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Short communication

Irrigation of pepper plant (*Capsicum* sp.) with water containing acrolein

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Abstract

MAGNACIDE® H herbicide (a.i. acrolein (2-propenal)) is an aquatic herbicide applied through underwater injection into agricultural irrigation canals for the control of submerged aquatic weeds. In support of the products national registration in Argentina, additional information was required pertaining to the potential persistence of acrolein residuals in plants irrigated with water treated with this herbicide. Pepper plants (*Capsicum* sp.) were treated through irrigation with the maximum application rate permitted (15 $\mu l\,l^{-1}$) on the label and at double the maximum application rate (30 $\mu l\,l^{-1}$), under controlled environmental conditions in a greenhouse. Reverse phase high-performance liquid chromatography (RP-HPLC) with fluorescence detection was the method used to determine acrolein. Parts of the plant (leaves, stems, roots and fruit) as well as soil were collected in order to determine potential residual levels of acrolein. The low values of acrolein measured in all samples were reduced to undetectable levels within a few hours, resulting a half-life of acrolein in pepper plants of 10.3 h. The plants did not exhibit any visible damage attributable to the aquatic herbicide. The most remarkable observation was that acrolein never persisted in any of the sampled plants. Therefore, it is safe to use water acrolein treated under controlled conditions through field application.

Keywords: 2-Propenal; Acrolein; Aquatic herbicides

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1. Introduction

Acrolein (2-propenal) herbicide is used in irrigation and drainage canals for the control of submerged aquatic weeds (Nordone et al., 1996a). Acrolein applied in this situation is highly toxic to fish and produces different kinds of effects in the aquatic (WSSA, 1994). The increasing use of chemicals in agriculture has resulted in widespread concern regarding pesticide residues entering the food supply (US National Research Council, 1993). Acrolein has been used in Argentina for the control of *Potamogeton pectinatus* L. in irrigation canals of the lower valley of the Río Colorado (39°23′S; 62°37′W) (Bentivegna, 2001). General physical properties of acrolein was descript (Anderson and Hood, 1962), but little information exists regarding the potential of acrolein to accumulate in food crops that have been irrigated with acrolein treated water. Due to this lack of information, the management of acrolein treated water is restricted and limited to situations where the application can be controlled. Reverse phase high-performance liquid chromatography (RP-HPLC) with florescence detection was the method used to determine acrolein as a florescent derivative in pepper plants, giving good limits of detection (Anderson, 1986).

The aim of this study was to establish the possibility of using acrolein-treated water for the crops irrigation, through the determination of acrolein levels in pepper plants (*Capsicum* sp.) after being irrigated with water treated with herbicide.

2. Materials and methods

The experiment was conducted in a greenhouse at the constant environment throughout the period of the test (relative humidity: 60%; temperature: $21 \pm 5\,^{\circ}C$). Pepper plants were planted in wooden boxes filled with soil, and irrigated with water treated with acrolein (96.1% Magnacide H″; Baker Petrolite) at three different concentrations (0, 0.25 and 0.50 mM). Control boxes (3) were irrigated with untreated water. Boxes ($1.60\,m \times 0.80\,m \times 0.40\,m$) were filled ($0.46\,m^3$) with clay loam soil (organic material: 1.72%; pH 7.5; salinity: $1.27\,dS\,m^{-1}$).

For each treatment, repetitions (3) with pepper plants (10) per box were made according to FAO protocol for a study of this type (FAO, 1987).

Predetermined quantities of acrolein were mixed with tap water (turbidity: 2.26 ± 0.21 Nephelometric Turbidity Units; pH 7.8 ± 0.1; electrical conductivity: $618.7 \pm 4.5\,mS\,cm^{-1}$; temperature: $18\,^{\circ}C$) in containers to obtain the correct dosing levels (as specified previously). The treatment mixture was made by adding the acrolein with a pipette to the containers. The exact amount of dosage in the treated water was measured by the colorimeter method developed by Kissel et al. (1978).

The application of the product to the plants was conducted when the plants had four true leaves. Each box was irrigated with (26 l) acrolein-treated water (0.25 and 0.50 mM) depending on the treatment. The control boxes were irrigated with untreated water in the same volume. One plant from each box was collected at 0.042 (1 h after treatment), 1, 2, 4, 8, 18, 30 and 70 days after treatment. At the day 70, plant, fruits and soil samples were taken.

