This is a "preproof" accepted article for Weed Science. This version may be subject to change in the production process, *and does not include access to supplementary material*. DOI: 10.1017/wet.2024.74

Short title: Chloroacetamide in cole crops

Improving Cole Crops Safety and Weed Control Response with Chloroacetamide and Oxyfluorfen Herbicides Combinations

Lynn M. Sosnoskie¹ and Thierry E. Besançon²

¹Assistant Professor (ORCID 0000-0002-1892-7934), Department of Horticulture, Cornell University, Geneva, NY, USA;

²Associate Professor (ORCID 0000-0003-0194-9675), Department of Plant Biology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ, USA.

Author for correspondence: Thierry E. Besançon, Department of Plant Biology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ, USA. E-mail: <u>thierry.besancon@rutgers.edu</u>

This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is unaltered and is properly cited. The written permission of Cambridge University Press must be obtained for commercial re-use or in order to create a derivative work.

Abstract

In 2022, trials were carried out in New Jersey and New York to assess the efficacy of weed management and the response of two cole crops to various herbicide combinations and rates. The experiments involved the application of S-metolachlor and micro-encapsulated (ME) acetochlor either alone or combined with oxyfluorfen. Different application timings of oxyfluorfen were tested in greenhouse and field studies. Results from the greenhouse trials showed that substituting S-metolachlor with ME acetochlor in over-the-top applied mixes with oxyfluorfen caused 15% to 22% less crop injury and increased seedling biomass by 33%. In field studies, nontreated plots exhibited significant weed growth, reaching up to 71% coverage 28 days after transplanting (DAT), whereas herbicide-treated plots exhibited weed cover at or below 10% by 28 DAT. Mixtures or sequential applications of oxyfluorfen and chloroacetamides achieved excellent control (\geq 99%) of the weed species complex compared to single applications of oxyfluorfen or chloroacetamides. However, applying both oxyfluorfen and a chloroacetamides post-transplanting, either as tank mixture or in sequence resulted in $\geq 19\%$ injury. Despite the effective weed control achieved with herbicide treatments, mixing herbicides post-transplanting reduced relative commercial yield by 46% to 94% compared to oxyfluorfen applied alone or followed by chloroacetamides. The findings from these experiments will inform regional crop safety guidelines and support potential modifications to oxyfluorfen labels regarding sequential applications with chloroacetamides.

Nomenclature: acetochlor, oxyfluorfen; S-metolachlor

Keywords: Cole crops, herbicide injury, sequential herbicide applications, vegetables, yield

In 2022, cabbage (*Brassica oleracea* var. *capitata* L.) and broccoli (*Brassica oleracea* var. *italica* L.) were grown on 25,500 and 59,700 ha, respectively, in the United States (US) with a combined crop value of almost US \$1.5 billion (USDA NASS 2024). Cole crops, many of which have shallow root systems and are short in stature, can be susceptible to weed interference, especially when it occurs early in the season (Bell 1995; Bellinder 2012; Chen et al. 2011; Fennimore et al. 2010; Latif et al. 2021; Sikkema et al. 2007a, b; Smart et al. 2001; Yu et al. 2018). Competing weeds can significantly reduce yield in cabbage, with an estimated mean loss of 54% across 44 studies conducted over 20 yr (M. VanGessel, personal communication). In broccoli, even low weed densities can exceed the crop's economic threshold, resulting in delayed harvest and reduced yield (Bell 1995; Latif et al. 2021). While competition for shared resources is a significant concern in cole crops production, weeds can also have substantial indirect impacts, including supporting populations of *Brassica* pests and pathogens, and serving as occupational hazards for agricultural workers during harvest (Al-Khatib et al. 1995; Bridges 1994; Chen et al., 2011; Dillard and Hunter 1986; Guerena 2020; McErlich and Boydston 2013).

Herbicides are essential for managing weeds in cabbage and broccoli cultivation, yet their efficacy is limited by the few registered products available and their narrow control spectrum (Sikkema et al. 2007b; Wyenandt et al. 2024). S-metolachlor [Weed Science Society of America (WSSA) group 15; chloroacetamide] and oxyfluorfen (WSSA group 14; diphenylether) are important preemergence (PRE) herbicide ai for the control of many weed species in cole crop production including pigweeds (Amaranthus spp.), hairy galinsoga (Galinsoga quadriradiata Cav.), nightshade species (Solanum spp.), common lambsquarters (Chenopodium album L.) and smartweed (Polygonum spp.) species as well as many annual grasses (Al-Khatib et al. 1995; Anonymous 2020; Besançon et al. 2020; Bhowmik and McGlew 1986; Cutulle et al. 2023; Pineda-Bermudez et al. 2023; Yu and Boyd 2018). Oxyfluorfen also received 24c Special Local Need labels for postemergence (POST) use after a minimum of two weeks after transplanting of broccoli, cabbage and cauliflower in several states, including NJ and NY (Anonymous 2021, 2022). Although S-metolachlor post-transplant (POST-T) followed by (fb) oxyfluorfen POST 14 d after transplanting (DAT) has been shown to be an effective combination, the sequential use of oxyfluorfen and chloroacetamide herbicides in the same season is discouraged because of injury potential in cole crops (Anonymous 2020; Bellinder 2012). Pineda-Bermudez et al. (2023) reported >20% cabbage and broccoli injury, which was characterized by stunting and necrosis, in

response to *S*-metolachlor POST-T at 0.72 kg ai ha⁻¹ followed by oxyfluorfen POST at 0.21 kg ai ha⁻¹. Similar results were observed by Bellinder (2012) who documented almost 30% cabbage injury. While cabbage and broccoli can recover from some early-season herbicide-induced damage, adverse weather conditions that reduce crop vigor may negatively impact later growth and head development (Sosnoskie, personal observation).

