PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Dynamics of Clomazone Formulations Under Different Applications Conditions

Dinâmica de Formulações de Clomazone em Diferentes Condições de Aplicação

TROPALDI, L.[1]*
BRITO, I.P.F.S.[2]
DIAS, R.C.[2]
TRINDADE, M.L.B.[3]
CARBONARI, C.A.[2]
VELINI, E.D.[2]

ABSTRACT - Maintenance of straw on the soil surface in sugarcane production areas can influence weed occurrence and herbicide dynamics. After application, considerable losses of clomazone can occur as a result of its physicochemical characteristics. For this reason, novel formulations have been developed. In addition to the conventional formulation, microencapsulated formulations are currently available. Thus, the aim of this study was to observe and compare the effect of clomazone formulations under different application conditions. For this purpose, the experiment was carried with 12 treatments in a completely randomized design, with four replications, using clomazone formulations (1,200 g a.i. ha^{-1}), on soils with different moisture levels, in the presence and absence of sugarcane straw, followed or not by rainfall simulation. The treatments were compared by observation of number of emerged plants and phytotoxicity at 7 and 14 DAA, as well shoot dry matter at 14 DAA for *Ipomoea nil*, *Urochloa decumbens* and *Panicum maximum*. In general, the microencapsulated formulation presented a better performance under the less suitable application conditions when compared to the conventional one. However, for the other conditions, both formulations had a similar performance.

Keywords: straw, herbicide, volatilization, *Ipomoea nil* (L.) Roth, *Urochloa decumbens* (Stapf.) R.D.Webster, *Panicum maximum* Jacq.

RESUMO - A manutenção de palha sobre a superfície do solo em áreas de produção de cana-de-açúcar pode influenciar a ocorrência de plantas daninhas e a dinâmica de herbicidas. Após a aplicação, perdas consideráveis da molécula clomazone podem ocorrer, principalmente devido às suas características físico-químicas. Por esse motivo, novas formulações foram desenvolvidas, e atualmente, além da formulação convencional, está disponível o microencapsulado. Assim, o objetivo deste estudo foi observar e comparar o efeito da formulação de clomazone em diferentes condições de aplicação. Para isso, o experimento foi implantado com 12 tratamentos, em delineamento inteiramente ao acaso com quatro repetições, utilizando-se formulações de clomazone (1.200 g i.a. ha^{-1}) em solos com diferentes níveis de umidade, na presença e ausência de palha de cana-de-açúcar, seguida ou não de simulação de chuva. Os tratamentos foram comparados por meio da observação do número de plantas emergidas e da fitointoxicação aos 7 e 14 DAA, além de massa seca da parte aérea aos 14 DAA, para ***Ipomoea nil, Urochloa decumbens*** *e* ***Panicum maximum****. De modo geral, a formulação microencapsulada apresentou melhor desempenho nas condições menos adequadas de aplicação, quando comparada com a convencional. No entanto, para as demais condições, ambas as formulações foram similares.*

Palavras-chave: palha, herbicida, volatilização, *Ipomoea nil* (L.) Roth, *Urochloa decumbens* (Stapf.) R.D.Webster, *Panicum maximum* Jacq.

*** Corresponding author:**
<l.tropaldi@unesp.br>

Received: November 8, 2017
Approved: April 12, 2018

Planta Daninha 2019; v37:e019187433

[1] Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrária e Tecnológicas, Dracena-SP, Brasil; [2] Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agronômicas, Botucatu-SP, Brasil. [3] Bioativa, Botucatu-SP, Brasil.

Doi: 10.1590/S0100-83582019370100076

INTRODUCTION

Sugar energy production is a prominent industry in Brazil, and sugar cane crops are grown on approximately 8.8 million hectares (Conab, 2017). Over time, the sugarcane production system has undergone significant changes; currently, it is complex and operated under a wide range of soil types, climate and agricultural practices (Velini et al., 2015; Monteiro and Sentelhas, 2017). Similarly, weed management has become even more complex, especially in the raw sugarcane system, because when straw mulch is maintained on the soil surface, it can affect both weed occurrence and herbicide effectiveness. The presence of straw mulch may initially reduce the occurrence of species considered to be sensitive to it, for example, signal grass (*Urochloa decumbens*), alexandergrass (*Urochloa plantaginea*) and crabgrass (*Digitaria* spp.). In other species, such as morning glory (*Ipomoea* spp.) (Correia and Durigan, 2004; Negrisoli et al., 2007), germination is stimulated and its frequency of plants is increased. In addition, the operations of straw collection for energy generation and/or straw management (e.g., windrowing) contribute to the loss of the suppressive effect on weed occurrence. Thus, availability and use of herbicides are necessary for weed control in sugarcane crops.