Leaves, stems and roots or fruits together were weighed (50 g) and homogenized with pure water (50 ml, Milli Q clay) in a Virtis apparatus (model N60K; The Virtis Company

Inc., Gardiner, NY) at 10,000 rpm (two times for 30 s). The resulting solutions were filtered using cotton mesh (80–100) and the filtrates centrifuged (3000 rpm, 10 min; Beckman J2-21 Centrifuge). Soil samples were taken at the same time as the plants and processed separately. Typically 10 g of soil was mixed with 20 ml of pure water, stirred for 10 min and filtered. Aliquots of the filtrate were processed as indicate for the plant extract.

Acrolein can be analyzed by HPLC (Nordone et al., 1997; Smith et al., 1995). As the fluorometric methods increase the sensitivity of detection limits when minimal concentrations have to be recorded (Alarcon, 1968; Kissel et al., 1978), we used both techniques according to detection limits.

A stable fluorescent derivative (hydrazones) detectable to low levels can be obtained by the reaction of fluorescent hydrazines with aldehydes (Anderson, 1986; Yan and Lin, 1997). The extracts obtained in the homogenized samples were reacted with Dansyl-hydrazine (D-100, Molecular Probes), and the corresponding conjugated Dansyl-hydrazones were analyzed directly by RP-HPLC with a fluorescence detector.

Volumes (0.2 ml) of solution containing acrolein standard (in suitable dilution) or samples of extract vegetation solution were acidified to 0.1 M with HCl and derivatizated by mixing them with Dansyl-hydrazine 3.8 nM in 1 M HCl (0.5 ml). After 10 min, 1 M NaOH (0.5 ml) was added to stop the reaction. The mixture in each case was stabilized with isopropyl alcohol (0.2 ml) and injected in the chromatographic column.

The equipment for HPLC was a Varian 5000 (Vista series) chromatograph equipped with fluorescence detector Fluorichrom (Varian), with EX: 340 nm; EM: 534 nm (Haugland, 1996).

For the separation of fluorescent compounds a RP-Nucleosil column 120-5/C-18 (Macherey-Nagel) was used with a mobile phase of methanol + water (78 + 22 by volume) at constant flow (1 ml/min) and temperature (40 °C). The peaks obtained were compared with the corresponding ones of Dansyl derived from an acrolein reference using their retention times (5.6 min). The quantification was measured on the basis of the area of the resolved peak (computer integration), comparing with a calibration curve constructed with an acrolein reference.

3. Results

A typical chromatogram of the extract of plants obtained from the second day of application in treated and untreated plants is shown in Fig. 1. The retention times of the peak present in the chromatogram did not agree with the corresponding one to the Dansyl derived from acrolein shown as a reference. As the detected fluorescent derivatives seen were in both the treated and untreated samples, it is assumed that they are naturally occurring compounds, not an acrolein metabolite. The plants did not exhibit visible damage attributable to the aquatic herbicide in any growth stage.

Absolute acrolein concentrations found in the extracts of the plants are shown in Fig. 2. The samples that were taken 1 h post-treatment had a minute concentration of acrolein (18 ng g fresh tissue^{-1}). The maximum concentration of acrolein was measured in the samples taken on the first day post-treatment (48 ng g ft^{-1}). At the second day post-treatment, the highest registered concentration of acrolein in plants (2 ng g ft^{-1}) was measured in the

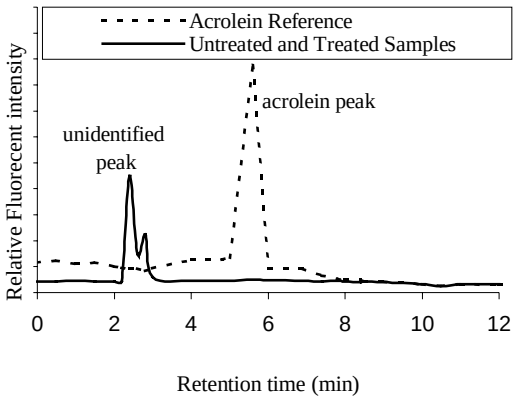

Fig. 1. Relative intensity of acrolein–Dansyl derivative in plant samples 2 days after treatment and untreated plant compared with the acrolein–Dansyl derivative reference.

