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Author for correspondence:
Ramon G. Leon, Department of Crop and Soil Sciences, Center for Environmental Farming Systems, and Genetic Engineering and Society Center, North Carolina State University, 4402C Williams Hall, Raleigh, NC 27695.
Email: rleon@ncsu.edu

Evaluation of imazapic and flumioxazin carryover risk for Carinata (*Brassica carinata*) establishment

Manuel E. Camacho[1], Travis W. Gannon[2], Khalied A. Ahmed[3], Michael J. Mulvaney[4], Joshua L. Heitman[5], Aziz Amoozegar[6] and Ramon G. Leon[7]

[1]Graduate Student, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA; current: Centro de Investigaciones Agronómicas (CIA), Universidad de Costa Rica, San Pedro, Costa Rica; [2]Associate Professor, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA; [3]Research Associate, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA; [4]Associate Professor, Department of Plant and Soil Sciences, Mississippi State University, Starkville, MS, USA; [5]Professor and University Faculty Scholar, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA; [6]Professor, Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC, USA and [7]Professor and University Faculty Scholar, Department of Crop and Soil Sciences, Center for Environmental Farming Systems, and Genetic Engineering and Society Center, North Carolina State University, Raleigh, NC, USA

Abstract

Carinata (*Brassica carinata* A. Braun) is a potential crop for biofuel production, but the risk of injury resulting from carryover of soil herbicides used in rotational crops is of concern. The present study evaluated the carryover risk of imazapic and flumioxazin for carinata. Label rates of imazapic (70 g ai ha^{-1}) and flumioxazin (107 g ai ha^{-1}) were applied 24, 18, 12, 6, and 3 mo before carinata planting (MBP). The same herbicides were applied preemergence right after carinata planting at 1X, 0.5X, 0.25X, 0.125X, 0.063X, and 0X the label rate. When either herbicide was applied earlier than 3 MBP, there was no difference in plant density compared with the nontreated control. Carinata damage was <25% when flumioxazin or imazapic was applied at least 6 MBP in Clayton, NC (sandy loam soil), while in Jackson Springs, NC (coarser-textured soil and higher precipitation), at least 12 MPB were needed to lower plant damage to <25%. Preemergence application of 0.063X each herbicide decreased plant density by 40%, with damage reaching >25%. Quantification of herbicide residues in both soils showed that imazapic moved deeper in the soil profile than flumioxazin. This was more evident in Jackson Springs, where 0.68, 3.52, and 7.77 ng of imazapic g^{-1} soil were detected (15- to 20-cm depth) when the herbicide was applied at 12, 6 and 3 MBP, respectively, while no flumioxazin residues were detected at the same soil depths and times. When residues were 7.78 and 6.90 ng herbicide g^{-1} soil in the top 10 cm of soil for imazapic and flumioxazin, respectively, carinata exhibited at least 25% damage. Rotational intervals to avoid imazapic and flumioxazin damage to carinata should be between 6 and 12 MBP depending on soil type and environmental conditions, with longer intervals for the former than the latter.

Introduction

Carinata (*Brassica carinata* A. Braun) was originally cultivated in northeast Africa and was recently introduced into other countries, including Canada (Rakow and Getinet 1998), Australia, New Zealand (Rahman et al. 2018), Italy (Cardone et al. 2003), Spain (Gasol et al. 2007; Martínez-Lozano et al. 2009), and India (Thakur et al. 2019). This crop has become a prospective winter rotational crop for the southeastern region of the United States due to its potential use for livestock feed and large-scale biofuel production (Kumar et al. 2020; Mulvaney et al. 2019). This crop presents desirable agronomic characteristics such as abiotic stress tolerance, the potential to grow during the winter under southeastern U.S. climatic conditions, and seed-shattering resistance, which increases harvest efficiency (Kumar et al. 1984; Rakow and Getinet 1998; Raman et al. 2017; Yang et al. 2010; Zanetti et al. 2013). In addition, compared with other oilseed species, carinata seeds present higher quantities of long-chain fatty acids (e.g., erucic acid), which are preferred to generate high-energy fuels with less energy input (Kumar et al. 2020; Mulvaney et al. 2019). Due to carinata's recent introduction as a rotational winter crop in the United States, there is limited information about the agronomic practices needed to attain high yields sustainably (Mulvaney et al. 2019). Specifically, there are few reports about weed control and carinata tolerance to herbicides (Ethridge et al. 2021; Leon et al. 2017).

Weed pressure and management are among the main challenges for ensuring productive and profitable cropping systems (Bridges 1994). In the United States, herbicides have become the most commonly used tool to address these problems, due to their effectiveness and ease of

Table 1. Selected soil physical properties assessed for two different locations in North Carolina.

Site	Horizon	Depth	Bulk density	Total porosity	K_s[a]	Clay	Silt	Sand
		cm	g cm^{-3}	cm^3 cm^{-3}	cm h^{-1}		%	
Clayton	Ap	0–29	1.65	0.38	0.72	4	8	88
	Bt	29–60	1.52	0.42	0.53	44	8	48
Jackson Springs	Ap	0–30	1.41	0.47	23.67	6	5	89
	E	30–58	1.67	0.37	15.93	6	5	89

[a]K_s, saturated soil hydraulic conductivity.

implementation. However, the persistence of herbicide residues in soil and potential carryover (Hollaway et al. 2006; Palhano et al. 2018) that could result in toxicity and rotational crop yield losses (Rector et al. 2020) are important concerns for growers considering alternative crops. This is especially true when there are knowledge gaps about plant-back restrictions, which is the case for carinata.

Currently in the southeastern United States, crop rotations include soybean [*Glycine max* (L.) Merr.], peanut (*Arachis hypogaea* L.), and cotton (*Gossypium hirsutum* L.) grown from spring to fall (Johnson et al. 2001). In these rotational systems, the preemergence herbicides imazapic, an acetolactate synthase (ALS) inhibitor, and flumioxazin, a protoporphyrinogen oxidase (PPO) inhibitor, have been widely used for effective control of dicotyledonous weed species to maintain target crop yields (Berger et al. 2012; Ferrell and Vencill 2003a; Matocha et al. 2003). Although PPO inhibitors have low residuality, depending on application timing in summer crops, this class of herbicides can potentially affect the following winter crops. In the southern United States, flumioxazin can be applied relatively late in the spring or early summer in double-crop soybeans (Hay et al. 2019), as well as postemergence layby in cotton (Ferrell et al. 2007; Ferrell and Vencill 2003b). These late applications might not allow enough time for herbicide degradation, resulting in increased carryover risk for winter crops (Price et al. 2020). For instance, plant damage and yield reduction have been reported in cotton due to imazapic carryover after peanut production (York et al. 2000). However, their use in the southeastern United States has increased to complement herbicide programs that require controlling weed species that have evolved resistance to ALS-inhibiting herbicides and glyphosate (Scarabel et al. 2007; Steckel 2007). With ALS inhibitors, growers suspected that uneven carinata stands and plant damage, including stunting, chlorosis, and flower abortion, in fields previously treated with imazapic and chlorimuron could be due to carryover.

To address growers' concerns about the limited information available on the risk of herbicide carryover for carinata, particularly for imazapic and flumioxazin, we conducted the present study focusing on three objectives: (1) assess the potential carryover risk of two residual herbicides (imazapic and flumioxazin) for carinata establishment, (2) characterize the movement and behavior of imazapic and flumioxazin in the soil, and (3) relate soil herbicide concentration with carinata planting and establishment safety.