Acetochlor (WSSA group 15; chloroacetamide) is registered for use in alfalfa (*Medicago sativa* L.), field corn (*Zea mays* L.), and soybean (*Glycine max* L.), among other crops, for the residual control of many common and troublesome grass and broadleaf weeds including barnyardgrass [*Echinochloa crus-galli* (L.) P.Beauv.], foxtails (*Setaria* spp.), *Panicum* spp., pigweeds, common lambsquarters, and *Galinsoga* spp (Lingenfelter et al. 2024; Van Wychen, 2022). Acetochlor can be formulated as an emulsifiable concentrate (EC) (Harness®; Bayer CropScience, Research Triangle Park, NC) or as a microencapsulated (ME) product (Warrant®; Bayer CropScience, Research Triangle Park, NC). Unlike the EC, the ME formulation has the ai enclosed in a matrix of polymers that slows the release of the herbicide (Guo et al. 2014). A few studies have demonstrated that ME acetochlor provides greater crop safety compared to the EC formulation when applied POST over foliage (Fogleman et al. 2018; Godwin 2017).

Few studies have explored the effectiveness of chloroacetamide herbicides in cole crops, including ME formulations that could enhance crop safety. Identifying herbicides that are safer to cabbage and broccoli, particularly when used in combination with oxyfluorfen, would greatly benefit growers by improving weed control efficacy. Preliminary greenhouse screenings conducted in NY and NJ showed reduced cabbage injury when ME acetochlor was applied POST in combination with oxyfluorfen or sulfentrazone as compared to *S*-metolachlor and EC acetochlor (Sosnoskie and Besançon, unpublished data). Thus, the objectives of this study were 1) to compare the weed control efficacy and crop safety of ME acetochlor and *S*-metolachlor, an EC, both alone and in combination with oxyfluorfen, and 2) to determine the optimal application sequence of chloroacetamide herbicides in relation to oxyfluorfen while still achieving satisfactory weed control.

Materials and Methods

Greenhouse experiments. Greenhouse trials were conducted in 2022 at Cornell AgriTech in Geneva, NY (42.87° N, 77.03° W), and at the Rutgers Philip E. Marucci Center for Blueberry and Cranberry Research in Chatsworth, NJ (39.42°N, 74.30°W). Cabbage 'Padoc' and broccoli 'Emerald Crown' cultivars were seeded in NY whereas cabbage 'Botran' and broccoli 'Imperial' cultivars were used in NJ. Five seeds were planted in 10-cm-square pots containing a commercial growing medium (SunGro[®] Horticulture Professional Growing Mix; Agawam, MA) and hand watered daily. Once emerged, plants were thinned to a single plant per pot and weekly fertilized with Jack's Professional General-Purpose 20-20-20 fertilizer (JR Peters, Inc., Allentown, PA) to provide a nitrogen concentration of 750 ppm. Greenhouses were set to a constant temperature of 20 C (\pm 2 C) with a 16 h d length in NY and a 12 h d length in NJ. Natural lighting was supplemented with high pressure sodium lamps equidistantly placed above the bench to deliver a photosynthetically active radiation flux density of 640 µmol·m⁻²·s⁻¹.

Herbicides treatments were applied over the top (OTT) of cabbage and broccoli plants at the 2to 3-leaf stages, on February 11 and March 25 at the NJ location, and on February 14 and April 18 at the NY site. Applications were made using a single-nozzle track spray chamber (DeVries Manufacturing, Hollandale, MN) equipped with an 8002EVS flat-fan Teejet® nozzle (TeeJet, Glendale Heights, IL) in New York, and a CO₂ backpack sprayer fitted with two XR8004VS nozzles (TeeJet Technologies, Glendale Heights, IL) spaced 46 cm apart in New Jersey. Both systems were calibrated to deliver 187 L ha⁻¹ at 103 kPa. Treatments included oxyfluorfen (GoalTender®; Nufarm Inc., Alsip, IL) at 210 g ha⁻¹ alone or mixed with *S*-metolachlor (Dual Magnum®; Syngenta Crop Protection LLC, Greensboro, NC) at 1,420 g ha⁻¹ or ME acetochlor (Warrant®; Bayer CropScience, St. Louis, MO) at 1,430 g ha⁻¹. Both chloroacetamide herbicides were also applied separately at the same rates. A nontreated control was included for comparison. The experiment was arranged in a randomized complete block design with ten replications per treatment; the study was conducted twice in time at each location.

Field experiments. In 2022, experiments to evaluate weed control efficacy and crop safety of *S*-metolachlor or ME acetochlor tank mixed or in sequence with oxyfluorfen in cabbage and broccoli were conducted at Cornell AgriTech in Geneva, NY and at the Rutgers Agricultural Research and Extension Center in Bridgeton, NJ (39.523611°N, 75.201944°W). Soil in Geneva was a Honeoye loam (fine-loamy, mixed, semiactive, mesic Glossic Hapludalfs) with 38% sand,

44% silt, 18% clay, 2.5% organic matter, and a pH of 6.3. The Bridgeton site was a Chillum silt loam soil (fine-silty, mixed, semiactive, mesic Typic Hapludults) with 54% sand, 28% silt, 18% clay, 2.4% organic matter content, and a pH of 5.7.

Prior to transplanting, all fields were cultivated to remove any emerged weeds and cultipacked to prepare a smooth bed for transplanting. Cabbage variety 'Padoc' and broccoli variety 'Emerald Crown' were used at both locations. In NY, the plots measured 7.6 meters in length and 3.1 meters in width. Each plot consisted of two rows of broccoli alongside two rows of cabbage, spaced 76.2 centimeters apart, with individual plant spacing within rows set at 51 centimeters. In NJ, the plots were slightly larger, measuring 9.1 meters in length and 3.1 meters in width, with the same arrangement of crops and spacing as in NY. Cole crops were manually transplanted into bare ground flat beds on May 10 in NY and on August 4 in NJ. All transplants had three to four leaves upon planting, and their root balls were buried at least 5 centimeters deep at both locations. Both sites received 1.5 centimeters of supplemental irrigation on the day of planting to aid in transplant establishment and to incorporate soil-applied herbicides. Additional irrigation was provided as necessary throughout the growing season in NJ, while the NY site relied primarily on rainfall. Dry conditions in Geneva (Table 1) did necessitate a second irrigation event after transplanting to promote crop development. Pest and crop management practices, including fertilization and insect and disease control, followed local guidelines at both sites (Wyenandt et al. 2024).

