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RESEARCH PAPER

Formulation and adjuvant effects on the absorption and translocation of ^{14}C-clethodim in wheat (*Triticum aestivum* L.)

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A new formulation of clethodim {(E,E)-(±)-2-(1-[([3-chloro-2-propenyl]oxy)imino]propyl)-5-(2-[ethylthio]propyl)-3-hydroxy-2-cyclohexen-1-one} is labeled for the control of grasses and volunteer grass crops, including glyphosate-resistant corn. The effects of the formulation (new: 0.12 kg L^{-1} and current: 0.24 kg L^{-1}) and adjuvants (ammonium sulfate [AMS], crop oil concentrate [COC] or both) on the absorption and translocation of the ^{14}C-clethodim was determined at 1, 4, 12, 24, 48, and 72 h after treatment (HAT) in wheat under greenhouse conditions. The absorption of the ^{14}C-clethodim with the 0.12 kg L^{-1} formulation was higher than that with the 0.24 kg L^{-1} formulation, especially at 24 HAT and beyond, regardless of the presence or absence of an adjuvant. The addition of an adjuvant increased the absorption of the ^{14}C-clethodim with the 0.12 kg L^{-1} formulation at all harvest times, except at 72 HAT. However, there were no differences in the ^{14}C-clethodim absorption among the adjuvants added to the 0.12 kg L^{-1} formulation, except at 48 and 72 HAT. Most of the ^{14}C-clethodim remained in the treated leaf independent of the formulation or adjuvant. The formulation did not have an impact on the distribution of the absorbed ^{14}C-clethodim; however, the presence of an adjuvant increased the movement of the ^{14}C-clethodim out of the treated leaf. Most of the absorbed ^{14}C-clethodim remained in the treated leaf and a negligible amount translocated to the root. These results demonstrated the improved absorption of clethodim with a formulation containing half of the active ingredient (0.12 kg L^{-1}) and the inclusion of both AMS and COC.

Keywords: absorption, adjuvant, clethodim, formulation, translocation.

INTRODUCTION

Growers in the USA have rapidly adopted herbicide-resistant crops, especially glyphosate-resistant (GR) crops, and have planted ~87% of soybean (*Glycine max* [L.] Merr.), 61% of cotton (*Gossypium hirsutum* L.), and 26% of corn (*Zea mays* L.) hectares to GR varieties in 2005 (USDA 2005). Volunteer grass crops, especially volunteer GR corn, pose real management challenges (in soybean and cotton). Traditionally, volunteer grass crops and grass weeds have been controlled by postemergent graminicides, such as clethodim {(E,E)-(±)-2-(1-[([3-chloro-2-propenyl]oxy)imino]propyl)-5-(2-[ethylthio]propyl)-3-hydroxy-2-cyclohexen-1-one}.

Clethodim is registered in cotton, peanut (*Arachis hypogaea* L.), soybean, and various other broadleaf crops (Anonymous 2005). It belongs to the cyclohexanedione chemical family of herbicides, which are potent inhibitors of the enzyme, acetyl-coenzymeA carboxylase (ACCase, EC 6.4.1.2) (Burton *et al.* 1987). Clethodim is commercially available as a 0.24 kg ai L^{-1}, emulsifiable concentrate (EC) formulation. A newer formulation of clethodim with half the active ingredient (0.12 kg L^{-1})

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will be commercialized soon for the control of grasses and volunteer grass crops, including GR corn (Smith J., 2004, personal communication). Clethodim is generally applied with an adjuvant, crop oil concentrate (COC) alone or in combination with a nitrogen source (e.g. ammonium sulfate [AMS]) for maximum efficacy (Anonymous 2005). The 0.12 kg L^{-1} formulation potentially will be tank-mixed with glyphosate in the near future and glyphosate (660 g L^{-1} formulation, Monsanto, St Louis, MO, USA) cannot be mixed with a formulation requiring COC. The AMS improved the control of selected annual grasses by clethodim, despite the presence of broadleaf- and sedge-controlling herbicide treatments (Burke *et al.* 2004) that have been shown to antagonize the graminicidal activity of clethodim (Burke & Wilcut 2003). Adjuvants improve the herbicide's efficacy (Hatzios & Penner 1985; Wanamarta & Penner 1989a) by increasing the herbicide's absorption (Hull *et al.* 1982; Wanamarta & Penner 1989b). The performance of adjuvants is influenced by the herbicide with which they are used, the weed species, water quality, and prevailing weather conditions (Hull *et al.* 1982; McWhorter 1982; Hatzios & Penner 1985). Characterization of the influence of clethodim and adjuvants on ^{14}C-clethodim absorption would lead to a better understanding and efficient use of clethodim in grass weed management. The objective of this research was to determine the effect of the formulation and adjuvants on the absorption and translocation of clethodim in wheat, which was used as a model grass.

