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The Phytotoxic Potential of the Terpenoid Citral on Seedlings and Adult Plants

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Citral is a monoterpene commonly found as volatile component in many different aromatic plants. Although many studies have identified the presence of citral in phytotoxic essential oils, this work determines for the first time the potential herbicidal effect of citral on weeds. The use of citral against weeds and crops resulted in the potential for the management of barnyardgrass, redroot pigweed, and ribwort. Clear morphological differences were observed between adult thale cress plants exposed to citral in two different application methods: spraying and watering. Citral-sprayed and citral-watered thale cress plants showed completely different effects after treatment, suggesting that foliar or root absorption can determine the effectiveness of this compound. This work demonstrates that citral is effective not only on seedling metabolism but also on adult plants by inhibiting growth and development altering the plant oxidative status.

Nomenclature: Citral, 3,7-dimethyl-2,6-octadienal, CAS 5392-40-5; barnyardgrass, *Echinochloa crus-galli* L. (Beauv.) ECHICRU; redroot pigweed, *Amaranthus retroflexus* L. AMARE; buckhorn plantain, *Plantago lanceolata* L. PLANLAN; thale cress, *Arabidopsis thaliana* L. ARATH.

Key words: Monoterpene, spraying, watering, phytotoxicity, weed management.

Weed control has always been an important area of study due to the negative influence that weed species have on crop productivity. The overuse of synthetic herbicides has resulted in the increase of soil and water contamination, loss of biodiversity and the appearance of many herbicide-resistant species, which makes weed control even more difficult. Although this could have been the trigger for seeking alternatives to synthetic chemical control, the appearance of genetically engineered herbicide-resistant crops has resulted in a dramatic increase in the use of synthetic chemicals, rather than a decrease (Owen 1997). Moreover, previous studies, mainly done with commercial herbicides, have demonstrated the increasing sensitivity or resistance of different treated plants to the applied herbicides depending on the application procedure (deBoer et al. 2011; Concenço et al. 2009).

Plants produce a large number of phytotoxins, which could potentially be used as herbicides (Duke et al. 2002). In the last few years, many efforts have been devoted to determine their mechanisms of action, a difficult task due to the many possible molecular targets they have. This apparently overwhelming task does, however, also present an opportunity for discovering new sites of action (Dayan et al. 2000), which will also depend on how the compound is applied, since very different effects may be found after watering (compound enters through the root) or spraying (direct contact with the aerial part of the plant). In the same way, foliar and root absorption and the translocation rate between aerial and subterranean plant organs can change and determine the effectiveness of the compound (Lycan and Hart 2006).

In recent years, several different molecules, such as terpenes, have been investigated and tested as new natural herbicides. Both the monoterpenes and the sesquiterpenes have been extensively studied, and some of them, such as the lactones, have even been proposed for use as natural herbicides (Macías et al. 1999). Citral is a mixture of the isomeric acyclic monoterpenes geranial (*trans*-citral or citral A) and neral (*cis*-citral or citral B) (Chaimovitsh et al. 2010; Djorjdevic et al. 2008; Dudai et al. 1999) in a 7 : 3 ratio (Dikusar et al. 2008). It is a plant compound that is present as a volatile component of the essential oils from many different aromatic plants (Djorjdevic et al. 2008) such as lemongrass (*Cymbopogon* spp.), which has between 65 and 85% citral in its essential oils; the lemon tea-tree (*Leptospermum liversidgei*; R. Baker & H. G. Smith), which has 80%; and lemon verbena (*Aloysia citrodora* Paláu), which has about 35% citral in the essential oils (Rabbani et al. 2006). Although no extensive works have been made on citral biodegradation and volatilization in the soil, it is known that citral may volatilize from water surfaces with estimated half-lives of between 1.2 and 12 d and is expected to show high mobility in the most soils (Griffin et al. 1999).

Chaimovitsh et al. (2010, 2011) recently showed the capacity of citral to change the normal growth of wheat radicles and *Arabidopsis* cells through microtubule disorganization, demonstrating a strong phytotoxic activity of this monoterpene. However, although many studies have identified the presence of citral in phytotoxic essential oils, no experiments have been made up to now to determine the potential herbicide effect of citral on weed management. Testing different weeds and crops can reveal more precisely the potential herbicide nature of citral. In the same way, no studies have been found in the literature with citral-treated adult plants, even when the effects and mechanisms of action of citral can be completely different in adult plants than in seedling metabolism.

Therefore, the purpose of this work was to demonstrate the phytotoxic potential of citral to different weeds and crops and to study the effects that the exogenous application of this monoterpene has on the metabolism of adult plants of thale cress, a model species widely used in phytotoxicity bioassays. In order to define the phytotoxic and metabolic effects of citral into the plant, parallel experiments were carried out with citral-spraying or citral-watering thale cress plants.

Materials and Methods

The phytotoxicity of the naturally occurring compound citral was evaluated, in crops and weeds species, with germination (germination rate and germination kinetics) and growth (radicle length) bioassays. Barley (*Hordeum vulgare* L. 'Reading UK'), wheat (*Triticum aestivum* L.

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'Havana'), corn (*Zea mays* L. 'Mataró'), rice (*Oryza sativa* L.; supplied by International Rice Research Institute, Los Baños, Philippines), soybean (*Glycine max* (L.) Merr. 'Isidor'), and lettuce (*Lactuca sativa* L. 'Great Lakes') were selected as crop species, and redroot pigweed, barnyardgrass, common purslane (*Portulaca oleracea* L.), ribwort, field bindweed (*Convolvulus arvensis* L.), and wild oat (*Avena fatua* L.), all from Herbiseed (Twyford, UK), were selected as associated weeds.

Pregermination treatments were applied in order to break dormancy and synchronize germination. Barley was soaked in water for 5 d at 40 C and then for another 5 d at 4 C; soybean was soaked in water for 6 h at room temperature; redroot pigweed, barnyardgrass, and common purslane were soaked in water for 15 d at 4 C; field bindweed was incubated with 96% H_2SO_4 for 30 min and then washed thoroughly with tap water for 5 min; and wild oat seeds were treated with 1% bleach and 0.2% KNO_3 after the cupule, lemma, and palea were removed. No pregermination treatments were applied to lettuce, wheat, corn, rice, and ribwort. Due to differences in growth, morphology, and life cycle, the experiments were designed differently for and adapted to each of the species to evaluate the effects of citral.

Citral (W230316 Sigma-Aldrich Co. LLC., St. Louis, MO), was prepared in ethanol (EtOH) and diluted in distilled water to the concentrations tested in the bioassays: 0, 50, 100, 200, 400, 800, and 1200 μM to a final concentration of EtOH of 0.1%.

Germination Bioassays. Between 24 and 50 seeds were placed in petri dishes (9-cm diam). The seeds were placed on Whatman 3 MM filter paper and dampened with distilled water + EtOH control or treatment solutions. The amount of solution (4 to 6 ml) was dependent on the size of the seeds. After the seeds were sown, the dishes were placed in a growth chamber under controlled conditions of light intensity, temperature, relative humidity, and photoperiod. The conditions were as follows: 35 C in dark for redroot pigweed; a 16/8-h light/dark cycle at 16/26 C for common purslane, barnyardgrass, and barley; a 8/16-h light/dark cycle at 22 C for ribwort; a 12-h dark cycle at 20/30 C for field bindweed; 27 C in dark for corn and rice; a 16/8 -h light/dark cycle at 16/26 C for wheat and wild oat; a 16/8-h light/dark cycle at 26/20 C for lettuce; and 27 C in the dark between two layers of filter paper moistened with the solution for soybean. Light was applied with a photosynthetic active radiation (PAR) of 75 μmol photons m^{-2} s^{-1}. The bioassays were triplicated in time and space, and every time five to eight replicates per citral concentration consisting of a petri dish with 24 to 50 seeds was used. The number of seeds per petri dish and replications per treatment depended on the size of the seeds. Eight replicates with 24 seeds each were used for large-seeded species, whereas five replicates with 50 seeds each were used for small-seeded species.

The number of germinated seeds was counted at the following times: every 3 h for 24 h for lettuce, every 4 h for 48 h for wheat, every 6 h for 48 h for corn, every 12 h for 96 h for barley, every 2 h for 10 h for rice, every 6 h for 96 h for soybean, every 2 h for 22 h for redroot pigweed, every 4 h for 16 h for barnyardgrass, every 12 h for 60 h for ribwort, every 24 h for 168 h for common purslane, every 6 h for 24 h for field bindweed, and every 12 h for 72 h for wild oat. A seed was considered to have germinated when the radicle protruded through the seed coat to a length of approximately 1 to 2 mm.