The trial was structured as a split-plot design comprising four replications. Herbicide treatments were designated as the main plots, while cole crop species served as the subplots. Single herbicide treatments consisted of oxyfluorfen (GoalTender[®]; Nufarm Inc., Alsip, IL) at 560 g ha⁻¹ applied PRE-T 24 h before transplanting (PRE-T), and *S*-metolachlor (Dual Magnum[®]; Syngenta Crop Protection LLC, Greensboro, NC) at 1,420 g ha⁻¹ and ME acetochlor (Warrant[®]; Bayer CropScience, St. Louis, MO) at 1,430 g ha⁻¹ applied OTT within 24 h of transplanting (POST-T). Tank mixed treatments included *S*-metolachlor at 1,420 g ha⁻¹ or ME acetochlor at 1,430 g ha⁻¹ plus oxyfluorfen at 560 g ha⁻¹ POST-T. One set of sequential treatments included oxyfluorfen (560 g ha⁻¹) PRE-T followed by (fb) *S*-metolachlor (1,420 g ha⁻¹) or ME acetochlor (1,430 g ha⁻¹) or ME acetochlor (1,430 g ha⁻¹) applied POST-T fb oxyfluorfen at 280 g ha⁻¹ 14 DAT (POST). A nontreated weedy control was included for comparison purposes. The

chosen application rates correspond to labeled recommendations with respect to soil texture and organic matter content. At both locations, treatments were applied using a CO₂ backpack sprayer calibrated to deliver 187 L ha⁻¹. Booms were fitted with two XR11002VS nozzles (TeeJet Technologies, Glendale Heights, IL) spaced 48 cm apart in NY, and four with XR8004VS nozzles (TeeJet Technologies, Glendale Heights, IL) set 46 cm apart in NJ.

Data collection: For the greenhouse trial, ratings included a visual evaluation of crop necrosis and stunting 14 d after treatment (DAT) using a scale ranging from 0% (indicating no visible damage or stunting) to 100% (indicating plant death or complete lack of growth). Aboveground plant biomass was collected individually 14 DAT, placed into paper bags, dried at 65 C for 96 h, and subsequently weighed.

Crop injury assessments were conducted at 14, 21, and 28 DAT. Injury, which primarily consisted of crop stunting with some minimal leaf burn, was rated on a scale from 0% (indicating no visible damage or stunting) to 100% (indicating plant death). Weed cover, a visual estimate of the percentage of plot area covered with weeds, was also evaluated at 14, 21, and 28 DAT using a scale ranging from 0% (no weed cover) to 100% (soil completely covered by weeds). In NY, at 14 and 28 DAT, individual weed plants were counted in a 0.25 m² quadrat positioned in the direct center of each cabbage and broccoli subplot within each herbicide treatment whole plot. For both locations, aboveground weed biomass was collected from two 0.25 m² quadrats placed in the center of each crop subplot at harvest. A single harvest occurred at both locations when a majority of cabbage and broccoli plants were considered U.S. No.1 according to USDA grades and standards (USDA 2006; USDA 2016). In NY and NJ, cabbage and broccoli were harvested on July 13 (65 DAT) and October 6 (63 DAT), respectively. At both locations, mean head wt was determined by averaging the data from 10 adjacent heads per row of cabbage and broccoli.

Statistical Analysis. Because of unequal variances, weed cover and crop injury data were arcsine square root transformed prior to analysis and back transformed for presentation of the data (Grafen and Hails 2002). Data were subjected to ANOVA using PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc., Cary, NC). Cole crop species and herbicide treatments were considered fixed effects whereas locations, runs nested within location, and replications nested within location by run were treated as random effects. When crop by herbicide treatment interactions were significant, data were separately analyzed by crop. In the absence of significant

interaction, data were combined over fixed effects and means comparisons between treatments were performed using Tukey's honest significance test at (α = 0.05). For the field experiments, orthogonal contrasts were used to evaluate differences for 1) the solo application of oxyfluorfen PRE-T or chloroacetamides POST-T compared to mixed and sequential applications (hereafter grouped under combined applications), 2) the solo application of oxyfluorfen PRE-T compared to both chloroacetamide herbicides applied alone POST-T, and 3) EC S-metolachlor compared to ME acetochlor in combined applications. A *P* ≤ 0.05 significance level was used for analysis of all orthogonal contrasts.

Results and Discussion

Greenhouse study.

Crop injury. The interaction between herbicide treatment and cole crop was significant for both crop necrosis (P = 0.024) and stunting (P = 0.0020). Thus, broccoli and cabbage data were examined separately (Table 2). Broccoli necrosis 14 DAT was minimal (\leq 3%) for both *S*-metolachlor and ME acetochlor OTT, when applied alone, but was significantly greater (7%) in response to oxyfluorfen. When mixed with oxyfluorfen, the EC formulation of *S*-metolachlor caused greater necrotic injury on broccoli (19%) than the ME formulated acetochlor (4%). The stunting response of broccoli to *S*-metolachlor and ME acetochlor OTT, when applied alone, was minimal (\leq 2%) compared to oxyfluorfen (11%). ME acetochlor in a tank mixture with oxyfluorfen caused less broccoli stunting (5%) than *S*-metolachlor (24%). For cabbage, necrosis was minimal for *S*-metolachlor, ME acetochlor, and oxyfluorfen when applied alone OTT (\leq 5%); ME acetochlor caused less burning (1%) to cabbage leaves as compared to a similar application of *S*-metolachlor (4%). Substituting *S*-metolachlor with ME acetochlor in a tank mix with oxyfluorfen reduced necrosis of cabbage seedlings from 27% to 6%. All OTT herbicide treatments resulted in \leq 6% cabbage stunting, except for *S*-metolachlor plus oxyfluorfen, which resulted in 26% stunting.

Crop relative dry biomass. There were no interactions between herbicide treatments and cole crops with respect to dry biomass, expressed as a percent of the nontreated check, so broccoli and cabbage data were combined (Table 2). *S*-metolachlor and ME acetochlor, applied alone, did not significantly reduce dry biomass compared to the nontreated control. Conversely, oxyfluorfen OTT reduced crop biomass 23% when applied alone and 44% when combined with

S-metolachlor. When oxyfluorfen was tank-mixed with ME acetochlor, crop biomass was reduced 17%.

Field study.

