

Enlist volunteer corn affects the crop development and seed quality of Enlist soybean

Alcimar Spindola Mazon¹ , Joanei Cechin^{2,3,*} , Cristiano Piasecki² , Juliano Gazola² ,
Jonas Rodrigo Henckes⁴ , Geri Meneghello¹ , Dirceu Agostinetto² 

1. Universidade Federal de Pelotas  – Fitotecnia – Ciência e Tecnologia de Sementes – Pelotas (RS), Brazil.
2. Universidade Federal de Pelotas  – Fitossanidade – Pelotas (RS), Brazil.
3. Universidade Estadual de Ponta Grossa  – Departamento de Fitotecnia e Fitossanidade – Ponta Grossa (PR), Brazil.
4. Universidade Estadual de Maringá  – Fitotecnia – Maringá (PR), Brazil.

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***Corresponding author:** joaneicechin@yahoo.com.br

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ABSTRACT: Enlist™ technology is a new trait on soybean and corn conferring tolerance to auxinic-type (2,4-D choline) and graminicides (aryloxyphenoxypropionates – FOPs) herbicides. However, the occurrence of volunteer corn tolerant to 2,4-D and FOPs can become a significant weed on soybean cropping systems, affecting the crop yield and reducing seed quality. This research aimed to evaluate herbicides to Enlist™ volunteer corn control and their effects on plant development, crop yield, and physiological seed quality of Enlist™ soybean. Field experiment was performed in a complete randomized block experimental design with four replicates, using a density of 10 plants·m⁻² of Enlist™ volunteer corn. Treatments consisted of herbicides applied at 3–4-leaves stage of volunteer corn with haloxyfop-P-methyl, clethodim, pinoxaden, cloransulam-methyl and imazethapyr regarding to a control with and without volunteer corn. The development and yield components of Enlist™ soybean were evaluated and physiological seed testing performed on soybean's seeds. Clethodim controlled Enlist™ volunteer corn, whereas haloxyfop-P-methyl, pinoxaden, and cloransulam-methyl showed insufficient control levels (30–35%) at 30 days after spray. Imazethapyr exhibited 84% of control of Enlist™ volunteer corn and injured the Enlist™ soybean, affecting its seed viability. Enlist™ volunteer corn interferences negatively the soybean crop yield, yield components, and affects the plant development pattern.

Key words: acetyl CoA carboxylase inhibiting herbicides, synthetic auxins, weed interference, seed analysis.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is the leading agricultural commodity for Brazilian farmers and trade balance, with major exported volumes that meet 29% of the world's demand (Giraudó 2020). Good soybean management practices, including high-quality seeds and adequate weed control, are essential to increase the crop yield. For example, weed interference can reduce up to 94% of soybean yield, depending on the weed species and coexistence period (Zandoná et al. 2018). In contrast, seeds with higher quality can increase approximately 15% the grain yield (Ebony et al. 2020). Otherwise, crop-weed interference can reduce seed quality due to physiological changes and its effects on photoassimilates production fill seeds (Carciochi et al. 2019). Roundup Ready® technology developed in the 1990s for soybean and available later for corn contributed to weed control, reducing costs, and increasing the grain yield (Benbrook 2016). After the commercial release, grain yield increased approximately 4-fold, whereas herbicides use reduced 75% between 1995 to 2013 (Benbrook 2016). This scenario based on repeated glyphosate application favored the selection of weed-resistant populations, and new traits with herbicide-resistance can provide new management strategies, aiming the control of weed herbicide-resistant populations (Duke 2020).

