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Effect of Water Potential, Temperature, and Soil Microbial Activity on Release of Starch-Encapsulated Atrazine and Alachlor

Brian J. Wienhold* and Timothy J. Gish

ABSTRACT

Starch encapsulation has been proposed as a method for controlling the rate at which pesticides are released into the soil. Relatively little is known about what environmental factors influence controlled release. A series of laboratory studies were initiated to improve our understanding of how water potential, temperature, and soil microbial activity influence rate of release of starch-encapsulated atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) and alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)-acetanilide). Water potential, imposed using polyethylene glycol, significantly influenced swelling of the starch matrix and rate of release of both herbicides. At a water potential of 0 MPa, complete release required 21 d for atrazine and 7 d for alachlor. As water potential declined, so did rate of release. At a water potential of -1.5 MPa, less than 50% of the encapsulated atrazine and less than 80% of the encapsulated alachlor had diffused out of the starch matrix after 28 d. Temperature also influenced rate of release of both herbicides. At 35°C nearly three times more atrazine and two times more alachlor was released from starch granules than at 15°C at all sampling times. Soil microbes increase the rate of release. After 21 d there was a twofold increase in the percentage of atrazine released from starch granules applied to nonsterile soil compared with granules applied to sterile soil. Effect of soil microbes on rate of alachlor release was apparent only at early times. After 5 d there was a 20% increase in the percentage of alachlor released from starch granules when microbes were present compared with release from starch granules applied to sterile soil. After 14 d essentially all of the alachlor had been released from starch granules applied to either sterile or nonsterile soil. The different influences that water potential, temperature, and soil microbes have on rate of release between atrazine and alachlor are likely due to differences in water solubility of atrazine (30 mg L^{-1}) and alachlor (240 mg L^{-1}).

INCREASED AWARENESS concerning the potential for agricultural chemicals to serve as sources for groundwater contamination has increased the level of research directed at understanding pesticide behavior (Burkart et al., 1990). Modification of pesticide behavior by encapsulating the chemicals in a starch matrix is one experimental approach receiving increased attention (Wing et al., 1987). Starch encapsulation has been shown to reduce volatilization (Schreiber et al., 1978) and leaching (Gish et al., 1991) losses of some herbicides.

Starch encapsulation modifies pesticide behavior by controlling the rate of release of the encapsulated compound (Baur, 1980; Schreiber and White, 1980; Wing et al., 1987). Our knowledge of potential factors influencing rate of release is limited to the chemical nature of the starch matrix (Coffman et al., 1984; White and Schreiber, 1984; Wing et al., 1987, 1988) and the nature of the chemical being encapsulated (Baur, 1980; Schreiber and White, 1980) with little knowledge of what effect environmental variables have on rate of release.

Release of a starch-encapsulated compound is governed mainly by a diffusion process. When starch granules are applied to the soil they imbibe water, swell, and the encapsulated compound diffuses out of the starch matrix. As a consequence, environmental factors influencing diffusion and uptake of water by the starch granules should influence rate of release. Temperature is known to influence diffusion, and water potential may be one measure of the availability of water to the starch granule.

Amylase (EC 3.2.1.1) is an enzyme that breaks down starch. Treatment of starch-encapsulated pesticides with amylase has been shown to enhance diffusion of the encapsulated compound from the starch matrix (Trimnell et al., 1985); however, the role that amylase produced by soil microbes may play in rate of release has not been evaluated. The objective of this research was to evaluate the effect of water potential, temperature, and soil microbial activity on rate of release of starch-encapsulated atrazine and alachlor. Atrazine and alachlor are widely used herbicides that exhibit differences in water solubility (30 mg L^{-1} for atrazine and 240 mg L^{-1} for alachlor) and should, therefore, exhibit differences in rate of release from the starch matrix (Baur, 1980). Field investigations are currently being conducted in several states to evaluate the effect of starch encapsulation of atrazine and alachlor on groundwater issues (M. M. Schreiber, 1990, personal communication). Results of the present study should have immediate application in helping interpret results of field studies.