Materials and Methods

Carryover Study

Field Experiment

Field experiments were conducted between 2017 and 2019 at the Central Crop Research Station in Clayton, NC, USA (35.670°N, 78.490° W) and the Sandhills Research Station in Jackson Springs, NC, USA (35.186° N, 79.669°W). Soils series were Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) and Candor sand (sandy, kaolinitic, thermic Grossarenic Kandiudults), respectively. The Norfolk loamy sand in Clayton had an Ap horizon (i.e., surface soil layer; 0- to 29-cm depth) with loamy sand, pH 5.1, and 0.41% total carbon (TC), and a Bt horizon (i.e., subsurface layer; 29 to 60 cm) with clay texture, pH 5.2, and 0.24% TC. The Candor sand in Jackson Springs had an Ap horizon (0 to 30 cm) with sand texture, pH 5.8, and 1.5% TC, and an E horizon (i.e., mineral subsurface layer; 30 to 58 cm) sand texture, pH 4.6, and 0.1% TC (Table 1).

Daily average values for solar radiation, air and soil temperatures, evapotranspiration, and precipitation were obtained from the automatic weather stations (ECONET, North Carolina State Climate Office) that were located within 1 km from each experimental site (Figure 1).

Risk of carinata damage due to carryover was studied using imazapic (Cadre®, BASF, Research Triangle Park, NC, USA) and flumioxazin (Valor® SX, Valent U.S.A., Walnut Creek, CA, USA). Imazapic (70 g ai ha^{-1}) and flumioxazin (107 g ai ha^{-1}) were applied to bare ground at 24, 18, 12, 6, and 3 mo before carinata planting (MBP). The area was maintained fallow with regular glyphosate applications. Herbicides were applied using a CO_2-pressurized backpack sprayer with flat-fan spray nozzles (XR11002VS, TeeJet®, Spraying Systems, Wheaton, IL, USA). This equipment was calibrated to deliver 187 L ha^{-1} of solution at 214 kPa of pressure. A nontreated control was included for comparison. Herbicides were incorporated with 1 cm of overhead irrigation in Jackson Springs the same day of the application. In Clayton, applications were done to incorporate the herbicide with rainfall events occurring within 48 h after application.

Each individual treatment was applied to 9-m^2 plots. The plots were planted with 100 seeds of carinata 'Avanza 641' along a 1.0-m-long furrow in the middle of the plot. Stand counts were performed 30, 57, and 103 d after planting (DAP) to evaluate the effect of herbicide carryover on crop emergence and survival. The field trial was conducted as a randomized complete block design with four replications in both locations.

Greenhouse Bioassay

One week after planting carinata, two undisturbed soil cores (4.5-cm diameter and 61-cm length) were taken from each plot and inserted in clear polyethylene sleeves using a hydraulic probe equipped with a quick-release cutting head (Giddings Machine, Windsor, CO, USA). Soil cores were separated into two groups: (1) cores for greenhouse bioassays and (2) cores for further herbicide residue analysis. Both groups were stored separately at −12 C until analysis. Bioassays were conducted as randomized complete block designs with four replications using cores from Clayton and Jackson Springs.

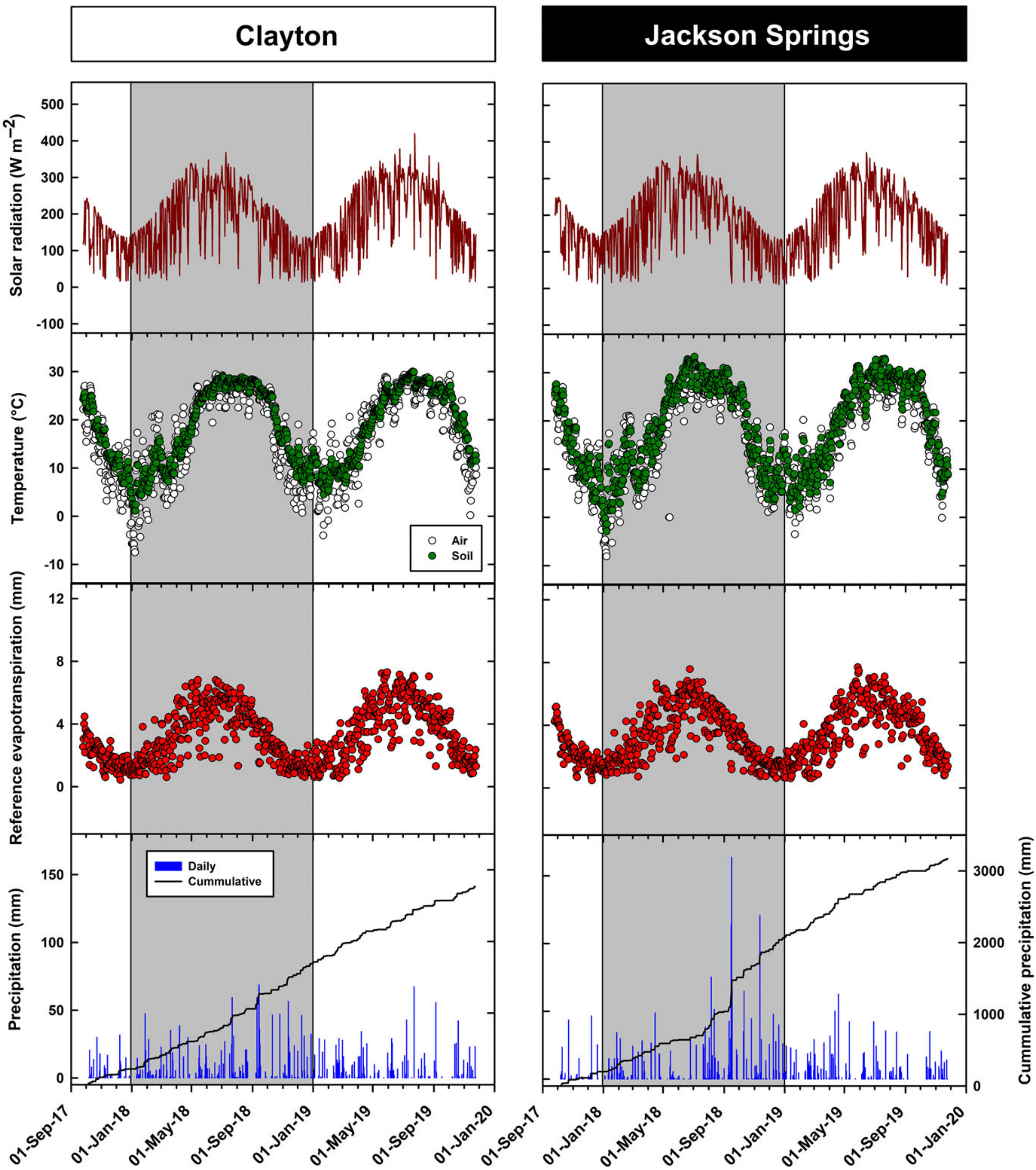

Figure 1. Soil and climatic variables assessed for two different locations in North Carolina where carinata trials were conducted during a period of 2 yr. Data points and bars represent daily average values. Data were collected from the first application at 24 mo before planting until soil core collection.

Soil cores from the first group were transferred to the greenhouse at 102 d after collection, placed horizontally, and fixed to a specially designed bench to avoid rolling. A 2-cm-wide opening was carefully cut along the sleeves without disturbing the soil using a Dremel tool (Dremel, Racine, WI, USA). A 0.5-cm-deep furrow was dug into the exposed soil, and carinata seeds were planted every 2 cm, starting from 0-cm depth to the end of the core. Soil cores were irrigated three times per day to maintain soil moisture at favorable levels for seed germination and plant growth. Daily mean temperature in the greenhouse was 24 C and ranged from 19 to 29 C.

