Biological Evaluation of Pesticides Released from Temperature-Responsive Microcapsules

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The pesticides diazinon, trifluralin, and alachlor were successfully encapsulated into temperature-responsive Intelimer microcapsules. The pesticides were differentially released into aqueous solutions at predictable rates in response to temperature. The response of corn (*Zea mays* L.) insect pests *Diabrotica balteata* LeConte (banded cucumber beetle) and *Diabrotica virgifera* LeConte (western corn rootworm) with the insecticide diazinon confirmed the temperature activity relationship of the encapsulated formulations compared to a commercial formulation. Encapsulation of trifluralin reduced corn injury in response to soil applications and reduced the need for immediate soil incorporation compared to the commercially used emulsifiable concentrate formulation. Encapsulated alachlor prolonged the control of large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and reduced leaching in soil compared to an emulsifiable concentrate formulation.

INTRODUCTION

Concern for the environment has increased interest in reducing the amount of pesticide applied per hectare and increasing pesticide safety (Thomas, 1990). This can be accomplished by changing the formulation of currently used pesticides to prolong their effectiveness, thus reducing the need for high initial doses or multiple applications (Seaman, 1990). Encapsulating pesticides into an inert matrix can provide protection from degradation and can prevent volatilization or leaching losses (Riggle and Penner, 1990). Past encapsulation technology has provided few pesticide formulations that possess desirable environmental characteristics and still effectively control targeted pests. Weed, insect, and disease pests are most active when soil or air temperatures reach a critical level. By developing a pesticide capsule that protects the pesticide from degradation and transfer processes until this critical temperature is reached, it is possible to reduce the total amount of pesticide needed. The objectives of this research were to demonstrate the temperature-controlled pesticide release characteristics of Intelimer polymer (Stewart, 1989) microcapsule formulations of three widely used pesticides, trifluralin [2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)-benzenamine], diazinon [*O*,*O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate], and alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide], and to evaluate the biological activity of each compared to that of commercial formulations.

EXPERIMENTAL PROCEDURES

Preparation of Microcapsules. Technical grade diazinon (87.5%), trifluralin (96%), and alachlor (90%) were obtained from Ciba Geigy, Griffin Corp., and Makteshim Agan, respectively. The active ingredient was combined with the polymer and an isocyanate and heated. The resulting oil mixture was dispersed in water and the particle size adjusted from 10 to 100 μm. An amine was added to the water phase, and mixing was continued for 1 h. The melt transition temperature of the microcapsules was measured by differential scanning calorimetry. Release rates, designated later as either fast or slow, were controlled by changing the polarity of the Intelimer polymer, the cross-link density of the wall, and the amount of wall material in the capsule. The final content of diazinon, trifluralin, and alachlor in the formulations was determined by dissolution in tetrahydrofuran, separation of the pesticide from the polymer, and analysis by gas chromatography.

Pesticide Release Rate As Affected by Temperature. The release rate of each pesticide from the various temperature-release microcapsules was determined by placing enough of each formulation to equal 7500 μg of active pesticide into 100-mL glass vials filled with deionized water for diazinon or alachlor and ethanol/water (1:1 v/v) for trifluralin. The vials were placed in a constant-temperature bath at the selected temperature, and sample aliquots were removed periodically for analysis by UV spectrophotometry (246 nm for diazinon and 279 nm for trifluralin) or liquid chromatography/UV detection (219 nm for alachlor). Release rates were conducted for up to 32 days at temperature below, at, and/or above the melt temperature for the capsule. The procedure was conducted three times for each pesticide formulation.

Biological Activity Evaluation of Diazinon Formulations. Two experiments were conducted to determine the biological efficacy of selected diazinon formulations. In the first, a slow and fast release capsule with a melting point of 25 °C was compared to a commercial formulation of diazinon (14 G) (14% active granular). Enough of each was mixed with a dry soil to achieve either a 2.0 or 2.5 ppmw concentration of diazinon. The soil was placed in a chamber at 20 °C. At the end of weeks 1, 2, and 4, four replicates of 100 g of soil treated with the various formulations were removed. The soil was placed in cups with a sprouted corn (*Zea mays* L.) seed and 10 larvae of *Diabrotica balteata* (banded cucumber beetle), a corn insect pest. Four replicates of untreated soil were also used for comparison. After 4 days, the soil was sifted and the number of live larvae was determined. After the 4-week sample was taken, the temperature was increased to 32 °C. Soil samples were taken after weeks 5, 6, and 8 and were processed the same as described above. The data were analyzed by dividing the number of dead larvae per cup by the number of live larvae that had survived in the nontreated soil and multiplying by 100. An analysis of variance was conducted on the data with the means compared by LSD at the 0.05 level of probability.