The total germination (G_T), germination speed (S, which indicates the number of seeds germinated between two measurements), and speed of accumulated germination (AS, which describes the accumulated number of seed germinated in each measurement period) were calculated in order to determine both the speed and the rate of germination (Chiapusio et al. 1997; Dias 2001; Thermina and Rukhsana 2005):

- $G_T = 100 \cdot N_T/N$, where T indicates the last observation and N is the total number of seeds sown.
- $S = n_1 + (1/2)n_2 + (1/3)n_3 + \ldots + (1/T)n_T$, where $n_t = (N_t - N_{t-1})/N_T$ (with $N_0 = 0$) is the number of seeds that germinated between observations $t - 1$ and t, expressed as a proportion of all seeds that germinated.
- $AS = N_1 + (1/2)N_2 + (1/3)N_3 + \ldots + (1/T)N_T$, which can be seen proportional to a weighted average speed in which the weights increase with time since sowing.

Although the G_T index is useful for a global interpretation of germination—inhibition, stimulation or no effect—it ignores any possible delays in germination, since it only considers the final results. It is therefore not sensitive enough for studying such a complex physiological process as germination. Each index provides different interpretations of the effects, and no one index can, on its own, precisely reflect all the effects there are on germination (Chiapusio et al. 1997).

Growth Bioassays. Pregerminated seeds in water were used in order to independently analyze the processes of germination and growth and thus prevent any bias in the measurement of the phytotoxic effect (Hoagland and Williams 2004). Between five and eight replicates were used per concentration. For each, between 15 and 25 pregerminated seeds, with approximately 2 mm of radicle, were placed in the dishes with the treatment. These dishes were then kept in a growth chamber under the same conditions as for germination. Once the bioassay was finished, the dishes were frozen at −20 C in order to prevent the seedlings from growing before measurement. Root length was measured for all the seedlings and a dose-response curve was prepared. The concentration of citral that causes 50% inhibition of germination rate (IC_{50}), concentration of citral that causes 80% inhibition (IC_{80}) and lowest complete inhibition concentration values were obtained.

Experiments on Adult Plants. Thale cress (ecotype Columbia, Col-0) seeds were sterilized in 50% EtOH solution and 0.5% NaOCl solution with 0.01% Triton solution, both for 3 min, and then washed three times with distilled water. After sterilization, the seeds were kept in 0.1% agar at 4 C for 72 h. Seeds were then sowed in petri dishes (145 cm^2) at an approximate density of one seed every 2 cm^2. The thale cress seedlings were grown in a plant agar medium with a mix of macro- and micronutrients (Murashige-Skoog, Sigma-Aldrich, USA) and 1% sucrose as supplement. These dishes were kept in a growth chamber for 15 d at a constant temperature of 22 ± 2 C (SE) and a PAR of 75 μmol m^{-2} s^{-1}.

After this time, one seedling per pot was transferred to an individual pot 5 cm in diam and 6 cm high containing inert perlite moistened with a half-strength Hoagland nutrient

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solution. The pots were placed in a growth chamber at 22 ± 2 C with a photoperiod of 8 h of light and 16 h of darkness (120 μmol m^{-2} s^{-1}) for 7 days. During this time the seedlings were watered twice a week with a half-strength Hoagland nutrient solution. The treatment with citral began when the plants had grown and reached five fully expanded leaves, and the watering was done every other day.

Citral was diluted in EtOH (0.1%) and water was added to the concentrations tested in this experiment: 0, 300, 600, and 1,200 μM (these values correspond to 0, 0.287, 0.574, and 1.149 kg ha^{-1}, respectively). These treatment solutions were applied by watering the perlite or spraying the plants to determine the differing effects of entry through the root compared with damage by contact.

Plants with similar size and number of leaves were selected; 20 plants were used for concentration and method of application (watering/spraying). During 21 d, citral in half-strength Hoagland solution was applied by watering every 2 d. For the spraying treatment, plants were sprayed daily, at 10:00 A.M., with citral dissolved in distilled water (0.001% Tween-20 solution) until all the leaves were covered with small droplets but before the point of runoff, and watered every other day with a half-strength Hoagland nutrient solution. EtOH at 0.1% was added to all the solutions, including the control. The experiment was duplicated in time and space.

After 21 d, shoots were harvested, washed thoroughly with tap water, dried, and used directly for measurements of fresh/dry biomass, C:N content, and elemental analysis, or frozen at −80 C for later measurements of free proline, lipid peroxidation, total proteins, and photosynthetic pigments. As well, after treatment application, several measurements were taken, both before and after harvesting.

Measurement of Chlorophyll a *Fluorescence*. Measurements of the chlorophyll *a* fluorescence emitted by three whole treated plants were recorded every 2 d during the 21 d of treatment with the Maxi-Imaging-PAM Chlorophyll Fluorescence System fluorometer from Walz (Effeltrich, Germany). The same three plants per treatment were measured throughout the experiment. The F_v (variable fluorescence) F_0 (dark fluorescence yield) and F_m (maximal fluorescence yield) parameters were measured after keeping the plants in the dark, and the values of photochemical and nonphotochemical quantum yield were obtained, including the effective quantum yield of the photosystem II (PSII) photochemical reactions (ϕ_{II}), the measurement of regulated energy dissipation in the form of heat (ϕ_{NPQ}), the measurement of nonregulated energy dissipation (fluorescence emitted) (ϕ_{NO}), the maximum PSII efficiency (F_v/F_m), and the estimated electron transport rate (ETR) (Bilger and Björkman 1990; Demmig-Adams et al. 1995; Genty et al. 1989; Kramer et al. 2004). After dark adaptation, when all the reaction centers were open, a saturation pulse of light was emitted to obtain the F_v/F_m. The plants were then monitored during 5 min of exposure to actinic light (110 μmol m^{-2} s^{-1}) to allow photosynthetic activity. Light saturation pulses of 2,700 μmol m^{-2} s^{-1} were applied at intervals of 20 s to obtain the previously described photochemical and nonphotochemical parameters. Fifteen measurements were obtained for each parameter at each measuring time, which yielded a kinetic plot for each parameter along time. The integral value of the area was obtained for all of these graphs. The value represented in the graphs of fluorescence for each time is the average area calculated from the kinetic measurement for three replicates in every treatment. This area highlights the magnitude of change in time and can be used to observe the total value of the trend. The comparison of these parameters between treatments will be essential for understanding the plant photosynthetic level and its capacity to withstand unfavorable situations.

Stomata. This measurement was made in vivo before harvesting to determine the frequency and density of stomata and the degree of stomatal opening on adult thale cress plants. The number of open and closed stomata in a 1-cm^2 surface area was counted for both sides of the leaf after collodion printing (Long and Clements, 1934). The measurements were made in four replicates per treatment.

Dry Weight/Fresh Weight. The aerial parts of five thale cress plants were weighed and oven-dried at 70 C for 72 h. After this time the samples were weighed again and the ratio of dry biomass to fresh biomass was obtained.

Elemental Analysis. A 3-mg aliquot of dried shoots was used to determine the total C, N, H, and S, using a Fisons elemental analyzer, Model EA 1108 CHNS (Beberly, MA), which has a measurement range of 100 ppmw, with a detection limit of 10 ppm. Calculations were made to obtain the C:N ratio. Other elements, such as Ca^{2+}, Cu^{2+}, Al^{3+}, Fe^{2+}, Na^{+}, P, K^{+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}, were analyzed by using an inductively coupled plasma optical emission spectrometer (model 4300DV, Perkin Elmer Optima, Waltham, MA). The results obtained are shown in mg g^{-1} of dry weight.

Measurement of Photosynthetic Pigments. The total amounts of chlorophyll *a*, chlorophyll *b*, and carotenoids were analyzed and calculated according to Wellburn (1994) in four independent biological replicates at the end of the experiment. The data were given in micrograms of protein per gram of dry weight. The carotenoid:chlorophyll ratio was calculated as an indicative measurement of the physiological status of the plants. In general, this index increases in anomalous or limiting situations due to an increase in the concentration of carotenoids (photoprotection) or a reduction in the chlorophylls (photodegradation), both indicative of a plant response to a changing factor (Filella et al. 1995).

Measurement of Total Proteins. This measurement was made in adult thale cress plants with the Bradford method according to Pedrol and Ramos (2001) in four independent biological replicates at the end of the experiment. Bovine serum albumin was used as a standard. Protein content was calculated per gram of dry wt.

