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Volatility of clomazone formulations under field conditions[1]

Volatilidade de formulações de clomazone em condições de campo

Fabio Schreiber[2]; Luis Antonio de Avila[3]; Ananda Scherner[4]; Diogo da Silva Moura[5]; Alfran Tellechea Martini[6]

Abstract - Volatilization plays an important role in the process of herbicide dispersion in the environment. The physicochemical characteristics of the clomazone molecule indicate its volatility potential. The present study aimed to evaluate the volatilization rate of three clomazone formulations under field conditions using indicator plants and wind tunnels. Transparent polyethylene tunnels were placed on a sorghum (*Sorghum bicolor*) field parallel to the prevalent wind direction, and the herbicide formulations were applied in plastic trays containing 10 kg of sieved soil and placed at the center of the tunnel. The experiment was arranged in a slip-plot design with four replications. The main plots were represented by different formulations of the herbicides: Gamit 360 CS®, Gamit 500 EC® and Gamit Star®, and control plots without herbicide application. The sub-plots represented the distances from the application site: 1, 2, 3, 4, 5, 6 meters. Herbicide injury in sorghum was then assessed at different days after application. The symptoms of all formulations were more intense in the plants that were closer to the application site, and were even more severe in the prevailing wind direction. In general, regardless of the application distance, Gamit 360 CS® caused less injury to the sorghum. It is concluded that among the formulations assessed, Gamit 360 CS® has a lower volatilization rate and, consequently, lower contamination risk of neighboring crops and the environment. The other two formulations have higher volatility potential.
Keywords: bioindicator; phytotoxicity; Gamit®; wind tunnels

Resumo - A volatilização representa um processo importante no deslocamento de agrotóxicos para o ambiente. As características físico-químicas da molécula do clomazone indicam potencial de volatilidade. O presente estudo tem o objetivo de avaliar a volatilização de três formulações de clomazone em condições de campo por meio de plantas bioindicadoras com o uso de tuneis de vento. Túneis de polietileno transparente foram alocados sobre a cultura do sorgo (*Sorghum bicolor*), onde as formulações do herbicida foram aplicadas no centro destes. O experimento foi arranjado em parcelas subdivididas, com quatro repetições. As parcelas foram representadas pelas diferentes formulações do herbicida: Gamit 360 CS®, Gamit 500 EC® e Gamit Star®, além da testemunha sem aplicação de herbicida. As subparcelas foram caracterizadas pelas distâncias em

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[2] Pós Doutorando em Fitossanidade, Departamento de Fitossanidade da UFPel, Pelotas, Rio Grande do Sul, Brasil. E-mail: <schreiberbr@gmail.com>.
[3] Professor na Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brasil. E-mail: <laavilabr@gmail.com>.
[4] Doutoranda em Fitossanidade, Departamento de Agroecologia, Slagelse, Dinamarca. E-mail: <ananda.scherner@agro.au.dk>.
[5] Doutorando em Fisiologia Vegetal, Departamento de Botânica, Universidade Federal de Pelotas (UFPel), Pelotas, Rio Grande do Sul, Brasil. E-mail: <diogodasilvamoura@yahoo.com.br>.
[6] Doutorando em Engenharia Agrícola da Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brasil. E-mail: <alfrantm@gmail.com>.

relação ao local de aplicação: 1, 2, 3, 4, 5, 6 metros a favor e contra a direção predominante do vento. Foi então realizada avaliação de toxicidade em diferentes dias nas plantas de sorgo após a aplicação dos herbicidas. Os sintomas de todas as formulações foram mais intensos nas plantas próximas do local da aplicação, sendo esses ainda mais acentuados ao longo da linha onde a direção do vento foi predominante. No geral, independente da distância, o Gamit 360 CS® causou menor toxicidade a bioindicadora. Conclui-se que dentre as formulações avaliadas, Gamit 360 CS® apresenta menor volatilização e consequentemente menor é o risco de contaminações de culturas vizinhas e do ambiente. As outras duas formulações possuem maior potencial de volatilização.
Palavras-chaves: bioindicador; fitotoxicidade; Gamit®; túneis de vento

Introduction

Clomazone is a selective herbicide that belongs to the isoxazolidinone chemical group. It acts directly in the photosynthesis process, inhibiting the deoxyxylulose phosphate synthase enzyme, responsible for the synthesis of isoprenoids, basic precursors of carotenoids (Ferhatoglu and Barret, 2006), which have the role of protecting chlorophyll from photo-oxidation. Therefore, a typical symptom of this chemical group is whitening of the leaves (Senseman, 2007).

