, (b) bioactivity, and (c) other considerations such as ease of synthesis. Laboratory testing could logically be coordinated closely with field leaching and efficacy tests, i.e., using the same soil. Whereas all Beltsville studies used a typical medium textured soil, future work might best utilize coarse textured soils.

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מרכז סיגרם למדעי
הקרקע והמים

BIOASSAY TESTS OF TRIFLURALIN MICROCAPSULES

by

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ABSTRACT

Biotoxicity of two encapsulated trifluralin formulations (Trif. 5 and Trif. 13) was tested relative to that of analytical trifluralin applied to Rehovot soil (sandy loam). *Setaria verticulata* was used as a test plant.

The herbicide formulations were mixed in dry soil samples, 200 gr each, in six different doses up to 0.5 ppm and five replications of each treatment (90 pots in total) were carried out. Plants were grown for 16 days in growth chambers under controlled irrigation, illumination and temperature. Eighteen pots with no herbicide used as control, were distributed in a prescribed network to examine the overall effect of some environmental factors (light, temperature, wind, and humidity) on growth conditions in various positions of the growth chamber. Successful seedlings (out of 16 seeds) were counted and upon harvest, fresh and dry weight in each pot was measured. The soil was recropped twice, 2 and 5 months, after the first harvest to test the incubation effect on the phytotoxicity of the different formulations.

The first harvest indicated a significantly higher efficacy of two encapsulated trifluralin formulations, with Trif.13 being more effective than Trif. 5. GR_{50} values estimated graphically were 0.077, 0.132, and 0.240 ppm for the Trif. 13, Trif. 5 and analytical trifluralin, respectively. The second crop showed decreased bioactivity of the formulated trifluralin while the unformulated trifluralin remained as active. The third crop did not indicate statistically significant differences for the two trifluralin microcapsules. In this case, only the analytical trifluralin and Trif. 13 indicated a low degree of phytotoxicity for high application rate (0.5 ppm), while for lower application rates, no reduction of growth was observed by the various

treatments. Plant growth was as high or even higher than that of the control plants.

Introduction

Detailed review of the various application studies of pesticide encapsulation is presented in another report by Helling and Pillai (1985). The objectives of this study were to test the efficacy of formulated versus unformulated trifluralin and examine the slow release characteristics of two microencapsulated herbicide formulations as reflected by their biological activity. Trifluralin was chosen as a test chemical for being an effective herbicide on the one hand, and for having some problematic properties, on the other. The properties with which we were concerned are: volatilization, low solubility in water, strong adsorption to soil and consequently immobility in soil. It is conceivable that in case of very small microcapsules, the trifluralin can be applied to soil by the irrigation water instead of mechanically which is advantageous. In the present study, microcapsules were too large to be mobile in test soil which includes a significant fraction of clay. Therefore, the bioassay test may serve in this case as an indicator with regard to two aspects of pesticide application: (a) the homogeneity of its distribution in soil and (b) the microencapsulation effect on its bioactivity and persistence in soil.

Materials and Methods

The soil used in this study is Rehovot red brown sand, taken from the farm of the Faculty of Agriculture of the Hebrew University, in Rehovot. The soil is loamy sand by classification, and moderately alkaline, pH ~ 7.5, with almost no organic matter. The soil was crushed and passed through a 2 mm sieve. Particle size distribution of the soil in dry

sieving is given in another report on mobility of microcapsules in saturated soil. The soil particle density was 2.53 gr/cm³. Cone-shaped, 200 cm³ plastic cups perforated at the bottom were utilized with 200 gr of dry soil in each. The soil bulk density in the cups was 1.47, ~1.7, and 1.48 gr/cm³ before the first, second and third sowing, respectively. The plastic cups were painted black on the outside to prevent photodegradation of the herbicide or fast development of microbial populations in the soil.

Two microencapsulated formulations of trifluralin, marked as Trif. 5 and Trif. 13, which were relatively easy for dispersion in water, were used for the bioassay test. The active ingredient (AI) of the microcapsules were 59% and 51.8%, respectively. Details of the microcapsule formulations are reported by Dr. A. Markus.