In the second experiment, the biological activity of a fast release formulation of the 25 °C release capsule of diazinon was compared to that of the 14 G commercial formulation of diazinon. Enough of each formulation was measured into soil to achieve a 1.0 ppmw concentration of diazinon. Each treatment was placed in a chamber at 20 °C. To determine the biological activity of each formulation over time, the same method as described in the first experiment was used except that larvae of *Diabrotica virgifera* (western corn rootworm) were used. Samples were removed from

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the chamber and assayed on the day of experiment initiation and 1, 2, and 4 weeks after initiation. The temperature in the chamber was raised to 30 °C after the 4-week sample, and additional samples were taken 5, 6, and 8 weeks after experiment initiation.

Biological Activity Evaluation of Trifluralin Formulations. In two experiments, the biological activity of various encapsulated trifluralin formulations was compared to that of a commercially available emulsifiable concentrate (EC) trifluralin formulation. The first experiment was conducted in a field containing a Sharpsburg silt loam soil (fine, montmorillonitic, mesic Typic Argiudoll) to evaluate the response of corn and two weed species to 0.56 and 1.12 kg of ai/ha rates of the fast and slow release trifluralin capsules with either 20 or 30 °C melting points. These treatments were compared to the same rates of the EC formulation of trifluralin. All treatments were applied in 165 L of water/ha with a hand-held spray boom that was powered by CO_2. Plots were 3 × 9 m, and the treatments were replicated three times in a randomized complete block design. Within 1 hour of application, all treatments were incorporated into the soil to a depth of 7.5 cm using a power-driven rototiller. On the same day of application, four rows of corn were planted in each plot. The experimental area was irrigated with 2.5 cm of water 3 days after experiment initiation. The corn and two weed species, barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.] and redroot pigweed (*Amaranthus retroflexus* L.), began to emerge within 14 days of experiment initiation. Corn plant counts (number of plants per 1 m of row from the middle two rows of each plot) and visual evaluations of corn injury and weed control were made 29 days after experiment initiation. Visual evaluations were made on a 0–100 scale with 0 being no injury to the corn or no weed control when compared to the plants growing in untreated plots and 100 being no corn or no weed emergence. All data were subjected to analysis of variance with means compared by LSD at the 0.05 level of probability.

The second experiment was established in the field to evaluate the effect of delayed soil incorporation of various trifluralin formulations on the control of giant foxtail (*Setaria faberi* Herrm.). The same trifluralin formulations as used in the first experiment were applied at either 0.84 or 1.12 kg of ai/ha to a Sharpsburg silty clay loam soil (fine, montmorillonitic, mesic Typic Argiudoll). Treatments were replicated 4 times in 3 × 9 m plots and arranged in a split–split plot design with the main effect being trifluralin rate, the subeffect being trifluralin formulation, and the sub-subeffect being the time of soil incorporation. In half of the treated plots, a power-driven rototiller was used to incorporate the herbicide formulations to a depth of 6 cm on the day of application. The remaining plots were not tilled until 52 days after application. Seventeen days after the latter tillage (12 weeks after the herbicide treatments had been applied), visual evaluations of giant foxtail control were made. Data were analyzed as previously described.

Biological Activity Evaluation of Alachlor Formulations. Fast and slow alachlor release formulations of capsules with a melting temperature of 20 °C and the commercial EC alachlor formulation were mixed with a sandy loam soil to achieve alachlor concentrations of 2.0 and 3.0 ppmw. Eight replications of each treatment were prepared by pouring 350 g of the treated soil into plastic cups, watering until the soil was thoroughly moistened, and placing all of the cups into a temperature-controlled lighted growth chamber set at 15 °C. In four of the replications, annual ryegrass (*Lolium multiflorum* Lam.) was planted into the soil on day 14 after experiment initiation. The soil was watered as necessary. The annual ryegrass was visually evaluated for alachlor injury on day 28. On day 30, the soil was thoroughly mixed within each cup of the remaining four replications of each treatment and large crabgrass seeds were planted. The soil was rewatered and the temperature raised to 25 °C. After 14 days, large crabgrass emergence and growth were visually evaluated for response to alachlor. This process was repeated on days 44 and 58. Data were analyzed as previously described.