MATERIALS AND METHODS

Plant materials

The seeds of wheat (Hard red spring wheat; variety: "Alsen"; North Dakota State University, Fargo, ND, USA) were planted in 4 cm diameter by 20 cm long plastic cones (Stuewe & Sons, Corvallis, OR, USA) containing a mixture of soil (Bosket sandy loam, fine–loamy, mixed, thermic Mollic Hapludalfs; pH = 8.2, 0.5% organic matter, cation exchange capacity = 16.7 meq 100 g^{-1}, 51.3% sand, 37.1% silt, 11.6% clay) and potting mix (Jiffy Products of America, Batavia, IL, USA) 1:1 by volume. The plants were subirrigated as needed. After emergence, the seedlings were thinned to one plant per cone. Two weeks after emergence, the plants were fertilized with a nutrient solution (W. R. Grace and Company, Fogelsville, PA, USA) containing 200 p.p.m. each of N, P_2O_5, and K_2O. The plants were grown outdoors during either September–October 2004 or March–April 2005 at the Southern Weed Science Research Unit, U.S. Department of Agriculture, Stoneville, MS, USA. The outdoor conditions were 27/16 ± 3°C day/night temperatures and natural light of a 13 h photoperiod. For all experiments, the uniform plants with four-to-five leaves were transferred from outdoors to a greenhouse for acclimatization 2 days prior to the ^{14}C-clethodim application. The greenhouse was maintained at 25/20 ± 3°C day/night temperatures with natural light supplemented by sodium vapor lamps to provide a 13 h photoperiod (300 μmol m^{-2} s^{-1}) from the application of the ^{14}C-clethodim-based treatments until harvest.

Absorption and translocation

^{14}C-Clethodim (^{14}C label on the 4- and 6-positions of the cyclohexane ring, 98% purity, specific activity of 2.12 GBq $mmol^{-1}$; Valent USA Corporation, Walnut Creek, CA, USA) in acetonitrile was mixed with a commercial 0.12 kg L^{-1} or 0.24 kg L^{-1} EC formulation of clethodim and selected additives (Valent USA Corporation, Walnut Creek, CA, USA) to obtain the treatment solution. The ^{14}C-clethodim treatment solution was prepared by diluting ^{14}C-clethodim in commercial formulations of clethodim with and without adjuvants to give a final concentration of 0.1 kg in 190 L of water. The wheat plants were treated with 0.12 kg L^{-1} clethodim formulation at 0.1 kg alone or in combination with AMS (Platte Chemical Company, Fremont, CA, USA) at 2.8 kg, COC (Helena Chemical Company, Collierville, TN, USA) at 1% (v/v) or COC at 1% (v/v) plus AMS 2.8 kg, and 0.24 kg L^{-1} clethodim formulation at 0.1 kg alone or in combination with COC (1% v/v). Each plant received 6.7 kBq of ^{14}C-clethodim in a total volume of 10 μL. The treatment solutions were applied with a microsyringe to the adaxial leaf surface of the third true leaf (blade) of wheat plants of 20 cm height (four-to-five-leaf stage) as four 2.5 μL droplets. The plants were harvested 1, 4, 12, 24, 48, and 72 h after treatment (HAT) and were divided into the treated leaf, the rest of the shoot, and the root. The treated leaves were excised and washed by gentle shaking for 20 s in 5 mL 50% aqueous methanol to remove the ^{14}C-clethodim remaining on the leaf surface. The harvested parts were wrapped in a single layer of tissue paper (Kimberly Clark Corporation, Roswell, GA, USA), placed in glass vials, and oven-dried at 60°C for 48 h. The oven-dried plant samples were combusted in a biological oxidizer (Packard Instrument Company, Dowers Grove, IL, USA), and the evolved $^{14}CO_2$ was trapped in 10 mL Carbosorb E (Packard Instrument Company, Meridian, CT, USA) and then 12 mL Permaflour E^+ (Packard Instrument Company, Meridian, CT, USA). Two 1 mL aliquots of each leaf wash were mixed with a 10 mL scintillation cocktail

(ICN, Costa Mesa, CA, USA). The radioactivity from the leaf washes and oxidations was quantified using liquid scintillation spectrometry (Packard Instrument Company, Dowers Grove, IL, USA). The experiment was conducted twice using three replications per treatment.