Weed coverage, control, and biomass. Prevalent species at the Geneva site included common ragweed, common lambsquarters, ladysthumb (*Persicaria maculosa* Gray), prostrate knotweed (*Polygonum aviculare* L.), and other *Polygonum* species, grouped under smartweed spp., as well as annual grasses including foxtails (*Setaria* spp.) and barnyardgrass. Pigweeds and crabgrasses were present in low numbers in the NY trial. Dominant species at the Bridgeton site included hairy galinsoga, common lambsquarters, and annual grasses including stinkgrass [*Eragrostis cilianensis* (All.) Vignolo ex Janch.] and goosegrass [*Eleusine indica* (L.) Gaertn.].

For all observation timings, weed cover and weed control were significantly different for herbicide treatment (P < 0.0001), but not for crop (P = 0.9499) nor the interaction between herbicide treatments and crops (P = 0.8603); consequently, data were combined over crops (Tables 3 and 4). Weed cover in the nontreated check was 9, 20, 35, and 71% at 7, 14, 21, and 28 DAT, respectively. Weed cover was $\leq 3\%$ at 7 and 14 DAT for all herbicide treatments (data not shown. At 21 and 28 DAT, weed cover ranged from 5-10% where *S*-metolachlor and ME acetochlor were applied alone POST-T; this is higher than the cover ratings for all other treatments, which did not exceed 3% (Table 3). For all observation timings and tank mixes, no differences were observed between the *S*-metolachlor- and the ME acetochlor-containing programs with respect to weed cover. Tank mixes and sequential herbicide programs of oxyfluorfen with chloroacetamides were more effective at reducing estimated weed cover compared to the single ai chloroacetamides treatments.

All herbicide treatments provided $\geq 97\%$ control of common lambsquarters, common ragweed, smartweed spp., hairy galinsoga, and annual grasses 7 and 14 DAT (data not shown). Tank mixes and sequential applications of oxyfluorfen with either *S*-metolachlor or ME acetochlor provided $\geq 99\%$ control of all weed species up to 28 DAT. At 21 DAT (data not shown) and 28 DAT, oxyfluorfen applied alone PRE-T provided better control of common lambsquarters (100%) than solo POST-T applications of both chloroacetamides (Table 4). For both observation timings, *S*-metolachlor provided significantly better control of common lambsquarters (98 and 94%) than ME acetochlor (92 and 84%). At 28 DAT, smartweed control was significantly higher with oxyfluorfen PRE-T (96%) compared to POST-T applications of

S-metolachlor (92%) and ME acetochlor (89%). Similar trends were observed for common ragweed; oxyfluorfen applied alone controlled common ragweed 97% compared to 90 and 89% for S-metolachlor and ME acetochlor, respectively. Excellent grass control (\geq 97%) was observed with all treatments, including oxyfluorfen PRE-T. All treatments containing the chloroacetamide herbicides completely controlled (100%) hairy galinsoga; by comparison, oxyfluorfen applied alone PRE-T controlled galinsoga 87% (data not shown). Oxyfluorfen PRE-T, applied alone, provided better control of common lambsquarters, common ragweed, and smartweeds 28 DAT than chloroacetamides. There were no differences between the three single ai for control of grasses or hairy galinsoga. With few exceptions, orthogonal contrast analyses showed the tank mixes and sequential herbicide programs were more effective at controlling weeds than using these herbicides singly. No differences in weed control were observed between the *S*-metolachlor-containing programs and the ME acetochlor-containing programs.

Overall weed density 28 DAT and biomass at harvest were both affected by herbicide treatments (P < 0.0001) but not by crops (P = 0.7937 and P = 0.1871, respectively), nor the interaction between herbicide treatments and crops (P = 0.0589 and P = 0.9748, respectively); as such data were averaged over crops (Table 4). Overall, total broadleaf density 28 DAT in NY was reduced 89-99% by all herbicide treatments relative to the nontreated control (246 plants m⁻²), although the single ai programs were significantly less effective than tank mixes and sequential programs averaging 20 and 3 plants m⁻², respectively (data not shown). Grass density was 216 plants m⁻² in the nontreated control. Higher grass density was noted for the solo PRE-T application of oxyfluorfen (9 plants m⁻²) as compared to *S*-metolachlor POST-T (5 plants m⁻²), as well as the tank mix and sequential herbicide programs (≤ 2 plants m⁻²). Grass densities in the solo POST-T ME acetochlor treatment (8 plants m⁻²) did not differ, significantly, from solo applications of oxyfluorfen or *S*-metolachlor (data not shown).

Compared to the nontreated control (1,320 g m⁻²), weed biomass collected at harvest was reduced 91% to \geq 99% in response to all herbicide treatments (Table 4). Among herbicides, solo POST-T applications of *S*-metolachlor (118 g m⁻²) and ME acetochlor (60 g m⁻²) were the least effective herbicide treatments for suppressing weed growth season-long, followed by oxyfluorfen PRE-T (33 g m⁻²). Tank mixes and sequential applications of oxyfluorfen and *S*-metolachlor or ME acetochlor reduced biomass levels to \leq 13 g m⁻². Orthogonal contrast analyses demonstrated that, given the weed populations present at the NY site, oxyfluorfen

applied alone PRE-T was more effective at reducing weed biomass than solo application of chloroacetamides. Orthogonal contrast analyses also confirmed the need to mix oxyfluorfen and chloroacetamide herbicides for maximizing weed biomass reduction (99%) compared to single ai (82%).

Crop Injury and Yield. Observed injury, which was characterized by stunting and some leaf burn, was affected by herbicide treatment at 14, 21, and 28 DAT (P < 0.0001) and crop 14 DAT (P< 0.0001), but not the interaction between the two factors (P \ge 0.0558) (Table 5). Averaged over cabbage and broccoli, S-metolachlor POST-T was the most injurious treatment of the solo applied ai. S-metolachlor caused 4, 8 and 3% stunting at 14, 21, and 28 DAT, respectively; injury from oxyfluorfen PRE-T and ME acetochlor POST-T did not exceed 2% at any observation timing. Orthogonal contrast analyses indicated greater crop injury for herbicide programs where oxyfluorfen was used in combination with the chloroacetamides compared to ai singly used. However, the order in which chemistries were applied affected the amount of damage sustained by the crop. When oxyfluorfen PRE-T was followed by S-metolachlor or ME acetochlor POST-T, maximum observed injury did not exceed 9 and 4% (21 DAT), respectively; at 28 DAT, visible crop damage was minimal (2-3%). Sequential applications where S-metolachlor and ME acetochlor POST-T were followed by oxyfluorfen POST 14 DAT injured cabbage and broccoli up to 31 and 25%, respectively (21 DAT); at 28 DAT, injury was 28% and 21%. POST-T tank mixing of oxyfluorfen and chloroacetamides caused \geq 19% injury 14 DAT, but no more than 5% 28 DAT. Orthogonal contrast analyses suggest that combinations of S-metolachlor with oxyfluorfen were more injurious to cabbage and broccoli than combinations of ME acetochlor with oxyfluorfen. Averaged over herbicide treatments, greater injury was observed for broccoli compared to cabbage, but only 21 DAT.