Measurement of Lipid Peroxidation. The level of lipid peroxidation was indirectly determined by measuring the increase in the levels of malondialdehyde (MDA), a by-product of lipid peroxidation, which is used as an indicator of membrane injury caused by a change in the plant's oxidant status. The MDA measurement was estimated according to Hodges et al. (1999) in four independent biological replicates

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Table 1. Values in percentage of the control of total germination, speed of germination, and speed of accumulated germination for weed and crop species. The experiment was conducted by triplicate. Bold type represents significant inhibition at $Pp < 0.05$ compared to the control. Bold italic numbers represent significant stimulation at $Pp < 0.05$ compared to the control.

	Germination index											
	Weed species						Crop species					
	Barnyard grass	Common purslane	Wild oat	Redroot pigweed	Field bindweed	Ribwort	Lettuce	Barley	Soybean	Rice	Corn	Wheat
Total germination												
0 µM	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
50 µM	95.5 ab	97.6 a	107.4 a	100.5 a	123.7 b	**78.9** b	109.1 a	92.3 ab	99.0 a	100.1 a	99.1 a	102.5 a
100 µM	88.8 ab	96.9 a	102.7 a	93.0 a	104.6 a	82.2 ab	107.1 a	**81.3** b	116.8 a	98.7 a	99.1 a	102.5 a
200 µM	101.2 a	101.2 a	90.2 a	**85.1** b	96.3 a	**71.1** b	**81.8** b	**71.6** bc	129.5 a	101.5 a	101.6 a	102.5 a
400 µM	**84.2** b	90.4 a	98.1 a	**87.9** b	113.1 a	**56.7** b	**29.3** c	**69.5** c	161.5 a	97.5 a	96.7 a	101.6 a
800 µM	**59.9** c	91.6 a	88.2 ab	**71.6** b	105.5 a	**16.7** c	**1.0** d	**50.2** d	86.3 a	95.6 a	93.4 a	100 a
1.2 mM	**33.3** d	90.4 a	**49.9** b	**48.4** c	100.5 a	**17.8** c	**0.0** d	**18.1** e	116.4 a	**79.2** b	95.9 a	100.8 a
IC_{50}	820 µM	Insensitive	1.5mM	1.2mM	Insensitive	546 µM	405 µM	708 µM	Insensitive	Low sensitive	Insensitive	Insensitive
Speed of germination												
0 µM	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
50 µM	99.6 a	112.5 a	102.2 a	100.2 a	127.9 a	**79.4** b	92.6 a	97.6 a	107.0 a	99.1 a	***165.6*** b	107.0 a
100 µM	93.5 ab	140.0 b	98.7 a	94.1 ab	100.2 a	**64.0** b	**79.6** b	**80.9** b	117.7 a	100.4 a	***182.8*** c	94.8 ab
200 µM	107.9 a	114.0 a	112.1 a	**79.3** b	113.8 a	**58.1** b	**54.1** c	**69.3** c	146.6 a	103.6 a	***164.1*** c	**86.5** bc
400 µM	**87.6** b	97.0 a	76.4 ab	**78.3** b	93.1 a	**35.7** c	**16.3** d	**67.5** c	146.7 a	99.3 a	106.3 a	**79.9** c
800 µM	**58.6** c	96.5 a	**38.6** b	**65.9** c	94.9 a	**9.6** d	**0.5** e	**50.6** d	92.2 a	92.4 ab	103.1 a	**76.4** c
1.2 mM	**33.5** d	96.8 a	**20.0** b	**37.8** d	86.2 a	**9.0** d	**0.0** e	**20.2** e	100.1 a	**83.0** b	**89.1** d	**76.6** c
Speed of accumulated germination												
0 µM	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
50 µM	93.2 ab	104.9 a	103.9 a	97.9 a	128.7 a	**79.3** b	85.1 ab	99. 8 a	113.4 a	96.1 a	***162.7*** b	105.7 a
100 µM	**78.9** c	100.2 a	100.9 a	95.9 a	101.0 a	**61.5** b	**66.5** b	**80.2** b	112.7 a	99.9 a	***188.1*** c	92.6 ab
200 µM	**87.4** b	108.2 a	113.9 a	**70.5** b	114.3 a	**55.9** bc	**39.2** c	**67.2** c	147.2 a	104.1 a	***177.6*** c	**80.9** b
400 µM	**82.3** b	95.5 ab	79.2 ab	**65.8** bc	90.5 a	**31.5** c	**7.8** d	**66.7** c	148.7 a	98.3 a	110.5 a	**71.7** c
800 µM	**68.9** c	**91.7** b	**36.6** b	**55.9** c	94.5 a	**7.7** d	**0.3** e	**49.7** d	96.0 a	89.9 a	98.5 a	**67.8** d
1.2 mM	**33.6** d	93.1 ab	**14.6** c	**24.7** d	86.4 a	**6.2** d	**0.0** e	**20.2** e	101.6 a	81.2 a	**85.1** d	**68.3** cd

[a] Numbers within rows followed by same lowercase letters indicate no differences among treatments, while different lowercase letters indicate differences among treatments.

at the end of the experiment. MDA content was calculated per gram of dry wt.

Measurement of Free Proline. Free proline content was calculated according to the procedure described by Bates et al. (1973) on three independent biological replicates per treatment at the end of the experiment. Proline concentration was read at 520 nm and calculated from a calibration line constructed with pure proline standards. Values were expressed as percentage of dry wt.

Statistical Treatment. The experiments were duplicated (for adult plants) or triplicated (for bioassays) in space and time, and the data from each were subjected to ANOVA in SPSS 15.0 (IBM, USA). No significant interactions with experimental repetition were detected; thus data from each experimental repetition was combined. After a Kolmogorov-Smirnov test for nonnormality and Levene's testing for heteroscedasticity, the statistical significance of differences among group means was estimated by ANOVA followed by least significant difference tests for homoscedastic data, and by Tamhane's T2 test for heteroscedastic data.

Results

Germination and Growth. G_T of weeds treated with citral showed a very strong significant inhibitory response in ribwort, barnyardgrass, and redroot pigweed, with G_T of wild oat below 50% for the highest concentrations when compared to the control (Table 1). As well, a significant delay in germination was observed as citral concentration increased—as shown by S and AS—in redroot pigweed, barnyardgrass, wild oat, and ribwort. Actually, ribwort showed the most significant inhibitory values. Common purslane and field bindweed were the only noninhibited weeds.

Germination of crop species was less affected by citral. The only relevant significant inhibitory effects were found on the G_T percentage and on S for the crop species lettuce and barley (Table 1). This effect was especially significant in lettuce, which showed total inhibition of germination at 1,200 µM citral concentration with an IC_{50} of 405 µM. However, rice had a low sensitivity to citral, showing strong stimulatory effects on germination kinetics, but only slight inhibitory effects of less than 20% could be detected at the highest concentration tested (1,200 µM). Soybean and corn were insensitive to citral and neither stimulation nor inhibition could be detected at any of the concentrations tested, for any of the calculated germination parameters.

Growth bioassays showed a similar pattern, with stronger effects on weeds than crops, and stronger effects in radicle length than in germination rate. Table 2 highlights the highly significant inhibitory effect of citral on radicle growth of barnyardgrass, redroot pigweed, and ribwort, even at concentrations as low as 50 µM. The lowest IC_{50} and IC_{80} values (Table 2) were found for the weeds ribwort, barnyardgrass, and redroot pigweed. By contrast, common purslane

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Table 2. Root length of weed and crop species. Bold numbers represent significant inhibition at Pp < 0.05 compared to the control. Bold italic numbers represent significant stimulation at $P < 0.05$. The concentrations of citral that cause 50 and 80% inhibition of germination rate (IC_{50}, IC_{80}) and R^2 values for the dose-response curve from each target species are also included. The experiment was repeated three times with five to eight replications each.