Autoradiography

Wheat plants of 20 cm height were treated with 13.4 kBq of ^{14}C-clethodim in a total volume of 10 μL, as described in the absorption and translocation experiments. After harvest, the plants were mounted on white glossy paper, avoiding contact of the treated leaf with other parts of the plant, pressed (order of stacking in the press from bottom to top: metal plate, mounted plant, piece of foam rubber, a wire screen, second metal plate, and press held together by large, metal binder clips on all four sides), and dried at 60°C for 48 h. The dried plants were exposed to X-ray film (Eastman Kodak Company, Rochester, NY, USA) for 4 weeks. After exposure, the film was developed (Eastman Kodak Company, Rochester, NY, USA) and fixed (Eastman Kodak Company, Rochester, NY, USA). The experiment was conducted once using two replications per treatment. The plants not treated with ^{14}C-clethodim were used as the control.

The data from all the experiments, except autoradiography, were analyzed by subjecting them to ANOVA. As a result of a lack of interaction between the experiments by treatment, the data from the duplicated experiments were pooled. The data from the absorption and translocation experiments were then subjected to regression analysis.

RESULTS AND DISCUSSION

Absorption and translocation

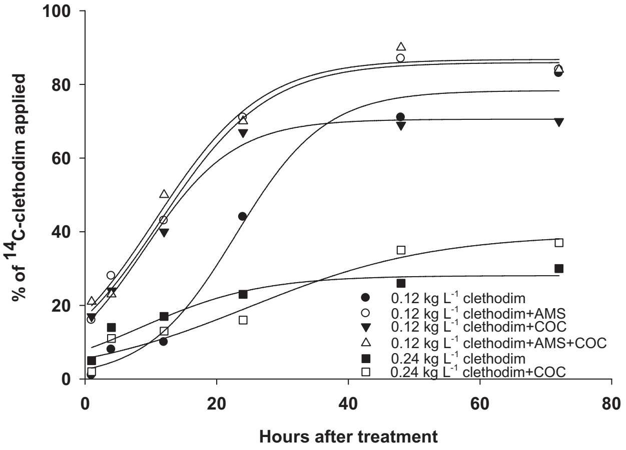

Fig. 1. Absorption of ^{14}C-clethodim applied to wheat with a height of 20 cm. The ammonium sulfate (AMS) was applied at 2.8 kg ha^{-1} and the crop oil concentrate (COC) was applied at 1% (v/v). Regression equations: (●), $y = 78.3/(1 + \exp[-(x - 22.9)/6.7])$, $r^2 = 0.99$, $P = 0.0015$; (○), $y = 85.9/(1 + \exp[-(x - 11.5)/8.1])$, $r^2 = 0.99$, $P = 0.0005$; (▼), $y = 70.5/(1 + \exp[-(x - 9.0)/6.7])$, $r^2 = 0.99$, $P = 0.0008$; (△), $y = 87/(1 + \exp[-(x - 10.8)/8.0])$, $r^2 = 0.99$, $P = 0.0013$; (■), $y = 28.1/(1 + \exp[-(x - 8.5)/9.1])$, $r^2 = 0.92$, $P = 0.0211$; (□), $y = 39.3/(1 + \exp[-(x - 25.1)/13.9])$, $r^2 = 0.95$, $P = 0.0109$.