Plant damage due to herbicide residues was visually estimated at 45 DAP. Three variables were evaluated: (1) closest distance from the soil surface in which damage was observed, (2) farthest distance from the soil surface in which damage was observed, and (3) visually estimated plant damage in the region between the closest and the farthest distance from the soil surface where damage was observed, with 0% = no damage, 50% = deformed, and 100% = missing or dead seedling.

Preemergence Herbicide Rate Study

Parallel to the first study, a second field experiment and greenhouse bioassay were conducted to evaluate the effect of decreasing rates of flumioxazin and imazapic in carinata when applied preemergence. These experiments were conducted as previously described for the carryover study, but imazapic and flumioxazin were applied at planting (0 MBP) at 1X, 0.5X, 0.25X, 0.125X, and 0.068X their recommended rates, 70 and 107 g ha^{-1}, respectively. In addition, soil cores were collected for the greenhouse bioassay and further herbicide residue analysis as described for the carryover study.

Herbicide Residues in Soils

A third study was conducted to evaluate flumioxazin and imazapic movement and behavior in soils from Clayton and Jackson Springs and further associate their soil residues with plant establishment and damage for carinata. This study was conducted using second group of soil cores taken from the field trials previously mentioned in both carryover and preemergence herbicide sections.

Soil Sample Preparation

Soil cores were moved to a lab bench and allowed to thaw for 8 h. These soil cores were horizontally dissected into four soil-depth segments: 0 to 5, 5 to 10, 10 to 15, and 15 to 20 cm. To avoid cross contamination, putty knives used for segmenting were decontaminated between cuts using ammonia:water (2:1 v/v) solution and dried using disposable paper towels. Each soil segment was homogenized by adding 200 g of pulverized dry ice and passing it through a soil grinder SA-45 with a 2.0-mm-sieve screen (Global Gilson, Lewis Center, OH, USA).

Herbicide Residue Analysis

Flumioxazin was extracted from corresponding samples by combining 20 g of processed soil with 25 ml of acetonitrile (Optima® LC/MS, Fisher Chemical, Fair Lawn, NJ, USA) in high-density polyethylene conical containers (225 ml). These containers were shaken for 45 min at 200 oscillations min^{-1} in an orbital shaker (KS501, IKA Works, Wilmington, NC, USA) and further centrifuged for 10 min at 3,500 rpm (Allegra 6KR centrifuge, Beckman Coulter, Indianapolis, IN, USA). A 10-ml aliquot of supernatant was collected from each soil sample, and 1 ml of this aliquot was filtered using a 0.45-μm PTFE membrane (VWR International, Radnor, PA, USA). The aliquot was then analyzed using high-performance liquid chromatography–mass spectrometry (Agilent-6120 Infinity, Agilent Technologies, Wilmington, DE, USA) coupled with a rapid-resolution high-definition column (Agilent ZORBAX RRHD SB-C18, Agilent Technologies).

For imazapic extraction, the same protocol was implemented using 25 ml of methanol (Optima® LC/MS, Fisher Chemical) instead of acetonitrile. The corresponding analyte concentrations were quantified using peak area measurements (OpenLAB CDS ChemStation, v. C.01.04, Agilent Technologies). For flumioxazin, the limit of quantification was 2.03 ng g^{-1} of dry soil, and the limit of detection was 1.01 ng g^{-1} of dry soil; for imazapic, those limits were 0.67 and 0.34 ng g^{-1} of dry soil, respectively. In addition, 20 g of soil were taken from each processed sample to estimate gravimetric soil moisture content (g g^{-1}) following Topp and Ferré's (2002) procedures to report data on a dry soil mass basis.

Recovery values of the total herbicide applied were calculated for each soil sample analyzed, using the analyte amount extracted and nominal application rates (1X) for each herbicide: 70 and 107 g ha^{-1} for imazapic and flumioxazin, respectively, and the decreasing fraction rates as mentioned in the preemergence herbicide rate application study. This calculation was performed using the following equation:

$$\%\ \text{recovery} = \left[\frac{\text{Total analyte recovered per soil sample (ng)}}{\text{Total analyte applied per soil sample (ng)}}\right] \times 100 \qquad [1]$$

The limits of quantification and detection were also expressed as recovery percentage of total applied. For flumioxazin, the limit of quantification was 1.36% and the limit of detection was 0.68% of total applied; for imazapic, those limits were 0.69% and 0.34% of total applied, respectively. Fortification recovery control of imazapic for soil samples ranged from 89% to 103% for Jackson Springs soil and from 93% to 99% for Clayton soil. The recovery controls of flumioxazin varied from 90% to 103% for Jackson Springs soils, and from 84% to 95% for Clayton soils.

Statistical Analysis

For the herbicide carryover study, an ANOVA was performed for plant density and damage using PROC GLIMMIX, where the factors location (L), herbicides (H), time of application (T), and their corresponding interactions were considered fixed effects, while factor block was considered random. Treatments were compared with the nontreated control using the Dunnett test with a significance level of $\alpha = 0.05$. Quadratic plateau models (nonlinear regression) were fit for herbicide carryover risk based on the results obtained from the greenhouse bioassay. Plant damage was selected as the dependent variable (y) and the herbicide time of application before planting (MBP) as the independent variable (x). These quadratic plateau models were fit as follows:

$$y = \begin{cases} y = a + bx + cx^2, & \textit{if } x \le x_0 \\ y_0, & \textit{if } x > x_0 \end{cases} \qquad [2]$$

where a, b, and c are the intercept, the linear coefficient, and the quadratic coefficient, respectively; x_0 is the critical value occurring at the intersection of the quadratic response; and y_0 is the plateau.

An ANOVA was performed for plant density and damage within the preemergence herbicide application study using PROC GLIMMIX, where location (L), herbicide (H), and preemergence rate (R), and their corresponding interactions were considered fixed effects, while block was considered random. For the preemergence herbicide rate studies under both field and greenhouse conditions, quadratic plateau models (Equation 2) were fit for plant density and plant damage as dependent variables (y), and the independent variable (x) was preemergence rate (R).

For the herbicide residues assessed in the soil samples, an ANOVA was performed using PROC GLIMMIX, where location (L), herbicide (H), time of application before planting (MBP), soil depth (SD), and their corresponding interactions were considered fixed effects, while factor block was set up as random. Means were separated using the Bonferroni test with a significance level of $\alpha = 0.05$.

Plant damage and plant population data were combined by herbicide. Quadratic plateau models (Equation 2) were fit to describe the behavior of plant damage or plant density change in response