	Weed species						Crop species					
Radicle length	Ribwort	Barnyard grass	Redroot pigweed	Common purslane	Wild oat	Field bindweed	Wheat	Rice	Corn	Barley	Lettuce	Soybean
0 μM	100	100	100	100	100	100	100	100	100	100	100	100
50 μM	98.2	**76.2*****	**90.1****	***118.1*****	***161.5******	99.9	***108.7****	***111.8******	107.9	99.9	**133.2*****	101.5
100 μM	**72.5***	**80.1***	**89.7****	***122.3******	***179.7******	110.1	107.1	***107.3*****	101.6	***116.3******	***129.9******	131.9
200 μM	**66.2****	**74.0*****	**75.7*****	***127.6******	***160.4******	102.9	98.4	100.2	**92.2****	***105.9****	***117.1******	***177.6******
400 μM	**43.0*****	**58.0*****	**59.9*****	96.8	***160.3******	85.4	**73.2*****	**88.1*****	**85.4*****	***108.8******	***102.4*****	***199.9******
800 μM	**29.0*****	**18.2*****	**53.4*****	**74.1*****	93.3	110.4	**18.4*****	**41.5*****	**94.6*****	**83.5*****	***108.1******	***181.0******
1.2 mM	**13.1*****	**2.3*****	**17.9*****	**62.0*****	**71.9*****	107.9	**10.1*****	**2.9*****	**83.2*****	**91.5****	96.2	124.2
IC_{50}	**307 μM**	**502 μM**	**581 μM**	**1.5 mM**	Low sensitive	Insensitive	**653 μM**	**707 μM**	Low sensitive	Insensitive	Insensitive	Insensitive
IC_{80}	**793 μM**	**885 μM**	**948 μM**	**> 2 mM**	Low sensitive	Insensitive	**981 μM**	**1.3mM**	Low sensitive	Insensitive	Insensitive	Insensitive
R^2	0.974	0.975	0.987	0.873			0.941	0.974				

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

was a species with "low sensitivity" to citral since the radicle length was never inhibited more than 30% and IC_{50} and IC_{80} values did not lie within the tested concentrations. Similarly, field bindweed (in which germination was also not affected) and wild oat showed low sensitivity and even resistance to citral in these bioassays (Table 2, insensitive species).

Contrary to the inhibitory values of weeds growth, crops showed a weak inhibition of radicle growth after citral treatment. Neither barley nor lettuce showed inhibition greater than 10% of the control, and the radicle length of corn was only inhibited by 18% at the highest concentration. Soybean was the species least inhibited by citral treatment. It showed not only tolerance to this compound but also a high rate of growth stimulation (by almost 100% at the 400 μM concentration). In addition, barley and lettuce also showed root growth stimulation at concentrations of between 50 and 800 μM of citral. Therefore, lettuce, soybean, barley, and corn were all considered to be citral-insensitive species. The exceptions were wheat and rice, which displayed highly significant inhibition of radicle growth as from citral concentrations of over 400 μM, although in wheat this inhibition was only found in the main root.

Phytotoxicity Experiments on Adult Plants. The phytotoxicity experiment was carried out over 21 d on thale cress plants. In the trial, two different methods of applying citral were tested: spraying and watering.

In general, plants watered with citral showed considerably reduced growth. This was homogeneous for all the plants undergoing the same treatment (Figure 1A). This reduction was especially drastic for plants watered with 1,200 μM citral, although the plants watered with the weakest concentration (300 μM) already showed a reduction in growth that was visible to the naked eye. A near-linear reduction in fresh biomass was observed in a citral-watered plug as the concentration of citral increased. There was also a significant reduction in the number of leaves starting from day 5 of citral-watering with concentrations of 600 and 1,200 μM (Figure 1B). Additionally, the plants watered with citral had 25% fewer stomata than the control (data not shown). Although citral-watered plants showed a 30% reduction in development and growth in comparison to the control, rosettes had a normal appearance, with no elongation of the petioles or apparent damage to the leaves such as chlorosis, necrosis, etc.

In the sprayed plants, plant responses were variable to the citral (Figure 1A). At all concentrations, some plants exhibited chlorosis and even necrosis, while some showed only a slight reduction in growth or no foliar damage. Moreover, the number of leaves showed no reduction until the last day of measurement (Figure 1B) and no differences in stoma density could be detected (data not shown).

The measurements of fresh and dry biomass of the treated plants showed a similar effect at all the concentrations studied, with high levels of inhibition, even at the lowest concentrations. Fresh and dry biomass was below 50% of the corresponding controls at all concentrations for both application methods tested. This reduction in dry biomass reached up to 90% in plants watered with 1,200 μM citral (Figure 2). However, the concentration of elemental nutrients in adult thale cress plants after 21 d of treatment was not affected (data not shown), except for calcium in citral-watered plants, which increased in direct proportion to the concentration of citral applied.

The citral application method affected the photochemical and nonphotochemical activity of the treated plants, especially F_v/F_m and ETR (Figure 3) and ϕ_{II}, ϕ_{NPQ}, ϕ_{NO} (Figure 4). Although an overall effect was observed in terms of the reduction of photosynthetic activity in watered as well as in sprayed thale cress plants (reduction in ϕ_{II}, reduction in ETR, increase in ϕ_{NO}, and reduction in q_L), the effect was earlier and more pronounced for plants sprayed than for plants watered with citral.

There was a significant reduction in the photochemical quenching, ϕ_{II}, between days 3 and 5 in citral-watered plants, which leveled out from day 11 (Figure 4). This reduction in the photochemical quenching was related to an increase in the values of ϕ_{NO} starting from day 7, which indicates an increase in nonregulated energy emission in the form of fluorescence. In turn, the ϕ_{NPQ} parameter was highly erratic, with increases and reductions that could be indicating the starting up of strategies of regulated emission in the form of heat that were ineffective, since, as mentioned above, an increase in ϕ_{NO} and a reduction in ϕ_{II} could be observed. The ETR values, however, only showed significant reductions as from day 15, when the plants had already been treated for 2 wk (Figure 3). On the other hand, the maximum PSII efficiency (F_v/F_m) was not affected for citral-watered plants (Figure 3). In this treatment, the chlorophyll *a* fluorescence emitted by plants

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A

B

Watering	Citral Concentrations (μM)		
	300	600	1200
1	82.8	86.2	72.4*
5	84.8	87.9	72.7*
9	86.1	83.3*	72.2*
13	81.4	79.1*	60.5***
17	97.9	85.1***	59.6***
21	103.7	79.6***	55.6***

Spraying	Citral Concentrations (μM)		
	300	600	1200
1	108	100	104
5	103.2	100	90.3
9	103.1	103.1	93.9
13	105.6	97.2	88.9
17	102.3	88.4*	76.8**
21	82.1**	69.6**	58.9***

Figure 1. (A) Pictures of thale cress adult plants after watering or spraying with citral. Control plants are shown at the left. (B) Values as percentage of the control and statistical significances of number of leaves per plant in thale cress plants treated with 300, 600, and 1,200 μM citral. Leaves were counted at 1, 5, 9, 13, 17, and 21 d after treatment. Asterisks indicate statistical differences compared to the control. * Pp < 0.05, ** Pp < 0.01, *** Pp < 0.001.

watered with 300 μM (the lowest concentration) was not affected by citral, and leaves showed values for photochemical and nonphotochemical rates of activity that were similar to those for the control.

For citral-sprayed plants, however, a rapid reduction in the ETR was observed, which was significant for all concentrations and was continuous for the highest concentration as from day 3 of the treatment (Figure 3). This reduction in the ETR was the earliest effect detected in these plants, since emission of fluorescence ϕ_{NO} increased as from day 5 of the treatment, and the photochemical quenching ϕ_{II} did not start to reduce until 9 d of spraying with citral. Once again, ϕ_{NPQ} displayed an erratic trend of stimulations and inhibitions (Figure 4). As well, the value of F_v/F_m in plants sprayed with the highest concentration of citral was altered in the last days of measurement, suggesting an effect on the PSII efficiency (Figure 3).

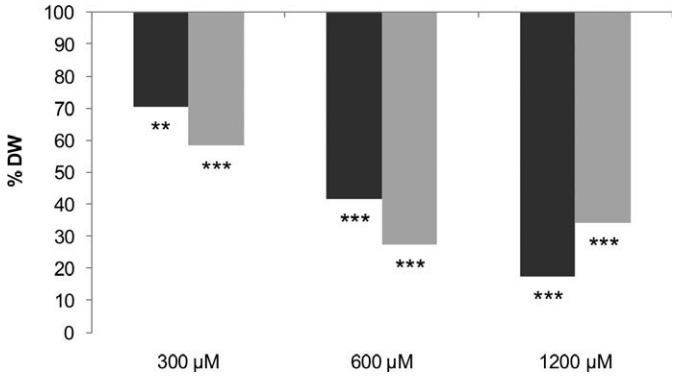

Figure 2. Dry weight (DW) in citral-watered or sprayed thale cress plants. Data are given in percentage of grams DW (g_{DW}) of the control. Asterisks represent statistical differences compared to the control. * Pp < 0.05, ** Pp < 0.01, *** Pp < 0.001.