The ^{14}C-clethodim absorption pattern over time could best be described by an equation of the form, $y = a/(1 + \exp[-(x - x_0)/b])$, where y is the amount of ^{14}C as a percentage of the amount of the ^{14}C-clethodim applied, a is the difference between the upper and lower response limits (asymptotes), x_0 is the time after treatment for a given measure of y, b is the slope of the curve around x_0, and x is the time after treatment, fitted to the raw data (Fig. 1). Considerable variation in the amount of ^{14}C-clethodim absorption was observed between the formulations and adjuvants within each harvest time. Irrespective of the presence or absence of an adjuvant, the ^{14}C-clethodim uptake with the 0.12 kg L^{-1} formulation was higher than with the 0.24 kg L^{-1} formulation, especially at 24 HAT and beyond (44–90% *vs* 16–37% of the amount of the ^{14}C-clethodim applied). Foliar-applied ^{14}C-fenoxaprop-ethyl absorption in wheat was 84% at 72 HAT (Lefsrud & Hall 1989). By 24 HAT, the ^{14}C-clethodim absorption in the wheat treated with the 0.12 kg L^{-1} formulation was 62–72% of the amount of the ^{14}C-clethodim applied in the presence of an adjuvant. In general, clethodim and other cyclohexanedione herbicides are rapidly absorbed (Wanamarta & Penner 1989a; Culpepper *et al.* 1999). For example, the ^{14}C-clethodim absorption in goosegrass increased from 36% of the ^{14}C-clethodim applied at 0.5 HAT to 89% of the ^{14}C-clethodim applied at 96 HAT (Burke & Wilcut 2003). The addition of an adjuvant caused a significant increase in the absorption of the ^{14}C-label with the 0.12 kg L^{-1} formulation at all harvest times (16–90% of the ^{14}C-clethodim applied in the presence of an adjuvant *vs* 1–71% of the ^{14}C-clethodim applied in the absence of an adjuvant), except at 72 HAT. The adjuvant type, AMS, COC, or AMS + COC, added to the 0.12 kg L^{-1} formulation did not affect the ^{14}C-label absorption, except at 48 and 72 HAT. Culpepper *et al.* (1999) reported that COC increased the ^{14}C-clethodim absorption in *Echinochloa crus-galli* L. (Beauv.) more than the non-ionic surfactant. The addition of AMS + COC to the 0.12 kg L^{-1} formulation consistently resulted in a

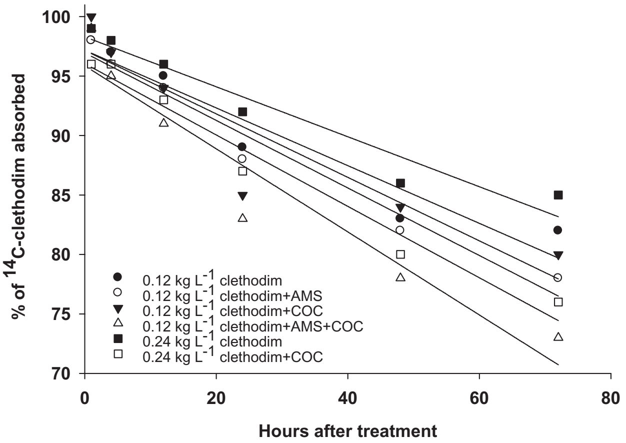

Fig. 2. Distribution of absorbed ^{14}C-clethodim in the treated leaves of wheat with a height of 20 cm. The ammonium sulfate (AMS) was applied at 2.8 kg ha^{-1} and the crop oil concentrate (COC) was applied at 1% (v/v). Regression equations: (●), $y = 97.2 - 0.24x$, $r^2 = 0.92$, $P = 0.0024$; (○), $y = 96.9 - 0.28x$, $r^2 = 0.97$, $P = 0.0004$; (▼), $y = 97.2 - 0.27x$, $r^2 = 0.85$, $P = 0.0085$; (△), $y = 95.9 - 0.35x$, $r^2 = 0.92$, $P = 0.0022$; (■), $y = 98.3 - 0.21x$, $r^2 = 0.94$, $P = 0.0014$; (□), $y = 96.1 - 0.3x$, $r^2 = 0.97$, $P = 0.0003$.

greater ^{14}C-clethodim uptake within each harvest time. Jordan *et al.* (1989) reported increased rates of sethoxydim (another cyclohexanedione herbicide) absorption in the presence of COC and AMS rather than COC alone in *Digitaria sanguinalis* L. (Scop.). Under our conditions, the addition of COC to the 0.24 kg L^{-1} formulation did not increase the absorption of ^{14}C-clethodim compared to the 0.24 kg L^{-1} formulation applied alone.