In Brazil, this herbicide has been commercialized under the trade names Gamit Star® and Gamit 500 EC® (800 and 500 g a.i. L^{-1}, respectively), both emulsifiable concentrated formulations. Today it is also marketed as Gamit 360 CS® (360 g a.i. L^{-1}) and formulated as a microencapsulated suspension. The microcapsules are particles, which contains the active ingredient in its inner core, protected by an outer layer of polymer of variable thickness (Memarizadeh et al., 2014). The microencapsulation of the herbicide ensures a more effective application of the product, minimizing losses by degradation, evaporation or dissolution and consequent runoff to water bodies, thus preventing environmental contamination (Nair et al., 2010).

Clomazone is considered moderately persistent in soil with half-life in aerobic and anaerobic conditions of 47.3 and 7.9 days (Tomco et al., 2010), respectively. In addition, some physicochemical properties of its molecule suggest that the herbicide can volatilize and be transported in the environment in the vapor phase. Examples of these properties include the vapor pressure (1.44×10^{-4} mm Hg at 25 ºC) and Henry's law constant (4.15×10^{8} atm m^3 mol^{-1}) (Senseman, 2007).

Schummer et al. (2010) detected clomazone in all samples collected from the atmosphere, which was predominantly in the gas form (83.4 %). Once in the atmosphere, the herbicide can be the transported over long distances (Pozo et al., 2009), and its subsequent deposition is unavoidable (Gavrilescu, 2005), resulting in environmental contamination and non-target crops injury. Other concern is related to human inhalation of this compound, which may cause adverse effects on health. Therefore, farmers and other people living in the surroundings of treated crops with this herbicide are constantly exposed to these risks (Ngoc et al., 2015).

Several volatilization chambers and systems have already been proposed by researchers, such as, the micrometeorological system (Majewski 1999), semi-fields (Van den Berg et al., 1999), and the wind tunnels (Bedos et al., 2002), with the last being the most common option. Wind tunnels or volatilization chamber have been widely used in studies evaluating herbicide drift and volatilization because it is very practical. This system produces a uniform airflow that aims to determine the movement of molecules through the air under controlled conditions of temperature and humidity. However, these tunnels are closed systems and have numerous disadvantages, for example, the establishment of microclimates and climate changes in their interior. Such changes include solar radiation, temperature, humidity, ventilation and condensation, which influence the transport of herbicides, compared to natural conditions (Shigaky and Dell, 2015). Consequently, the volatilization rate collected may not represent the magnitude of losses. Furthermore, the construction and maintenance of these tunnels are too costly and require specific spaces to house them (Moreira and Antuniassi, 2010).

An effective and low-cost method (Nunes and Vidal, 2009) of assessing the risks posed by pollutants present in ecosystems and detecting chronic or acute levels of air contamination is via bioindicator organisms. These animal or vegetable organisms have the potential to undergo alterations in their vital functions or chemical composition (Lam and Gray, 2003), indicating the presence of a given contaminant. Clomazone in the vapor phase has already been detected by bioindicator plants, as reported by Schreiber et al. 2013, and sorghum (*Sorghum bicolor*) is the most appropriate species.

However, little information on the behavior of clomazone in the vapor phase is found in the literature under field conditions, mainly due to the lack of reliable and economically viable experimental methods. For this reason, this study had the objective of assess the volatilization of three clomazone formulations under field conditions using indicator plants and wind tunnels.

Material and Methods

The experiment was conducted under field conditions from November to January in the 2011/2012 growing season and repeated in 2012/2013. It was arranged in a split-plot design with four replicates. The main plots were represented by different formulations of the clomazone herbicide: Gamit 360 CS® (360 L^{-1} g a.i., CS, FMC), Gamit 500 EC® (500 L^{-1} g a.i., EC, FMC) and Gamit Star® (800 L^{-1} g i.a., EC, FMC), and control plots without herbicide application. The sub-plots consisted of the distances from the application site: 1, 2, 3, 4, 5, 6 meters with or against the prevailing wind direction.

