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Uptake and Fate of Ethephon ([2-Chloroethyl]phosphonic Acid) in Dormant Weed Seeds[1]

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ABSTRACT

Although ethephon ([2-chloroethyl]phosphonic acid) is often used as a form of liquid ethylene in studies of seed germination, it is not known if ethylene evolved from ethephon in the seed is sufficient to elicit the desired response and/or if ethephon has a regulatory action that alone accounts for the response. For these reasons we studied the uptake and fate of [1,2-^{14}C]ethephon in dormant seeds of *Avena fatua*, *Sinapis arvensis*, *Thlaspi arvense*, and *Chenopodium album*. The radioactivity within the seeds was separated into a labile carbon-labeled ethephon/ethylene fraction (64–87%) and, following extraction in methanol-chloroform-water (12:5:3), into fractions associated with insoluble (12–29%) and soluble (3–8%) seed constituents. The radioactivity associated with seed constituents was reduced 5 to 75% by hot alkaline hydrolysis (2.5 N KOH, 70° C for 1 hour). Although a small portion of the ethephon (or metabolite of ethephon/ethylene) taken up by the seeds is tightly bound to the tissues, our results indicate that, at the appropriate external concentrations of ethephon, the amount of ethylene evolved from ethephon within the seeds is sufficient to produce the desired ethylene mediated responses. However, factors affecting the decomposition of ethephon must be considered in the decision as to whether to use ethephon as a liquid supply of ethylene.

Ethephon ([2-chloroethyl]phosphonic acid) decomposes above pH 4.0 to yield ethylene, phosphate, and a chloride ion (9, 14). Because ethephon degrades to ethylene, it is used widely in agriculture and research as a convenient means of administering ethylene (3). Ethephon is also an effective dormancy breaking compound for seeds of several species that respond to ethylene (1, 6). Despite the widespread use of ethephon as liquid ethylene in germination studies, little is known of the binding, uptake, or fate of ethephon in seeds. It is important to note that commercially available solutions of ethephon are acidic (pH about 2.3) and that ethephon will contribute to the buffer intensity of the test solution (diprotic acid, $pka_1 = 2.24$, $pka_2 = 6.97$; 2). In addition, ethephon degrades at a rate proportional to the fully dissociated species (dianion concentration) and the rate is markedly temperature dependent (2). In germination studies, relatively concentrated solutions of ethephon (1 mM) are often required to produce the desired response. Because these solutions are acidic, the rate of decomposition would be very low, particularly at the temperatures used in studies of seed germination (<40° C). This would suggest an active role for ethephon alone in addition to the ethylene effects, or that the rate at which ethephon decomposes within the seed is sufficient to maintain internal ethylene concentrations at levels high enough to mediate a response. In order to resolve these possibilities, we investigated the binding, uptake and decomposition of the ethephon in dormant weed seeds and report our findings here.

MATERIALS AND METHODS

Plant Material. Seed of *Avena fatua* L. (wild oats), *Sinapis arvensis* L. (wild mustard), *Thlaspi arvense* L. (stinkweed), and *Chenopodium album* L. (lamb's-quarters) were harvested from the University of Alberta Parkland Farm, Edmonton, Alberta in 1985 and stored in airtight glass containers in darkness at −24° C. Ethylene alone or combined with KNO_3 is effective in breaking seed dormancy in *C. album* and *A. fatua* but not *T. arvense* and *S. arvensis* (10, 11; JS Goudey, HS Saini, MS Spencer, unpublished data).

Labeled Ethephon and Determination of Radioactive Carbon. The [^{14}C]ethephon (4.1 mC/mol) was obtained from Mallinckrodt Chemical Works, St. Louis, and was provided as a gift by Union Carbide Agricultural Products Canada Ltd., Calgary. All liquid samples were solubilized in Aquasol II (NEN Research Products, Dupont Canada Inc.) and counted in a Searle Analytic Inc. Isocap/300 liquid scintillation system. Solid samples (200–300 mg) were oxidized in a model OX-300 Biological Material Oxidizer (R. J. Harvey Instruments Corp., Hillsdale, NJ) and the $^{14}CO_2$ combustion products trapped in R. J. Harvey Carbon 14 Cocktail. L-[1-^{14}C]Leucine was used as an internal standard to correct for quenching and differences in counting efficiencies between the scintillants.