The ^{14}C-clethodim remaining in the treated leaf over time could best be described by an equation of the form, $y = y_0 + ax$, where y is the amount of ^{14}C as a percentage of the amount of the ^{14}C-clethodim absorbed, y_0 is the intercept, a is the slope, and x is the time after treatment (Fig. 2). Irrespective of the formulation or adjuvant, most of the ^{14}C-clethodim (73–100% of the ^{14}C-clethodim absorbed) remained in the treated leaf (Fig. 2). Cyclohexanedione herbicides only translocate to a limited extent out of the treated leaf (Campbell & Penner 1987; Culpepper *et al.* 1999). ^{14}C-Clethodim translocation to the shoot over time could best be described by an equation of the form, $y = y_0 + ax$ (Fig. 3). The parameters of the equation have been explained earlier. The formulation did not have an impact on the distribution of the absorbed ^{14}C-clethodim. However, the presence of an adjuvant caused an increase in the movement of the ^{14}C-label from the treated leaf. The addition of COC + AMS

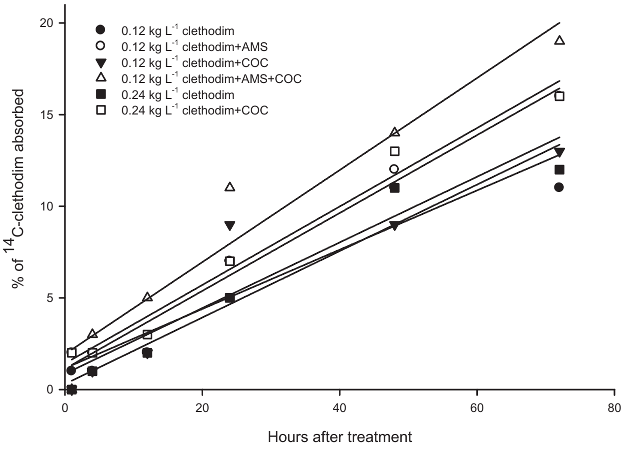

Fig. 3. Distribution of absorbed ^{14}C-clethodim in the shoots of wheat with a height of 20 cm. The ammonium sulfate (AMS) was applied at 2.8 kg ha^{-1} and the crop oil concentrate (COC) was applied at 1% (v/v). Regression equations: (●), $y = 1.16 + 0.16x$, $r^2 = 0.88$, $P = 0.0052$; (○), $y = 1.13 + 0.21x$, $r^2 = 0.98$, $P = 0.0002$; (▼), $y = 0.86 + 0.18x$, $r^2 = 0.87$, $P = 0.0062$; (△), $y = 1.93 + 0.25x$, $r^2 = 0.94$, $P = 0.0013$; (■), $y = 0.3 + 0.18x$, $r^2 = 0.95$, $P = 0.0009$; (□), $y = 1.42 + 0.21x$, $r^2 = 0.98$, $P = 0.0002$.

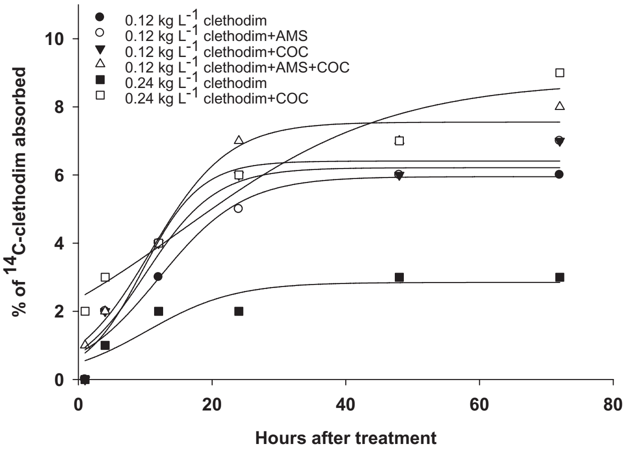

Fig. 4. Distribution of absorbed ^{14}C-clethodim in the roots of wheat with a height of 20 cm. The ammonium sulfate (AMS) was applied at 2.8 kg ha^{-1} and the crop oil concentrate (COC) was applied at 1% (v/v). Regression equations: (●), $y = 5.9/(1 + \exp[-(x - 12.1)/6.3])$, $r^2 = 0.96$, $P = 0.0094$; (○), $y = 6.2/(1 + \exp[-(x - 10.1)/5.3])$, $r^2 = 0.92$, $P = 0.0207$; (▼), $y = 6.4/(1 + \exp[-(x - 9.6)/4.3])$, $r^2 = 0.96$, $P = 0.0085$; (△), $y = 7.6/(1 + \exp[-(x - 10.9)/5.8])$, $r^2 = 0.98$, $P = 0.0017$; (■), $y = 2.8/(1 + \exp[-(x - 10.3)/6.5])$, $r^2 = 0.88$, $P = 0.0432$; (□), $y = 8.7/(1 + \exp[-(x - 15.5)/15.7])$, $r^2 = 0.96$, $P = 0.0074$.