Additionally, the differences observed in the fluorescence parameters between the two application methods were reflected in spatial differences in the fluorescence emitted by the treated plants. Thus, plants sprayed with citral showed greater fluorescence emission at the edges of the older leaves, while the vascular bundles and the youngest leaves in the rosette remained intact (Figure 5). In contrast, plants watered with citral showed almost no reduction, and the pattern for all the leaves in the rosette was very similar to the control. On the other hand, as mentioned above, the "citral-sensitive" plants in the spraying treatments showed many areas with considerable reduction in photochemical quenching, whereas "citral-resistant" plants kept almost all their rosette intact after spraying with citral.

The same pattern was obtained for both treatment methods, in the content of photosynthetic pigments (Figure 6). For the lowest concentrations (300 and 600 μM) there was an increase in pigment concentration (chlorophyll *a*, chlorophyll *b*, and carotenoids), which was more pronounced for chlorophyll *b*, whereas for the highest concentration (1,200 μM) a significant reduction was seen. This was more obvious for citral-watered than for citral-sprayed plants. By contrast, a highly significant

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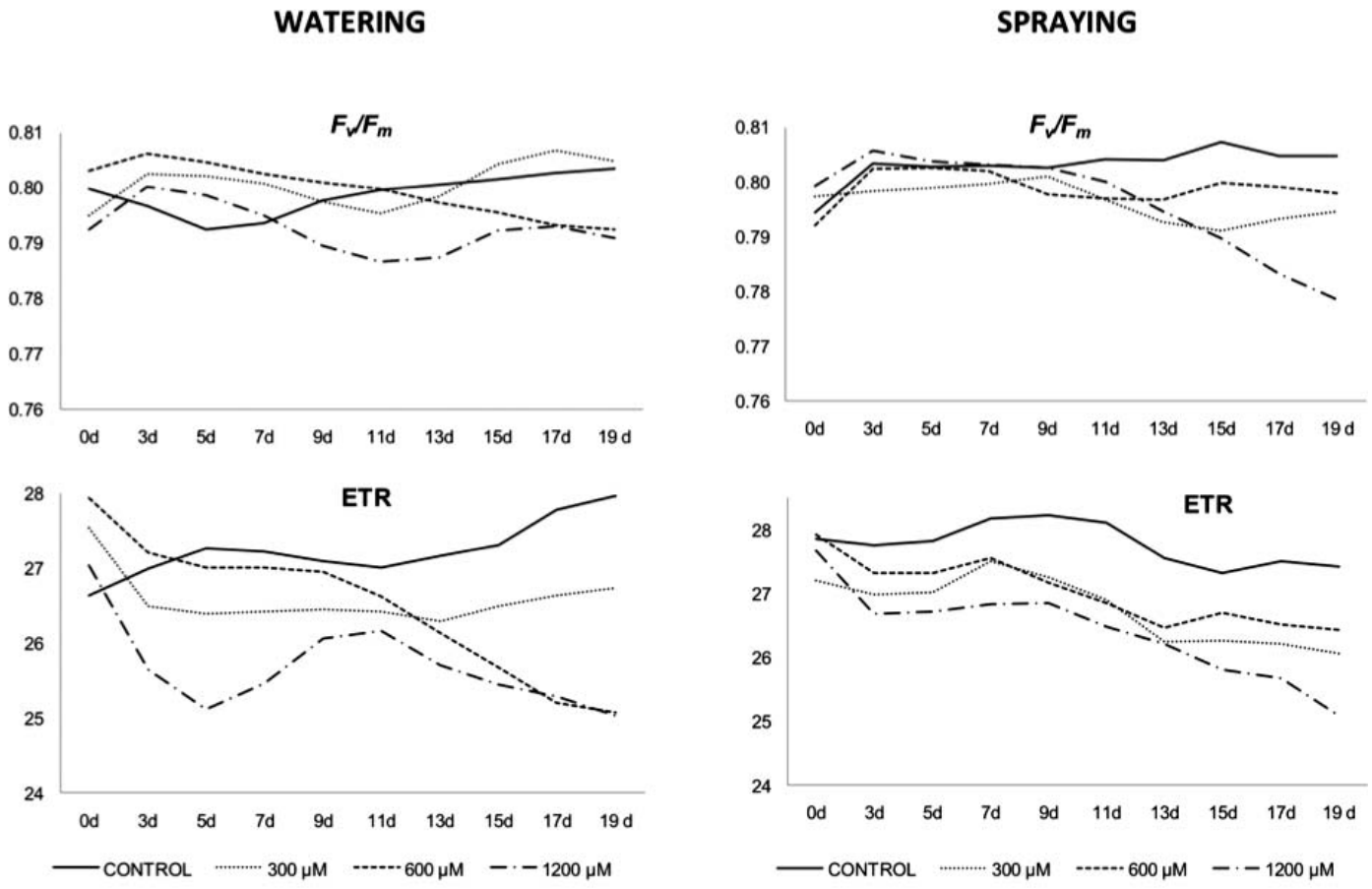

days	F_v/F_m - watering			ETR - watering		
	300	600	1200	300	600	1200
0						
3						
5	+++	+++	+++			--
7	++	++				-
9						
11						
13			-			
15				-	-	--
17					--	-
19			-		--	--

days	F_v/F_m - spraying			ETR - spraying		
	300	600	1200	300	600	1200
0						
3	+			--	-	--
5	-				-	--
7		+	+	-		-
9					-	--
11			-	--	-	--
13			--	--		--
15			--	-		-
17			--		--	---
19			---	--	-	---

Figure 3. Values of maximum quantum efficiency of dark-adapted photosystem II (PSII), maximum PSII efficiency (F_v/F_m), and apparent electron transport rate (ETR) in whole thale cress plants after watering or spraying with 300, 600, or 1,200 µM citral. Fifteen measures were obtained for each parameter at each measuring time in whole plants, and values integrated afterwards. The integral value of the area was obtained from for each parameter at every time. Tables show the statistical significance of positive (+) or negative (−) differences with respect to untreated plants: + or −, Pp < 0.05; ++ or − −, Pp < 0.01; +++ or − − −, Pp < 0.001.

reduction of total proteins was observed for the plants watered with the highest citral concentration, whereas plants sprayed with citral showed the complete opposite pattern, with a significant increase in protein content in plants treated with 1,200 µM (Figure 7A).

For measurements of lipid peroxidation and MDA content, a significant increase of 35% was observed in plants sprayed with any of the citral concentrations, whereas in citral-watered plants there was a very small increase (less than 10%) in lipid peroxidation in plants treated with concentrations of 600 and 1,200 µM (Figure 7B).

Finally, sprayed plants showed an increase in accumulated proline with values of over 300% in comparison with the control. Plants watered with citral also had higher values (190%) in comparison with the control for the concentration of 1,200 µM (Figure 7C).