MATERIALS AND METHODS

Water Potential

Herbicides used in this study were encapsulated in pearl cornstarch using a jet-cooked method (Wing et al., 1987). Starch-encapsulated atrazine contained 11.1% active ingredient (a.i.) and alachlor contained 10.1% a.i. Starch granules 0.4 to 1.2 mm in diam. were used.

Effect of water potential on swelling of the starch matrix was assessed by placing 0.5 mL of dry starch-encapsulated atrazine in a graduated centrifuge tube. Ten milliliters of solution of a known water potential was added and the volume occupied by the starch granules was noted at 5, 10, 15, 30, 60, 120, and 300 min. Water potential was adjusted by adding either NaCl or polyethylene glycol 6000 (PEG) (Michel and Kaufmann, 1973) to deionized water to achieve water potentials of 0, -0.5 , -1.0 , and -1.5 MPa. The experiment was replicated three times.

Effect of water potential on rate of pesticide release from the starch matrix was assessed by placing starch-encapsulated atrazine ($500\text{ }\mu\text{g a.i.}$) or alachlor ($500\text{ }\mu\text{g a.i.}$) in a glass jar containing 25 mL of solution of known water potential. Water potential was adjusted by adding sufficient PEG to deionized water to achieve water potentials of 0, -0.5 , -1.0 , and -1.5 MPa. Samples were left undisturbed until sampled for solution pesticide concentration. The experiment assessing atrazine rate of release was replicated three times and the experiment assessing alachlor rate of release was replicated five times. Solution concen-

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trations were determined after 1, 5, 8, 14, 21, and 28 d. Solution concentration was expressed as a percentage of chemical added to the jars. An arcsine transformation was performed on the percentage release data and analysis of variance was used to detect significant differences in rate of release as a function of time and water potential (Sokal and Rohlf, 1981).

Temperature

Effect of temperature on swelling of the starch matrix was assessed by placing 0.5 mL of dry starch-encapsulated atrazine in a graduated centrifuge tube. Ten milliliters of solution of known water potential and temperature was added and the volume occupied by the starch granules was noted after 24 h. Water potential was adjusted by adding PEG to deionized water to achieve water potentials of 0 and -1.0 MPa at temperatures of 15, 25, and 35 °C. The experiment was replicated three times.

Effect of temperature on rate of pesticide release from the starch matrix was assessed by placing starch-encapsulated atrazine (500 μg a.i.) and alachlor (500 μg a.i.) in a glass jar containing 25 mL of solution of known water potential and temperature. Water potential was adjusted by adding sufficient PEG to deionized water to achieve water potential of 0 and -1.0 MPa at temperatures of 15, 25, and 35 °C. All volumetric measurements were made at 25 °C, and temperatures were maintained by incubating the jars in a constant temperature bath. Samples were left undisturbed until sampled for solution pesticide concentration. The experiment was replicated three times. Solution concentrations were determined after 1, 5, and 8 d. Solution concentration was expressed as a percentage of chemical added to the jars. An arcsine transformation was performed on the percentage release data and analysis of variance was used to detect significant differences in rate of release as a function of temperature (Sokal and Rohlf, 1981).