Standard solutions of [^{14}C]ethephon were shown previously to contain a number of radioactive and nonradioactive inpurities (8). We examined the purity of our aqueous [^{14}C]ethephon standard by heating for 1 h at 70° C aliquots in flasks containing 2.5 M KOH (final concentration) and sealed with serum stoppers. Although ethephon is completely degraded under these conditions (2), the labeled impurities are unaffected (8). The presence of ethylene in the head space was confirmed with a Hewlett Packard model 5880 A gas chromatograph and, after purging the flasks with hydrocarbon free air overnight (5), the levels of radioactivity present in solution were not significantly different from background levels. Hence, the presence of any radioactive impurities in our [^{14}C]ethephon was not considered an important source of experimental error.

Uptake Experiments. For the uptake experiments, the seeds (1 g dry weight) were imbibed in darkness at 20° C for 24 h in 20 ml plastic scintillation vials containing 10 ml of distilled water and 0.6 μC of [^{14}C]ethephon (final concentration of 9.75 μM). The vials were continuously rotated (end over end) during this period. Levels of radioactive carbon present in the imbibing solutions were measured before and after the incubation period. After 24 h of imbibition, the seeds were washed three times with distilled water and samples removed for analysis of radioactive

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carbon. Fresh weights were measured of seeds that had been imbibed in distilled water containing unlabeled ethephon, washed, then blotted dry between paper towels.

Partitioning of Radioactivity within the Seed. Seeds imbibed in [^{14}C]ethephon for 24 h were washed with distilled water, then placed in one well containing 2 ml of 0.1 N HCl in a split bottom 25 ml flask. The other well contained 1 ml of 0.25 M mercuric perchlorate in 2.0 M perchloric acid to trap [^{14}C]ethylene (15). Ethylene is not evolved from ethephon in 0.1 N HCl and thus the [^{14}C]ethylene trapped in the perchloric acid was from degradation of ethephon within the seeds. The efficiency of this method to trap [^{14}C]ethylene in our experimental system was >95%. The flasks were sealed and after 24 h of continuous agitation on a rotary shaker in darkness, the levels of radioactivity present in the mercuric perchlorate, seeds, and extracting solutions were measured as described above. The remaining seed was air dried, ground using a mortar and pestle, and extracted three times with methanol-chloroform-water (12:5:3) according to Dickson (4) then centrifuged at 1000*g* for 10 min. The supernatant layers were removed and the pellets air dried. Samples from both fractions were subjected to hot alkaline hydrolysis to degrade [^{14}C]ethephon. The pellets were reextracted in 2.5 N KOH, heated at 70°C for 1 h, centrifuged, the supernatant layer removed, and the pellet dried. The liquid fractions were made basic with KOH (pH > 12) before heating. Levels of radioactivity in the solution and solid phases were measured as described above.

Determination of Rate Constants for the Decomposition Reaction. Rate constants for the decomposition of ethephon at 20° C and at pH values from 5 to 7 were estimated from the rate of ethylene evolved. In these experiments, 5 ml of 0.1 mM ethephon were placed in 50 ml calibrated flasks capped with serum stoppers. Ethylene levels in the head space were measured over time with a Hewlett Packard 5880A gas chromatograph (5). Ethylene present in the solution phase was included in calculations of the rate constants.