After some experiments were carried out on wheat, oats and setaria, it was found that setaria verticulata is very sensitive and most suitable as a test plant.

The efficacy of the two microencapsulated formulations were tested on a comparative basis with unformulated analytical trifluralin and untreated soil. Each of the three materials was applied to soil at 6 concentrations: 0.025, 0.050, 0.075, 0.100, 0.250, 0.500 ppm (on weight basis) with 5 replications of each treatment. In total, 90 cups were used for this purpose. In addition, 18 cups of untreated soil were used as control. As application rate was quite low, careful mixing through several dilution steps was followed. Soil fraction that passed through 250 sieve (ASTM, No. 60, 250 MIC) was used to form 3 stocks, 10 gr each, with 1000 ppm active ingredient of Trif. 5, Trif. 13 and analytical trifluralin. Samples of these stocks were used to form new stocks of 50 and 5 ppm by adding the necessary amount of the soil fine fraction. From these stocks, certain

quantities were withdrawn and blended with the 200 gr soil in the pots to yield the prescribed trifluralin AI concentration.

Seeds were screened and only good seeds were selected for sowing. Sixteen seeds were planted in each pot in square matrix of 4 x 4, 1 cm below soil surface. The distance between adjacent plants was about 1.2 cm. Planting was carried out a day after irrigation when the soil was still wet.

The room temperature during the experiment was 26-28°C with 12 hour day length. A Neon light was fixed approximately 50 cm above the pots.

Irrigation was applied at two day intervals, using distilled water. The pots were wet to saturation degree of 60% (based on porosity determined before the first sowing). Fertilizers were applied twice, 1/2 Hougland each time, through the irrigation water before sowing and one week after.

Upon harvest, the number of plants in each pot was counted to calculate the percentage of good seedlings. Fresh weight and dry weight, following 48 hr oven drying at 70°C, were also measured.

Between crops, the pots were left to dry at room temperature. Before the second recropping, the soil in the pots was not treated mechanically in order to avoid structure disturbance and additional mixing compared to the first crop. As a result, seedling was more erratic than in the first crop and higher values of standard deviation were observed. Before the third sowing, the hard structure was broken and the soil was loosened to a bulk density approximately equal to that of the first crop.

The pots were randomly distributed in the growth chamber except for the untreated pots which were used for control. The 18 cups were given a specific position on the board to allow observation of the pot position effect on plant growth. The arrangement of the various cups in the growth chamber is given in Fig. 1.

	Treatment Number	Trifluralin Concentration (ppm)
C-CONTROL	1	0.025
Trp-ANAL. TRIFLURALIN	2	0.050
Tr5-ENCAPSULATED TRIFLURALIN No.5	3	0.075
Tr13-ENCAPSULATED TRIFLURALIN No.13	4	0.100
	5	0.250
	6	0.500

Replications: a b c d e

C	Tr13 6 d	C	Trp 1 d	C	Tr13 5 c
Tr13 4 e	Trp 5 e	Tr5 2 c	Tr5 6 b	Tr13 1 b	Trp 1 c
Tr5 4 a	Tr13 1 c	Tr5 3 a	Trp 2 e	Tr5 5 e	Trp 4 e
C	Tr13 4 c	C	Tr13 4 b	C	Tr5 1 a
Tr5 5 b	Trp 5 b	Trp 2 a	Trp 2 c	Trp 6 e	Tr5 1 c
Trp 4 b	Tr13 3 c	Trp 5 c	Tr5 4 d	Trp 6 d	Tr5 2 a
Tr13 3 e	C	Tr5 2 d	C	Tr5 3 e	Tr13 6 e
Trp 1 a	Tr13 4 a	Trp 3 c	Trp 6 b	Trp 3 e	Tr13 1 e
Trp 3 a	Tr13 5 b	Tr5 2 b	Tr13 2 d	Tr13 2 b	Tr5 6 a
C	Trp 2 d	Tr13 6 c	Trp 4 c	Tr13 2 a	C
Tr5 5 d	Tr5 4 c	Tr13 2 e	Trp 1 e	Tr13 5 e	Tr13 3 b
Trp 6 a	Tr5 6 e	Tr5 3 b	Trp 4 d	Trp 1 b	Trp 4 a
Trp 5 d	C	Tr5 2 e	C	Tr13 3 d	Tr5 4 b
Tr5 3 d	Tr13 1 a	Trp 2 b	Trp 6 c	Tr13 5 d	Tr13 4 d
Trp 5 a	Tr5 5 c	Tr13 5 a	Tr13 2 c	Tr5 6 d	Tr5 4 e
C	Tr5 5 a	C	Tr5 3 c	C	Tr13 6 b
Tr13 3 a	Tr5 1 d	Trp 3 b	Tr5 6 c	Tr13 6 a	Tr13 1 d
C	Tr5 1 b	C	Tr5 1 e	C	Trp 3 d