Soil Microbes

To assess the effect of soil microbial activity on rate of pesticide release, an experiment was conducted to compare the rate of release of atrazine and alachlor from starch granules applied to sterile and nonsterile soil. Fifty grams of Monmouth loamy fine sand (Typic Hapludult) that had been air-dried and passed through a 2-mm sieve was placed in each of 60 buchner funnels. Thirty of these samples were then sterilized by autoclaving for 1 h on consecutive days. After autoclaving, all 60 soil samples (30 sterile and 30 nonsterile) were moistened with sterile distilled water and allowed to sit overnight before placing starch-encapsulated atrazine (300 μg a.i.) and alachlor (300 μg a.i.) on the soil surface. Samples were covered with aluminum foil to reduce evaporation. To maintain the soil in a moist condition (200 g H_2O kg^{-1}), water was sprinkled onto the soil surface 1, 5, 8, 14, 21, and 28 d after pesticide application. During each water application the buchner funnels were placed on a suction flask and a 1-kPa vacuum was applied to remove water in excess of the desired moisture content. After 1, 5, 8, 14, 21, and 28 d, 10 samples (two soil treatments and five replications) were randomly selected to determine the amount of pesticide that had been released. Starch granules were carefully removed from the soil surface and pesticide remaining in the granules was determined using an enzymatic pretreatment and methanol extraction procedure (Wienhold and Gish, 1991). Herbicide content was determined by gas chromatography. Release of the herbicide from the starch matrix was assumed to be the difference between the amount initially applied and the amount remaining at the time of sampling. Herbicide released was expressed as a percentage of that applied to the surface. The design of this experiment was a completely randomized

factorial with two soil treatments, six collection dates, and five replications. An arcsine transformation was performed on the percentage release data and analysis of variance was used to detect significant differences in rate of release as a function of time and soil treatment (Sokal and Rohlf, 1981).

Concentration of atrazine and alachlor was determined by solid phase extraction with C-18 sep-pak cartridges (Waters Associates, Milford, MA¹) (Nash, 1990). The chemicals were eluted from the cartridges with ethyl acetate and herbicide concentration determined using a gas chromatograph equipped with a nitrogen-phosphorus detector. Operating conditions of the gas chromatograph were: 30 m by 0.32 mm glass capillary column coated with 0.26 μm SPB-5; injector temperature of 200 °C, oven temperature of 150 °C, and detector temperature of 220 °C; He carrier gas at 2.5 mL min^{-1} . Trifluralin (α,α,α -trifluoro-2,6-dinitro-*N,N*-di-propyl-*p*-toluidine) was used as an internal indicator.

RESULTS AND DISCUSSION

Water Potential

Water potential had a differential effect on the degree of swelling exhibited by starch-encapsulated atrazine and was dependent on whether NaCl or PEG was used to adjust water potential (Fig. 1). Swelling of the starch granules was not affected by water potential when water potential was imposed using NaCl (Fig. 1A). Starch granules exposed to NaCl solutions swelled to 350% of their original volume within 2 h and exhibited little or no change in volume after 2 h. Swelling of the starch granules was markedly affected by water potential when water potential was imposed using PEG (Fig. 1B). Granules exposed to a water potential of 0.0 MPa swelled to 350% of their original volume while those exposed to a water potential of -0.5 MPa increased their volume only 70%, and granules exposed to lower water potentials swelled even less. Swelling of starch granules in solutions containing PEG at water potentials <0.0 MPa equilibrated within 1 h. The swelling behavior of the starch-encapsulated herbicides used in this study, when the granules were placed in water or NaCl solution, are similar to that reported by Wing et al. (1987) for starch-encapsulated thiocarbamates. The effect of water potential, imposed using PEG, on swelling of starch-encapsulated herbicides has not been previously reported.

We hypothesize that the differential swelling response described above is a function of the size of the hydrated molecule used to adjust water potential. In the case of NaCl, the hydrated sodium and chloride ions are small enough to enter the pores of the starch matrix (Schreiber and White, 1980) where a portion of the water from the hydration envelope is able to loosen the hydrogen bonds that crosslink the starch macromolecule (Wing et al., 1988) and the granule swells. In contrast, the hydrated PEG molecule is very large and is unable to enter the pores of the starch matrix, leaving only water that is unbound in the PEG solution to enter the starch matrix. The more PEG there is in the solution, the less unbound water there will be and swelling of the starch matrix will be reduced. In soil, as the water content declines, a larger percentage of the water is bound to colloids and less

¹ Trade names or company names are included for the benefit of the reader and imply no endorsement or preferential treatment of the product listed by the USDA.