Clomazone (2-[(2-chlorophenyl)methyl]-4.4-dimethyl-3-isoxazolidinone) stands out among the several herbicides recommended for sugarcane. It is considered to be a broad-spectrum herbicide when applied at preemergence or early post-emergence of monocot species and some eudicotyledons (Brasil, 2003). In terms of physical-chemical characteristics, clomazone is soluble in water (1,100 mg L^{-1} at 25 ^oC) and moderately volatile (vapor pressure of 1.92 x 10^{-2} Pa at 25 ^oC), and it may present considerable losses during and after application. Mervosh et al. (1995) found increased volatilization with increasing temperature; thus, at high temperatures, as found on the surface of straw mulch, losses can be intensified, increasing the potential of environmental contamination and poisoning of neighboring crops, as well as reducing the effectiveness of weed control.

To reduce pesticide losses, the development of new formulations has been receiving special attention (Parker et al., 2005). Microencapsulation is one of the techniques currently used for pesticide formulation with the purpose of minimizing problems resulting from losses and degradation (Sopeña et al., 2009; Wibowo et al., 2014). For example, the microencapsulated formulation of clomazone (Gamit 360 CS®) has been recommended for sugarcane cultivation (Brasil, 2003). Schreiber et al. (2015) found that the microencapsulated formulation of clomazone resulted in lower phytotoxicity to bioindicator species, because it is less volatile. Likewise, there was greater selectivity in the microencapsulated formulation of clomazone in several crops, such as maize, sorghum, rice (Schreiber et al., 2015), physic nut (Berté et al., 2015) and cassava (Scariot et al., 2013). In general, the lower phytotoxicity of a microencapsulated formulation has been attributed to the slow release of the active ingredient by the microcapsule.

As mentioned above, some studies have been developed to compare clomazone formulations, but none of them made a comparison between different application conditions, particularly for the sugarcane production system. Thus, the objective of the present study was to observe and compare the effect of microencapsulated and conventional formulations of clomazone - under different conditions of mulching, form of application and rainfall simulation - on three weed species, under the assumption that the effectiveness of the formulations depends on the conditions during and after herbicide application, as well as on the sensitivity of plants to the molecule.

MATERIAL AND METHODS

The study was conducted in a greenhouse at 27 $^oC \pm 2$ ^oC under sunlight. The experiment was set up with 12 treatments, in a completely randomized design with four replications. Different formulations of clomazone (1,200 g a.i. ha^{-1}) were applied on soils with different levels of moisture in the presence and absence of sugarcane straw mulch, with or without rainfall simulation. Table 1 shows the details of the treatments.

The experimental plots consisted of rectangular 4 L plastic pots (14 x 28 x 11 cm), filled with a mixture of three soils with different textures (clayey Distroferric Red Nitisol, medium-texture Dystrophic Dark Red Latosol and sandy Dystrophic Red Latosol), which resulted in a substrate

Table 1 **-** Description of the treatments carried out in the experimental units after application of clomazone formulations

Treatment	Description
1	Control without straw
2	Control with straw
3	Gamit 360 CS® on dry soil without straw and rainfall simulation at 1 DAA(1)
4	Gamit 360 CS® on dry soil, followed by straw mulch and rainfall simulation at 1 DAA
5	Gamit 360 CS® on moist soil, followed by straw mulch
6	Gamit 360 CS® on straw, with moist soil
7	Gamit 360 CS® on straw, with dry soil and rainfall simulation at 1 DAA
8	Gamit Star® on dry soil without straw and rainfall simulation at 1 DAA
9	Gamit Star® on dry soil, followed by straw mulch and rainfall simulation at 1 DAA
10	Gamit Star® on moist soil, followed by straw mulch
11	Gamit Star® on straw, with moist soil
12	Gamit Star® on straw with dry soil and rainfall simulation at 1 DAA

(1) Days after application.

with average texture and clay, silt and sand contents of 317, 96, and 587 g kg^{-1}, respectively. The results of chemical analysis were: organic matter, 19 g dm^{-3}; pH ($CaCl_2$ 0.01 mol L^{-1}), 4.1; P (resin), 9 mg dm^{-3}; K, Ca, Mg and H+Al, 1.2, 6.0, 6.0 and 52 $mmol_c$ dm^{3}, respectively; and saturation of 20%.