To determine how the various formulations would affect the movement of alachlor in soil due to water, a dried, sieved clay loam soil was packed into 7.6 cm (i.d.) × 37.5 cm plastic tubes that were capped on one end. The column had been horizontally sectioned into 2.5-cm segments, each connected by silicon sealer, and small gravel was placed in the capped bottom prior to the tubes being filled with soil. The cap had a small hole in it to allow for drainage. No soil was packed into the top 5 cm of each tube. The final bulk density of the soil was 1.2 g/cm^3. One pore volume (approximately 600 mL) of water was passed through each tube for conditioning. After 24 h, 2.5 cm of sand was placed on the soil surface of each tube and 3.34 mL of a 500 ppm solution of either the EC, 20 °C fast release, or 20 °C slow release alachlor formulations was added to the sand. Four replications of each formulation were placed into a temperature-controlled chamber set at either 15 or 25 °C. After 4 h, filter paper was placed on the soil surface and a constant head of water was placed on each tube until 600 mL of water had been added. The soil was allowed to drain for 12 h after addition of the last amount of water.

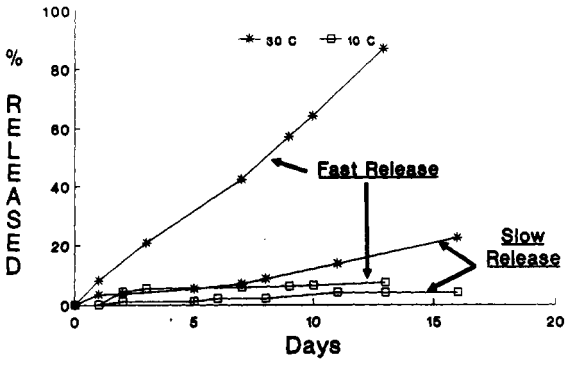

Figure 1. Release of diazinon into deionized water from the 30 °C melting point capsules for slow and fast release formulations at 10 and 30 °C. SD for the fast release capsules ranged from 0.97 to 1.6% at 10 °C and from 1.4 to 8.0% at 30 °C. SD for the slow capsules ranged from 0.2 to 1.2% at 10 °C and from 0.2 to 5.5% at 30 °C.

The tubes were removed from the temperature-controlled chambers and placed in a greenhouse at 25–32 °C. The 2.5-cm segments of each tube were separated by slicing each apart with a sharp knife. Each segment was placed horizontally on plastic so that the upper side corresponded with the top of the tube as it was sliced apart. Large crabgrass seeds were liberally sprinkled onto the upper surface of each segment, pressed into the soil, and covered lightly with sand. Plastic wrap was placed over all segments to prevent excessive drying of the soil. After 8 days, the large crabgrass was visually evaluated for alachlor injury to determine the depth of alachlor movement in each tube. If large crabgrass injury was detected in the first soil segment, it was assumed that the alachlor had moved out of the sand and at least 2.5 cm deep into the tube. The same was assumed for each segment where alachlor injury to the large crabgrass was observed. The greatest depth of alachlor penetration for each tube was recorded, and treatment means were compared by LSD at the 0.05 level of probability.

RESULTS AND DISCUSSION

The capsule formulations produced by this procedure contained approximately 25, 19, and 18% diazinon, alachlor, and trifluralin by weight, respectively. When the release rates of each formulation were evaluated, some of each pesticide was detected at the zero sample time, indicating some free or very rapidly released pesticide was in the formulation (data not shown). The amount detected at time zero was less than 9% of the pesticide in all formulations, except for the trifluralin 20 °C fast release formulation (15%). In the calculations to determine the rate of pesticide release from the capsules, the amount of pesticide detected at time zero was subtracted from the amount detected at each time interval. Temperature did not influence the amount of pesticide detected at time zero.