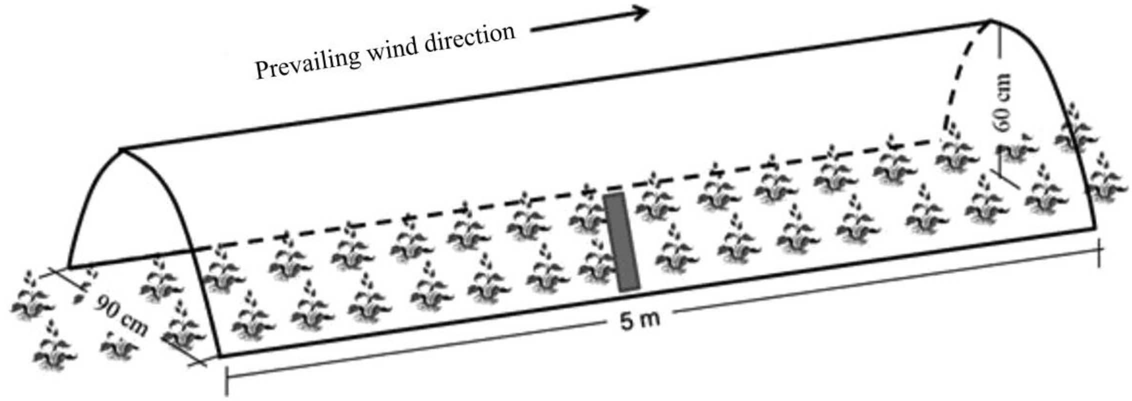
Wind tunnels were built with a 150-micron transparent polyethylene covering two rows of a sorghum crop. The laterals of the tunnels were fixed on the ground, with open ends, and the sides were in contact with the ground, not allowing lateral losses of the herbicide, and measured 0.90 m, 0.60 m and 5 m in width, height and length, respectively. The tunnels were assembled parallel to the prevailing wind direction, and the openings at both ends allowed the passage of the wind inside the structure (Figure 1A).

Climate conditions were monitored throughout the experiment period, using a Kestrel 4000® and Icel® thermo-hygro-anemometers. The device was placed inside the tunnel to measured and stored the air temperature, air relative humidity, wind speed and direction, every 20 seconds.

Sorghum (cultivar BRS 308) seeds were sown in an area of 1,800 m² (30 x 60 m) at rows spaced 0.45 m. Base fertilization was applied right before sowing, using 350 kg ha^{-1} of the 5-20-20 (N-P-K) formulation. When the plants were at the V_2-V_3 development stage, 16 wind tunnels (described previously) made of polyethylene were installed over the area, with spacing between each other to avoid influence between treatments. Each tunnel had only one of the herbicide formulations.

At the center of each tunnel it was placed a plastic tray filled with approximately 10 kg of sieved soil. The soil used in the trays had no previous herbicides application in the last five years, and was collected from the A horizon of an Albaqualf soil at the same site of the experiment. The soil characteristics were as follows: pH_{water} (1:1) = 5.1; CEC at pH7 = 5.4 $cmol_c$ dm^{-3}; Organic matter = 1.2 %; clay = 15 %; texture = 4 (sandy); Ca = 1.8 $cmol_c$ dm^{-3}; Mg = 1 $cmol_c$ dm^{-3}; Exchangeable Al = 0.2 $cmol_c$ dm^{-3}; available P = 4.3 mg dm^{-3}; exchangeable K = 30 mg dm^{-3}.

A.



B.