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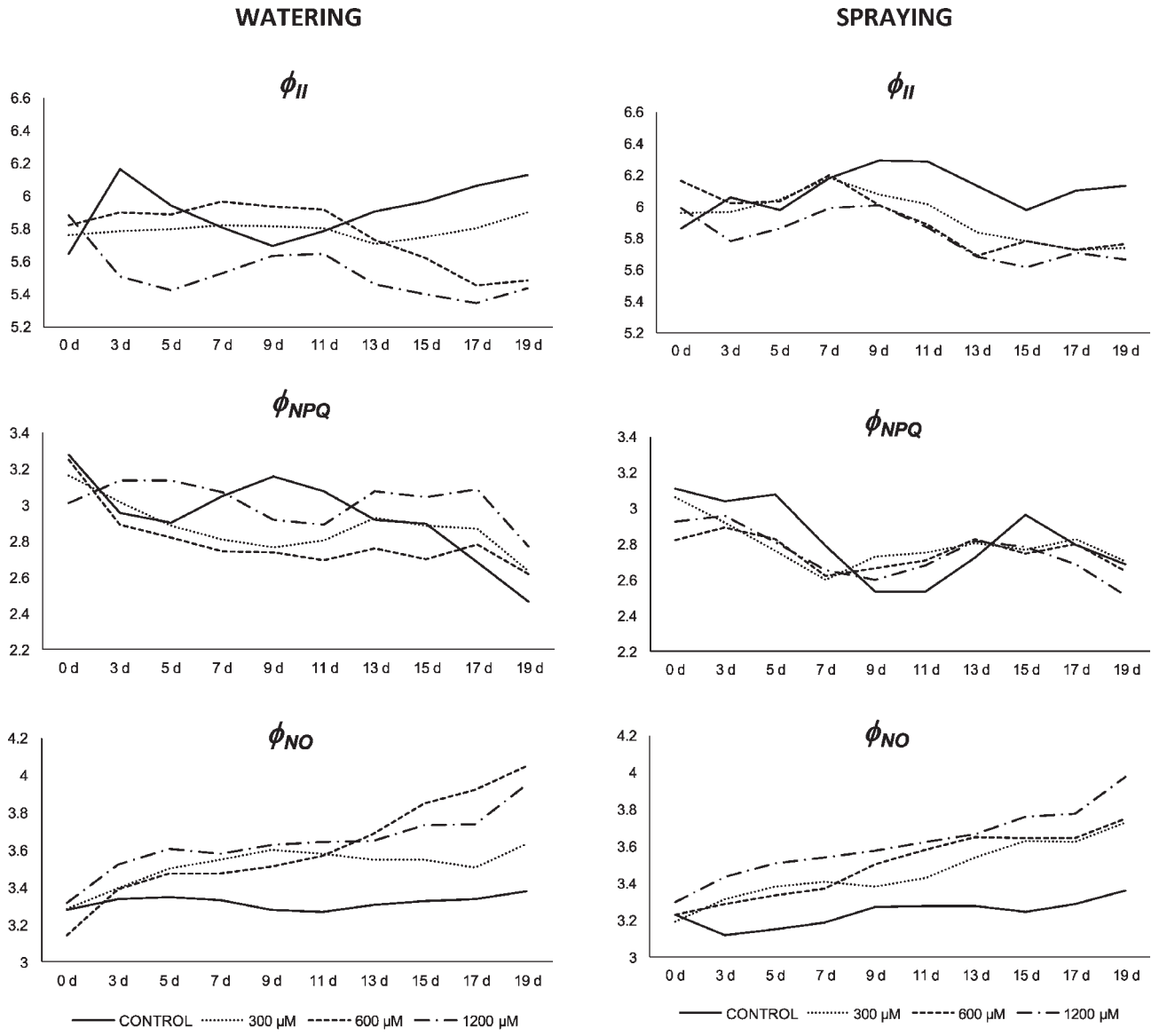

days	ϕ_{II} - watering			ϕ_{NPQ} - watering			ϕ_{NO} - watering		
	300	600	1200	300	600	1200	300	600	1200
0									
3			-						
5	-	-	−−	++	++				
7		+		−−	−−	-	+	+	+
9				−−	−−	−−			+
11	-	-	−			+		+	+
13			-	−−	−−			+	+
15		-	−	+	-	+++		+	+
17		−−	−					++	++
19		−−	−	++	+++	+++	+	++	++

days	ϕ_{II} - spraying			ϕ_{NPQ} - spraying			ϕ_{NO} - spraying		
	300	600	1200	300	600	1200	300	600	1200
0									
3			-				++		+
5	-	-	−−	++	++		+++	++	+++
7		+		−−	−−	-	++	+	+++
9				−−	−−	−−			++
11	-	-	−			+		+	++
13			-	−	−−		++	+	+++
15		-	−	+	-	+++	+	+	+
17		−	−			++	++	++	+++
19		−	−	++	+++	+++	++	++	+++

Figure 4. Values of the effective photochemical quantum yield of photosystem II (ϕ_{II}), the quantum yield of light-induced nonphotochemical quenching (ϕ_{NPQ}), and the quantum yield of all photosynthetically active photon fluxes other than ϕ_{NPQ} and ϕ_{II} (ϕ_{NO}) in whole thale cress plants after watering or spraying with 300, 600, or 1,200 µM citral. Fifteen measures were obtained for each parameter at each measuring time in whole plants, and values integrated afterwards. The integral value of the area was obtained from for each parameter at every time. Tables show the statistical significance of positive (+) or negative (−) differences with respect to untreated plants: + or −, Pp < 0.05; ++ or − −, Pp < 0.01; +++ or − − −, Pp < 0.001.

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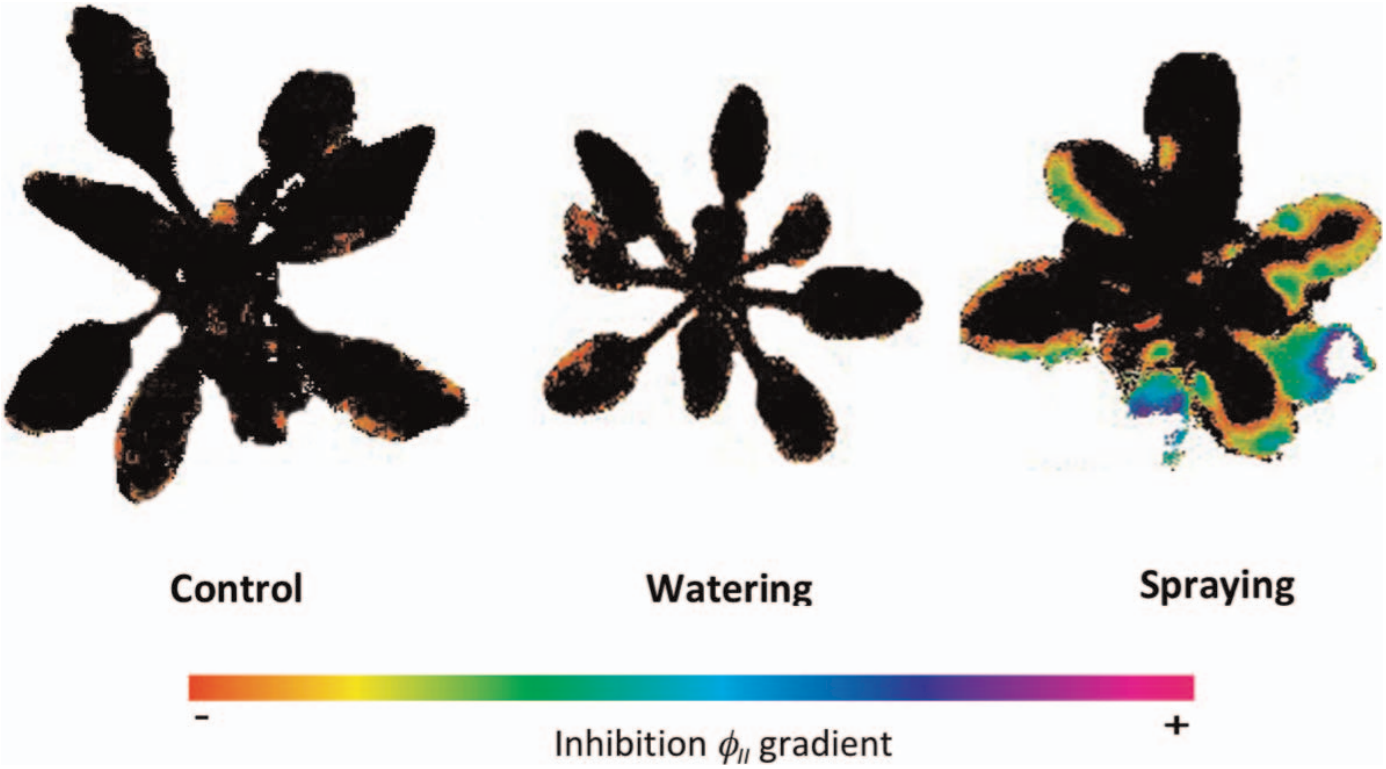

Figure 5. Images of fluorescence emission in thale cress plants after control, 1.2 mM citral-watering or 1.2 mM citral-spraying treatments. Maximum photosystem II efficiency (F_v/F_m) inhibition is represented in red for minimum values and in green for maximum values. Black color represents no inhibition of F_v/F_m compared to the control. (Color for this figure is available in the online version of this paper.)

Discussion

Germination and Growth. The germination and growth bioassays confirmed the phytotoxicity of the monoterpene citral on the seedlings' metabolism, which affected germination and radicle length in most of the tested species. Both G_T and the S after citral treatment depended on the target species.

The G_T index showed a similar pattern in most of the species tested, decreasing as citral concentration increased. However, this was not the case for wheat, soybean, and corn in the crop group and common purslane and field bindweed in the weed group, which were not affected. Moreover, the results of G_T did not always correlate with the data regarding S and AS, as each index provides different interpretations of the effects on seed germination, and no one index can precisely reflect all of them (Chiapusio et al. 1997).