Relative commercial yield (RCY) expressed as a percentage of the nontreated control was affected by herbicide treatment (P = 0.0356) but not by crop (P = 0.1442), nor by the interaction between herbicide treatment and crop (P = 0.9440); as such data are averaged over crops. Averaged over cabbage and broccoli, the commercial yield of the nontreated control was 384 g head⁻¹; when herbicides were applied, mean RCY across treatments was 846 g head⁻¹ (data not shown). Relative commercial yields were the highest when oxyfluorfen was applied PRE-T singly or followed by chloroacetamides POST-T ($\geq 262\%$). Weeds were well controlled by these treatments and visible crop injury, in the form of stunting and leaf necrosis, was minimal. Two of

the lowest RCYs occurred when the chloroacetamides were applied POST-T singly, which were the two treatments with the highest level of weed biomass; the RCY for ME acetochlor was greater (220%) than S-metolachlor (175%), likely due to the lower weed biomass measured for this treatment. Relative commercial yields for the POST-T tank mixes of oxyfluorfen with Smetolachlor or ME acetochlor and the sequential treatments where POST-T applications of Smetolachlor and ME acetochlor were followed by POST applications of oxyfluorfen ranged from 205-253%. While effective for weed control, these treatments also caused the greatest amount of early season stunting and leaf burn observed in trial.

Cole crops are sensitive to crop-weed competition, particularly early in the season when transplants are still becoming established. Latif et al. (2012) found that the critical control period for transplanted broccoli lies between 15 and 30 DAT (2021). Similarly, Weaver (1984) found that the critical weed control period for transplanted cabbage was between 21 and 35 DAT. For vegetable crops, labor costs constitute a significant portion of total production expenses compared to other commodities. Consequently, herbicides play a crucial role in weed management in cabbage and broccoli production systems. In our studies, oxyfluorfen, *S*-metolachlor, and ME acetochlor applied alone and in combination provided \geq 84% weed control across species, significantly reduced weed cover, and prevented weed biomass accumulation relative to the nontreated control. The performance of *S*-metolachlor and oxyfluorfen against common and troublesome weeds in cole crop production has been documented in numerous studies (Bhowmik and McGlew 1986; Chomas and Kells 2004; Cutulle et al. 2019; Li et al. 2016, Pineda-Bermudez et al. 2023; Soltani et al. 2014, Soltani et al. 2018). ME acetochlor has not been investigated for weed control in cole crops although its weed control efficacy has been demonstrated in other crops (Ferebee et al. 2019; Jhala et al. 2015).

The minor injury observed in response to *S*-metolachlor applied alone to cabbage and broccoli in both greenhouse and field trials is consistent with previous reports describing POST-T safety in cole crops (Bellinder and Warholic 1988; Bellinder et al. 1989; Reis et al. 2017; Sikkema et al., 2007a; Yu and Boyd, 2018). Oxyfluorfen safety, when applied PRE-T and POST, in cole crops has also been documented (Bhowmik and McGlew 1986; Cutulle et al. 2019; Pineda-Bermudez et al. 2023; Sikkema et al. 2007b). The stunting and necrosis observed with oxyfluorfen in the greenhouse study was not completely unexpected given label warnings

about possible injury to cole crops following POST treatments (Anonymous 2021, 2022) and the fact that plant cuticles were likely thinner in the more protected greenhouse environment.

Like *S*-metolachlor, no significant injury nor biomass reduction was observed when ME acetochlor was applied singly OTT. The safety of ME acetochlor, relative to EC herbicide formulations, has been explored in other cropping systems. For POST treatments, EC acetochlor injured rice (*Oryza sativa* L.) 23 and 18% at 2 WAT and 4 weeks after flooding, respectively, whereas injury from the ME formulation was 11% for both observation timings (Fogleman et al. 2018). Bellinder and Warholic (1988) compared EC and ME formulations of alachlor to metolachlor and other chloroacetamide herbicides. Although they observed variable, and sometimes extreme (up to 60%), injury in their trials, they did not detect significant differences among the ai with respect to their potential to cause injury or reduce yields.

Tank mixes or sequential applications that incorporate multiple modes of action offer a broader spectrum of weed control and are recommended for managing herbicide resistance (Norsworthy et al. 2012). All combinations of *S*-metolachlor and acetochlor with oxyfluorfen were effective for weed control in this study, although there was significant variability with respect to crop injury potential. Like Bellinder (2012) and Pineda-Bermudez et al. (2023), all treatments that include oxyfluorfen POST-T or POST caused significant injury to both cabbage and broccoli and reduced yields. Oxyfluorfen applied PRE-T and fb *S*-metolachlor or ME acetochlor combined both excellent weed control and good crop safety. The comparison of *S*-metolachlor with ME acetochlor did not substantially impact crop injury potential in the field despite promising greenhouse results. This discrepancy could be attributed to various environmental factors in the field, like temperature or moisture stress, which might have offset the phytotoxicity advantages linked with the ME formulation.

Practical implications

Cabbage and broccoli are sensitive to weed competition, particularly early in the season when transplants are not fully established. This study investigated the possible use of ME acetochlor in place of *S*-metolachlor to reduce crop injury in tank mixes and sequential applications. Greenhouse trials demonstrated ME acetochlor resulted in less crop injury than *S*-metolachlor when tankmixed with oxyfluorfen; however, these results were not supported in field trials. *S*-metolachlor and oxyfluorfen are important tools for managing weeds PRE and POST in cole

crop production. *S*-metolachlor and oxyfluorfen have complimentary control spectrums, however, label language advises against using both in a single season due to injury concerns. Results from this study showed that oxyfluorfen PRE-T followed by *S*-metolachlor or ME acetochlor POST-T provided good crop safety and was effective at suppressing the local weed communities. Growers with late spring or fall-planted crops may find this sequence beneficial for the management of weed species that can emerge continuously throughout the summer, such as hairy galinsoga, or for managing pigweed species that have evolved herbicide resistance to POST herbicides. Additionally, the number of POST herbicide options labeled for use on cole crops remain limited, justifying the need to optimize residual weed control through sequential applications as demonstrated in this study.