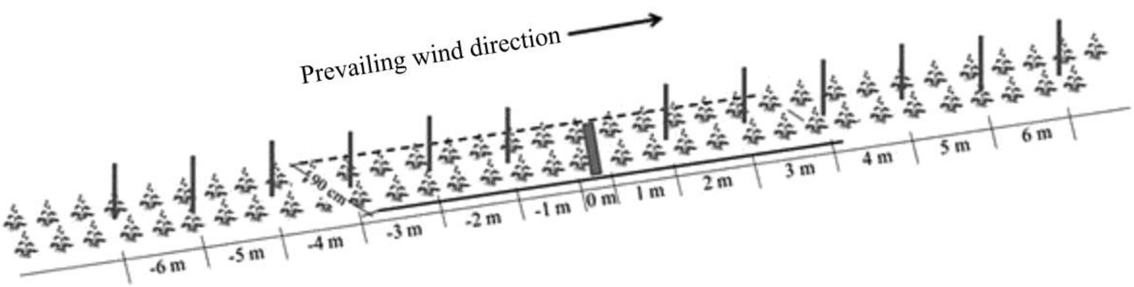


Figure 1. Schematic plot arrangement, showing the tray allocation containing soil treated with herbicide (represented by the gray rectangle) (A) and stakes placed one meter apart from each other, from the herbicide application point (B).

After installing the tunnels and the trays inside them, the different herbicides formulations were applied uniformly on the soil surface using graduated pipettes. The dose applied was of 960 g a.i. ha^{-1}, i.e. in the treatment with Gamit 360 CS®, Gamit 500 EC® and Gamit Star® the doses corresponded to 2.7 L ha^{-1}, 1.92 L ha^{-1} and 1.2 L ha^{-1}, respectively.

The herbicide treatments were applied using a CO_2-pressurized backpack sprayer equipped with fan tips nozzles (XR 110.015), regulated at a pressure of 181 kPa and calibrated to deliver 150 L ha^{-1} of the spray solution. At the time of application, the air temperature was 13°C and 15°C during the 2011/2012 and 2012/2013 growing seasons, respectively. The wind speed, at both growing seasons, was an average of 1.5 km h^{-1}. Soil moisture was maintained at 10 Pka.

The use of trays had the purpose of preventing the absorption of the herbicide by the plants root system; it was only intended to assess the volatilization effect. The experimental site was also marked every 1 m from the point of the herbicide application. The positive numbers were arranged with the prevailing wind direction, and the negative ones in the opposite wind direction (Figure 1B) to evaluate the gradual injury caused by the herbicide along the culture rows.

It was accessed plant injury (phytotoxicity) by the herbicide, which was measured on the sorghum plants at 2, 5, 10, 14, 20 and 24 days after application of the herbicide (DAH) on the soil, using a percent scale from zero to 100, where zero represented the absence of symptoms (without whitening effect) and 100 the plants death.

Data were subjected to analysis of variance ($P \leq 0.05$). Mean were compared using the confidence interval at 95%.

Results and Discussion

There was an interaction (Table 1) between the formulations and the distances from the point of application relating to the injury effect at 2, 5, 10, 14, 20 and 24 DAH in both experimental periods (replicates).

Table 1. Summary of the analysis of variance for injury in sorghum plants (at 2, 5, 10, 14, 20 and 24 days after application of the herbicides) in the 2011/2012 and 2012/2013 growing seasons.

Model	DOF[1]	2		5		10	
2011/2012		MQ[2]	F	MQ	F	MQ	F
Formulation (A)	3	579	29.0*	3559	45.2*	1842	28.0*
Residues (a)	12	20.0	-	78.7	-	65.8	-
Distance (B)	11	410	65.6*	2478	124*	2035	153*
A x B	33	78.6	12.6*	380	19.1*	282	21.3*
Residues (b)	132	6.26	-	19.8	-	13.3	-
Total	191	-	-	-	-	-	-
Model	**DOF**	**14**		**20**		**24**	
2011/2012		MQ	F	MQ	F	MQ	F
Formulation (A)	3	688	43.1*	248	16.6*	53.1	4.90*
Residues (a)	12	15.9	-	15.0	-	10.8	-
Distance (B)	11	871	176*	366	73.3*	88.2	22.6*
A x B	33	132	26.7*	64.0	12.8*	22.0	5.65*
Residues (b)	132	4.93	-	5.00	-	3.89	-
Total	191	-	-	-	-	-	-
Model	**DOF**	**2**		**5**		**10**	
2012/2013		MQ	F	MQ	F	MQ	F
Formulation (A)	3	387	22.0*	2003	13.4*	5635	109*
Residues (a)	12	17.5	-	149	-	51.7	-
Distance (B)	11	242	53.8*	1491	107*	2786	493*
A x B	33	65.1	14.4*	221	15.9*	452	80.0*
Residues (b)	132	4.50	-	13.9	-	5.64	-
Total	191	-	-	-	-	-	-
Model	**DOF**	**14**		**20**		**24**	
2012/2013		MQ	F	MQ	F	MQ	F
Formulation (A)	3	743	162*	179	7.98*	31.3	1.70*
Residues (a)	12	4.57	-	22.4	-	18.4	-
Distance (B)	11	623	300*	335	42.4*	44.4	8.95*
A x B	33	107	51.9*	54.3	6.87*	9.45	1.91*
Residues (b)	132	2.07	-	7.91	-	4.95	-
Total	191	-	-	-	-	-	-