In the experimental units, three weed species were sown; they are commonly found in areas of sugarcane production: morning glory [*Ipomoea nil* (L.) Roth], signal grass [*Urochloa decumbens* (Stapf.) R.D. Webster] and buffalo grass (*Panicum maximum* Jacq.]. The seeds were mixed to the soil to be only in the central part of the pots, at depths varying from 0 to 4 cm. To this end, a smaller rectangular container was used to delimit the distribution of seeds. It was filled with a mixture of soil and seeds, while the rest of the pots contained soil only (Figure 1). Seeds were respectively used in the following amounts: 0.3 g for morning glory, 0.3 g for signal grass and 0.1 g for buffalo grass. These quantities were previously determined for the occurrence of high-density weed populations.

Figure 1 **-** Experimental units. (A) soil at the bottom of the pot, with a mixture of soil+seeds inside the container at the center; (B) soil near the edge of the pot, with a mixture of soil+seeds inside the container at the center; (C) pot with soil and soil+seeds at the time of application of treatments.

Clomazone (Gamit Star® and Gamit 360 CS®) was applied with a stationary sprayer, in a closed room, fitted with a spraying bar containing four XR 110.02 tips (Teejet, Jacto Máquinas Agrícolas AS, Pompeia, SP, Brazil), spaced 0.5 m apart and positioned 0.5 m above the targets. The system was operated with a travel speed of 3.6 km ha^{-1}, spray volume of 200 L ha^{-1} and constant pressure of 150 kPa, pressurized by compressed air. At the time of application, temperature and relative air humidity were 24 °C and 74%, respectively, and the edges of the pots were protected to prevent the herbicide from entering the pots after rainfall simulation (Figure 2).

In the treatments in which straw mulch was used before or after herbicide application (T1, T4, T5, T6, T7, T9, T10, T11 and T12), it was added at a volume of 5 t ha^{-1}. This quantity is sufficient to intercept almost 100% of the herbicides applied (Cavenaghi et al., 2007), and it causes little interference in weed germination. This is the worst condition that can occur when

Figure 2 - (A) experimental units arranged for herbicide application; (B) moment of herbicide application; (c) pot with protected edges and distribution of sugarcane straw mulch.

straw mulch is used. The presence of 5 t ha^{-1} is enough to virtually maximize all the negative effects in terms of herbicide dynamics, without presenting positive effects as far as weed control is concerned (Martins et al., 1999).

For the treatments in which rainfall simulation was performed after herbicide application (T3, T4, T7, T8, T9 and T12), the same sprayer was used, but with a second bar, composed of eight high-flow TK-SS-20 spray tips, spaced 0.5 cm apart, maintained at 1.4 m above the target and operated to produce total rain depth of 20 mm.

Except for rainfall simulation, which characterizes the different treatments, subsurface irrigation was performed in the pots to prevent movement of the herbicide applied on the straw. For this reason, a perforated hose was previously tucked inside the pots before sowing to perform irrigation, when necessary, by injecting water directly on the ground with the help of a syringe.

The effect of formulations under different conditions was checked by means of evaluations of emergence and phytotoxicity at 7 and 14 days after application (DAA) and of shoot dry matter of weeds at 14 DAA. Emergence was determined by counting the number of plants emerged during these periods. and phytotoxicity scores were assigned, in comparison with the treatment without herbicide application. Zero was assigned for absence of symptoms and 100% for plant death (SBCPD, 1995). Shoot dry matter (SDM) was determined by collecting only the living tissue of plants, followed by drying to constant weight in a forced air circulation oven at 60 ^{o}C, with subsequent measurement with a precision scale.