to the 0.12 kg L^{-1} formulation or the addition of COC to the 0.24 kg L^{-1} formulation caused a significant translocation of the ^{14}C-label away from the treated leaf at 24, 48, and 72 HAT. There was a dramatic increase in the ^{14}C-label movement to the rest of the shoot at 24 HAT and beyond (5–19%), irrespective of the formulation or adjuvant. The translocation of the ^{14}C-clethodim in *Eleucine indica* was 8.3% of the applied ^{14}C into the rest of the shoot at 96 HAT (Burke & Wilcut 2003). The addition of COC + AMS to the 0.12 kg L^{-1} formulation or the addition of COC to the 0.24 kg L^{-1} formulation caused a significantly greater accumulation of the ^{14}C-label in the rest of the shoot at 24, 48, and 72 HAT. This indicates that these adjuvants facilitate the availability of higher levels of clethodim for movement to the site of action, that is, actively growing meristematic regions. The ^{14}C-clethodim translocation to the root over time could best be described by an equation of the form, $y = a/(1 + \exp[-(x - x_0)/b])$ (Fig. 4), where y is the amount of ^{14}C as a percentage of the ^{14}C-clethodim absorbed. The other parameters of the equation have been explained earlier. When the ^{14}C-clethodim was applied as the 0.12 kg L^{-1} formulation plus COC + AMS or as the 0.24 kg L^{-1} formulation plus COC, its translocation to the root was highest compared to the other treatments at all harvest times, except 12 HAT.