Acknowledgements

The authors would also like to express their appreciation for technical support provided by Erin Hitchner, Craig Austin, Elizabeth Maloney, Melissa McClements, and Wesley Bouchelle, as well as technical help from farm crews at RAREC and Cornell Agritech. Thank you to the Network for Environment and Weather Applications, part of New York State Integrated Pest Management at Cornell University, and The Office of the New Jersey State Climatologist at Rutgers University for the weather data. No conflicts of interests have been declared.

Funding

The authors acknowledge funding support for this research by the New York Cabbage Research and Development Program, Vegetable Growers Association of New Jersey, and the Rutgers New Jersey Agricultural Experiment Station. This work was also supported by the Specialty Crops Research Initiative, National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2016-51181-25402.

Competing Interest

Competing interests: The author(s) declare none.

References

- Al-Khatib K, Libbey C, Kadir S (1995) Broadleaf weed control and cabbage seed yield following herbicide application. HortScience, 30:1211-1214
- Anonymous (2020) GoalTender® herbicide label. Nufarm Americas Inc. https://www.cdms.net/ldat/ldGT4000.pdf Accessed: May 22, 2024
- Anonymous (2021) GoalTender® herbicide Special Local Need label for NY. Nufarm Americas Inc. <u>https://www.cdms.net/ldat/ldGT4016.pdf</u>. Accessed: September 18, 2023
- Anonymous (2022) GoalTender® herbicide Special Local Need label for NJ. Nufarm Americas Inc. https://www.cdms.net/ldat/ldGT4018.pdf. Accessed: September 18, 2023
- Bell CE (1995) Broccoli (*Brassica oleracea* var *botrytis*) yield loss from Italian ryegrass (*Lolium perenne*) interference. Weed Sci 43:117-120
- Bellinder RR (2012) Evaluating new herbicides for cabbage. <u>http://www.hort.cornell.edu/expo/proceedings/2013/Cole%20Crops/Cole%20Crops%20Belli</u> <u>nder%20Weeds.pdf</u>. Accessed: September 18, 2023
- Bellinder RR, Warholic DT (1988) Evaluation of acetanilide injury and its potential for yield reductions in cabbage, *Brassica oleracea* L. Weed Technol. 2:350-354.
- Bellinder RR, Wilcox-Lee D, Senesac A, Warholic DT (1989) Response of early-maturing cabbage (*Brassica oleracea* var. *capitata*) to metolachlor. Weed Technol 3: 463-466
- Besançon TE, Wasacz MH, Carr BL (2020) Weed control and crop tolerance with *S*-metolachlor in seeded summer squash and cucumber. Weed Technol 34: 849-856
- Bhowmik PC, McGlew EN (1986) Effects of oxyfluorfen as a pretransplant treatment on weed control and cabbage yield. HortScience 111:686-689
- Bridges DC (1994) Impact of Weeds on Human Endeavors. Weed Techol. 8:392-395
- Chen M, Shelton AM, Hallett RH, Hoepting CA, Kikkert JR (2011) Swede midge (Diptera: Cecidomyiidae), ten years of invasion of crucifer crops in North America. J Econ Entomol 104:709-716
- Chomas, AJ, Kells, JJ (2004) Triazine-resistant common lambsquarters (*Chenopodium album*) control in corn with preemergence herbicides. Weed Technol 18:551–554
- Cutulle M, Campbell H, Couillard DM, Ward B, Farnham MW (2019) Pre-transplant herbicide application and cultivation to manage weeds in southeastern broccoli production. Crop Prot 124:104862

- Dillard HR, Hunter JE (1996) Association of common ragweed with Sclerotinia rot of cabbage in New York State. Plant Dis 70:26-28
- Fennimore SA, Tourte L, Rachuy JS, Smith RF, George C (2010) Evaluation and economics of a machine-vision guided cultivation program in broccoli and lettuce. Weed Technol 24:33-38
- Ferebee JH, Cahoon CW, Besançon TE, Flessner ML, Langston DB, Hines TE, Blake HB, Askew MC (2019) Fluridone and acetochlor cause unacceptable injury to pumpkin. Weed Technol 33:748-756.
- Fogleman M, Norsworthy JK, Barber T, Gbur, E (2018) Influence of formulation and rate on rice tolerance to early-season applications of acetochlor. Weed Technol 33:239-245
- Godwin Jr, JA (2017). Evaluation of very-long-chain fatty acid-inhibiting herbicides in Arkansas rice production. Master's Thesis. Fayetteville AR. University of Arkansas. <u>https://www.proquest.com/dissertations-theses/evaluation-very-long-chain-fatty-acidinhibiting/docview/1991534335/se-2?accountid=10267</u> Accessed: May 25, 2024
- Guerena M (2020) Cole crops and other brassicas: organic production. National Center for Appropriate Technology ATTRA Sustainable Agriculture. Accessed: February 10, 2023
- Guo Y, Yang Q, Yan W, Li B, Qian K, Li T, Xiao W, He L. (2014) Controlled release of acetochlor from poly (butyl methacrylate-diacetone acrylamide) based formulation prepared by nanoemulsion polymerisation method and evaluation of the efficacy. International Journal of Environmental Analytical Chemistry 94:1001-1012
- Jhala AJ, MS Mayank, Willis JB (2015) Weed control and crop tolerance of micro-encapsulated acetochlor applied sequentially in glyphosate-resistant soybean. Can J Plant Sci 95: 973-981
- Latif A, Jilani MS, Baloch MS, Hashim MM, Khakwani AA, Khan QU, Saeed A, Mamoon-ur-Rashid M (2021) Evaluation of critical period for weed crop competition in growing broccoli crop. Sci Hortic 287:110270
- Li, Z, Van Acker, RC, Robinson, DE, Soltani, N, Sikkema, PH (2016) Halosulfuron tank-mixes applied PRE in white bean. Weed Technol 30:57–66
- Lingenfelter D, Wallace J, VanGessel M, Vollmer K, Besancon T, Flessner M, Singh V, Chandran R, Kumar V, Sosnoskie, L (2024) Mid-Atlantic field crop weed management guide. <u>https://extension.psu.edu/mid-atlantic-field-crop-weed-management-guide Accessed</u> <u>March 12</u>, 2024.