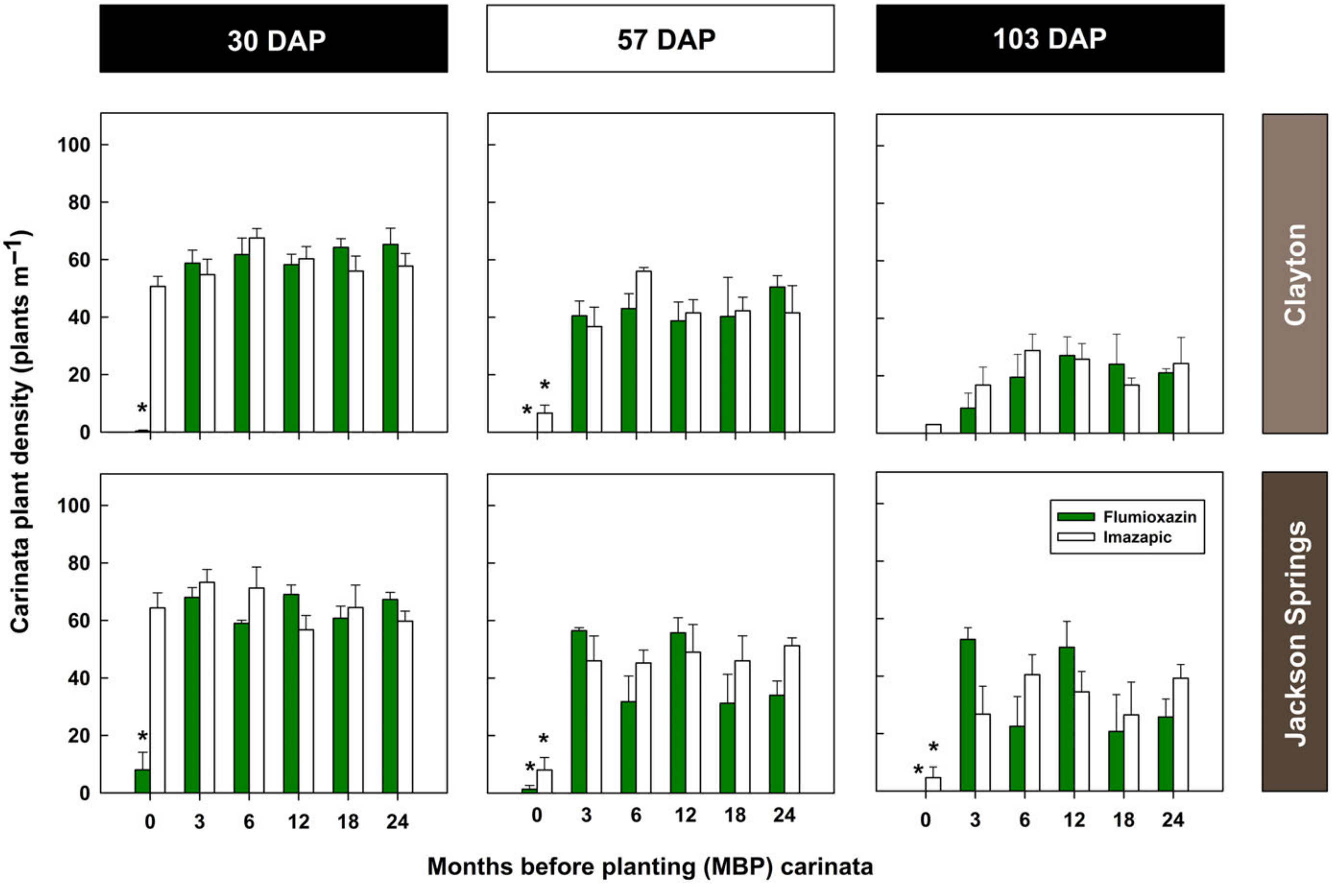

Figure 2. Carinata population density (plants per meter of row) in response to application interval (months before planting, MBP) using two herbicides at two locations in North Carolina. Evaluations were conducted 30, 57, and 103 d after carinata planting (DAP). Error bars represent the standard error of the mean ($n = 4$). An asterisk (*) indicates significant differences compared with the nontreated control according to a Dunnett test (P-value < 0.05). Imazapic and flumioxazin were applied at 70 and 107 g ha^{-1}, respectively.

to soil herbicide concentration (ng g^{-1} of soil). From these models, maximum herbicide concentration thresholds were set at 25% carinata damage or population density reduction. This arbitrarily chosen percent is within the range of tolerable density reductions without impacting yield (Mulvaney et al. 2019), and plants suffering 25% damage tend to recover to levels of nontreated plants (Leon et al. 2017).

Data were analyzed with SAS (v. 9.4, SAS Institute, Cary, NC, USA). All regression models were fit using the package EASYNLS in R Studio (R v. 4.0.4, 2021-02-15) "Lost Library Book" (R Studio Team 2015), and further optimized using PROC NLIN in SAS.

Results and Discussion

Carryover Herbicide Effects on Carinata under Field and Greenhouse Conditions

Carinata crop stands under field conditions exhibited a decreasing trend during the three evaluations performed after planting. This behavior has been previously described for this plant species as "self-thinning" due to intraspecific competition among the emerged plants (Mulvaney et al. 2019; Seepaul et al. 2021). Reductions in population density are not necessarily a major problem for production, because carinata has a high degree of compensatory ability in response to density changes by modifying the level of branching of the plant. Thus, maximum yield can be achieved under a large range of plant densities (Seepaul et al. 2021). High densities will have more plants with fewer branches and inflorescences, while low densities will result in plants with abundant branching and reproductive structures. Therefore, as long as growers use high planting densities, yield goals can still be achieved even if there is some level of reduction in carinata crop stand resulting from herbicide carryover. However, it is the combined effect of herbicides on plant density and plant damage that represents the greatest risk to production, as observed with imazapic in other rotational crops (e.g., corn [*Zea mays* L.] and cotton; Ulbrich et al. 2005; York et al. 2000). In this regard, imazapic residues and corresponding concentration thresholds for plant damage and reduction in plant density in the present study were similar to those reported in the literature for yield reductions in cotton with other herbicides of the imidazolinone family such as imazaquin (Barnes et al. 1989).

Carinata exhibited self-thinning, evidenced as reduction in plant density across treatments for Clayton (from 62 plants m^{-1} at 30 DAP to 27 plants m^{-1} at 103 DAP) and Jackson Springs (from 68 plants m^{-1} at 30 DAP to 52 plants m^{-1} at 103 DAP).

Flumioxazin applied preemergence (0 MBP) in both locations presented the lowest plant density at 30 DAP, 0.33 and 8 plants m^{-1}, respectively, which was considerably lower than the corresponding nontreated control (62 plants m^{-1} in Clayton and 68 plants m^{-1} in Jackson Springs; Dunnett test P < 0.0001). Interestingly, 27 d later (57 DAP), imazapic presented similar effects on plant density at both

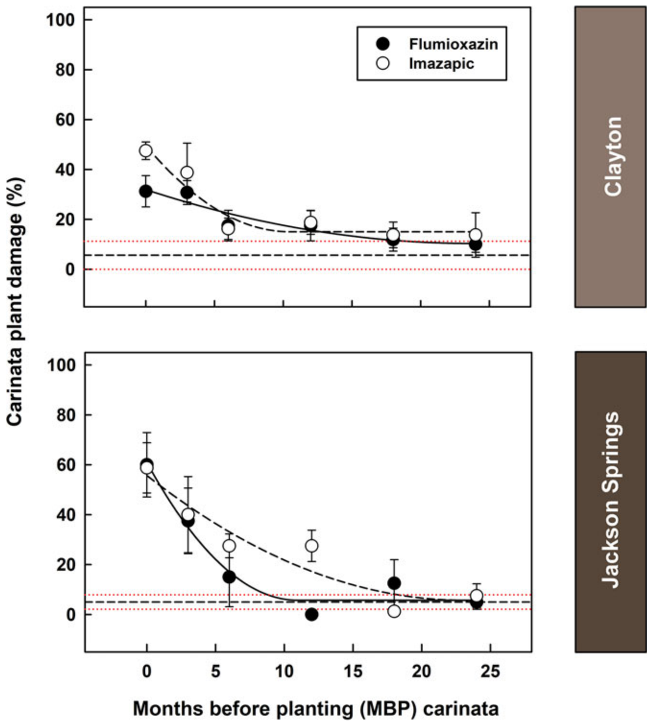

Figure 3. Plant damage from two locations in North Carolina in response to application interval before carinata planting using two herbicides in carinata. Black solid and dashed curved lines represent the best-fit model for imazapic and flumioxazin, respectively. Error bars represent the standard error of the mean ($n = 4$). Horizontal dashed black line indicates the average plant damage (due to frost) observed in the nontreated control, and dotted red lines represent standard error.

locations. Thus, both herbicides exhibited the lowest carinata density among preemergence treatments (Figure 2) after 2 mo with values of <5 plants m^{-1} (both Jackson Springs and Clayton) for flumioxazin and 8.0 and 6.7 plants m^{-1} (Jackson Springs and Clayton, respectively) for imazapic. Those crop densities represented considerable reductions compared with nontreated controls (53 plants m^{-1} in Clayton and 54 plants m^{-1} in Jackson Springs; Dunnett test $P < 0.0001$). Also, when imazapic or flumioxazin was applied at 3 MBP or at longer application intervals (e.g., 12 to 24 MBP), plant density values did not differ from the nontreated control, regardless of location (Figure 2). This last result could suggest a carinata plant-back not earlier than 3 MBP if imazapic or flumioxazin was previously used in other rotational crops (e.g., cotton, soybean).