Figure 1. Schematic plan of pots position in the growth chamber.

Results and Discussions

Figure 2 shows pictures of the plants taken 8 days after sowing of the first crop. The upper group are pots treated with Trif. 13. Each row represents different application rate with increasing order from left to right. The row on the extreme left is the control. The AI concentration in the other rows are 0.025, 0.05, 0.075, 0.100, 0.250, 0.5, respectively. The middle group represents treatment by Trif. 5 while the lower group was treated with analytical trifluralin. Both groups were arranged in the same order as the upper group. Observing the rows right to the center, it is possible to see that the phytotoxicity of Trif. 13 was higher than the other formulation with the analytical trifluralin being the least efficient in that stage.

Figure 3 is a picture of the various treatments taken on the 16th day, a few hours before harvest. The pots set up is the same as in Fig. 2. The higher effectiveness of Trif. 13 is obvious.

Figure 4 shows the efficacy of the three formulations illustrated by the comparison with the control at the upper horizontal row. The AI concentration in soil of the three treatments is identical and equal to 0.075 ppm.

Figures 5 and 6 are the same as Fig. 4 but with active ingredients concentration equal to 0.25 and 0.50 ppm, respectively. The three figures clearly indicate the relative toxicity of the three treatments: Trif. 13>Trif. 5>Anal. The differences are very clear in Fig. 5 (AI concentration=0.25) where Trif: 13 yields complete kill, Trif. 5 drastic oppression of growth and the analytical trifluralin diminished growth approximately by 50% of control. For high AI, application rate of 0.5 ppm

(a)

(b)

(c)

Figure 2. The plants 8 days after sowing of first crop: (a) Trif. 13; (b) Trif. 5, and (c) Anal. Trifluralin.

(a)

(b)

(c)

Figure 3. The plants 16 days after sowing - before harvest of first crop.
 (a) Trif. 13; (b) Trif. 5, and (c) Anal. Trifluralin.

Figure 4. Plants before harvest of first crop. Upper row is the control. Lower three rows treated with 0.075 AI, Trif. 13, Trif. 5 and Anal. Trifluralin, respectively.

Figure 5. As in Figure 4 with AI=0.25 ppm.

Figure 6. As in Figure 4 with AI=0.50 ppm.

both formulated and unformulated were effective.

Based on the data measured during the first harvest, we found a quite high correlation between fresh and oven dry weight including all pots used in the experiment (Fig. 7). Consequently, only fresh weight has been considered in the following as no significant information is added by the dry weight. It may be worth noting that while on the overall a linear function represents well the relationship between dry and fresh weight, there is a large number of points below the regression curve for low fresh weight (high application rate of herbicide) which may indicate that the ratio of dry to fresh weight decreases for the affected plants.

The qualitative results of Figs. 2 to 6 are quantified in Figs. 8 to 15. Figure 8 shows the fresh weight (of all 5 replications) relative to fresh weight of control as a function of active ingredient concentration. The higher efficacy of the microencapsulated formulation is obvious. In the case of the unformulated trifluralin there seems to be no effect at low concentration (AI concentration <50 ppb).

Trifluralin affects cell division and consequently good seedling as well. Therefore, this variable was considered to be of interest.