The data were subjected to analysis of variance and the averages were compared by the t-test ($p \leq 0.05$); the levels of significance were determined for the contrasts between the control treatment and the other treatments with the use of t-distribution. Furthermore, the relationship between the different formulations for the same condition was analyzed by means of linear correlations. Data analyses were performed using Microsoft Excel spreadsheets (2013) and the SAS® University Edition.

RESULTS AND DISCUSSION

Tables 2, 3 and 4 show the mean number of plants emerged at 7 and 14 DAA and shoot dry matter (SDM), at 14 DAA of *Ipomoea nil*, *Urochloa decumbens* and *Panicum maximum* for each study condition, respectively. The analysis of number of plants and SDM showed that the treatments interfered in the establishment of all the plant species.

Both the conventional and the microencapsulated formulations - applied over dry soil without straw mulch and rainfall simulation (T3 and T8), or on dry soil with straw mulch and rainfall simulation (T4 and T9), or even on moist soil with straw mulch but without rainfall simulation (T5 and T10) - resulted in the smallest numbers of plants, mainly at 14 DAA, in all the study species. Similarly, these treatments also caused greater reductions of SDM at 14 DAA (Tables 2, 3 and 4). The effects observed after application of clomazone are due to the process of inhibition of carotenoid synthesis, which results in the symptom of bleaching of leaf tissues (Hess, 2000; Ferhatoglu and Barrett, 2006; Dayan and Zaccaro, 2012). In this study, the seedlings were bleached at emergence, and tissue necrosis occurred only after exposure to sunlight, leading to seedling death, as evidenced by a reduction in the number of plants at 14 DAA.

Table 2 - Number of plants and shoot dry matter (SDM) of *Ipomoea nil*

Treatment	Number of plants		SDM 14 DAA(1)	
	7 DAA	14 DAA	(g)	% of control
T1	27.25	32.50	1.01	100.00
T2	37.80	36.50	1.67	100.00
T3	30.75	9.25	0.29	28.63
T4	25.50	8.75	0.27	16.02
T5	16.25	9.50	0.32	19.23
T6	26.50	24.25	0.61	36.49
T7	25.00	12.25	0.28	17.02
T8	27.00	7.75	0.19	19.33
T9	23.75	7.50	0.21	12.95
T10	18.75	5.00	0.16	9.75
T11	28.00	19.00	0.49	29.16
T12	30.50	27.75	0.70	42.08
F treatments	129.75**	23.31**	32.13**	
T1 vs T2	121.45**	1.52ns	35.43**	
T1 vs T3,8	4.73*	73.0**	64.40**	
T2 vs T4,5,6,7,9,10,11,12	335.61**	81.32**	238.64**	
CV(2) (%)	4.62	27.52	30.19	
LSD(3) – T-test at 5%	1.23	4.65	0.15	

(1) Days after application; (2) Coefficient of variation; (3) Least significant difference by the t-test at 5% of probability; *, ** Significant at 5 and 1% probability, respectively; ns Non-significant at 5% probability.

Table 3 - Number of plants and shoot dry matter (SDM) of *Urochloa decumbens*

Treatment	Number of plants		Dry weight 14 DAA(1)	
	7 DAA	14 DAA	(g)	% of control
T1	106.25	159.00	1.51	100.00
T2	77.80	138.75	1.18	100.00
T3	90.75	0.25	0.00	0.00
T4	42.75	0.00	0.00	0.00
T5	55.25	0.00	0.00	0.00
T6	68.25	90.50	0.38	32.59
T7	61.00	15.25	0.04	3.54
T8	93.00	1.50	0.00	0.00
T9	48.00	0.00	0.00	0.00
T10	56.75	0.00	0.00	0.00
T11	67.75	79.50	0.35	29.45
T12	61.00	70.25	0.22	18.99
F treatments	5.31*	106.63**	180.11**	
T1 *vs* T2	6.22*	6.23*	37.00**	
T1 *vs* T3,8	2.48ns	507.25**	1057.26**	
T2 *vs* T4,5,6,7,9,10,11,12	8.86**	308.60**	687.88**	
CV(2) (%)	21.50	24.78	24.70	
LSD(3) – T-test at 5%	15.09	11.62	0.07	

(1) Days after application; (2) Coefficient of variation; (3) Least significant difference by the t-test at 5% of probability; *, ** Significant at 5 and 1% probability, respectively; ns Non-significant at 5% probability.