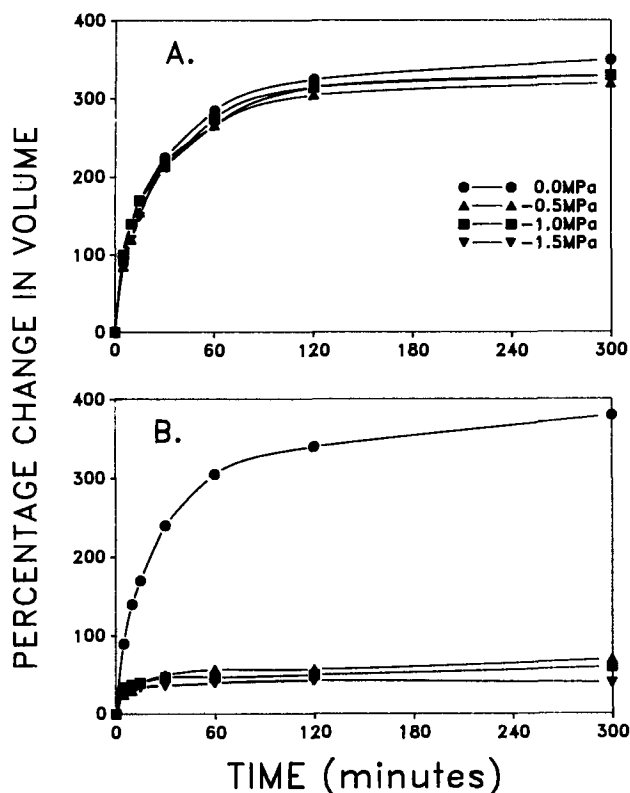


Fig. 1. Effect of water potential imposed using (A) NaCl and (B) polyethylene glycol on swelling of starch-encapsulated atrazine. Error bars are smaller than symbol size.

unbound water will be present; hence, PEG simulates matrix potential in soil (Zur, 1966; Graham-Bryce, 1967). Since the objective of this study was to assess factors that may influence pesticide release kinetics from the starch granule in the soil environment, we are most interested in the affect of matrix potential on the starch granule; hence, only matrix potential was imposed during the rate of release study.

The solution concentration as a function of time increased for both atrazine ($P < 0.001$) and alachlor ($P < 0.001$) regardless of water potential (Fig. 2). However, water potential significantly affected the rate at which atrazine ($P < 0.001$) and alachlor ($P < 0.001$) diffused from the starch matrix, with rate of release declining as water potential declined (Fig. 2). After 8 d, nearly three times more atrazine had been released from starch granules exposed to a solution with a water potential of 0 MPa than from granules exposed to a solution with a water potential of -1.5 MPa. The solution concentration of atrazine had reached equilibrium after 21 d at a water potential of 0 MPa with complete release of atrazine from the starch matrix. At lower water potentials, solution concentrations continued to increase, suggesting that the chemical was still being released from the starch granule. At a water potential of -1.5 MPa, less than 50% of the atrazine had diffused from the starch matrix after 28 d (Fig. 2A).

Alachlor diffused from the starch matrix more quickly than did atrazine. After 5 d essentially all of the alachlor had been released from the starch matrix at water potentials of 0 and -0.5 MPa (Fig. 2B). As water