Diazinon Release. At 10 °C, very little diazinon in the 30 °C melting point capsules was released from either the fast or slow release formulation (7.4 and 4%, respectively) (Figure 1). After 13 days at 30 °C, the fast release

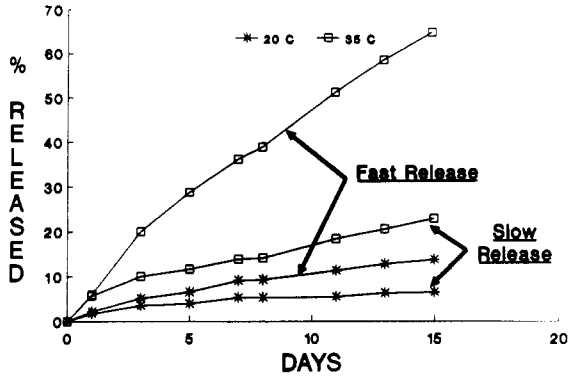

Figure 2. Release of trifluralin into ethanol/water (1:1) from the 30 °C melting point capsules for slow and fast release formulations at 20 and 35 °C. SD for the fast release capsules ranged from 0.2 to 0.7% at 20 °C and from 0.5 to 4.6% at 35 °C. SD for the slow release capsules ranged from 0.3 to 1.0% at 20 °C and from 0.5 to 7.2% at 35 °C.

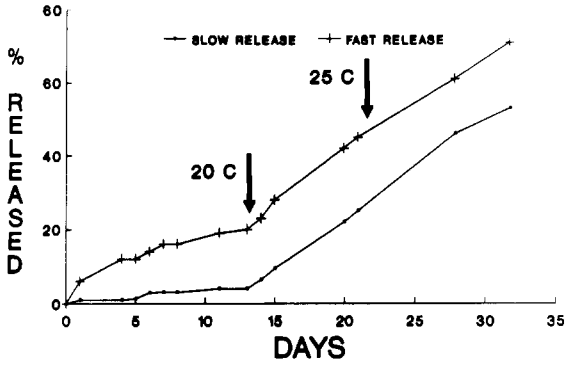

Figure 3. Release of alachlor into deionized water from the 20 °C melting point capsules for slow and fast release formulations. Temperature was 10 °C from day 0 to day 13, 20 °C from day 13 to day 21, and 25 °C from day 21 to day 32. SD ranged from 0.5 to 1.6% with the slow release capsules and from 0.4 to 3.5% with the fast release capsules.

formulation had released 86.8% (±8% SD) of the diazinon compared to 22.5% (±5.5% SD) from the slow release formulation after 16 days. These results demonstrate not only the temperature release response of the capsules but also that the release rate above the trigger temperature can also be controlled in the laboratory.

Trifluralin Release. Trifluralin was released similarly to diazinon when the 30 °C melting point capsules were maintained at 20 °C, with less than 10% of the trifluralin being released from either the fast or slow release formulations (Figure 2). Increasing the temperature correspondingly increased the overall release of trifluralin from both formulations, with the fast formulation releasing up to 64.7% (±2.5% SD) at 35 °C after 15 days compared to 22.8% (±7.2% SD) of the slow release formulation. A similar response was observed with the 20 °C melting point capsules (data not shown). The active ingredient of the capsule seems to affect the release rate from the capsules, with the trifluralin 30 °C fast formulation having a release rate of 7.5 μg/h when held at 30 °C (release curve not shown) compared to 20.9 μg/h for the diazinon 30 °C fast formulation held at the same temperature (Figure 1).

Alachlor Release. The slow release, 20 °C melting point capsules did not release more than 6.5 (±0.7% SD) of the encapsulated alachlor during the 13-day period when the temperature was held below 20 °C (Figure 3). The fast release formulation allowed more alachlor to be released during the same period (20 ■ 1.5% SD). When the temperature was changed to 20 °C on day 13, the rate of release from both formulations increased dramatically

Table I. Control of *D. balteata* with Various Formulations of Diazinon and Temperature Regimes

formulation	concn, ppm	% control *n* weeks after treatment[a]					
		n = 1	*n* = 2	*n* = 4	*n* = 5	*n* = 6	*n* = 8
30 °C slow	2.0	0	2	8	89	97	79
30 °C slow	2.5	0	5	8	92	97	89
30 °C fast	2.0	0	0	3	100	100	79
30 °C fast	2.5	0	58	35	100	94	97
14 G	2.0	29	28	24	84	97	44
14 G	2.5	57	73	73	100	84	56
LSD (0.05)		19	21	14	19	25	18

[a] Temperature was maintained at 20 °C for the first 4 weeks after treatment and then switched to 32 °C.