Enlist™ technology is a new tool available for farmers in several countries to control glyphosate-resistant broadleaf weeds, conferring tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D choline), glyphosate, and ammonium-glufosinate herbicides (Wright et al. 2010; Skelton et al. 2017). The tolerance to 2,4-D choline was obtained by the insertion of the aryloxyalkanoate dioxygenase (*AAD-12 V1*) coding-gene. Thus, *AAD-12* protein catalyzes the conversion of 2,4-D to 2,4-dichlorophenol (2,4-DCP) using sugar molecule to detoxify it into a non-phytotoxic compounds (Wright et al. 2010). On the other hand, the double mutant of 5-enolpyruvylshikimate 3-phosphate synthase (EPSPs) and phosphinothricin acetyltransferase (*pat*) confers tolerance to glyphosate and ammonium-glufosinate, respectively (Wright et al. 2010). Furthermore, Enlist™ technology can be found in corn from the insertion of the *AAD-1 V3* gene that encodes the *AAD-1* protein. This protein catalyzes the breakage of the chemical bond between oxygen and carbon, conferring tolerance to acetyl CoA carboxylase (ACCCase) herbicides of the aryloxyphenoxypropionates (FOPs) chemical group. It allows the control of grasses in Enlist corn's post-emergence (Gonzalez et al. 2018).

In several countries, especially in Brazil, agricultural production systems cultivate soybean after corn in the same season, a practice known as off-season (Petter et al. 2015). In this case, propagules (seeds and kernels) of Enlist™ corn can be lost during the harvest, originating individual or clumps of volunteer corn for the subsequent crop, reducing the soybean yield (Marca et al. 2015; Petter et al. 2015). The highly competitive ability of volunteer corn was reported in Roundup Ready® soybean with an economic threshold for individual plants of 0.45 plants·m⁻² and approximated to zero for clumps (Piasecki and Rizzardi 2018). Alternative chemical control includes ACCCase inhibiting herbicides performed during the initial development stages (V₂-V₄) to ensure adequate control (Pertile et al. 2018).

Considering the negative effects of crop-weed interference, seed's physiological quality can also be affected due to the physiological changes and lower photoassimilates translocation for the seeds, reducing the 1,000-seed weight and amount of reserves and proteins (Marcos-Filho 2015). Volunteer corn plants are undesirability in soybean, especially in scenarios with an increase of glyphosate-resistant and greater Enlist™ technology adoption. Thus, approaches are needed to evaluate herbicides effectiveness in Enlist™ volunteer corn control and measuring the interference effects on plant development and seed quality of Enlist™ soybean.

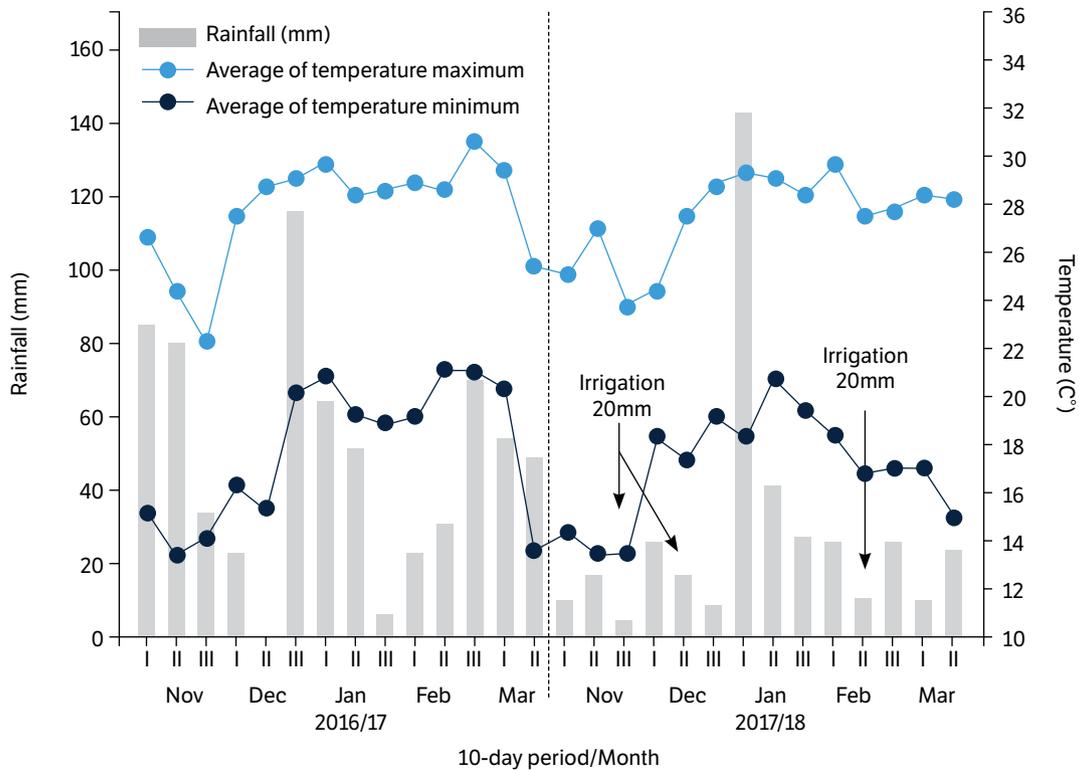
This research aimed to evaluate herbicides to control Enlist™ volunteer corn and their effects on plant development, crop yield, and physiological seed quality of Enlist™ soybean.