Table 2 clearly shows that the presence of straw mulch was crucial for an increase in the number of emerged plants of *I. nil* (38.7% to 12.5% 7 DAA and to 14 DAA) in the presence of 5 t ha^{-1} of sugarcane straw. This effect has been widely reported in the literature. Importantly, for some species, there is no physical impediment to germination and emergence of weeds in

Table 4 - Number of plants and shoot dry matter (SDM) of *Panicum maximum*

Treatment	Number of plants		SDM 14 DAA(1)	
	7 DAA	14 DAA	(g)	% of control
T1	61.00	135.75	0.24	100.00
T2	43.20	112.25	0.24	100.00
T3	58.25	0.00	0.00	0.00
T4	15.00	0.00	0.00	0.00
T5	26.50	0.00	0.00	0.00
T6	21.75	44.25	0.04	18.99
T7	24.50	0.75	0.00	0.00
T8	66.25	0.00	0.00	0.00
T9	22.75	0.00	0.00	0.00
T10	24.00	0.00	0.00	0.00
T11	18.00	22.00	0.02	9.41
T12	19.00	10.25	0.01	4.27
F treatments	38.10**	170.43**	83.86**	
T1 vs T2	18.15**	121.46**	<0.01ns	
T1 vs T3, 8	0.11ns	4.74*	383.30**	
T2 vs T4,5,6,7,9,10,11,12	46.99**	335.61**	475.46**	
CV(2) (%)	17.92	26.81	42.87	
LSD(3) – T-test at 5%	6.05	7.37	0.02	

(1) Days after application; (2) Coefficient of variation; (3) Least significant difference by the t-test at 5% of probability; *, ** Significant at 5 and 1% probability, respectively; ns Non-significant at 5% probability.

the presence of quantities of up to 20 t ha^{-1} of straw, which is in line with the findings of Negrisoli et al. (2007), who found that germination of *I. grandifolia* was stimulated in areas covered with straw. Similar results were also reported in the study by Correia and Durigan (2004). These authors also found an increase in the number of plants of *I. quamoclit* emerged on the basis of increasing quantities of straw. Therefore, weeds whose germination is higher in areas with straw currently cause problems that tend to increase in production systems that maintain a layer of straw on the soil surface.

Table 5 shows the data of phytotoxicity for *I. nil*, *U. decumbens* and *P. maximum*. For *I. nil* at 14 DAA, treatments T3, T5, T7, T8, T9 and T10 presented phytotoxicity values equal or close to 50%, demonstrating its low susceptibility to clomazone, regardless of formulation or condition assessed. Conversely, the grass species, i.e., both *U. decumbens* and *P. maximum*, presented a well-defined and intense pattern of susceptibility. The treatments T3, T4, T5, T8, T9 and T10 were more effective in terms of phytotoxicity, causing the death of most of the seedlings.

Generally, another relevant point is the higher levels of intoxication of treatments containing the microencapsulated formulation, in comparison to the conventional formulation, particularly when the herbicides were applied on straw on moist soil (T6 *vs.* T11) or dry soil (T7 *vs.* T12), both without rainfall simulation, respectively. After application, herbicides are usually exposed to various types of loss (degradation or movement through the environment). Depending on their intensity, these processes can interfere in herbicide effectiveness (Bloomfield et al., 2006; Arias-Estévez et al., 2008). Very likely, the conventional formulation may have been less toxic because losses occurred more quickly than for the microencapsulated formulation, because the molecule is readily available. By contrast, the microencapsulated formulation presents a slow release (Whorton, 1995).

In this way, it is assumed that toxicity of both formulations, when they were applied over the straw mulch (T6 and T7 or T11 and T12, respectively), may have been due to absorption of the molecule in the form of vapor by weeds, as supported by the work of Schreiber et al. (2015). These authors reported that volatilization of clomazone led to higher phytotoxicity rates in sorghum, maize and rice in hermetically sealed boxes, and/or by direct contact of the molecule (deposited

Table 5 - Phytotoxicity scores at 7 and 14 DAA for *Ipomoea nil*, *Urochloa decumbens* and *Panicum maximum*