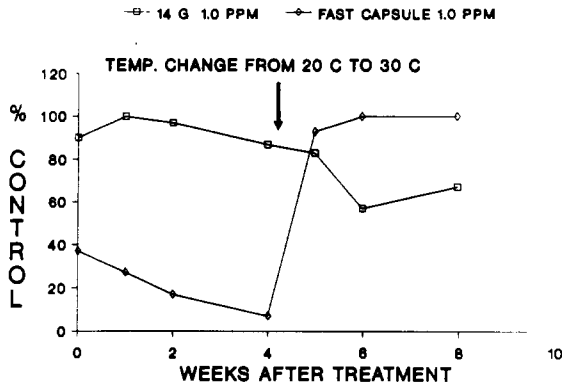

Figure 4. Control of *D. virgifera* with 30 °C melting point capsules of the fast diazinon release formulation and the 14 G diazinon formulation as affected by temperature over time. The temperature was changed after the 4-week sample date from 20 to 30 °C.

(Figure 3); however, the rate of release was not different between the two formulations and did not change when the temperature was increased to 25 °C on day 21.

Biological Activity of Diazinon Formulations. Control of *D. balteata* during the first 4 weeks after treatment in soil that had been incubated at 20 °C was significantly less with the diazinon 30 °C melting point capsule formulations than with the 14 G formulation (Table I). When the temperature was raised to 32 °C after the 4-week sample date, larvae control increased for all formulations, to 84 and 100% control for the 5- and 6-week evaluation dates, respectively. Eight weeks after treatment, the fast and slow release diazinon capsule formulations at both concentrations resulted in better larvae control than the 14 G formulation, 89 and 97%, respectively, for the capsule formulations vs 56% for 14 G.

The results of the second experiment were similar to those of the first. Control of *D. virgifera* was less with the diazinon fast release capsule formulation than with the 14 G formulation (38 to 5% vs 90 to 100%, respectively) for the first 4 weeks of the experiment when the soil was maintained below the melting point of the capsules (Figure 4). When the temperature was increased to 30 °C after week 4, control of the larvae with the fast release capsule formulation increased to above 98% for the 5-, 6-, and 8-week samples, while control with the 14 G formulation fell to 60%.

The response of the insect larvae to the diazinon capsule formulations supports the temperature release data and verifies the potential of these formulations to provide longer control of these insects at lower concentrations of active ingredient than the conventional 14 G formulation.

Biological Response to Trifluralin Formulations. Corn is a large-seeded grass that is moderately susceptible to trifluralin and was most injured by the 1.12 kg/ha rate

Table II. Response of Corn (*Z. mays*) and Two Weed Species to Various Formulations of Trifluralin Applied in the Field

formulation	rate,[a] kg/ha	corn injury, %	corn stand reduction, %	visually evaluated control giant foxtail, %	visually evaluated control redroot pigweed, %
20 °C fast	0.56	20	15	99	100
20 °C fast	1.12	70	40	100	100
20 °C slow	0.56	27	16	99	100
20 °C slow	1.12	60	36	98	100
30 °C fast	0.56	17	6	94	94
30 °C fast	1.12	33	19	98	98
30 °C slow	0.56	17	2	98	100
30 °C slow	1.12	13	3	98	100
EC	0.56	23	9	99	100
EC	1.12	70	61	99	100
LSD (0.05)		26	21	3	2

[a] All treatments were incorporated into the soil within 1 h of application.

Table III. Control of Giant Foxtail with Various Formulations of Trifluralin When Incorporated Immediately after Application or When Incorporation Was Delayed by 52 Days in the Field

formulation	rate, kg/ha	giant foxtail control,[a] % immediate incorp	giant foxtail control,[a] % delayed incorp
20 °C slow	0.84	83	31
20 °C slow	1.12	91	68
20 °C fast	0.84	84	39
20 °C fast	1.12	91	43
30 °C slow	0.84	64	65
30 °C slow	1.12	69	69
30 °C fast	0.84	71	63
30 °C fast	1.12	85	71
EC	0.84	86	38
EC	1.12	95	33
LSD (0.05) (rate × formulation × incorporation)		13	

[a] Giant foxtail control was visually estimated 17 days after the delayed incorporation (69 days after herbicide application).

of each trifluralin formulation in the first experiment (Table II). Corn injury and stand reduction were significantly reduced by the 30 °C formulations at the 1.12 kg/ha rate when compared to the EC formulation at the same rate (Table II). At the 0.56 kg/ha rate, none of the formulations caused significant injury. All of the treatments gave excellent control of the weeds in the experiment. When the trifluralin treatments were incorporated into the soil immediately after application, the EC and 20 °C formulations controlled giant foxtail similarly at both rates when evaluated 69 days after treatment (Table III). The 30 °C slow release formulation did not control the giant foxtail as well as the EC or the 20 °C formulations at comparable rates. The 30 °C fast release formulation gave slightly better control than the 30 °C slow release formulation. However, when the trifluralin formulations were not incorporated until 52 days after treatment, all of the 30 °C formulations gave significantly better giant foxtail control than the EC formulation (63 to 71% vs 33 to 38%, respectively). Except for the 1.12 kg/ha rate of the slow release formulation, the 20 °C melting point capsules controlled the giant foxtail similarly to the EC formulation when soil incorporation was delayed.