[1]Degrees of freedom; [2]Mean squares; *significant at 5% probability level by F-test

Symptoms of injury were also observed two DAH for all formulations. The injury level caused by Gamit 500 EC® and Gamit Star® in general did not indicate a significant difference between each other. The injury effect caused by Gamit 360 CS® was lower compared to the other formulations (Figures 2 and 3), mainly in the plants grown near the herbicide application site (1 m away). These results corroborate those found by Schreiber et al., (2015), who evaluated volatilization of three clomazone formulations in a greenhouse and observed that Gamit 360 CS® caused less injury to the indicator plant species.

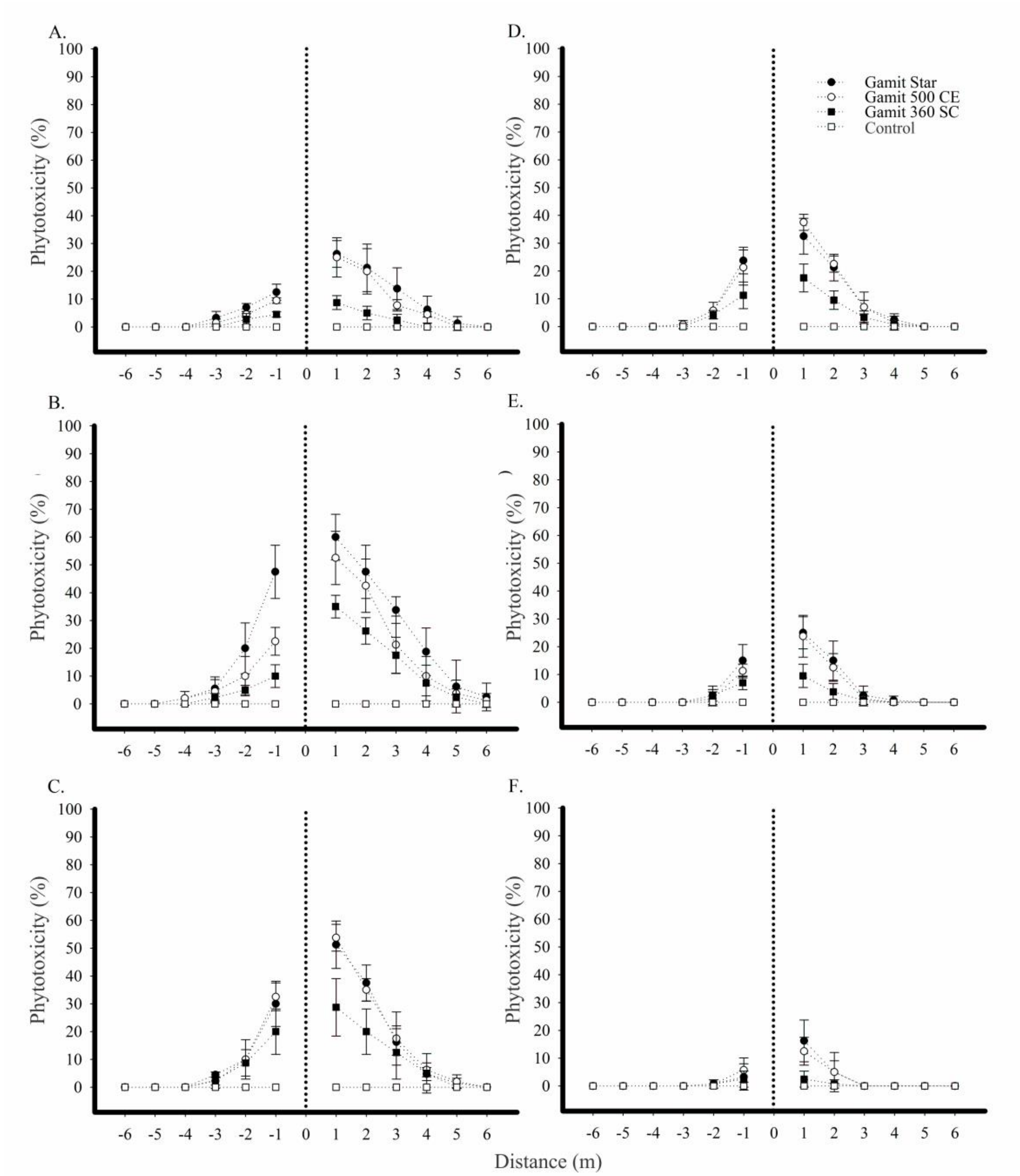


Figure 2. Phytotoxicity in sorghum plants during the 2011/2012 growing season at 2 (A), 5 (B) and 10 (C) 14 (D), 20 (E) and 24 (F) days after application at distances (m) from the point of application (0), of three clomazone formulations: Gamit 360 CS®, Gamit 500 EC® and Gamit Star®. Positive values represent the direction of the prevailing wind and the negative values the opposite direction. The dots represent the mean values of the replications, and the bars the respective mean confidence intervals at 95% probability level.

In other study conducted by Keifer et al. (2007), also in controlled conditions, it was determined that volatilization of microencapsulated clomazone formulations was 16% lower than the total volatilization of an emulsified concentrated formulation. Thus, the lower toxicity caused by Gamit 360 CS® to sorghum may be associated with the fact that this product is microencapsulated, releasing the herbicide slowly, in a controlled and continuous way (Campos et al., 2014), thus reducing its volatilization.