MATERIAL AND METHODS

Field experiment (geographic coordinates: 31°80'01"S, 52°50'38"W, and 12 m above the sea) was carried out during the 2016/17 and 2017/18 summer growing season (Figure 1 – environmental conditions), using a randomized complete block experimental design with four repetitions. The field soil was classified as Endoaquult (USDA Soil Taxonomy) and has the following characteristics performed at 0-10 cm layer depth: pH = 5.6, organic matter = 1.5%, clay = 16%, CEC_{pH7} = 6.7 cmolc·dm⁻³, Al³⁺ = 3.1 cmolc·dm⁻³, Ca⁺² = 6.6 mmolc·dm⁻³, Mg⁺² = 2.7 cmolc·dm⁻³, K⁺ = 0.23 mg·dm⁻³, and P = 9.9 g·dm⁻³. For the winter growing season, black oat was used as a cover crop that was burndown with glyphosate applied 30 days before the sowing at a rate of 1,080 g ae·ha⁻¹.

Enlist™ soybean (DAS-44406-6 lineage) was sowed at 32 plants·m⁻² in plots of 7.2 m², 45-cm rows spaced. Seeds were treated with pyraclostrobin + thiophanate-methyl + fipronil (Standak® Top) at the rate of 2 mL of commercial product for each kg of seed, and inoculated with *Bradyrhizobium japonicum* bacterium to nitrogen fixation. Base fertilization was calculated in accordance with the recommendations for the soybean crop, using 350 kg·ha⁻¹ of nitrogen, phosphorous, and potassium (NPK) formulation 02-20-20. F₂ progeny of Enlist™ volunteer corn (DAS-40278-9 lineage) was randomly sown and standardized at 10 individual volunteer plants·m⁻². Enlist™ seeds were obtained from Corteva Agriscience company with required authorization.

Post-emergent herbicides to control Enlist™ volunteer corn included haloxyfop-P-methyl (60 g ai·ha⁻¹, Verdict® Max, Corteva, Brazil), clethodim (108 g ai·ha⁻¹, Select® 240 EC, UPL, Brazil), pinoxaden (30 g ai·ha⁻¹, Axial®, Syngenta, Argentina), cloransulam-methyl (40 g ai·ha⁻¹, Pacto®, Corteva, Brazil), and imazethapyr (100 g ai·ha⁻¹, Vezir®, ADAMA Agricultural



Source: Estação Meteorológica da Empresa Brasileira de Pesquisa Agropecuária (Embrapa) Terras Baixas, Capão do Leão (RS), Brazil.

Figure 1. Environmental conditions during November to March 2016/17 and 2017/18 for the experiment with Enlist™ volunteer corn in Enlist™ soybean measured in each 10-day period. In the second year, three irrigations were performed with 20 mm each in November, December, and February to enhance the soil moisture content.

Solutions, Brazil) compared to the control without and with Enlist™ volunteer corn. Specific adjuvant was added for each herbicide, in accordance with the manufacturer's recommendations. Herbicides were applied at V₃-V₄ phenological stages of corn, using a CO₂ backpack sprayer equipped with four TeeJet AXI 110.15 spray nozzles, spaced at 0.5 m calibrated to deliver 120 L·ha⁻¹. The herbicide application was performed at air temperature of 21°C, 59% relative air humidity, and wind speed of 2.9 km·h⁻¹. After herbicide application, rainfall was not reported for seven days.