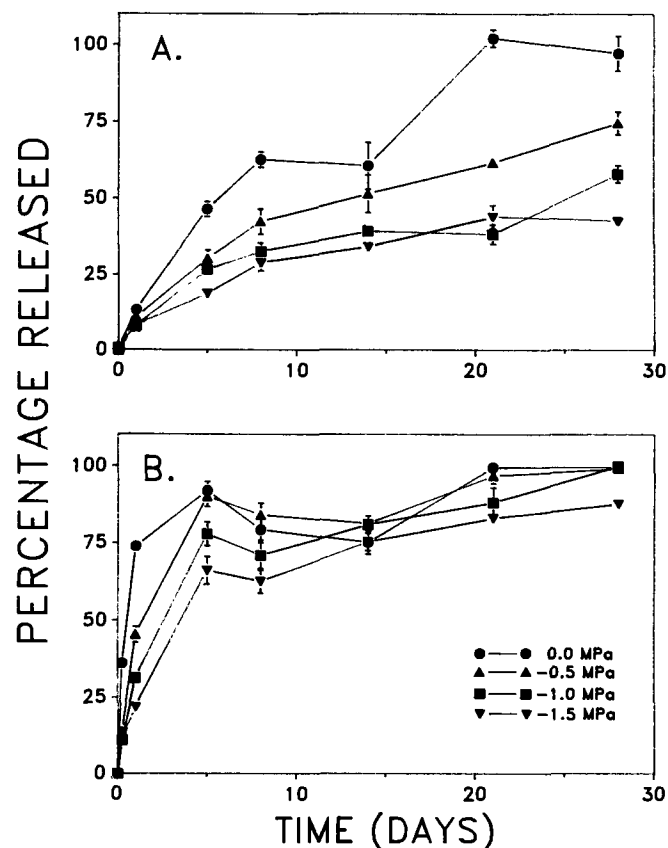


Fig. 2. Effect of water potential imposed using polyethylene glycol on percentage release of (A) atrazine ($LSD_{0.05} = 10\%$) and (B) alachlor ($LSD_{0.05} = 8.6\%$) from starch granules as a function of time. Error bars indicate 1 SE of the mean. If not shown, error bars are smaller than symbol size.

potential declined, the rate of release of alachlor from the starch matrix also declined; however, this effect was not as marked as with atrazine. At the lowest water potential used in this study, nearly 70% of the alachlor had been released after 5 d, and more than 75% of the alachlor was released from the starch matrix after 28 d (Fig. 2B).

There was a positive correlation between percentage change in volume as a function of water potential and percentage of atrazine released into solution as a function of water potential after 5 d ($r = 0.93$), 8 d ($r = 0.94$), and 28 d ($r = 0.85$). There was also a strong correlation between volume change and solution concentration of alachlor as a function of water potential after 5 d ($r = 0.61$), but after 8 d ($r = 0.40$) and 28 d ($r = 0.36$) the correlation was weak. Since atrazine is released slowly (relative to alachlor) and water potential influences were significant at all sampling times, the high correlation between swelling (Fig. 1B) and release of atrazine (Fig. 2A) is apparent at all sampling times. In contrast, alachlor is released more quickly and water potential influences were significant only at early times (until essentially all of the alachlor has been released), therefore, the correlation between swelling (Fig. 1B) and release of alachlor (Fig. 2B) is strong only at early times.

The effect of water potential on swelling and release characteristics of starch granules has not been previ-

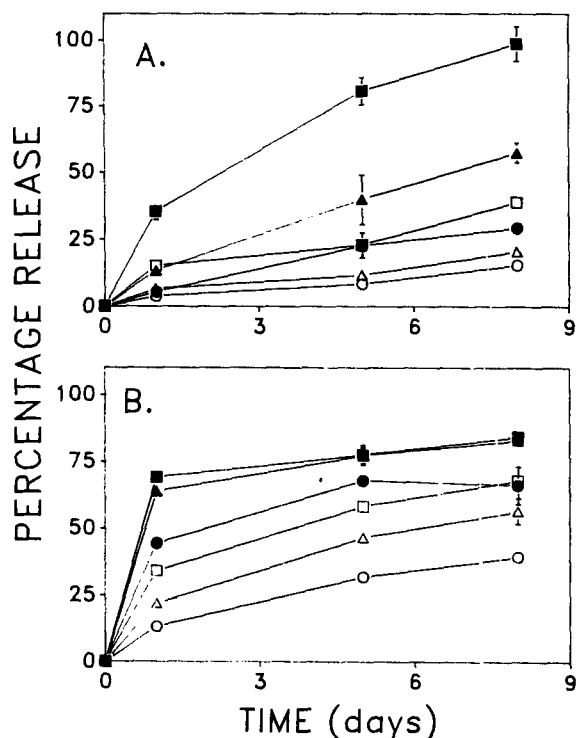


Fig. 3. Percentage release of (A) atrazine ($LSD_{0.05} = 9\%$) and (B) alachlor ($LSD_{0.05} = 7\%$) from starch granules as a function of time, temperature [15 (●), 25 (▲), and 35 °C (■)], and water potential [0 MPa (filled symbols) and -1.0 MPa (open symbols)]. Error bars indicate 1 SE of the mean. If not shown, error bars are smaller than symbol size.