In addition to plant density, carinata damage was assessed under greenhouse conditions (Figure 3). The highest value for plant damage was observed for both herbicides when applied at planting, regardless of location. In Clayton, imazapic and flumioxazin caused 48% and 31% damage, respectively; in Jackson Springs, these same herbicides caused 59% and 60% damage, respectively (Figure 3).

Plant damage decreased as the time between application and planting increased for both herbicides at both locations. This behavior was described using quadratic plateau regression models, for which R^2 ranged from 0.40 to 0.58 for flumioxazin and 0.50 to 0.64 for imazapic (Table 2). From those results (Figure 3), we identified the critical preplant intervals for application, such that carinata may not be negatively affected by herbicide residues. For example, carinata density may have remained high and stable when the herbicides were applied >6 MBP at both locations (Figure 3). In Clayton, the preplant interval to avoid a ≥25% damage by imazapic or flumioxazin was 6 MBP, while in Jackson Springs it was 12 MBP (Figure 3).

The results indicate that carryover issues might be noticeable during establishment or later. This latter scenario is likely to occur due to: (1) the persistence and mobility reported for the ALS-inhibitor herbicide family (de Assis et al. 2021; Marchesan et al. 2010); (2) subsequent root growth and interception of the metabolite at deeper soil horizons (Souza et al. 2020); and (3) the slow rate of the mechanism of action, which must first deplete amino acid seed reserves before symptoms of herbicide toxicity affect the plant (Webster and Masson 2001). This might explain why reductions in plant density due to imazapic, in contrast to flumioxazin, were not observed at 30 DAP but were evident at 57 DAP. This same behavior has been also observed in cotton, where plants did not show damage or mortality until 14 d after imazapic application (Grey et al. 2005).

Preemergence Herbicide Rate Effects on Carinata under Field and Greenhouse Conditions

Except for imazapic assessed at 30 DAP at both locations, there was a decrease in carinata density as herbicide rate increased up to 1X rate for both herbicides (Figure 4). Imazapic's effect on plant density was evident only until 57 DAP at both locations, clearly showing that imazapic's effect on carinata was slower than that of flumioxazin. This decreasing trend in plant density in response to herbicide rate was best described with quadratic plateau regression models, with R^2 ranging from 0.80 to 0.90 for flumioxazin and 0.52 to 0.78 for imazapic (Table 3). These regression models included the critical inflection point indicating the rate above which plant density reached a minimum and did not change with further increases in herbicide rate. For instance, in Jackson Springs at 57 DAP, the critical rates for imazapic and flumioxazin were 48.3 g ha^{-1} (0.69X) and 21.4 g ha^{-1} (0.20X), respectively. The critical values in Clayton were 14.7 g ha^{-1} (0.21X) and 18.19 g ha^{-1} (0.17X) for imazapic and flumioxazin, at the same evaluation date, respectively (Figure 4). However, even at the lowest evaluated rates (6.68 and 4.38 g ha^{-1} for imazapic and flumioxazin, respectively), both herbicides caused 50% to 60% reductions in plant density compared with the nontreated control (Figure 4).

Carinata damage increased exponentially as rate increased for both herbicides (Figure 5) until reaching a point at which further increments did not change damage. Regression models fit to describe this pattern presented R^2 values from 0.36 to 0.69 for imazapic and from 0.36 to 0.57 for flumioxazin (Table 4). The herbicide rate to cause 25% damage was 5.25 and 6.30 g ha^{-1} (0.075X and 0.09X) for imazapic in Clayton and Jackson Springs, respectively, and was 5.35 and 10.70 g ha^{-1} (0.05X and 0.10X) for flumioxazin in Clayton and Jackson Springs, respectively (Figure 5).

Total Herbicide Recovery from Soils for Imazapic and Flumioxazin

When imazapic or flumioxazin was applied at 12 and 18 MBP at the recommended label rate (1X), the recovered herbicide amounts from soil at both location soils were <2%. As the application interval decreased to 6 and 3 MBP, residue recovery in the soil increased for both herbicides, although it was greater for imazapic than flumioxazin at both 3 and 6 MBP (Table 5). For instance, in Jackson Springs, imazapic recovery was 7.96% and 3.61%, for 3 and 6 MBP, respectively, at a soil depth between 15 and 20 cm (Table 2).

Table 2. Regression model and fit parameters to predict carinata damage in response to interval between applications and planting.[a]

Location	Herbicide	*a*	*b*	*c*	R^2	*F*	pr > *F*	AIC[b]
Clayton	Imazapic	49.67 ± 6.11	−6.62 ± 1.17	0.09 ± 0.08	0.50	7.2	0.0042	198.41
	Flumioxazin	31.94 ± 3.48	−1.81 ± 0.27	0.04 ± 0.01	0.40	10.49	0.0007	184.54
Jackson Springs	Imazapic	55.70 ± 5.30	−4.33 ± 0.40	0.09 ± 0.01	0.64	17.58	<0.0001	194.56
	Flumioxazin	60.95 ± 8.34	−10.06 ± 1.61	0.46 ± 0.11	0.58	14.38	0.0001	213.38

[a] $y = \begin{cases} y = a + bx + cx^2, & if\ x \le x_0 \\ y_0, & if\ x > x_0 \end{cases}$, where *y* is damage in percent; *x* is application interval (months before planting); *a*, *b*, and *c* are the intercept, the linear coefficient, and the quadratic coefficient, respectively; x_0 is the critical value occurring at the intersection of the quadratic response; and y_0 is the plateau. These results are complementary to Figure 3. *a*, *b*, and *c* values: ±SE.
[b] AIC, Akaike information criterion.