RESULTS

Decreases in the levels of radioactivity present in the imbibing solutions were measured after 24 h of imbibition and ranged from 2 ± 1% for *T. arvense* to 11 ± 3% for *S. arvensis*. At this time, the pHs of the imbibing solutions were 5.8 to 6.2. At pH 6 and 20° C, the measured rate constant for the decomposition reaction was 1×10^{-6} s^{-1}. This value is consistent with rates approximated from the results of Biddle *et al.* (2), which were obtained at higher temperatures (>30° C) using manometric techniques to measure ethylene evolution. Based on our rate constant, approximately 18% of the added ethephon would have decomposed after 24 h. Solutes, such as sugars, leached from the seeds may have influenced the decomposition reaction by forming stable configurations with the ethephon present in solution (8).

In all cases the amount of radioactivity incorporated by the seeds was significantly less than expected based on the volume of solution taken up (weight gain, Table I). This suggests that ethephon was partially excluded from the seed tissues. Previous studies have shown that the uptake of ethephon applied to plant surfaces is very limited (8, 13). The distribution in seed imbibed in [^{14}C]ethephon for 24 h, is presented in Table II. The seeds were extracted in an acidic solution to prevent further breakdown of ethephon and allow comparison of levels of labeled carbon in the form of ethylene to that present as ethephon. Total recoveries ranged from 82% for *C. album* to 96% for *S. arvensis*. The presence of carbon labeled ethephon in the extracting solution was confirmed by the same method used to check the purity of the [^{14}C]ethephon standard ("Materials and Methods"). The presence of labeled ethylene in the mercuric perchlorate solution was confirmed in a similar fashion: aliquots were placed in flasks containing concentrated HCl to liberate the ethylene complexed with mercury (15) and, after purging the solutions overnight with hydrocarbon free air, the radioactivity remaining in solution was not significantly different from background levels. More than 65% of the total radioactivity recovered was in the form of carbon-labeled ethephon/ethylene.

Table I. *Uptake of ^{14}C-Ethephon by Dormant Weed Seeds*
The results are from four separate experiments.

Seed	Seed Weight		Uptake	
	Dry	Imbibed	Calculated[a]	Measured
	mg/seed		*cpm/seed*	
A. fatua	26	39	1886	571 ± 92
S. arvensis	3.0	5.2	311	31 ± 5
T. arvense	1.09	1.76	96	4.7 ± 1.2
C. album	0.9	1.24	46	4.8 ± 2.0

[a] Based on weight gain after 24 h of imbibition.

Table II. *Distribution of Radioactivity in Seeds Imbibed in Solutions of ^{14}C-Ethephon for 24 h*

Species	Radioactivity			
	^{14}C-Ethephon[a]	^{14}C-Ethephon[b]	Seeds[c]	
			Insoluble	Soluble
	% of total recovered[d]			
A. fatua	77	7	13 (10)	3 (1)
S. arvensis	33	51	12 (11)	4 (3)
T. arvense	29	39	29 (21)	3 (1)
C. album	44	21	27 (8)	8 (2)

[a] Radioactivity recovered in the 0.1 N HCl extracting solution. [b] Radioactivity recovered in the mercuric perchlorate solution. [c] Radioactivity bound to seed constituents separated following extraction in methanol-chloroform-water into insoluble and soluble fraction. Radioactivity remaining after treatment with 2.5 N KOH and heat (70°C for 1 h) are bracketed. [d] Maximum relative standard deviation was ± 5% of the reported values.

The amount of radioactivity recovered in the seeds after incubation in 0.1 N HCl for 24 h was not significantly decreased with longer incubations (>72 h) or decreased following successive incubations in fresh solutions of 0.1 N HCl (data not shown). This indicates that a portion of the [^{14}C]ethephon incorporated by the seeds is tightly bound to internal sites and/or that ^{14}C-ethephon or the decomposition product [^{14}C]ethylene is metabolized by the seed tissues. In order to evaluate these possibilities, the seeds were extracted with methanol-chloroform-water (12:5:3) and the levels of radioactivity present in the insoluble and soluble fractions measured. The insoluble residues contained over 75% of the total radioactivity recovered (mostly proteins, nonstructural carbohydrates, hemicellulose, cellulose). Little activity was lost following hot alkaline hydrolysis (2.5 N KOH; 70° C for 1 h) for all species except *A. fatua*. Roughly 69% of the radioactivity recovered in the residue of *A. fatua* was lost following alkaline hydrolysis. Larger decreases (up to 75%) were measured in the supernatant fractions following treatment with KOH and heat (Table II). These results suggest that a portion of the labeled carbon associated with seed constituents is present in the form of ethephon or ethylene. However, additional study is required to identify the true nature of these labeled compounds.