ously evaluated. Our results suggest that a matric potential may play an important role in herbicide release when the herbicides are applied in the starch-encapsulated formulation. While the method used to encapsulate a herbicide in the starch matrix, the type of starch used in the encapsulation procedure, and size of starch granules used are variables that have been shown to exert a significant influence on release characteristics (Wing et al., 1987, 1988), these variables were invariant in this study. Differences in release behavior between the two chemicals used in this study are likely due to differences in solubility between alachlor (240 mg L^{-1}) and atrazine (30 mg L^{-1}).

Temperature

Temperature affected the degree of swelling exhibited by starch-encapsulated atrazine. The swelling response of starch granules exposed to water potentials of 0 and -1.0 MPa at a temperature of 25 °C was the same as that presented in Fig. 1B. The effect of temperature on swelling was similar at both water potentials used in this experiment. At a temperature of 15 °C starch granules swelled 20% less, and at 35 °C starch granules swelled 20% more than they did at 25 °C.

The solution concentration as a function of time increased for both atrazine ($P < 0.001$) and alachlor ($P < 0.001$), regardless of temperature (Fig. 3). However, temperature affected the rate at which atrazine ($P < 0.001$) and alachlor ($P < 0.001$) diffused from the

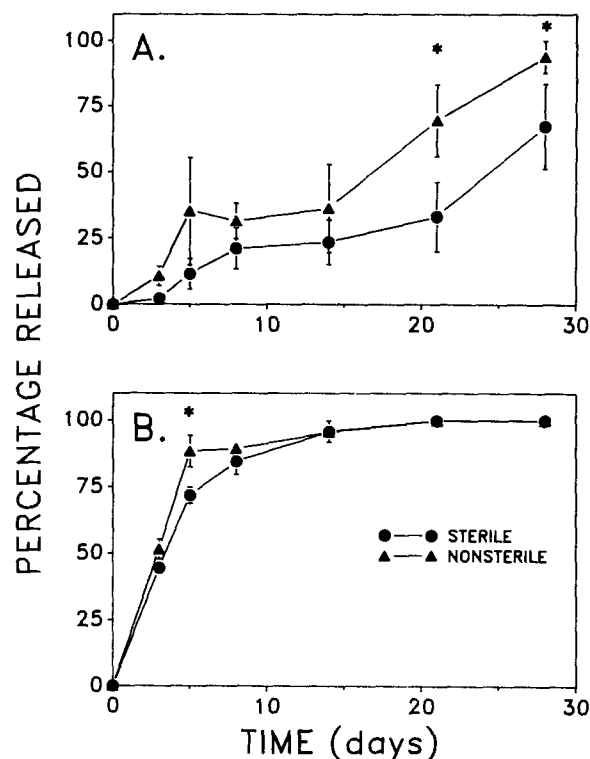


Fig. 4. Effect of soil microbes of percentage release of (A) atrazine and (B) alachlor from starch granules as a function of time. Error bars indicate 1 SE of the mean. If not shown, error bars are smaller than symbol size. *Denotes means significantly different by $LSD_{0.05}$ for that sampling period.

starch matrix, with rate of release declining as temperature declined (Fig. 3). After 8 d atrazine had been completely released from starch granules incubated at 0 MPa and 35 °C, while only 25% had been released from starch granules incubated at 0 MPa and 15 °C (Fig. 3A). At -1.0 MPa 30% of the atrazine had been released from starch granules incubated at 35 °C, and <20% was released from starch granules incubated at 15 °C after 8 d (Fig. 3A).