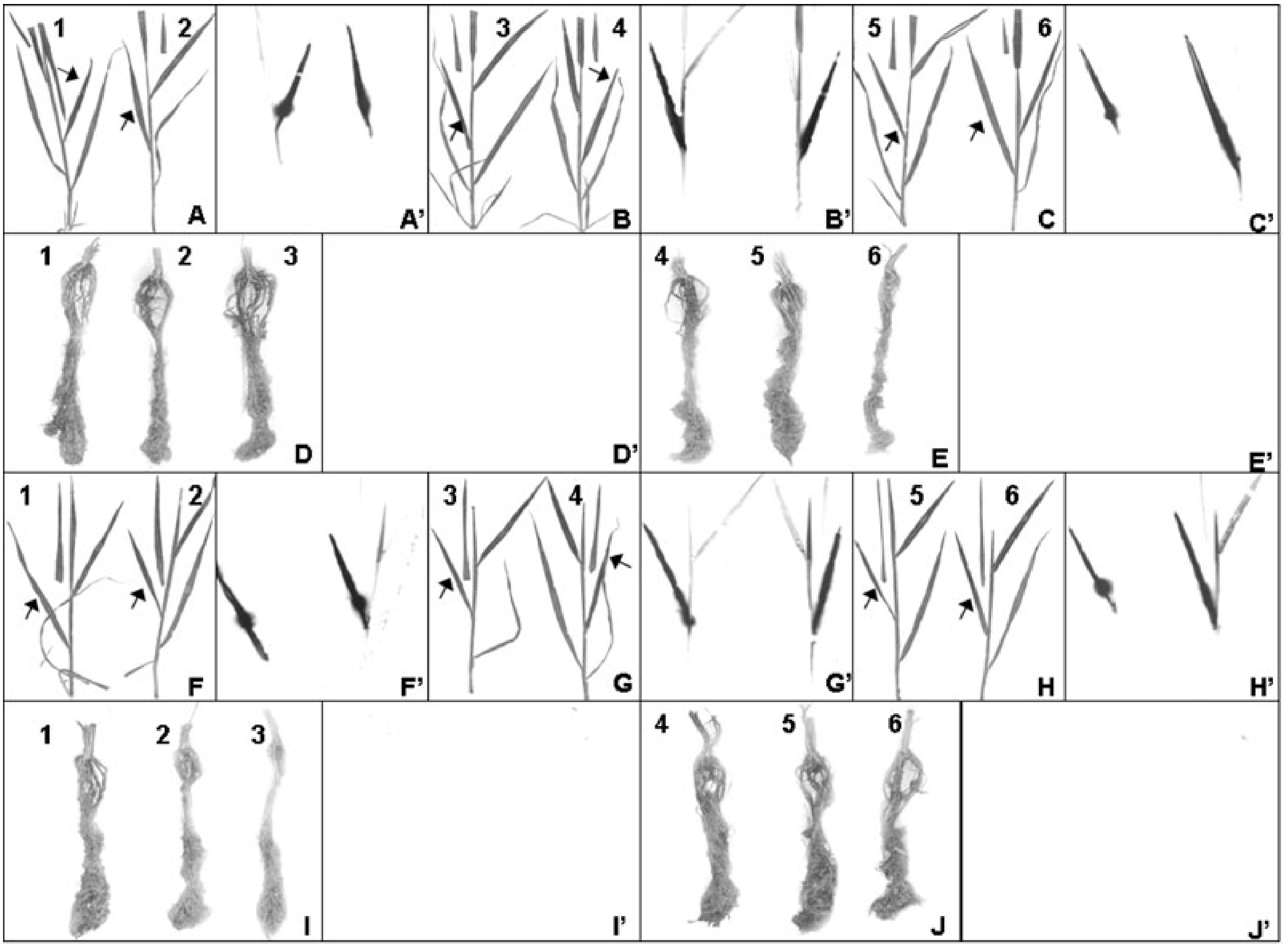

Fig. 5. Translocation of ^{14}C-clethodim applied in a solution of 0.12 kg L^{-1} or 0.24 kg L^{-1} formulation to wheat with a height of 20 cm at 24 h (first and second rows) and 72 h (third and fourth rows) after treatment. The arrows indicate the treated leaves. Panels A to J and A′ to J′ represent wheat plant specimens and their corresponding autoradiograms, respectively. Numbers 1, 2, 3, 4, 5, and 6 represent ^{14}C-clethodim applied as 0.12 kg L^{-1}, 0.12 kg L^{-1} + ammonium sulfate (AMS), 0.12 kg L^{-1} + crop oil concentrate (COC), 0.12 kg L^{-1} + AMS + COC, 0.24 kg L^{-1}, and 0.24 kg L^{-1} + COC, respectively.

Autoradiography

The X-ray autoradiograms of the wheat are represented in Fig. 5. ^{14}C-Clethodim applied to the third true leaf of the wheat plants, which were 20 cm in height, did not translocate appreciably to all parts of the plants. Of the absorbed ^{14}C-label, most remained in the treated leaf, as ascertained by quantization in the translocation study (Fig. 2). The distribution of the ^{14}C-label that translocated out of the treated leaf was restricted to the rest of the shoot (Fig. 5A′,B′,C′,F′,G′,H′) and a negligible quantity of the ^{14}C-label translocated to the root (Fig. 5D′,E′,I′,J′). An increase in the intensity of the image in the autoradiograms indicated that the ^{14}C-clethodim accumulation increased in the rest of the shoot from 24–72 HAT (Fig. 5A′ *vs* F′, B′ *vs* G′, C′ *vs* F′) with no apparent change in the translocation to the roots from 24–72 HAT (Fig. 5D′ *vs* I′, E′ *vs* J′). The addition of an adjuvant to the clethodim formulations of 0.12 kg L^{-1} and 0.24 kg L^{-1} caused an increase in the absorption of the ^{14}C-clethodim (Fig. 5) compared to the ^{14}C-label absorption when the clethodim formulation was applied alone to the wheat plants. The enhanced absorption of ^{14}C-clethodim in the presence of an adjuvant has indirectly contributed to the increased translocation of the ^{14}C-label in the rest of the shoot compared with that of the formulation alone, visibly more evident at 72 HAT (Fig. 5F′,G′,H′).

In summary, the 0.12 kg L^{-1} formulation of clethodim, with half the active ingredient concentration, was absorbed to a greater extent than the 0.24 kg L^{-1} formulation. The addition of COC + AMS can significantly increase the absorption of the 0.12 kg L^{-1} formulation. Ahrens (1994) noted that ultraviolet (UV) light degrades clethodim. Therefore, the addition of COC + AMS to the 0.12 kg L^{-1} formulation potentially could increase the herbicide's efficacy by decreasing the exposure of clethodim to UV light. In other studies, AMS improved the control of selected annual grasses by clethodim, despite the presence of broadleaf- and sedge-controlling herbicide treatments (Burke *et al.* 2004) that have been shown to antagonize the graminicidal activity of clethodim (Burke & Wilcut 2003).

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