DISCUSSION

Most of the [^{14}C]ethephon taken up by weed seeds was recovered as carbon-labeled ethephon/ethylene. The remaining

radioactivity was associated with insoluble and soluble seed constituents. This labeled carbon may be in the form of ethephon, ethylene, or a metabolite of ethephon/ethylene. Hence, our results provide no evidence to indicate that ethephon has it's own regulatory action. We have, however, shown that ethephon is degraded within the seeds, which is consistent with the belief that ethephon probably acts via ethylene.

In order to determine if the amount of ethylene evolved from ethephon was sufficient to mediate the desired response, we examined the effects of ethylene gas and ethephon on germination of *C. album*. Unbuffered solutions of 0.7 mM ethephon (pH 4.3) or 10 $\mu l \cdot L^{-1}$ ethylene gas induced maximal germination of *C. album* when given in combination with 10 mM KNO_3 (10). If gaseous ethylene is uniformly distributed within the aqueous phase of the seed, exposure to 10 $\mu l \cdot L^{-1}$ ethylene would give a final concentration of 0.02 $pmol \cdot seed^{-1}$ based on the volume of water imbibed. When imbibed in 0.7 mM ethephon, each seed would contain 22 pmol of ethephon. At pH 5.5 (pH of a distilled water extract of *C. album*) and 20° C, the rate constant for the decomposition reaction was 0.5×10^{-6} s^{-1}. Under these conditions it would take 13 min to achieve an internal ethylene concentration of 0.02 $pmol \cdot seed^{-1}$. Although much of the ethylene evolved from ethephon inside the seed would rapidly diffuse from the seed, our calculations indicate that the amount of ethephon present within the aqueous phase of the seed alone would maintain internal ethylene concentrations at levels sufficient to initiate germination of *C. album*. In addition, since the seed usually remains in contact with the imbibing solution during a germination experiment, and uptake is controlled primarily by diffusive forces (most of the ethephon taken up is easily displaced), the ethephon concentration within the seed would likely remain constant in equilibrium with the external concentration. We examined this further by imbibing seeds in unbuffered and buffered (50 mM phosphate buffer, pH 2) solutions in the presence of 10 mM KNO_3 and ethylene supplied as a gas (10 $\mu l \cdot L^{-1}$) or as ethephon (0.7 mM). Buffering the solutions at pH 2 prevented the decomposition of ethephon present in the filter paper and thus any evolution of ethylene from ethephon had to occur within the seed. The phosphate buffer alone had no affect on germination and did not influence ethephon uptake. After 7 d of incubation in darkness at room temperature, no significant differences in germination frequencies were measured. The germination frequencies for the gaseous ethylene and ethephon treatments were 95 ± 2% and 88 ± 5% at pH 2 and 93 ± 2% and 92 ± 4% at pH 6, respectively. Thus, the amount of ethylene evolved from ethephon within *C. album* seed is sufficient to induce germination.

Although uptake of ethephon by seeds is limited, our results indicate that the amount of ethylene evolved from ethephon taken up under conditions routinely used in germination studies is sufficient to produce the desired ethylene mediated responses. However, the decision as to whether to use ethephon in germination studies as a convenient means of administering ethylene requires some consideration of factors that can influence the decomposition reaction, such as temperature, pH (2), relative humidity (7), and the presence of solutes that form stable complexes with ethephon (8). These factors may have an important influence on the results, particularly in studies on seed of *C. album* where the sensitivity of the seed to ethylene changes with time (12).

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