Alachlor was released from the starch matrix more rapidly than was atrazine. At a water potential of 0 MPa, nearly 70% of the alachlor was released from starch granules incubated at 25 and 35 °C after 1 d (Fig. 3B). At 15 °C, <50% of the alachlor was released from starch granules incubated at 0 MPa for 1 d (Fig. 3B). At a water potential of -1.0 MPa, two time more alachlor was released from starch granules incubated at 35 °C than from starch granules incubated at 15 °C at all sampling times, and complete release had not occurred after 8 d (Fig. 3B).

These results suggest that temperature may play an important role in herbicide release when the herbicides are applied in the starch-encapsulated formulation. Rate of release will be especially slow when starch-encapsulated herbicides are applied to cool, dry soil surfaces.

Microbial Activity

Release of atrazine ($P < 0.001$) and alachlor ($P < 0.001$) as a function of time increased in both sterile and nonsterile soil (Fig. 4). Soil microbe activ-

ity significantly increased the rate of release of both atrazine ($P < 0.003$) and alachlor ($P < 0.02$) (Fig. 4). After 28 d more than 90% of the encapsulated atrazine was released when soil microbes were present compared with less than 70% when soil microbes were excluded (Fig. 4A). The effect of microbial activity on alachlor rate of release is significant only at early times (Fig. 4B). Essentially all of the alachlor was released from the starch matrix by the 14th d in sterile or nonsterile soil. Microbes were able to utilize starch as an energy source, and in doing so, broke down the starch matrix and increased the rate of release. Microbial activity in the vicinity of starch granules has been observed (Schreiber et al., 1988), and microbial enzymes have been shown to increase the release rate of starch-encapsulated trifluralin (Trimnell et al., 1985). However, the magnitude of the effect microbes may have on release rate has not been reported previously. The starch granules were exposed to a water potential of 0 to -0.5 MPa at the soil surface. However, the rate of release of atrazine and alachlor in the sterile soil (Fig. 4) is slightly less than that in a -0.5 MPa solution (Fig. 2). This is most likely due to limited contact between the starch granules and the soil solution compared with the complete contact in the aqueous solutions. Soil microbes increased the percentage of herbicide released by 15 to 30%, with the effect being greatest for atrazine, which has a slower rate of release (Fig. 4A). The magnitude of the increase in herbicide release, even with limited contact between the starch granules and the soil solution, suggests that soil microbial activity may play an important role in release kinetics of starch-encapsulated pesticides.

Results presented here suggest that water potential, temperature, and microbial activity exert a significant effect on release of starch-encapsulated atrazine and alachlor. Complete release of these herbicides may not occur for one to several weeks after application. These results have environmental and agronomic implications. From an environmental perspective, controlled release should increase the length of time the herbicide is available for adsorption by the soil, resulting in less of the pesticide being available for volatilization (Schreiber et al., 1978) and leaching losses (Gish et al., 1991) at early times.

From an agronomic perspective, activity of herbicides applied in the starch-encapsulated formulation may be less than that of commercial formulations at early times. Efficacy may be especially poor when starch-encapsulated herbicides are applied to coarse-textured soils where water holding capacity is poor or when they are applied to a cool, dry soil surface. Studies using finer-textured soils have shown that differences in efficacy between formulations is short lived, disappearing after 1 or 2 wk, and persistence of starch encapsulated herbicides is greater compared to commercial formulations (Coffman and Gentner, 1980; Coffman et al., 1984).

CONCLUSIONS

1. Starch encapsulation results in slow release of the encapsulated herbicide. Atrazine, the less soluble

herbicide used in this study, is released more slowly than the more soluble alachlor.

2. Water potential exerts a significant effect on rate of herbicide release. Rate of herbicide release declined with declining water potential.

3. Temperature exerts a significant effect on rate of herbicide release. Rate of herbicide release declined with declining temperature.

4. Soil microbes are able to ameliorate the effects of water potential by digesting the starch matrix.

5. Herbicide activity may not be realized for up to 3 wk after application of starch-encapsulated herbicides. To maintain efficacy, starch-encapsulated formulations may have to be applied prior to planting.

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