Figure 9 shows the average percentage of good seedling for each treatment as a function of the AI concentration. Absolute rather than relative values were used to indicate the percentage of good seedling in the control pots which was 90%. No systematic trend was observed at low herbicide concentration. Higher values of good seedling were observed in the case of using unformulated trifluralin at low doses (25 and 100 ppb). The order of toxicity was again Trif. 13 >Trif. 5 >Anal.

Randomness in good seedling as a result of genetic, physiological and physical variables not investigated in this study, may have introduced noise in the effect of the trifluralin application on total fresh weight.

Figure 7. Fresh weight versus dry weight of all treatments of first crop.

Figure 8. Fresh weight relative to control of all plants in each treatment of the first crop as a function of AI concentration in soil.

ACTIVE TREFLAN CONCENTRATION (ppb)

Figure 9. Percentage of good seedling of first crop as a function of the trifluralin AI concentration in the soil.

In order to distinguish somehow between good seedlings and growth, the average value of fresh plant weight was calculated relative to plant weight of control (Fig. 10). The decrease of these values with increase of AI concentration is in this case more consistent than in Figs. 8 and 9, though the order of efficiencies of the three different trifluralin products did not change.

As mentioned above, the replications of untreated soil were larger than other treatments (18:5). They were placed in the growth chamber as described in Fig. 1 to observe the effect of position on growth condition. Such an effect was expected because of nonuniformities of environmental conditions (on the microscale of plant size) over the growth areas, including wind intensity and direction, temperature, humidity and light intensity. Figure 1 shows that the pots were set up in 6 rows and 18 columns. The control pots were marked by C. Using the plant fresh weight of the control as an indicator, the average values for rows and columns were calculated and drawn in Fig. 11. It is noticeable that the variation in the longitudinal direction represented by column average values (stars) is systematic, with significantly heavier weight of the plant in the center. The variation of the line (or row) average value (pluses) is less systematic. To these points, we fitted polinoms of the second and third order, dotted and dashed lines, respectively, with minimum square deviation as criteria for best fit. For each pot, a correction factor was determined according to its position on the board (line and column) using the corresponding polinom values (correction factor for each pot being equal to the multiple of the maximum values of the two polinoms divided to the values of the polinoms at the corresponding position of the pot). The corrected plant fresh weight as function of active trifluralin

Figure 10. Average plant fresh weight relative to control as a function of the trifluralin AI concentration in the soil (first crop).

Figure 11. Average plant fresh weight of the control pots on first crop at the different lines (pluses) and columns (stars).

concentration is shown in Fig. 12. The fluctuation (experimental errors), displayed by various curves, were minimized by accounting for the position or the environmental effects. The curves related to Trif. 13 and Trif. 5 are now a monotonically decreasing function in the complete AI concentration range. The differences in shape between these two curves and the curve related to the unformulated trifluralin may indicate differences in uniformities of herbicide distribution in the soil. This idea is seconded by the results of Fig. 13 showing the calculated coefficient of variance of the growth reduction for each treatment as a function of AI concentration in soil. The difference is very large for low trifluralin concentration; almost 5 times higher in the case of unformulated trifluralin. At high concentration the differences are not that significant. We may conclude therefore that significantly better distribution is achieved when applying formulated trifluralin than analytical trifluralin.

An empirical exponential function

$$y = ae^{-x^n}$$

was used to represent the experimental results of Fig. 12, where y is the plant relative fresh weight, x is active trifluralin concentration in soil and a and n are characteristic parameters to be determined by best fit procedure. The parameters a and n, and the correlation factor for three curves are listed in Table 1. There is a significant difference between r^2 calculated for the formulated and the unformulated trifluralin indicating again the smaller variability of the results in case of the microcapsules application. The fitted function and data are shown in Fig. 14. The agreement between empirical curve and the experimental result is quite good. Using the continuous functions of Table 1, the active trifluralin

ACTIVE TREFLAN CONCENTRATION (ppb)

Figure 12. Corrected average plant fresh weight, relative to control of first crop as a function of trifluralin concentration in the soil.

Figure 13. Coefficient of variation of reduction in fresh weight relative to control as a function of AI concentration in the soil (first crop).