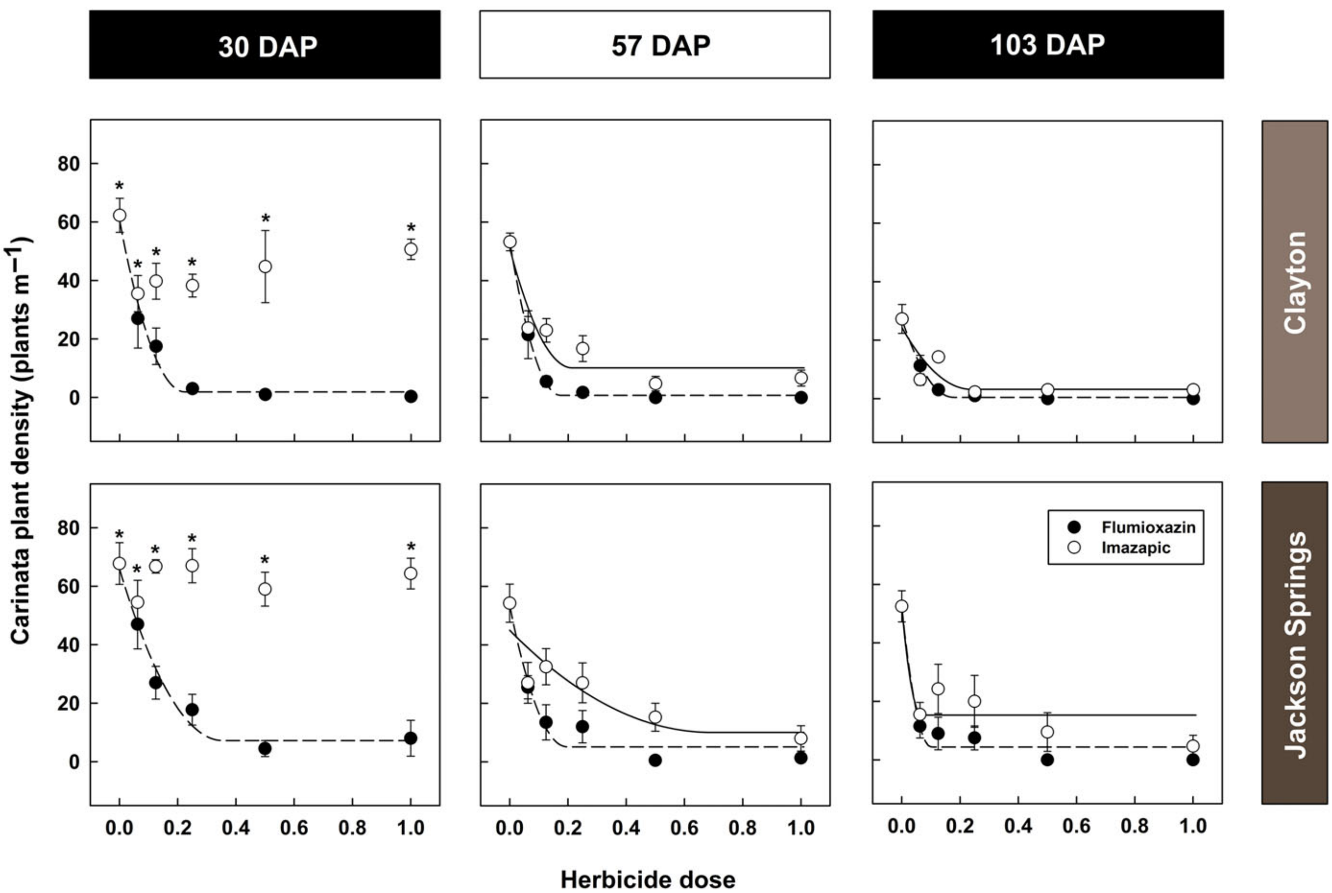

Figure 4. Effect of increasing rates (as fractions of the recommended rate, 1X) of two herbicides on carinata population evaluated in two locations in North Carolina. Black solid and dashed lines represent the best-fit model for imazapic and flumioxazin, respectively. Evaluations were conducted at 30, 57, and 103 d after carinata planting (DAP). An asterisk (*) indicates that no regression model presented a good fit for these data. Error bars represent the standard error of the mean ($n = 4$). Full rates (1X) for imazapic and flumioxazin were applied at time zero using recommended label rates of 70 and 107 g ha^{-1}, respectively.

Conversely, flumioxazin remained in the top 5 cm of the soil, with a small movement down to 10-cm depth as observed for 3 and 6 MBP in both locations. In Clayton, flumioxazin recovered residues at a soil depth of 5 to 10 cm were 2.08% for 3 MBP and 3.05% for 6 MBP. Flumioxazin recovery from soil Jackson Springs ranged from 3.90% to 2.14% for 3 and 6 MBP, respectively (Table 5). Herbicides from the imidazolinone family are persistent in soil, and under optimum conditions, they can remain in the soil for extended periods ranging from 371 to 705 d after application (Marchesan et al. 2010). Imazapic has been described as a highly persistent herbicide in soil, with slow rates of degradation and minimal volatilization (Aichele and Penner 2005; Ulbrich et al. 2005).

Soil adsorption affinity expressed as K_d for imazapic is 0.10 to 0.23 in soils with textural classes ranging from clay to loamy sand (Goldwasser et al. 2021). In weathered soils of Brazil (Ultisols and Oxisols), K_d for imazapic was 0.25 to 0.052 for sandy clay loams and loamy sands, respectively (de Assis et al. 2021). These low K_d values, coupled with high solubility (2,150 mg L^{-1}), make

Table 3. Regression model and fit parameters to predict carinata density in response to herbicide rate in two North Carolina locations.[a]

Evaluation[b]	Location	Herbicide	*a*	*b*	*c*	R^2	*F*	pr > *F*	AIC[c]
		Imazapic[d]							
30 DAP	Clayton	Flumioxazin	60.37 ± 4.97	−523.60 ± 69.44	1,171.80 ± 73.49	0.82	46.82	<0.00001	179.47
		Imazapic[d]							
	Jackson Springs	Flumioxazin	66.09 ± 4.76	−332.40 ± 46.02	469.20 ± 64.08	0.80	41.22	<0.00001	183.20
		Imazapic	50.84 ± 4.01	−379.80 ± 55.87	886.00 ± 58.16	0.78	34.64	<0.0001	169.63
57 DAP	Clayton	Flumioxazin	53.05 ± 3.21	−604.00 ± 44.02	1,743.40 ± 42.74	0.90	88.56	<0.0001	159.45
		Imazapic	44.99 ± 4.22	−101.10 ± 33.54	73.17 ± 78.48	0.52	10.63	0.0007	188.77
	Jackson Springs	Flumioxazin	53.52 ± 4.42	−491.70 ± 61.09	1253.80 ± 61.56	0.80	40.61	<0.0001	174.43
		Imazapic	24.24 ± 3.06	−176.70 ± 42.93	370.10 ± 46.73	0.62	16.23	<0.0001	157.08
	Clayton	Flumioxazin	27.15 ± 2.24	−303.50 ± 30.76	861.20 ± 30.01	0.83	47.52	<0.0001	142.91
103 DAP	Jackson Springs	Imazapic	52.50 ± 6.91	−1,097.10 ± 162.50	8,082.30 ± 141.10	0.54	11.98	0.0004	190.90
		Flumioxazin	52.50 ± 4.13	−947.80 ± 98.29	4,669.40 ± 88.30	0.84	53.8	<0.0001	167.17

[a] $y = \begin{cases} y = a + bx + cx^2, & \text{if } x \le x_0 \\ y_0, & \text{if } x > x_0 \end{cases}$, where *y* is carinata plant density; *x* is herbicide rate relative label rate (0 to 1); *a*, *b*, and *c* are the intercept, the linear coefficient, and the quadratic coefficient, respectively; x_0 is the critical value occurring at the intersection of the quadratic response; and y_0 is the plateau. These results are complementary to Figure 4. *a*, *b*, and *c* values: ±SE.
[b] DAP, days after planting.
[c] AIC, Akaike information criterion.
[d] No regression model presented good fit for this data.