The control of Enlist™ volunteer corn plants and Enlist™ soybean sensitivity were evaluated at 10, 20, and 30 days after application (DAA), through visual assessments from 0 to 100% – 0% refers to the absence of phytotoxic effects and 100% to the complete death of the plants. Enlist™ soybean plant height (PH), height of first pod insertion (HFPI), number of internodes and braches per plant (INP and BNP) were measured at the full maturity. Soybean PH was measured with a rule from the soil surface to the tip of the last fully expanded leaf in 10 random plants per plot. Enlist™ soybean was harvested in an area of 3.5 m² to calculate the grain yield, and 10 plants were used to quantify the yield components (pods number per plant, seeds per plant, 1,000-seed weight). Samples were threshed, cleaned, and weighed in a precision analytical balance, at a standard seeds moisture of 13%. The 1,000-seed weight was performed after samples cleaned, using eight subsamples containing 100 seeds each. A sample portion from each plot was stored in a cold chamber at 10°C for 30 days to perform the seed physiological analysis.

Seed testing to evaluate the physiological quality was performed at a seed laboratory, using a completely randomized design with four replicates of 50 Enlist™ soybean seeds each. Seed physiological analysis was measured by the germination test and first germination count, accelerated aging, tetrazolium seed testing, and field emergence, following the official methodology described in the Brazilian rules for seed testing (Brasil 2009). For germination testing (GT), seeds were distributed over two sheets of germitest paper previously moistened with distilled water at an amount equivalent to 2.5-fold the paperweight, and placed in a biochemical oxygen chamber demand (BOD) regulated at 25°C and 12 h in light. The first germination count (FGC) of normal seedlings was performed at five days after setting up the germination test. In contrast,

the final germination was obtained at eight days after, with the values expressed in percentage. Accelerated aging (AA) was carried out in transparent boxes, using 200 seeds distributed in a single layer under lifted wire mesh. The boxes were filled with 40 mL of distilled water, closed and transferred to a BOD at 41°C for 72 h (Brasil 2009). Afterwards, the seed germination test was conducted equally of the FGC methodology.

Tetrazolium seed testing (TST) was performed using two subsamples of 50 seeds each by repetition. Seeds were put in a paper previously moistened with distilled water for 16 h and placed in a germination camera at 25°C. Then, seeds were transferred to glass cups with a 0.075% tetrazolium solution, maintained for 3 h in the dark inside an incubator at 40°C to form the embryo color. Seed viability and vigor were evaluated in a magnifying glass, with values expressed in percentage. Field emergence (FE) under natural conditions was carried out in raised beds, using two subsamples of 50 seeds each by repetition. Soybean seeds were sown manually to a depth of 2 cm in a wet soil. The normal seedlings count was evaluated 21 days after sown, with values expressed in percentage (Nakagawa 1999).

Data were analyzed for normality by the Shapiro-Wilk test and subjected to variance analysis ($p < 0.05$). When significant, means were compared by Duncan's test ($p < 0.05$) using the Statistical Analysis System software (SAS, version 9.0). Data were analyzed together due to absence of significant differences between growing seasons.

RESULTS AND DISCUSSION

Insufficient control of Enlist™ volunteer corn was found after application of haloxyfop-P-methyl, pinoxaden, and cloransulam-methyl herbicides, regardless of the evaluated period, with values lower than 35% (Table 1). Clethodim exhibited the highest control of Enlist™ volunteer corn with levels greater than 91% during the evaluated period, whereas imazetapyr controlled 84% of volunteer corn at 30 DAA (Table 1). Studies have been reported the application of ACCase

Table 1. Control (%) of Enlist™ volunteer corn and injury (%) on Enlist soybean after exposure to post-emergence herbicides evaluated at 10, 20 and 30 DAA. 2021.