- McErlich AF, Boydston RA (2013) Current State of Weed Management in Organic and Conventional Cropping Systems. Young SL, Pierce FJ (eds) in Automation: The Future of Weed Control in Cropping Systems. Pages 11-34
- Norsworthy JK, Ward SM, Shaw DR, Llewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and Recommendations. Weed Sci 60 :31-62
- Pineda-Bermudez L, Besançon TE, Sosnoskie LM (2023) Sulfentrazone crop safety and efficacy in cabbage and broccoli. Weed Technol. 37:569-577
- Reis MR, Melo CAD, Raposo TP, Aquino RFBA, Aquino LA (2017) Selectivity of herbicides to cabbage (*Brassica oleracea* var. *capitata*). Planta Daninha 35.
- Sikkema PH, Soltani N, Deen W, Robinson DE (2007a) Effect of S-metolachlor application timing on cabbage tolerance. Crop Prot 26:1755-1758
- Sikkema PH, Soltani N, Robinson DE (2007b) Responses of cole crops to pre-transplant herbicides. Crop Prot 26:1173-1177
- Smart JR, Brandenberger L, Makus D (2001) Cabbage (*Brassica oleracea* L.) response to sulfentrazone for broadleaf weed control. J Veg Crop Prod 7:97-108
- Soltani, N, Nurse, RE, Sikkema, PH (2014) Weed management in kidney bean with tank mixes of *S*-metolachlor, imazethapyr and linuron. Agric Sci 5:611–617
- Soltani N, Brown L, Sikkema PH (2018) Control of glyphosate-resistant common ragweed in corn with preemergence herbicides. Can J Plant Sci 98: 959-962
- [USDA-AMS] United States Department of Agriculture Agricultural Marketing Service (2006) United States Standards for Grades of Italian Sprouting Broccoli. <u>https://www.ams.usda.gov/sites/default/files/media/Broccoli%2C_Italian_Sprouting_Standa_ rd%5B1%5D.pdf</u>. Accessed August 24, 2024
- [USDA-AMS] United States Department of Agriculture Agricultural Marketing Service (2016) United States Standards for Grades of Cabbage. <u>https://www.ams.usda.gov/sites/default/files/media/CabbageStandards.pdf</u>. Accessed August 24, 2024[USDA NASS] US Department of Agriculture National Agricultural Statistics Service (2024) 2012 Census of Agriculture. Washington, DC: US Department of Agriculture. <u>https://www.nass.usda.gov/AgCensus/.</u> Accessed: March 12, 2024

- Van Wychen L (2022) 2022 Survey of the most common and troublesome weeds in broadleaf crops, fruits & vegetables in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. https://wssa.net/wp-content/uploads/2022-Weed-Survey-Broadleaf-crops.xlsx Accessed: March 12, 2024
- Weaver SE (1984) Critical period of weed competition in three vegetable crops in relation to management practices. Weed Res 24:317-325
- Wyenandt CA, van Vuuren MMI, Hamilton GC, Hastings PD, Owens D, Sánchez E, VanGessel MJ (2024) 2024/2025 Mid-Atlantic Commercial Vegetable Production Recommendations. <u>https://njaes.rutgers.edu/pubs/publication.php?pid=e001</u>. Accessed: March 15, 2024
- Yu J, Boyd NS, Dittmar PJ (2018) Evaluation of herbicide programs in Florida cabbage production. HortScience 53:646-650

Month	Geneva		Bridgeton					
Montin	2022	30-yr avg	2022	30-yr avg				
		m	ım					
May	42	83	127	89				
June	117	92	71	106				
July	18	95	51	113				
August	23	89	55	124				
September	27	83	101	112				
October	5	97	150	96				
November	39	65	91	80				
Total	271	604	646	720				

Table 1. Average monthly rainfall in 2022 and 30-yr monthly rainfall average for Geneva, NY and Bridgeton, NJ.

Table 2. Effect of OTT herbicide treatments on cabbage and broccoli injury and relative dry biomass 14 DAT for greenhouse experimentations conducted at Geneva, NY, and at Chatsworth, NJ, in 2022.

Treatment ^a	Rate	Broccoli				Cab	RDW				
Treatment	Raic	Necrosis ^b		Stunting		Necrosis		Stunting			
	g ai ha ⁻¹	% nontreated control									
S-metolachlor	1,420	3	c	2	cd	4	b	3	b	91	ab
ME acetochlor	1,420	0	d	0	d	1	c	4	b	92	a
Oxyfluorfen	210	7	b	11	b	5	b	6	b	77	c
S-metolachlor + oxyfluorfen	1,420 + 210	19	а	24	а	27	a	26	a	56	d
ME acetochlor + oxyfluorfen	1,420 + 210	4	bc	5	bc	6	b	4	b	83	bc

^aAbbreviation: DAT, d after treatment; OTT, over the top; RDW, relative dry weight.

^bMain effect means within a column followed by the same letters are not significantly different from each other according to Tukey's honest significance test ($P \le 0.05$).

Table 3. Effect of herbicide treatments and timing of application on weed coverage in cabbage and broccoli field trials conducted at Geneva, NY, and Bridgeton, NJ, in 2022.