Table 1. Parameters of the empirical function $y=ae^{x^{-n}}$ derived by best fit procedure.

	a	n	r ²
Anal. Trif.	7.25×10^{-4}	1.252	0.862
Trif. 5	1.33×10^{-2}	0.808	0.984
Trif. 13	1.30×10^{-2}	0.908	0.986

Table 2. The active trifluralin concentration in soil leading to growth reduction of 50 and 90% (GR₅₀ and GR₉₀ , respectively).

	GR ₅₀	GR ₉₀
Anal. Trif.	240	624
Trif. 5	132	586
Trif. 13	77	294

ACTIVE TRIFLURALIN CONCENTRATION (ppb)

Figure 14. Data (points) and fitted curves describing the corrected relative plant fresh weight as a function of the trifluralin AI concentration in the soil.

concentrations causing 50% and 90% growth reduction were calculated (Table 2).

The soil was recropped 2 months after the first harvest. In the meantime the soil had been dried and hardened. As a result, the same amount of irrigation lead to considerably higher saturation and aeration deficiency. The soil hardness seriously affected seedling. The analyses of the results followed the same procedure applied for the first crop.

Figure 15 shows the relative fresh weight as a function of the active trifluralin concentration for the three forms of applied trifluralin. Two main properties can be observed: (a) Significant variation of the results for low values of trifluralin concentration. All three curves show considerably higher fresh weight for applicate rate of 75 ppb. (b) The distances between the three curves, representing differences in bioactivity, diminished compared to the first harvest result. Both microencapsulated formulation seems to have lost bioactivity compared to the unformulated trifluralin. Similar trend was observed in Fig. 16 showing the percentage of good seedling. The resemblance between the shape of the curves in Figs. 15 and 16 indicate that the percentage of good seedling may partly explain the relative fresh weight data of Fig. 15. For example, the significantly higher values of good seedling at 75 ppb than the control explain why the relative fresh weight at this concentration is higher than 100%. When the single plant fresh weight is considered (Fig. 17), the shapes of the curves at low AI concentration is different to the corresponding ones in Fig. 15. In the case of Trif. 13, the relative plant weight is smaller than 100% showing more systematically the effect of the formulated herbicide.

If we assume that the percentage of good seedling was indeed affected

Figure 15. Fresh weight relative to control of all plants in each treatment of the second crop as a function of AI concentration in the soil.

ACTIVE TRIFLURALIN CONCENTRATION (ppb)

Figure 16. Percentage of good seedlings of second crop as a function of the trifluralin AI concentration in the soil.

Figure 17. Average plant fresh weight relative to control as a function of the trifluralin AI concentration in the soil (second crop).

by the mechanical condition of the soil, one may conclude from Fig. 17 that Trif. 5 lost more of its bioactivity than indicated by Fig. 15. While the formulated trifluralin suffered from considerable losses of their phytotoxicity, the unformulated trifluralin seems to be quite persistent in soil with no losses observed at the second crop (there is some gain at maximum concentration at 500 ppb).

Similar to Fig. 11, Figure 18 illustrates the effect of position of the plant fresh weight. In this case, the position effect seems to be more important. However, the fluctuation of the measured data around the fitted function is more pronounced for the columns than that observed in the first crop. In such a case, the application of the correction factor may not be as effective. Figure 19 shows the plant relative fresh weight using the correction factor based on results of Figure 18 (see discussion of the first harvest data for details). The curves were not smooth in this case as shown in Fig. 10.

The third crop was sown 5.5 months after application of the herbicide to soil. To improve seedling, the soil in the pots was loosened before sowing. No aeration problems were observed. Figures 20 to 23 show the results of the third harvest. The main trend of losing bioactivity as observed during the second harvest continued in the third crop. Figure 20 shows that low application rate of herbicide yielded higher production of fresh weight compared to control. Since in this case the percentage of good seedling is generally lower than that of the untreated soil (Fig. 21), there seems to be no retarding effect on growth of the pesticide application after 5.5 months. On the contrary, except at maximum application rate of 500 ppb, there is a general increase of growth (Fig. 22) though statistically insignificant. The phenomenon of increased growth at low active concentration of herbicides is known and generally explained

Figure 18. Average plant fresh weight of the control pots on second crop at the different lines (pluses) and columns (stars).