Treatment	7 DAA[(1)]			14 DAA		
	I. nil	*U. decumbes*	*P. maximum*	*I. nil*	*U. decumbens*	*P. maximum*
T1	0.00	0.00	0.00	0.00	0.00	0.00
T2	0.00	0.00	0.00	0.00	0.00	0.00
T3	52.50	81.25	83.75	52.50	98.75	100.00
T4	67.50	83.75	87.50	46.25	100.00	100.00
T5	67.50	86.25	88.75	52.50	100.00	100.00
T6	40.00	33.75	36.25	27.50	43.75	62.50
T7	62.50	68.75	100.00	52.50	72.50	86.25
T8	62.50	81.25	83.75	53.75	100.00	100.00
T9	73.75	85.00	87.50	52.50	100.00	100.00
T10	82.50	86.25	100.00	71.25	100.00	100.00
T11	52.50	33.75	51.25	40.00	22.50	70.00
T12	37.50	50.00	56.25	35.00	43.75	55.00
F treatments	142.32**	353.45**	332.10**	28.64**	679.06**	436.84**
C.V.[(2)] (%)	8.95	6.14	6.12	20.17	4.83	4.94
LSD[(3)] T-test at 5%	4.53	3.58	4.01	8.25	3.19	3.66

[(1)] Days after application; [(2)] Coefficient of variation; [(3)] Least Significant Difference by the T-test at 5% probability; ** Significant at 1% probability; [ns] Non-significant at 5% probability.

on the surface of the straw mulch) with the developing weeds. Volatilization may have even complemented the effect of the herbicide.

The conventional formulation (Gamit Star®), in particular, was found to be 12.5% more toxic than the microencapsulated formulation when it was applied over the straw mulch and there was no rainfall simulation (T6 *vs.* T11) for the species of critical control (morning glory) (Table 5). There was no pattern for the susceptible species (grasses). The conventional formulation caused 21.25% less phytotoxicity than the microencapsulated formulation for *U. decumbens* but 7.5% more phytotoxicity in *P. maximum*. Thus, as discussed previously, the higher intoxication rate of the conventional formulation in *I. nil* and *P. maximum* must have happened because the formulation produced more vapor that the microencapsulated formulation at the moment of greatest sensitivity of the plant species. The reason is that volatilization is one of the main ways in which pesticides with high vapor pressure move from the surface of the water and soil to the atmosphere. However, it may also have been strongly influenced by other factors, e.g., wind, temperature, physicochemical properties of the molecule, in addition to the surface on which the molecule is deposited (Locke et al., 1996).

Another point that can be speculated is the fact that guttation (a common event in these grass species) may have contributed to the dissolution of the capsule, making it available for absorption, while the conventional formulation had already suffered considerable loss of the molecule in the environment. However, these hypotheses still need to be tested to provide further insights into the effects observed.

Differences in the selectivity of clomazone formulations have been reported in several crops, such as maize, sorghum and rice (Schreiber et al., 2015), physic nut (Berté et al., 2015) and cassava (Scariot et al., 2013). In general, the greater selectivity of the microencapsulated formulation for the crops has been attributed to the slow release of the clomazone molecule. This hypothesis has been supported by the fact that the release of the contents of the microcapsule to the environment generally occurs by mechanical disturbances arising from temperature, pH, solubility, biodegradation and dissemination (Whorton, 1995). Thus, effects that are not desirable to crops are minimized while the herbicide remains effective for weed control.

Figure 3 shows the correlation between the microencapsulated and the conventional formulations of clomazone for all study species for each of the evaluated conditions There was a significant positive correlation of number of plants between the microencapsulated and the conventional formulations ($p<0.05$). Likewise, the parameter SDM was also significant. These