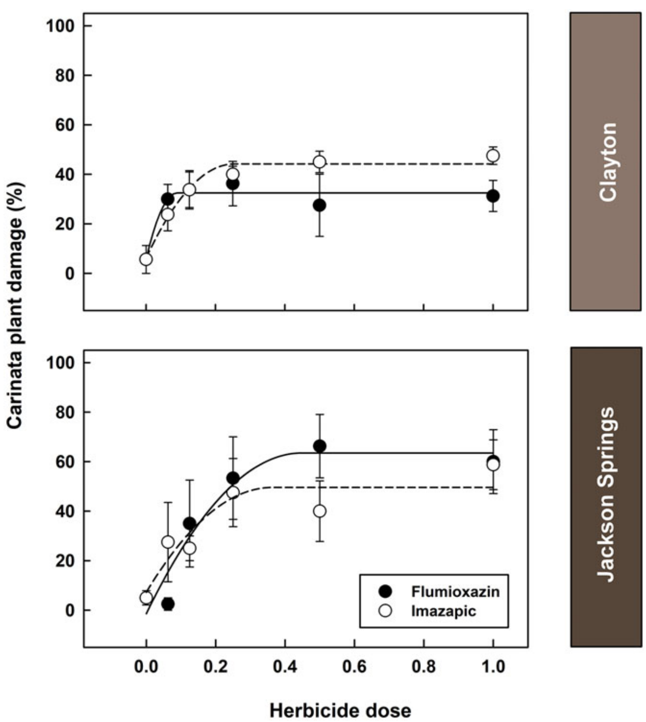

Figure 5. Plant damage from two locations in North Carolina to evaluate the effect of increasing rates (as fractions of the recommended rate, 1X) of two herbicides in carinata. Black solid and dashed lines represent the best-fit model for imazapic and flumioxazin, respectively. Error bars represent the standard error of the mean ($n = 4$). Full rates (1X) for imazapic and flumioxazin were applied using recommended label rates of 70 and 107 g ha^{-1}, respectively.

imazapic leaching possible, especially in coarse-textured soils with low organic matter content (de Assis et al. 2021; Neto et al. 2017). Similarly, there was higher imazapic recovery from soils in Jackson Springs (sand-textured soils and higher precipitation values) compared with Clayton (Table 1; Figure 1). Conversely, flumioxazin has higher adsorption affinity than imazapic. For instance, K_d values of 0.4 to 3.8 have been reported for soils with textural classes ranging from sandy clay loam to loamy sand (Ferrell and Vencill 2003a). In addition, flumioxazin is less mobile in soil, with a tendency to remain in the first 5 cm of the soil surface (Chen et al. 2021). Furthermore, its persistence in the soil is considerably lower than that reported for imazapic (Alister et al. 2008; Ferrell and Vencill 2003a). Flumioxazin degradation rate is affected by temperature, soil moisture, and organic matter, which influence microorganism activity and decrease the stability of this herbicide in the soil (Chen et al. 2021; Ferrell and Vencill 2003a). As microbial activity increases, flumioxazin's half-life and persistence decrease.

Herbicide Residue Damage and Survival Thresholds in Carinata

As concentration of herbicide residues recovered from the soil (ng g^{-1} of soil) increased, there was a corresponding increase in plant damage for both imazapic and flumioxazin (Figure 6). Regression models fit to describe this pattern in plant damage presented R^2 values of 0.42 and 0.35 for imazapic and flumioxazin, respectively. Using these models, we estimated that the concentration to cause at least 25% plant damage was 7.78 and 6.90 ng g^{-1} of soil for imazapic and flumioxazin, respectively.

The same approach was used to identify herbicide concentration thresholds that would decrease carinata density by 25% compared with the nontreated control. The corresponding regression models fit to describe the decrease in plant density, presented R^2 values of 0.36 and 0.39 for imazapic and flumioxazin, respectively (Figure 7). For flumioxazin, it was estimated that at 12.7 ng g^{-1} of soil, the plant density would decrease from 43 to 33 plant m^{-1} (corresponding to a 25% decrease in plant density). Meanwhile, this threshold corresponded to 14.7 ng g^{-1} of soil for imazapic (Figure 7).

Practical Considerations for Imazapic and Flumioxazin Use in Carinata-Cropping Systems

Our results highlight the importance of considering persistence and mobility of imazapic and flumioxazin when assessing plant damage and carryover effects on carinata. For instance, if a field bioassay is intended to determine whether the residues of imidazolinone herbicides are low enough to ensure safe carinata planting, it is crucial to consider soil properties and sampling depths (Horowitz 1976; Winton and Weber 1996). If imazapic was

Table 4. Regression model and fit parameters to estimate carinata damage in response to herbicide rate when applied preemergence.[a]

Location	Herbicides	*a*	*b*	*c*	R^2	*F*	pr > *F*	AIC[b]
Clayton	Imazapic	6.14 ± 4.01	299.67 ± 35.94	−590.05 ± 44.47	0.69	23.66	<.0001	183.90
	Flumioxazin	5.63 ± 7.12	597.70 ± 168.90	−3,323.24 ± 148.60	0.36	5.71	0.0109	192.23
Jackson Springs	Imazapic	7.24 ± 8.83	232.86 ± 85.96	−320.10 ± 120.00	0.36	6.48	0.0064	220.55
	Flumioxazin	−1.41 ± 9.70	289.08 ± 108.60	−321.81 ± 176.80	0.57	13.42	0.0002	214.23

[a] $y = \begin{cases} y = a + bx + cx^2, & \text{if } x \le x_0 \\ y_0, & \text{if } x > x_0 \end{cases}$, where *y* is damage in percent; *x* is herbicide rate relative label rate (0 to 1); *a*, *b*, and *c* are the intercept, the linear coefficient, and the quadratic coefficient, respectively; x_0 is the critical value occurring at the intersection of the quadratic response; and y_0 is the plateau. These results are complementary to Figure 5. *a*, *b*, and *c* values: ±SE.
[b] AIC, Akaike information criterion.

Table 5. Effect of the herbicide application interval before carinata planting on total recovery of two herbicides in soils from two locations in North Carolina.

			Recovery of the total applied[a]				
Location	Herbicide	Depth	0 MBP	3 MBP	6 MBP	12 MBP	18 MBP
		cm			%		
Clayton	Flumioxazin	0–5	79.34 B	4.15 HI	1.77 I	0.00 I	0.00 I
		5–10	0.00 I	2.08 I	3.05 HI	0.03 I	0.00 I
		10–15	0.00 I	0.00 I	0.01 I	0.00 I	0.00 I
		15–20	0.00 I	0.00 I	0.00 I	0.00 I	0.00 I
	Imazapic	0–5	93.72 A	23.86 E	8.74 GHI	0.46 I	0.00 I
		5–10	1.81 I	32.86 D	11.50 FGH	1.88 I	0.00 I
		10–15	0.00 I	0.70 I	1.65 I	1.15 I	0.28 I
		15–20	0.00 I	0.00 I	0.00 I	0.13 I	0.00 I
Jackson Springs	Flumioxazin	0–5	57.67 C	4.10 HI	2.28 I	0.00 I	0.00 I
		5–10	19.71 EF	3.90 HI	2.14 I	0.05 I	0.00 I
		10–15	0.00 I	0.17 I	0.05 I	0.00 I	0.00 I
		15–20	0.00 I	0.00 I	0.00 I	0.00 I	0.00 I
	Imazapic	0–5	73.28 B	8.49 GHI	1.42 I	0.48 I	0.00 I
		5–10	18.33 EF	14.35 FG	3.67 HI	0.91 I	0.00 I
		10–15	2.13 I	13.09 FG	3.30 HI	1.79 I	0.15 I
		15–20	0.00 I	7.96 GHI	3.61 HI	0.70 I	0.14 I

[a] Percent of nominal application rates for each herbicide: 70 and 107 g ai ha^{-1} for imazapic and flumioxazin, respectively. Means followed by same letter are not significantly different according to Bonferroni test ($P < 0.05$). MBP, months before planting carinata.

previously applied to a sandy soil, planting bioindicators in the field and determining safety simply based on the number of emerged seedlings could be misleading because of the downward movement of this herbicide in the soil, especially if precipitation is sufficient to leach this herbicide to deeper layers in the soil profile, as observed in Jackson Springs (Figure 1; Table 2). A better approach would be to run the bioassay using soil samples collected from a range of depths.