Citral did not affect the G_T of wheat and corn, but affected S, which was accelerated in the case of corn and slightly slowed down for wheat. Actually in some species, such as ribwort and barley, a significant slowing down was observed already at the lower concentrations, whereas in other species S was not affected or else it was only affected at high concentrations. Moreover, in general, all species that showed inhibition of G_T also showed a retardation in germination. The delay of seed germination must be considered in two different ways: it can be an interesting mode of action of natural compounds, as delaying weed germination beyond the optimum will reduce early vigor and make weed invasion slower and less timely, which can make the crop more competitive (Gibson et al. 2002), but it should be considered also in crop management when crop germination is delayed with the treatment, even when the delay seems unimportant.

Germination and root length IC_{50} values reflect the different sensitivity of these two processes to citral action. In general, treatment with citral had a more drastic effect on radicle growth in weeds than in crops, since only wheat and rice could be considered citral-sensitive species. In the weed group, by contrast, only wild oat and field bindweed could be considered citral-insensitive species, since the other species tested showed high levels of root length inhibition. These results reveal the strong phytotoxic activity of citral on plant metabolism, and show the potential of this monoterpene on weed management with promising applications in the tested species. Although to extrapolate these data to field situations, one must consider that citral concentration may vary with factors such as half-life of the compound in the field, biotic and abiotic soil factors, or environmental conditions (Dayan and Duke 2009; Inderjit, 2001), citral shows phytotoxic potential against weeds and its use should be further studied in integrated weed management in the crop–weed systems of corn–barnyardgrass, corn–redroot pigweed, soybean–redroot pigweed, and soybean–barnyardgrass, or against weeds that are typical of citrus orchards, such as ribwort and barnyardgrass.

In particular, the corn–barnyardgrass system could considerably benefit, since not only could the germination of barnyardgrass be slowed down and that of corn accelerated after citral treatment, which would give the crop an advantage over the weed, but also, growth of the weed is considerably inhibited, which could lead to great difficulties in its establishment in a cultivated corn field. Considering that barnyardgrass is one of the most abundant weeds in corn fields (Bosnic and Swanton 1997), an appropriate management of this species, which favors its displacement, is of special interest

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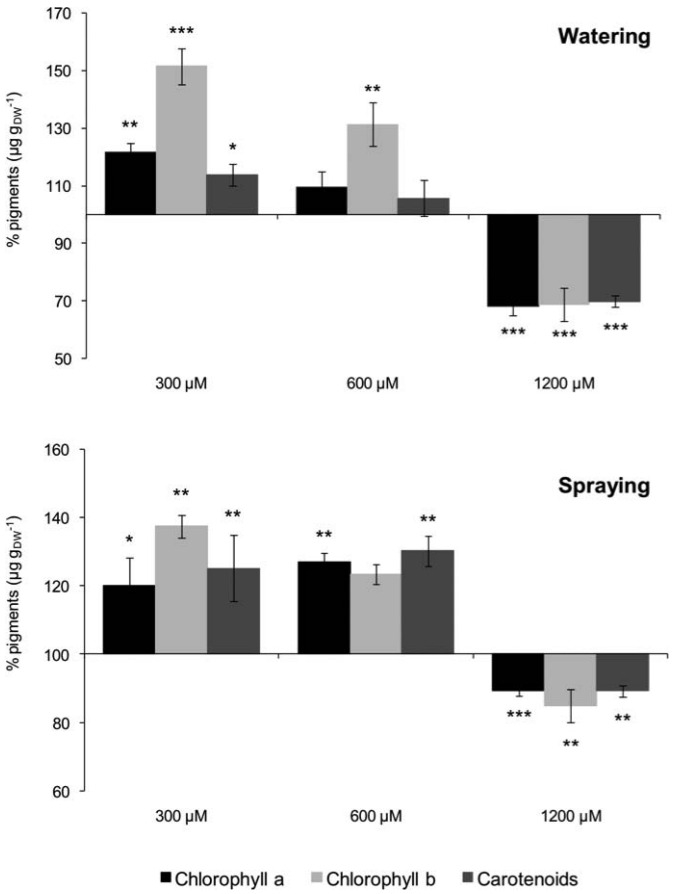

Figure 6. Chlorophyll *a*, chlorophyll *b*, and total carotenoid contents of adult thale cress leaves after 300, 600, or 1,200 µM citral watering or spraying, expressed as a percentage of the micrograms per gram dry weight (µg g_{DW}^{-1}) of untreated control plants after 21 d of treatment. Asterisks represent statistical significance with respect to controls: * $Pp < 0.05$, ** $Pp < 0.01$, *** $Pp < 0.001$.

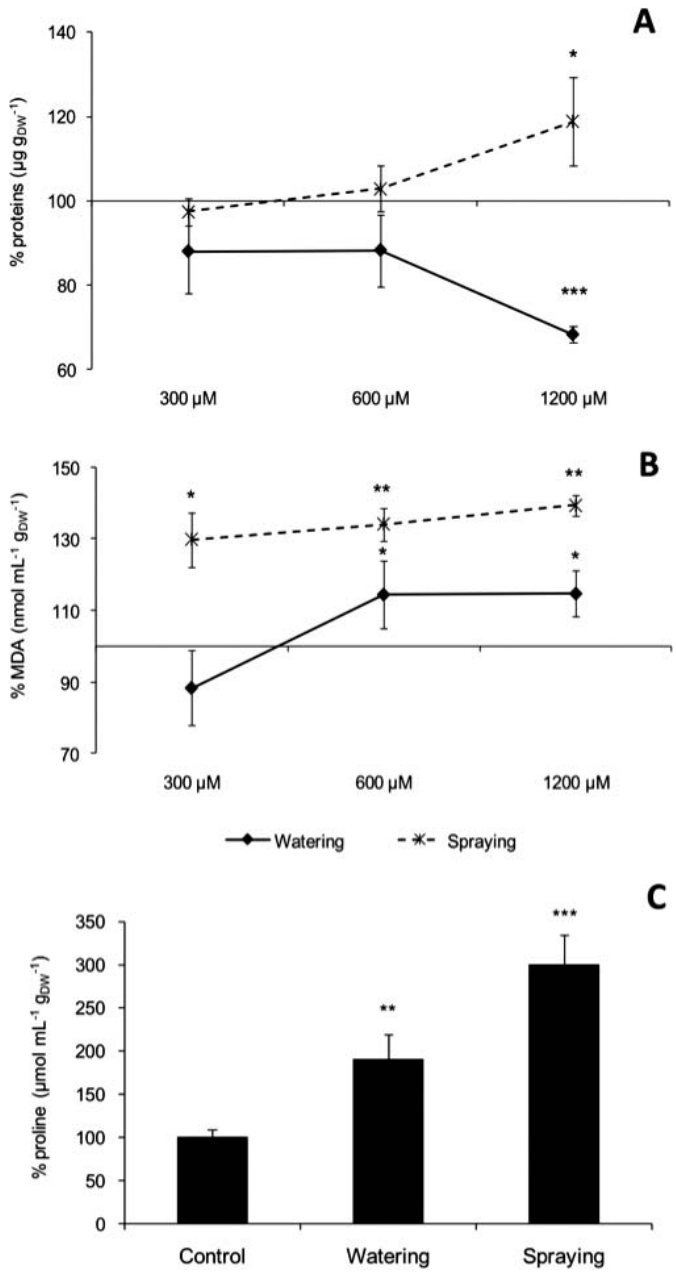

Figure 7. (A) Total protein content (µg$_{protein}$ g_{DW}^{-1}), (B) malonyldialdehyde content (nmol$_{MDA}$ $m.^{-1}$; dry weight basis), and (C) free proline content (µmol$_{PRO}$ ml^{-1}; dry weight basis) of leaves of thale cress watered or sprayed with 300, 600, or 1,200 µM citral after 21 d of treatment (percentages of untreated control plant values). Asterisks represent statistical significance of differences between treated and untreated plants: * $Pp < 0.05$, ** $Pp < 0.01$, *** $Pp < 0.001$.

for increasing the productivity of corn. As well, something similar can be stated about corn–redroot pigweed.

Another interesting system for study is the one of soybean and redroot pigweed or barnyardgrass. These two weed species are commonly found in cultivated fields of soybean, and interspecies competition between them may even occur. Losses in soybean productivity due to competition with redroot pigweed and barnyardgrass are over 50%; weed control would therefore be of great benefit from an agricultural point of view. Managing the crop–weed system with citral could be of special interest because soybean germination was not affected and growth was stimulated during the treatment, which could allow this crop (soybean) to compete better with the associated weeds redroot pigweed and barnyardgrass.