0.50 mM treatment. The most remarkable observation was that acrolein never accumulated in any of the sampled plants. The low values of acrolein measured in all samples were reduced to undetectable levels within a few hours, giving a half-life of acrolein in pepper plants of 10.3 h, irrespective of the initial concentration of acrolein applied. The average life of the product in the plant was calculated by dividing the curve of the regression of the concentration of Dansyl-derivative in the post-treatment abstracts (Fig. 3). The acrolein treatments (0.25 and 0.50 mM) showed the same profile of degradation of acrolein in the plants (Figs. 1–3). Eventually, samples of soil and fruit were analyzed and acrolein persistence was not detected.

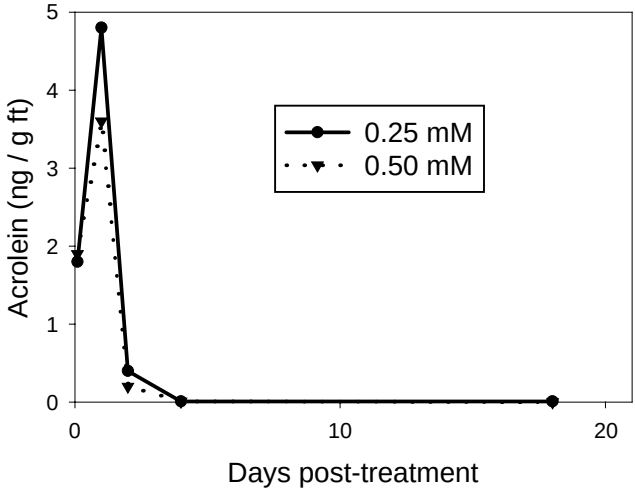

Fig. 2. Absolute quantity of acrolein in pepper plant samples up to 20 days after treatment (0.25 and 0.50 mM).

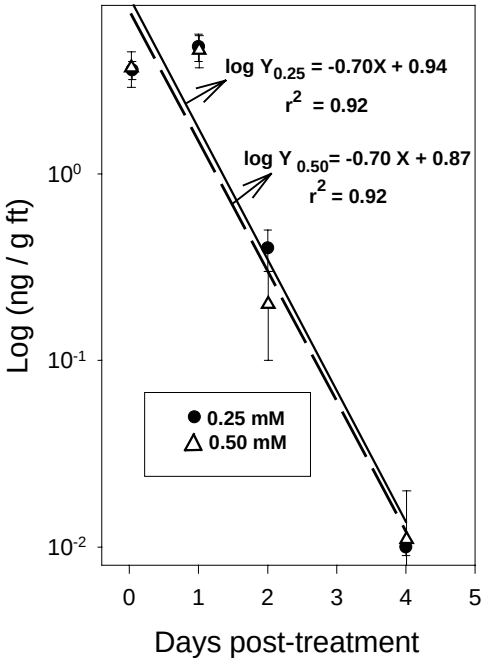

Fig. 3. Regression curve of acrolein residues in pepper plants as a function of time post-treatment.

4. Discussion

The limit of detection for the acrolein Dansyl derivatives was 0.3 pmol, equivalent to 2×10^{-4} μl l^{-1} of acrolein in the sample. The separation of the Dansyl derived by RP-HPLC allows the ability to differentiate the corresponding peak of acrolein from other derivatives (Fig. 1).

Acrolein in water can be reduced very quickly due to varying factors. The hydration is the most important factor that affects the degradation, follow by the volatility, dilution, adsorption and the influence of microorganisms (Smith et al., 1995; Nordone et al., 1996b) The rapid degradation of acrolein in water produces a quick diminution of the concentration of the chemical available for uptake by irrigated plants in relation to the initial content of the treatment.

Acrolein persistence was not observed in the plants, which implies that it was rapidly biodegraded and metabolized to water-soluble compounds (WSSA, 1994; WHO, 1992).

[^{14}C]-labeled acrolein was used to determine the fate of acrolein that was absorbed by the plants and became incorporated into own structures (Nordone et al., 1998). Lettuce plants irrigated at different frequencies with acrolein-treated water do not retain significant concentrations of the product in leaves, roots or surfaces of the plants (Nordone et al., 1997). Acrolein is poorly adsorbed and translocated by terrestrial plants (WSSA, 1994).

The half-life of acrolein in the pepper plants determined in this study (10 h) is similar to that determined by Smith et al. (1995) in investigations of aerobic or anaerobic degradation in the aquatic conditions.

According to this research, the water treated with acrolein can be used for irrigation because it does not show accumulation of the herbicide in the pepper plants.

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