Trifluralin EC formulations are not applied to the soil for weed control prior to corn emergence due to the potential for crop injury. These experiments have demonstrated that the 30 °C melting point capsules reduce corn injury caused by trifluralin applied prior to corn emergence without reduced weed control. Also, trifluralin EC formulations require immediate soil incorporation to avoid volatility and photodegradation losses from the soil surface following application, which results in reduced weed control (Humburg, 1989). The 30 °C melting point capsule extended the period of weed control effectiveness when soil incorporation was delayed.

Table IV. Response of Large Crabgrass (*D. sanguinalis*) and Annual Ryegrass (*L. multiflorum*) with Various Formulations of Alachlor in a Temperature-Controlled Growth Chamber

formulation	concn, ppm	% growth inhibition: annual ryegrass (15 °C) 14 DAT[a]	large crabgrass (25 °C) 30 DAT	44 DAT	58 DAT
20 °C fast	2.0	53	93	81	70
20 °C fast	3.0	88	99	99	73
20 °C slow	2.0	33	98	80	25
20 °C slow	3.0	40	98	98	19
EC	2.0	44	93	81	6
EC	3.0	81	100	99	59
LSD (0.05)		14	22	21	15

[a] DAT, days after initial treatment. The soil was maintained at 15 °C for 30 days. Annual ryegrass was planted on day 14. On day 30, the temperature was raised to 25 °C and the soil was planted with large crabgrass seed. Large crabgrass was replanted into cups on days 44 and 58.

Table V. Movement of Various Alachlor Formulations in Packed Soil Tubes at 15 and 25 °C

formulation	temp, °C	depth of leaching,[a] cm
20 °C fast	15	1.7
20 °C slow	15	0
EC	15	8.5
20 °C fast	25	5.9
20 °C slow	25	1.7
EC	25	19.5
LSD (0.05)		3.7

[a] Mean of the greatest depth where alachlor injury was observed.

Biological Response to Alachlor Formulations. The 20 °C slow release alachlor formulation caused the least amount of annual ryegrass injury from the day 14 planting date when the temperature was held at 15 °C (Table IV). The 20 °C fast release formulation and the EC formulation caused the same amount of annual ryegrass growth inhibition. The 20 °C fast release formulation of alachlor provided longer lasting large crabgrass control than the EC or 20 °C formulation when the temperature was raised to 25 °C, as indicated by the amount of growth inhibition at the 58-day planting date. Control of large crabgrass was similar for all formulations when evaluated at the 30- and 44-day planting dates.

Temperature greatly affected alachlor movement in soil with all of the alachlor formulations (Table V). Less alachlor movement occurred with both of the 20 °C melting point capsule formulations when compared to the EC formulation. At 15 °C, there was no movement of alachlor from the 20 °C slow release formulation and insignificant movement from the fast release formulation. Alachlor from the EC formulation moved to a depth of 8.5 cm at 15 °C. There was minimal movement of alachlor from the 20 °C slow release formulation at 25 °C (1.7 cm), while the 20 °C fast release capsule had increased movement to 5.9 cm. The greatest alachlor movement, 19.5 cm, occurred with the EC formulation at the 25 °C temperature. These results indicate that the release of alachlor is temperature dependent and that the rate of release can be controlled independently of the temperature switch of the capsule. Finally, the controlled release of alachlor significantly

reduces its ability to leach yet dispenses enough active ingredient for adequate weed control.

Conclusions. Diazinon, alachlor, and trifluralin were successfully encapsulated into formulations that differentially released their respective pesticides at predictable rates in response to temperature. The biological activity of the encapsulated pesticide formulations agreed with the aqueous release data. Pest control was extended with the encapsulated pesticide formulations under various conditions. It was demonstrated that the encapsulated formulation of alachlor reduced alachlor leaching in soil compared to the EC formulation. Laboratory and field biological data indicate that these formulations have the potential to reduce crop phytotoxicity and allow for extended pest control at lower application rates than are currently required from the commercial formulations evaluated in this research.

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