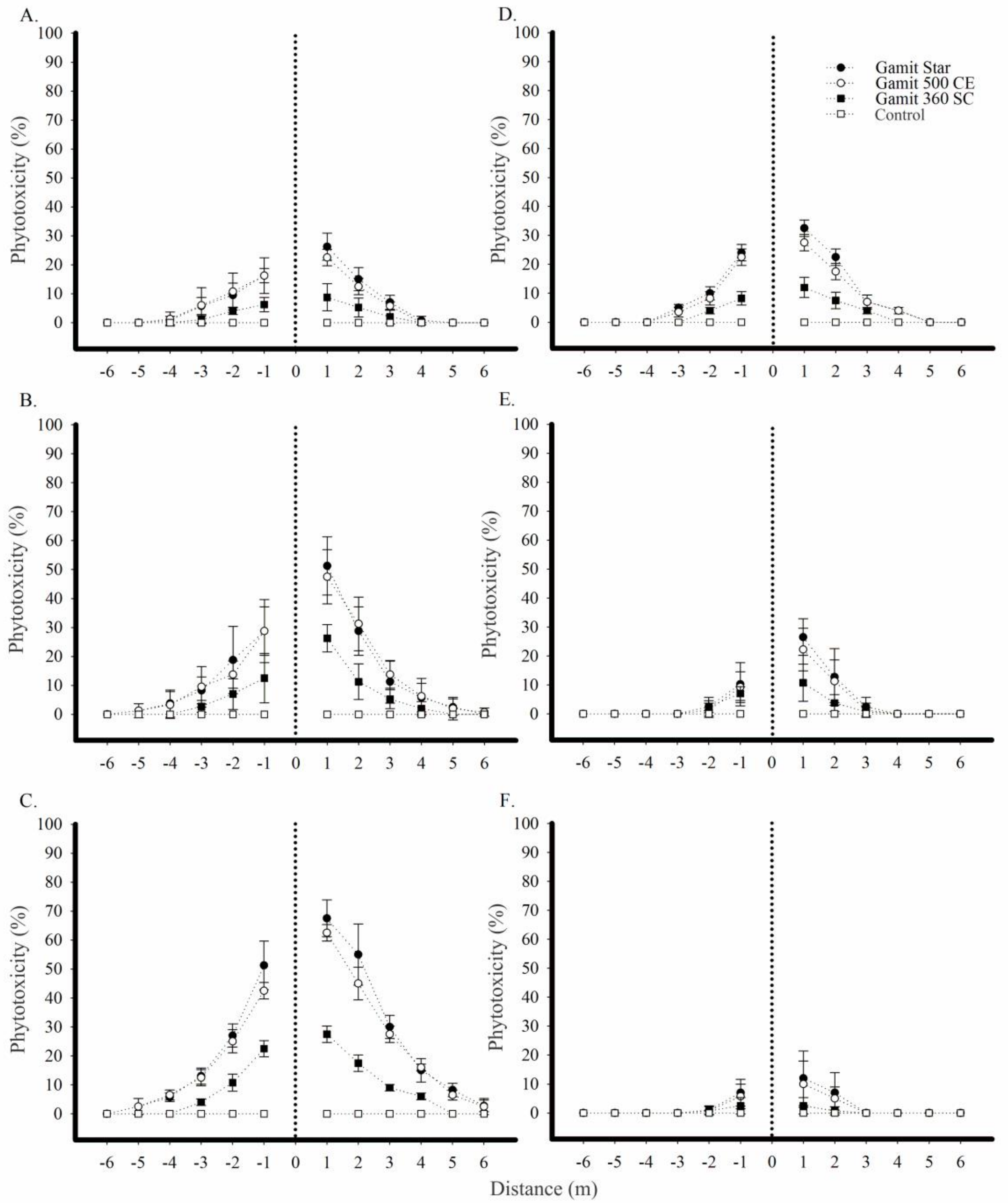


Figure 3. Phytotoxicity in sorghum plants during the 2012/2013 growing season at 2 (A), 5 (B) and 10 (C), 14 (D), 20 (E) and 24 (F) days after application at the respective distances (m) from the application (0) of three clomazone formulations: Gamit 360 CS®, Gamit 500 EC® and Gamit Star®. Positive values represent the direction of the prevailing wind, and the negative values the opposite direction. The dots represent the mean values of the replications, and the bars the respective mean confidence intervals at 95% probability.

At 24 DAH, the sorghum plants exhibited low herbicide injury symptoms (Figure 2F and 3F). The maximum level of sorghum toxicity was observed at 10 DAH in the 2012/2013 growing season, one meter from the trays and in the same direction of the prevailing wind, being 70% for the Gamit Star® formulation, 60% for Gamit 500 EC® and 30% for Gamit 360 CS® (Figure 3C). However, after this period, the plants continued growing and developing, with new leaves growing without apparent symptoms. Schreiber et al. (2015) found, in controlled environment, practically the same levels of toxicity of this study at 10 DAH, indicating similarity with the field results. However, at 24 DAH, the same authors reported high levels of injury, without recovery of the plants, possibly because it was a closed environment, not enabling the herbicide dissipation in the environment, a situation not found in the field conditions.

The plants closer to the herbicide treated tray showed the highest injury level. More intense symptoms were observed in plants that grown at a distance of up to one meter from the point of application and in the same direction of the prevailing wind (Figures 2 and 3). These injury levels decreased gradually with the increase of distance to the applied area.

At 5 DAH, symptoms were present in the plants that were farther away from the point of application, i.e. six meters (6) with the wind and five meters (-5) against the wind (Figure 2B and 3B), and at these distances toxicity was below 5%. The tunnels were five meters long, so the effect of the herbicide exceeded their limit. However, outside the tunnels, the herbicide was more vulnerable to dilution in the environment, which may have hindered more severe effects in farther locations.