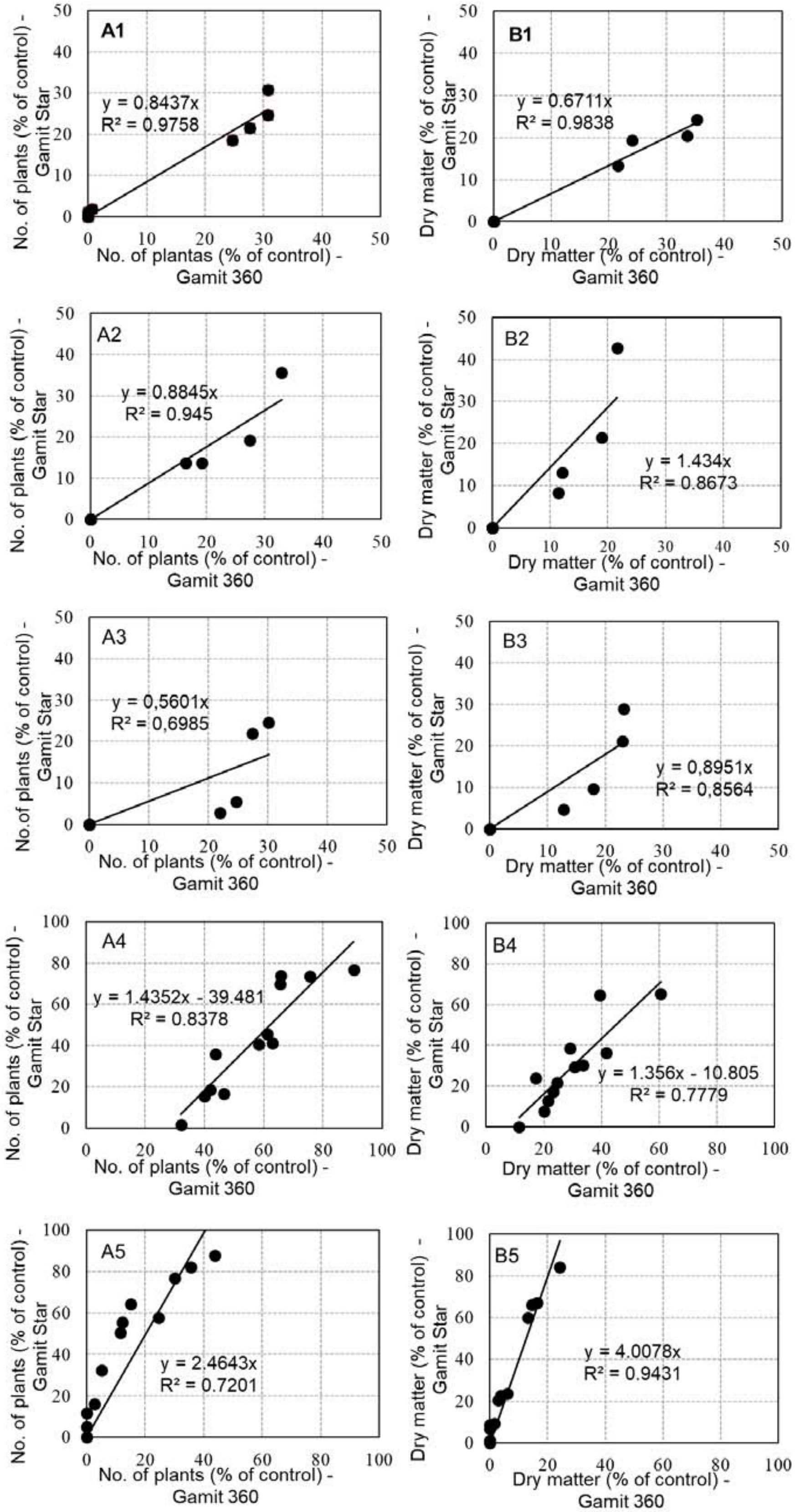

Figure 3 - Ratio between number of plants (A) and shoot dry matter (SDM) (B) of different species under the following conditions: 1) herbicide application on dry soil without straw and without rainfall simulation; 2) herbicide application on dry soil followed by straw mulch with rainfall simulation of 20 mm one day later; 3) herbicide application on moist soil followed by straw mulch and without rainfall simulation after application; 4) herbicide application on straw, with moist soil and without rainfall simulation; 5) herbicide application on straw, with dry soil and without rainfall simulation. Linear regression and correlation coefficient.

results indicate that there was a significant effect caused by different formulations on the evaluated parameters. Finally, it could be concluded that there is a significant relationship between the formulations for the parameter number of emerged plants. Importantly, in Figure 3, the equivalence graphs for each of the conditions allow to estimate how much the conventional formulation is equivalent to the microencapsulated formulation.

When effectiveness is compared between the clomazone formulations under different application conditions, the microencapsulated formulation usually showed a better performance in the less suitable conditions. However, for appropriate application conditions, both formulations had a similar performance.

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