ACTIVE TREFLAN CONCENTRATION (ppb)

Figure 19. Corrected average plant fresh weight, relative to control of second crop as a function of trifluralin concentration in the soil.

ACTIVE TREFLAN CONCENTRATION (ppb)

Figure 20. Fresh weight relative to control of all plants in each treatment of the third crop as a function of AI concentration in the soil.

Figure 21. Percentage of good seedlings of third crop as a function of the trifluralin AI concentration in the soil.

Figure 22. Average plant fresh weight relative to control as a function of the trifluralin AI concentration in the soil (third crop).

Figure 23. Coefficient of variation of reduction in fresh weight relative to control as a function of AI concentration in the soil (third crop).

either by direct or indirect effects on plants by affecting competing population on the available nutrient resources.

Figure 23 shows the calculated coefficient of variation of the growth reduction. The negative values result from increased growth relative to the untreated soil. The coefficient values are generally high and therefore the differences between the treatment with Trif. 13, Trif. 5 and the unformulated trifluralin observed in Fig. 21 are in most cases statistically insignificant.

Conclusions

Considerably higher bioactivity of the microencapsulated formulations than the analytical trifluralin, was observed during the first crop. Some differences were found also between the two microcapsule formulation with the Trif. 13 showing higher phytotoxicity. However, these differences may also have resulted from some inaccuracies in the determination of the active ingredient in the Trif. 5 microcapsules (being actually less than value used in our study). This possibility is based on later remeasurement different methods of Trif. 13 and Trif. 5 content of active ingredient. In these cases, the phytotoxicity of the two microencapsulated formulation would be rather similar mainly, in both cases considerably higher than that of the unformulated trifluralin during the first crop. However, both Trif. 13 and Trif. 5 displayed diminishing effectiveness in the second crop, 2.5 months after application of the herbicide to soil, while the unformulated trifluralin seems to be still as persistent in soil as in the first crop. In the third crop, all trifluralin applications seem to lose their bioactivity. The differences in residue effectiveness were rather insignificant.

The above results are somewhat different from those obtained by our colleague in the U.S.A. who carried out the bioassay test on silty loam soil and on a different test plant (oats). He generally found high persistence of the trifluralin bioactivity in soil after 3 and 6 months. He also found Trif. 13 to be active at initial rate after 3 months, 2-3x lower than unformulated trifluralin, while Trif. 5 seemed to lose bioactivity which is not in accordance with our findings. The differences may partly be explained by the differences in soil type, experimentally conditions and the significantly higher application rate (10-20 times higher concentration). We think, however, that no real difference exists between Trif. 13 and Trif. 5 bioactivity decay characteristics.

Based on the above results, it seems clear that the efficacy of the microencapsulated trifluralin was significantly higher during the first crop than that of the unformulated trifluralin. This result can not be attributed to the slow release property because in the case of slow release over a long period, the results should be expected to be the opposite to our findings. We therefore conclude that even though the active trifluralin in soil was higher in the case of the unformulated herbicide, its availability at the immediate vicinity of the roots, and thus its effectiveness, were considerably smaller. The main reason we can see is uniformity in soil. The trifluralin can be effective in very low rates when evenly spread. In our study, the trifluralin release in the soil was fast enough to maintain active level in relatively low rates of application. In the case of untreated formulation, the herbicide could not be dispersed as well and points of relatively highly concentrated material were probably formed in the soil, (source point) while other places remained with too low concentration. This phenomenon coupled with low solubility and low mobility prohibited even distribution in the soil.

Consequently, higher doses of unformulated trifluralin were required to achieve the same growth reduction observed when microencapsulated formulation was applied.

Concluding, we found the microencapsulation of such herbicide to be rather beneficial. Much better uniformity is obtained which enhances its effectiveness. Consequently, less active ingredient is required while minimizing its long term adverse effect.

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