Herbicide treatment	Rate (g ai·ha ⁻¹)	Control (%)		
		10 DAA	20 DAA	30 DAA
Control with volunteer corn	-	0.0 e*	0.0 e	0.0 e
Control without volunteer corn	-	100 a	100 a	100 a
Haloxyfop-P-methyl	60	8.0 e	12 d	14 d
Clethodim	108	91 b	98 a	100 a
Pinoxaden	30	24 d	19 d	16 d
Cloransulam-methyl	40	30 d	32 c	35 c
Imazetapyr	100	60 c	87 b	84 b
Coefficient of variation (%)		5.1	3.3	4.8
Herbicide treatment	Rate (g ai·ha ⁻¹)	Enlist soybean injury (%)		
		10 DAA	20 DAA	30 DAA
Control with volunteer corn	-	0.0 c	0.0 c	0.0 c
Control without volunteer corn	-	0.0 c	0.0 c	0.0 c
Haloxyfop-p-methyl	60	3.0 c	0.0 c	0.0 c
Clethodim	108	5.0 c	2.0 c	0.0 c
Pinoxaden	30	5.0 c	1.0 c	0.0 c
Cloransulam-methyl	40	10 b	8.0 b	6.0 b
Imazetapyr	100	19 a	16 a	13 a
Coefficient of variation (%)		19.9	11.5	8.1

*Same lowercase letters in the column did not differ by Duncan's test ($p < 0.05$); DAA: days after application.

inhibiting herbicides to control volunteer corn, mainly when performed at V₂-V₃ phenological stages to ensure better control levels (Chahal and Jhala 2015; Pertile et al. 2018). The reduced control of Enlist™ volunteer corn to haloxyfop-P-methyl was expected because its tolerance to that herbicide due to *AAD-1* enzyme insertion confers tolerance to AOPPs herbicides, reducing the herbicidal damage (Wright et al. 2010; Chekan et al. 2019). Reduced control was also found to pinoxaden, an active ingredient from phenylpyrazolin chemical group (DEN), controlling only 65% of the Enlist™ volunteer corn at 28 DAA (Striegel et al. 2020), suggesting that the *AAD-1* enzyme can also reduce the sensibility of Enlist™ corn to DEN. In contrast, imazethapyr exhibited 84% control of glyphosate-volunteer corn at 30 DAA (Marca et al. 2015).

Despite providing 84% of control, imazethapyr injured the Enlist™ soybean, with values up to 19%. On the other hand, haloxyfop-P-methyl, pinoxaden, and clethodim applied at labeled rate did not exhibit significant injury, with values lower than 5% (Table 1). The specific activity of ACCase-inhibiting herbicides on grasses occurs due to their selective inhibition of homomeric plastidic ACCase only found in monocots. In contrast, heteromeric plastidic or homomeric cytosolic forms found in soybean and other dicots are not inhibited by ACCase inhibitors (Kukorelli et al. 2013). On the other hand, cloransulam-methyl and imazethapyr inhibit the enzyme acetolactate synthase (ALS), dependent on metabolic degradation to provide rapid inactivation of herbicide molecule. Thus, lower injury levels of these herbicides in soybean involve differential activity of cytochrome P₄₅₀ enzymes complex followed by glutathione conjugation, leading to differential tolerance between soybean and weeds (Nandula et al. 2019).

Additionally, volunteer corn plants, when present in soybean crops, can interfere and cause damage to the plant development. Thus, chemical management of Enlist™ volunteer corn has greater importance to reduce the competition and ensure better conditions for soybean development. While the resources are limited in agricultural fields, crop-weed competition can change the light capture and perception in response to shading, affecting physiological processes and their development (Mckenzie-Gopsill et al. 2016). The presence of Enlist™ volunteer corn increased the Enlist™ soybean PH, especially for the treatments with reduced control by post-emergence herbicides evaluated (Table 2).

Table 2. Morphological parameters and yield components of Enlist™ soybean after the interference of Enlist™ volunteer corn. 2021.