Imazapic and flumioxazin are registered for preemergence control of dicotyledonous weed species in soybean, peanut, and cotton, and their use in the southeastern United States has been both extensive and intensive (Berger et al. 2012; Ferrell and Vencill 2003a; Matocha et al. 2003). Therefore, if carinata is incorporated as a winter third crop in an existing peanut–cotton rotation, selection of the appropriate preemergence herbicide will be critical to avoid herbicide carryover issues such as those described in the present study. For instance, the risk of carinata damage and reductions in plant density would be lower if flumioxazin was employed as the preemergence herbicide during the peanut or cotton cycle immediately preceding carinata. This type of rotational consideration has been used to ensure the safety of other Brassicaceae species. For example, daikon radish (*Raphanus sativus* L.) planted as a cover crop was affected by residual herbicides in peanut–cotton rotations, but imazapic reduced plant height more than flumioxazin (Price et al. 2020).

Soil properties should also be considered when selecting the proper herbicide as part of a well-designed crop rotation. For example, flumioxazin persistence in soil is highly dependent on organic matter and water content, which are directly involved in the microbial-mediated degradation of this herbicide (Chen et al. 2021; Glaspie et al. 2021). In addition, soil texture plays a major role in herbicides' sorption on soil particles and their bioavailability. As the clay fraction increases, the herbicide binds to clay, and its availability for microbial decomposition and mineralization decreases. Therefore, there will be considerable differences among soil textures for flumioxazin persistence (Ferrell and Vencill 2003a). Conversely, sandier soils with low organic matter content adsorb imidazolinone herbicides such as imazapic to soil particles less and have reduced microbial degradation, resulting in increased persistence and availability to damage crops (Marchesan et al. 2010). Therefore, when planning to grow carinata as a winter crop after rotational cotton or peanut, it is important to consider both soil physical and chemical properties and herbicide behavior in soil.

Our results provide a baseline for residue levels and application intervals that can be used to determine the risk of flumioxazin and imazapic carryover to carinata in sand- or loamy sand–

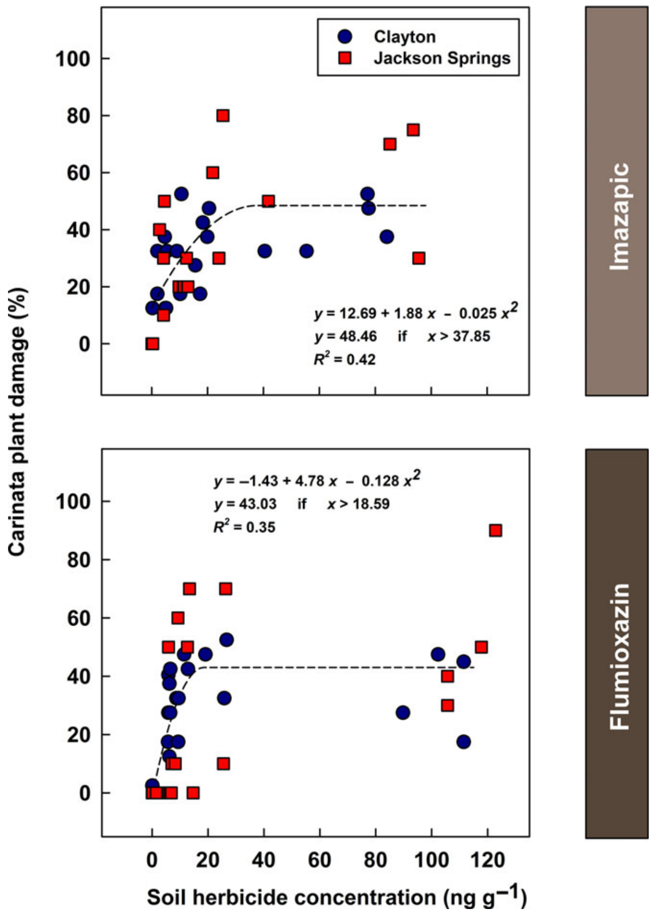

Figure 6. Soil herbicide recovered amount (expressed as ng ai g^{-1} of soil) and its effect on carinata plant damage observed at two locations in North Carolina. Black dashed lines represent the best-fit model selected for each herbicide.

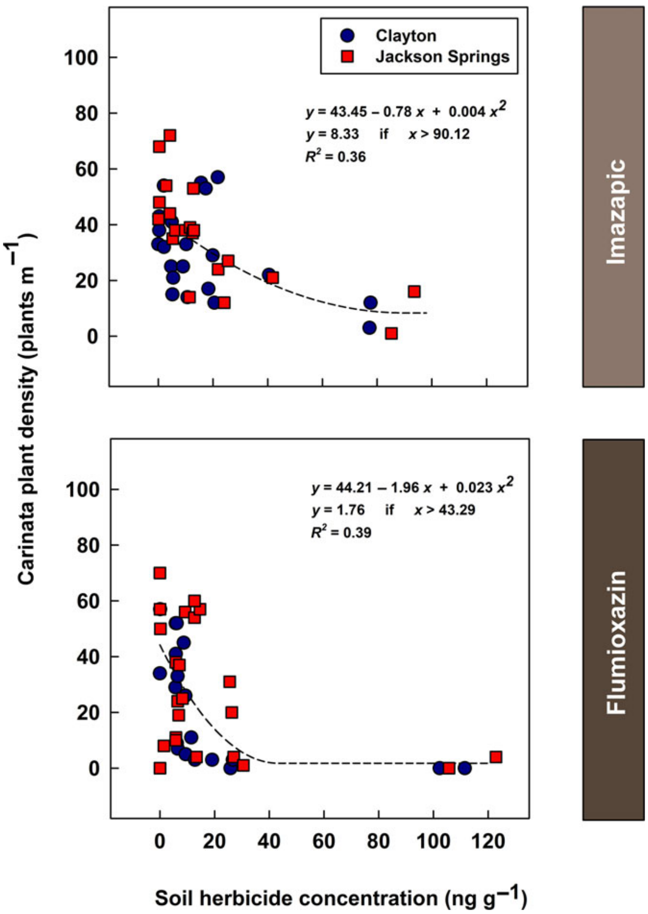

Figure 7. Soil herbicide recovered amount (expressed as ng ai g^{-1}) and its effect on carinata plant density assessed at two locations in North Carolina. Black dashed lines represent the best-fit model selected for each herbicide.

textured soils. Further research is needed on finer-textured soils (loam, silty loam, clay loam). It is important to caution that the present study focused on carinata seedling establishment. It will be necessary to confirm the safety of residue levels identified here during the entire growing season to ensure that yield is not adversely affected.

Carinata has recently been introduced as an alternative winter crop in the southeastern United States and may show promise for the diversification of crop rotations to manage herbicide-resistant weeds (Tiwari et al. 2021a, 2021b). However, concerns among growers about the risk of carryover of commonly used residual herbicides have hampered adoption of this crop.

Compared with Clayton, at Jackson Springs (where the cumulative precipitation and sand content were higher) imazapic was more persistent and moved to deeper layers within the soil, representing a risk to carinata plants even when applied at 6 MBP or at shorter intervals. Our results suggested that carinata can be planted safely if either imazapic or flumioxazin was applied at least 6 to 12 MBP, depending on soil and environmental conditions. When a peanut–cotton rotation incorporates carinata as winter crop, special caution must be taken to identify edaphic conditions as well as preemergence herbicide selection to avoid herbicide carryover and damage to carinata. Based on our results, the use of flumioxazin as a preemergence herbicide in the preceding summer crop is a better alternative than imazapic to ensure carinata safety.

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