Other interesting aspects of the use of citral on weed control are the significantly low IC_{50} and IC_{80} values of this compound, which are below the values for other natural compounds that are today recognized as promising metabolites for controlling weeds (2(3*H*)benzoxazolinone, sorgoleone, vanillin, etc.); the low persistence of the compound in the environment, which would avoid the risk of contamination after citral treatment; and the dose needed for an effective inhibition, which is similar to other POST synthetic herbicides broadly used in the field, such as paraquat (recommended dose from 0.3 to 2.0 kg ha^{-1}), diuron (recommended dose from 0.5 to 10 kg ha^{-1}) or PCP (recommended dose from 2 to 15 kg ha^{-1}).

Phytotoxicity on Adult Plants. Clear morphological differences were observed between adult thale cress plants exposed to the two different application methods. Sprayed plants showed an effect that differed between plants, with some citral-resistant plants (good growth, no chlorosis or necrosis, and same number of leaves), some citral-sensitive plants (smaller in size, many necrotic and chlorotic areas, and fewer leaves), and even some dead plants.

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By contrast, watered plants showed a pronounced and homogenous reduction in the size of all rosettes, which growth was reduced in more than 80% of the control at the highest concentration. This reduction in growth, however, was not associated with petiole elongation or presence of foliar damage involving chlorosis or necrosis. Moreover, the values of fresh and dry biomass showed a trend of gradual and significant reduction for both treatments. Plants watered with citral were therefore smaller and with fewer leaves, which suggests a global effect on the plant metabolism.

A reduction in photochemical quenching, ϕ_{II}, followed by an increase in the emission of fluorescence (ϕ_{NO}) was detected in plants watered with citral, but no reduction in the ETR appeared until the last days of the treatment, which indicates that the biochemical phase of photosynthesis was not directly affected by the exogenous application of citral. The nonphotochemical quenching was erratic, with increases and decreases throughout the treatment time. This suggests that the plants were not able to support the stress, and definitive mechanisms for dissipating the regulated energy (in the form of heat) did not start up (Maxwell and Johnson 2000). Additionally, both the spatial distribution of fluorescence and the inhibition detected in ϕ_{II} were highly homogeneous throughout the whole plant, and no physical damage appeared in the treated plants. The data for ETR, ϕ_{II}, and F_v/F_m suggest that photosynthesis is being reduced as a result of a general slowing down of the metabolism.

Chlorophyll *a*, chlorophyll *b* and carotenoids increased in 300 and 600 µM citral-treated plants, whereas at the highest concentration (1,200 µM) there was a highly significant reduction in these photosynthetic pigments. Although the role of carotenoids as accessory pigments is important for the plant, they also play a fundamental role as free radical scavengers, i.e., in protecting against photoinhibition (Havaux and Kloppstech 2001). This increase in carotenoids may be preventing the photodegradation of chlorophyll *a*, which would explain the high levels of chlorophyll *a* and *b* measured in these plants. Moreover, there was an increase in the amount of MDA and free proline at all concentrations, and a highly significant reduction in the percentage of proteins in comparison with the control at the 1,200 µM concentration. This increase in MDA and carotenoids suggests that the plants may be experiencing oxidation damage due to citral treatment.

Some of the symptoms observed in citral-watered plants, such as the reduction of the total number of leaves per plant, the decreased total growth, the increase in proline content, and the change in the total chlorophyll and carotenoid contents were previously recorded in abscisic acid (ABA)-treated plants (Gomathinayagam et al. 2009). ABA is a sesquiterpene that plays an important role as a plant hormone in several physiological processes in plant growth and development, including not only cellular division but also elongation, germination, and the opening and closing of stomata in response to environmental stress (Finkelstein and Rock 2002). The signal received by the plant when a signaling cascade is induced, mediated by these hormones, may lead to regulating the stomatal density and opening in order to control transpiration and ensure that as little water is lost as possible. In fact, the reduction in the stomatal density (like that observed in our plants) confers greater efficiency of water use on the thale cress leaves than the control of the stomatal opening and closing (Yu et al. 2008; Zhang et al. 2008).

Considering the previously mentioned symptoms in citral-watered plants, that citral is a monoterpene, and that its chemical structure is very similar to ABA, this may be a case of an effect of citral on the metabolism of thale cress that is similar to that exerted by ABA, whether because citral could mimic the action of ABA, or because it could generate a water stress in the plant, which initiates the ABA's signaling cascade. Future measurements of the levels of ABA in plants watered with citral could help to better elucidate its mechanism of action.

Regarding the citral-sprayed plants, the most relevant effects on fluorescence monitoring were an early reduction in ETR, which caused the photochemical quenching, and a constant reduction in ϕ_{II} at all concentrations, which corresponded to an increase in nonregulated energy in the form of fluorescence ϕ_{NO}, suggesting not only that the plant is in a stress situation, but also that no controlled energy emission mechanisms are being developed (Kramer et al. 2004). Moreover, the maximum PSII efficiency (F_v/F_m) caused highly significant levels of inhibition during the last few days of measurements in plants sprayed with the highest concentration of citral, suggesting the start of photoinhibition processes and therefore of physical damage to the citral-sprayed plants (Demmig-Adams et al. 1995; Maxwell and Johnson 2000).

As already stated, there were morphological differences between plants in response to the spraying treatment: ''resistant'' and ''sensitive'' plants. As the images of fluorescence emission showed, the damage—solely present in the sensitive plants—had a heterogeneous spatial distribution, being principally detected on the edges of fully developed leaves, whereas the central parts of the leaves (the vascular bundles) remained intact. This fluorescence may therefore be suggesting the initiation of early senescence processes (Martínez-Peñalver et al. 2011). In senescence, in order to prevent cell death and allow efficient nutrient mobilization from senescing leaves to other parts of the plant, the photosynthesis apparatus is systematically dismantled. This requires differential metabolic, spatial, and temporal adjustments (Wingler et al. 2004), allowing nutrient mobilization from affected to unaffected leaves. One of the first physiological changes in the senescence process is the reduction in the synthesis of Rubisco (Feller et al. 2008) and a slowing down of the biochemical phase, which could explain the rapid reduction in the ETR in the citral-sprayed plants (Lim et al. 2003).

Regarding the pigment content, increases in chlorophyll *a*, chlorophyll *b*, and carotenoids at the lower citral concentrations, and decreases in these pigments at the highest concentration were observed in citral-sprayed plants, which would support the reduction in ϕ_{II}, ETR, and F_v/F_m in plants sprayed with the highest citral concentration. The chlorotic areas in plants, which were sensitive to citral spraying, could be responsible for this overall reduction in pigments. In fact, the whitening of leaves with the loss of pigments, and specifically the chlorophylls, is one of the most characteristic symptoms of the programmed cell death, which takes place during induced senescence (Noodén et al. 1997). The detected increase in MDA content (lipid peroxidation) could be caused by an imbalance in the plant's oxidant status, since senescence in leaves can be also the consequence of a gradual deterioration of the plasma membrane due to increased lipid peroxidation resulting from reduced antioxidant enzymatic

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activities (Dhindsa et al. 1981; Van Acker et al. 2000). This oxidation damage was in turn corroborated by an increase in the percentage of proline, an "osmolyte habitually used by the plant" in response to different types of stress (Aspinall and Paleg 1981). Prior studies have suggested that the accumulation of proline might compensate for deficiencies in the water potential while ensuring the homoeostasis required for metabolic functions to continue (Hare and Cress 1997), stabilize subcellular structures, and eliminate free radicals as a result of the formation of long-lived chemical compounds (Hare and Cress 1997). This increase in proline could therefore be related to the damage caused to the membrane and the change in the membrane's permeability resulting from the increase in lipid peroxidation.

The data for ETR, ϕ_{II}, F_v/F_m, pigments, proline, and lipid peroxidation, together with the images of fluorescence, suggest contact damage and an associated process of senescence in plants sprayed with citral. Confirmation in forthcoming studies of the presence of oxidation damage and other parameters that are directly related to early senescence will permit validation of the hypothesis suggested here.

This work demonstrates that citral is effective not only on seedling metabolism but also on adult plants by inhibiting growth and development. Additional studies should be undertaken to understand the changes related to oxidative stress. Moreover, clear morphological differences were observed between adult thale cress plants exposed to citral spraying or watering, suggesting that foliar or root absorption can change and determine the effectiveness of this compound.

Acknowledgments

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