Many mathematical models use air temperature and wind speed to predict the volatilization rate of chemical compounds, such as those proposed by Lichiheb et al. (2013) and Personne et al. (2009). In these models, the rate of compounds volatilization increases significantly as these variables increase. Daily mean air temperature during the experimental period varied from 17 and 31°C. The 2012/2013 growing season was slightly warmer than 2011/2012 (Figure 4A). Daily mean wind speed ranged from 0.2 to 14 km h^{-1}, and this was higher in the 2011/2012 growing season than in 2012/2013 (Figure 4B).

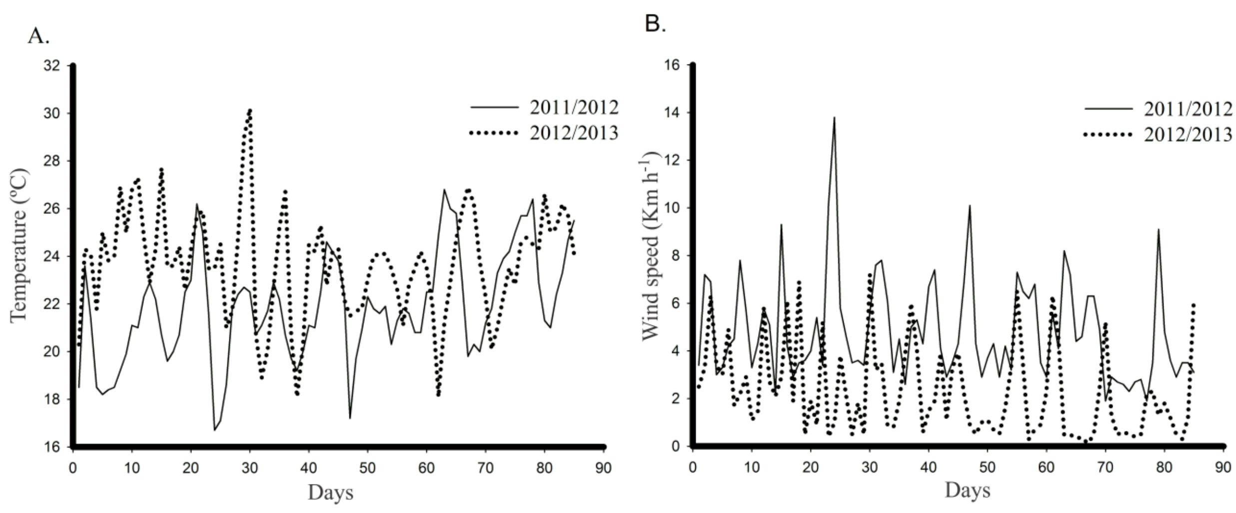


Figure 4. Daily mean air temperature (°C) (A) and daily mean wind speed (km h^{-1}) (B) during the experimental periods (2011/2012 and 2012/2013 growing seasons), after sowing sorghum, collected by thermo-hygro-anemometers.

In general, daily mean temperature and wind speed did not cause an apparent difference in the toxicity levels between the two growing seasons. However, it was clear that the wind had influence in the transportation of the volatilized herbicide as the most severe injury occurred with the prevailing wind. Obviously, the wind did not always blow in the same direction but oscillated during the experimental period, and for this reason injury occurred at both directions of the application point.

According to Mahugija et al. (2015), the wind direction and speed affect the distribution of herbicides into the atmosphere. The wind can carry these toxic molecules over long distances and then blow them back onto the ground or plants. This could explain the gradual toxicity symptoms along the sorghum crop lines, which diminished as the distance from the application sites increased, because while such molecules are carried by the wind, part of them is deposited and intercepted, inclusive by vegetation. Thus, the concentration of molecules available in the air was smaller as the distance increased and, consequently, the deposition of such molecules on the sorghum plants was smaller and toxicity levels were lower.

In general, the use of wind tunnel was efficient in the present clomazone volatilization evaluation. Although the experiment was conducted under field conditions, where climate conditions vary, the data obtained corroborate those found by Schreiber et al. (2015) under controlled conditions and it was consistent over the two growing season.

Conclusions

The Gamit 500 EC® and Gamit Star® formulations exhibited a similar volatilization potential.Among the formulations assessed, Gamit 360 CS® has a lower volatilization rate and, consequently, lower contamination risk of neighboring crops and the environment. The Gamit 500 EC® and Gamit Star® formulations have higher volatility potential.

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