Herbicide treatment	Rate (g ai·ha ⁻¹)	Morphological parameters			
		PH (m)	HFPI (cm)	INP	BNP
Control with volunteer corn	-	1.43 a*	29.8 a	17.2 b	2.6 c
Control without volunteer corn	-	1.25 c	28.4 a	21.1 a	4.0 a
Haloxyfop-P-methyl	60	1.42 a	24.6 b	17.1 b	2.0 c
Clethodim	108	1.33 b	23.9 b	22.4 a	4.2 a
Pinoxaden	30	1.46 a	26.0 b	17.5 b	2.4 c
Cloransulam-methyl	40	1.37 b	22.8 c	18.9 b	2.2 c
Imazetapyr	100	1.35 b	22.1 c	22.3 a	3.6 b
Coefficient of variation (%)		5.8	5.3	7.2	15.6
Herbicide treatment	Rate (g ai·ha ⁻¹)	Soybean yield components			
		PNP	SP	SW (g)	GY (kg·ha ⁻¹)
Control with volunteer corn	-	27.9 c*	71 c	179.3 a	868.5 c
Control without volunteer corn	-	86.3 a	196 a	166.4 c	3536.1 a
Haloxyfop-P-methyl	60	29.3 c	63 c	176.1 a	954.1 c
Clethodim	108	89.8 a	204 a	162.8 c	3268.4 a
Pinoxaden	30	30.2 c	66 c	179.2 a	839.8 c
Cloransulam-methyl	40	31.8 c	70 c	178.9 a	1172.6 c
Imazetapyr	100	61.4 b	138 b	171.5 b	2277.4 b
Coefficient of variation (%)		26.7	28.1	3.6	21.1

*Same lowercase letters in the column did not differ by Duncan's test ($p < 0.05$); PH: plant height; HFPI: height of first pod insertion; INP: internodes number per plant; BNP: branches number per plant; PNP: pod number per plant; SP: seeds per plant; SW: 1,000-seed weight; GY: grain yield.

Enlist™ soybean treated with haloxyfop-P-methyl and pinoxaden showed PH of 15% greater than control without Enlist™ volunteer corn. Additionally, PH of Enlist™ soybean was 10% higher after exposure to cloransulam-methyl, imazethapyr, and clethodim herbicides than control (weed-free), demonstrating the need for adequate management to avoid the adverse effects of corn-soybean competition. The coexistence of glyphosate-tolerant volunteer corn in competition with soybean increased 16% the PH, especially on varieties with short growth cycle and control performed later (Caratti et al. 2018). Morphological and physiological changes due to the neighbor's competition can occur between species in response to the light perception and its effects on antioxidant machinery, affecting the architecture of the canopy and photosynthetic efficacy (Benemann et al. 2020). At prior to harvest, HFPI of Enlist™ soybean was not affected significantly by corn-competition, with values greater than 28.4 cm (Table 2). In contrast, all herbicides applied on Enlist™ soybean reduced the HFPI, especially the imazethapyr, with values 28% lower than the control without Enlist™ volunteer corn and herbicide application (Table 2). HFPI is an important parameter used to regulate the harvester platform's cutting height to avoid grain losses, requiring a minimum pod height to cut-off greater than 15 cm in moderately declined fields (Andrade et al. 2016). Furthermore, herbicides can reduce the first pod's height, especially when it triggers high injury levels. For example, fomesafen and cloransulam-methyl were applied on Roundup Ready® soybean at a labeled rate reduced 22% the HFPI (Novakoski et al. 2020).

Other morphological changes can occur due to competition and injury caused by herbicides, affecting the development. Enlist™ soybean without competition caused by volunteer Enlist™ showed greater INP and BNP values. Plots treated with clethodim exhibited INP and BNP values statistically equal to control without volunteer corn (Table 2). However, lower control obtained by haloxyfop-P-methyl, pinoxaden, and cloransulam-methyl herbicides increased the corn-soybean interference effects and reduced the INP and BNP strongly, with values 18 and 55% lower than Enlist™ soybean without Enlist™ volunteer corn (Table 2). The corn-soybean competition due to inadequate control affects reproductive structures' development, with values up to 80% lower depending on the soybean cultivar used (Caratti et al. 2018). These results show the importance of ensuring highest levels of control of Enlist™ volunteer corn, reducing the interference, and protecting the grain yield potential.

Evaluating the grain yield components, Enlist™ soybean without Enlist™ volunteer corn or treated with clethodim showed higher PNP and SP, with values up to 68% greater than control with Enlist™ volunteer corn and other herbicides evaluated (Table 2). However, the SW of Enlist™ soybean without volunteer corn was 6% lower than control with volunteer Enlist™ corn or treated with haloxyfop-P-methyl (Table 2). Several results have reported substantial pods reduction in soybean under stressors agents like weed competition (Caratti et al. 2018; Piasecki and Rizzardi, 2018). An unfortunate outcome of control can favor the weed development, affecting the crop plant growth and reducing the available resources to photoassimilates production (Baucom 2019). The higher corn height reduces the light interception by soybean and changes the red:far-red ration detected by plant phytochromes, affecting the resource partitioning (Ballaré 2014). Crop-weed competition can increase oxidative stress and up-regulate the expression of photosynthetic apparatus genes (Benemann et al. 2020). Additionally, morphological changes under competition in soybean include lower internodes and branches number, reducing the reproductive buds, pods, and seeds produced per plant (Harre and Young 2020).

Enlist™ soybean with Enlist™ volunteer corn showed an average grain yield of 73% lower than the control without volunteer plants and 70% lower than the Enlist™ soybean treated with clethodim (Table 2). Furthermore, plots managed with imazethapyr controlled only 84% of the Enlist™ volunteer corn, reducing 36% of the soybean grain yield compared to the control, demonstrating that lower control levels can reduce the grain yield due to greater ability competitive (Table 2). Pertile et al. (2018) reported the higher competitive potential of Roundup Ready® volunteer corn on soybean, reducing the grain yield at low densities. Thus, Enlist™ soybean grain yield reflected the level of Enlist™ volunteer corn control, in which the clethodim herbicide provided adequate control in comparison of haloxyfop-P-ethyl, pinoxaden, imazethapyr, and cloransulam-methyl. Due to competition, the restricted growing conditions also limit the plant's ability to support seed demand, reducing the total photoassimilates and threshold values of the plant to set seeds produced. In contrast, the seed-seed partitioning can cause penalties for the seed weight, because it would have resource-limited during the grain filling (Carciochi et al. 2019). In agricultural systems, adverse effects of weed interference are broadly known, causing strong reduction of the grain yield, especially when the weed management is inadequate during the initial crop development.

Seed physiological quality of Enlist™ soybean was affected negatively due to the interference caused by volunteer plants, with G and FCC values 5% lower compared to the control without volunteer corn (Table 3). Similarly, plots treated with pinoxaden and cloransulam-methyl exhibited G values greater than 97%, whereas Enlist™ soybean was subjected to clethodim, pinoxaden, and haloxyfop-P-methyl herbicides and showed FCC values greater than 83% (Table 3). Lower soybean nutrient accumulation can be affected by weed competition, causing the lowest grain yield and seed weight (Harre and Young 2020). On the other hand, seeds with a larger size and weight can show greater seed germination and vigor than smaller seeds (Derre et al. 2017). Thus, seeds with higher reserves amount available to embryo development are essential to ensure the seedling establishment, good stand uniformity, and greater grain yield (Ebone et al. 2020). Although the FCC indicates the seed vigor, other deterioration processes can reduce the germination and vigor, generally identified through more consistent physiological seed testing (Kandasamy et al. 2020).

Based on AA and FE testing, Enlist™ soybean treated with haloxyfop-P-methyl, pinoxaden, and clethodim herbicides showed significant germination percentage, with values greater than 94%, and statistically similar to control without Enlist™ volunteer corn (Table 3). However, imazethapyr and cloransulam-methyl applied on Enlist™ soybean reduced the AA, whereas imazethapyr herbicide affected negatively the FE, reducing approximately 5% compared to the control (Table 3). Larger seed sizes have better embryo formation and significant reserve amounts, ensuring the successful emergence of seedlings (Derre et al. 2017). During the soybean seed germination, several metabolic proteins play a role-key to mobilize the reserves, especially in the carbohydrate metabolism and redox processes (Han et al. 2013), essential steps to begin the germination. In this way, seed vigor is a critical factor determining the initial seedling development, and ensures the initial plant-stand establishments (Ebone et al. 2020), a key-point to enhance the grain yield.

Evaluating the physiological parameters by TZ testing, vigor and viability of Enlist™ soybean were elevated to all herbicide treatments, except to imazethapyr, with values greater than 94% (Table 3). The lowest values of vigor

Table 3. Physiological seeds quality of Enlist soybean evaluated by germination (G), first germination count (FGC), accelerated aging (AA), field emergence (FE), and tetrazolium testing after herbicide treatment to control of Enlist™ volunteer corn. 2021.

Herbicide treatment	Rate (g ai·ha ⁻¹)	G (%)	FCC (%)	AA (%)	FE (%)
Control with volunteer corn	-	92 b*	79 b	92 b	95 b
Control without volunteer corn	-	97 a	84 a	95 a	99 a
Haloxyfop-P-methyl	60	93 b	86 a	95 a	99 a
Clethodim	108	92 b	83 a	94 a	97 a
Pinoxaden	30	98 a	86 a	94 a	99 a
Cloransulam-methyl	40	97 a	76 b	90 b	98 a
Imazetapyr	100	95 ab	73 b	90 b	94 b
Coefficient of variation (%)		1.8	7.1	2.7	2.0

Herbicide treatment	Rate (g ai·ha ⁻¹)	Tetrazolium testing (%)	
		Vigour	Viability
Control with volunteer corn	-	94 b*	96 a
Control without volunteer corn	-	97 a	98 a
Haloxyfop-P-methyl	60	98 a	99 a
Clethodim	108	98 a	98 a
Pinoxaden	30	98 a	99 a
Cloransulam-methyl	40	97 a	98 a
Imazetapyr	100	86 c	91 b
Coefficient of variation (%)		3.1	2.8

*Same lowercase letters in the column did not differ by Duncan's test ($p < 0.05$).

and viability found in Enlist™ soybean after exposure of imazethapyr possibly occurred by higher injury level of herbicide used to control the Enlist™ volunteer corn. Similar results were reported in soybean cultivar BRS 382CV (imidazolinone-tolerant – Cultivance® technology), Coodetec cultivar CD249 STS (sulfonylurea-tolerant soybean – STS technology) and Nidera cultivar NA5909RR (glyphosate-tolerant technology) managed in lowland areas, in which greater amount of imazapic+imazapyr herbicide residues from carry over caused significant plant injury and reduced the seeds physiological quality (Agostinetto et al. 2018), demonstrating negative effects of imidazolinone herbicides applied in soybean depending on injury level caused. Although imazethapyr is selective for soybean, the cleavage of ring aromatic from imidazolinones herbicides depends on cultivar differential metabolic ability to rapidly metabolize and inactivate the toxic compounds. It involves the enzymatic complex of cytochrome P₄₅₀ monooxygenases and glutathiones that require adenosine triphosphate (ATP) (Teclé et al. 1993). Otherwise, some factors like crop management, stress occurrence, and meteorological conditions during the seed development and maturation can influence seed quality, reducing the vigor (Rao et al. 2017).

According to the present study results, the control of Enlist™ volunteer corn must be appropriately performed to reduce the interference and protect the grain yield of Enlist™ soybean, assuring the production of seeds with better physiological quality. The recommended practices to control Enlist™ volunteer corn include the application of clethodim or other ACCase herbicides from cyclohexanedione (DIMs) chemical group, performed at initial development (up to V₄ phenological stage) to provide high control levels and reduce the losses of grain during the harvest, and rotation of the transgenic technologies available inside of the same area.

CONCLUSION

Clethodim is efficient to control Enlist™ volunteer corn and selective for Enlist™ soybean. Haloxyfop-P-methyl, pinoxaden, and cloransulam-methyl do not control Enlist™ volunteer corn, affecting negatively the Enlist™ soybean plant development and grain yield, but do not affect the physiological seed quality. Imazethapyr causes significant injury in Enlist™ soybean, reducing the seed viability by tetrazolium testing.

AUTHORS' CONTRIBUTION

Conceptualization: Mazon, A. S., Cechin, J., Piasecki, C. and Agostinetto, D.; **Methodology:** Mazon, A. S., Meneghello, G. and Agostinetto, D.; **Investigation:** Mazon, A. S., Gazola, J. and Henckes, J. R.; **Writing – Original Draft:** Mazon, A. S., Cechin J. and Piasecki, C.; **Writing – Review and Editing:** Cechin, J. and Piasecki, C.; **Supervision:** Meneghello, G. and Agostinetto, D.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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The authors declare no conflicts of interest.

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