#	Treatment ^a	Rate	Timing	21 DAT ^b		28 DAT		
		g ai ha ⁻¹			(%	%	
1	Nontreated control	0	-	35	a	71	a	
2	Oxyfluorfen	560	PRE-T	2	cd	3	c	
3	S-metolachlor	1,420	POST-T	5	bc	9	b	
4	ME acetochlor	1,430	POST-T	7	b	10	b	
5	Oxyfluorfen fb S-metolachlor	560 fb 1,420	PRE-T fb POST-T	1	d	2	cd	
6	Oxyfluorfen + S-metolachlor	560 + 1,420	POST-T	0	d	0	d	
7	S-metolachlor fb oxyfluorfen	1,420 fb 280	POST-T fb POST	1	d	1	cd	
8	Oxyfluorfen fb ME acetochlor	560 fb 1,430	PRE-T fb POST-T	1	d	2	cd	
9	Oxyfluorfen + ME acetochlor	560 + 1,430	POST-T	1	d	1	cd	
10	ME acetochlor fb oxyfluorfen	1,430 fb 280	POST-T fb POST	1	d	1	cd	
Orth	logonal contrast							
S	ingle ai (2-4) vs tank mixes and	< 0.0	001	< 0.0	001			
0	xyfluorfen (2) vs chloroacetamie	de (3-4) alone		0.043	36	0.002	25	
S	metolachlor (5-7) vs acetochlor	0.6294		0.7341				

^aAbbreviation: ai, active ingredient; DAT, days after transplanting; fb, followed by; ME, microencapsulated; POST-T, 1-day post-transplant application; POST, 14-days post-transplant application; PRE-T, pre-transplant application.

^bMain effect means within a column followed by the same letters are not significantly different from each other according to Tukey's honest significance test ($P \le 0.05$).

Table 4. Effect of herbicide treatments and timing of application on common lambsquarters, smartweed spp., common ragweed and grass weeds control and overall weed biomass fresh weigh 28 days after treatment in cabbage and broccoli field trials conducted at Geneva, NY, and Bridgeton, NJ, in 2022.

#	Treatment ^a	Data	Timina	Weed control								Weed biomass FWb	
# Treatment ^a		Rate Timing		CHEAL ^b		POLY spp. AMB			BEL Grasses		es		
		g ai ha ⁻¹					% .					g m ⁻²	-
2	Oxyfluorfen	560	PRE-T	100	a	96	b	97	a	97	b	1,320	a
3	S-metolachlor	1,420	POST-T	94	b	92	c	89	b	98	b	33	cd
4	ME acetochlor	1430	POST-T	84	c	89	c	90	b	98	b	118	b
5	Oxyfluorfen fb S-metolachlor	560 fb 1,420	PRE-T fb POST-T	99	a	100	a	99	a	100	a	60	bc
6	Oxyfluorfen + S-metolachlor	560 + 1,420	POST-T	100	a	100	a	100	a	100	a	6	d
7	S-metolachlor fb oxyfluorfen	1,420 fb 280	POST-T fb POST	100	a	100	a	100	a	100	a	4	d
8	Oxyfluorfen fb ME acetochlor	560 fb 1,430	PRE-T fb POST-T	100	a	100	a	99	а	99	ab	4	d
9	Oxyfluorfen + ME acetochlor	560 + 1,430	POST-T	100	a	100	a	99	a	100	a	13	cd
10	ME acetochlor fb oxyfluorfen	1,430 fb 280	POST-T fb POST	100	a	100	a	100	a	100	a	4	d
Orthogonal contrast													
Si	ngle ai (2-4) vs tank mixes and se	equential applie	cations (5-10)	< 0.00	001	< 0.00	001	< 0.0	001	< 0.00	01	0.0011	
Ox	xyfluorfen (2) vs chloroacetamid	e (3-4) alone		< 0.00	001	< 0.00	001	0.00	11	0.600	9	0.0001	
S-1	metolachlor (5-7) vs acetochlor (8-10) in combi	ned applications	0.937	73	1.000	0	0.272	26	0.536	2	0.0586	

^aAbbreviation: ai, active ingredient; AMBEL, common ragweed; CHEAL, common lambsquarters; DAT, d after transplanting; fb, followed by; FW, fresh weight; ME, microencapsulated; POL, smartweed; POST-T, 1-day post-transplant application; POST, 14-days post-transplant application; PRE-T, pre-transplant application.

^bCHEALand grasses were collected from both locations whereas AMBEL and smartweed were only collected from Geneva, NY.

^bMain effect means within a column followed by the same letters are not significantly different from each other according to Tukey's honest significance test ($P \le 0.05$).

щ	Transformer and ^a	Data	Timina	Injury ^b	y ^b							rcial
#	Treatment ^a	Rate	Timing	7 DAT	14 D	A T ^c	21 DA	ΑT	28 DA	Υ	yield	
Herbicide g ai ha ⁻¹				(%				% NT	'C		
2	Oxyfluorfen	560	PRE-T	1	1	c	1	d	1	cd	268	а
3	S-metolachlor	1,420	POST-T	1	4	b	8	bc	3	bc	175	с
4	ME acetochlor	1430	POST-T	0	2	bc	2	d	0	d	220	b
5	Oxyfluorfen fb S-metolachlor	560 fb 1,420	PRE-T fb POST-T	1	5	b	9	bc	3	bc	278	а
6	Oxyfluorfen + S-metolachlor	560 + 1,420	POST-T	2	24	a	15	b	5	b	223	b
7	S-metolachlor fb oxyfluorfen	1,420 fb 280	POST-T fb POST	1	2	bc	31	a	28	a	253	ab
8	Oxyfluorfen fb ME acetochlor	560 fb 1,430	PRE-T fb POST-T	1	3	bc	4	cd	2	b-d	262	а
9	Oxyfluorfen + ME acetochlor	560 + 1,430	POST-T	2	19	a	13	bc	5	b	218	b
10	ME acetochlor fb oxyfluorfen	1,430 fb 280	POST-T fb POST	1	2	bc	25	a	21	a	205	bc
Cro	p											
В	roccoli			1	5		13	а	6		242	
C	abbage			1	5		8	b	5		225	
Orth	nogonal contrast											
Single ai (2-4) vs tank mixes and sequential applications (5-10)		0.5005	< 0.0	0001	< 0.00)01	< 0.00)01	0.2473			
С	xyfluorfen (2) vs chloroacetamid	e (3-4) alone		0.8676	0.01	62	0.006	9	0.087	7	0.0120	
S	-metolachlor (5-7) vs acetochlor ((8-10) in combi	ined applications	0.0410	0.06	93	0.006	3	0.031	8	0.2274	

Table 5. Effect of herbicide treatments and timing of application on crop injury and commercial cole crop yield at harvest for field trials conducted at Geneva, NY, and Bridgeton NJ, in 2022.

^aAbbreviation: ai, active ingredient; DAT, d after transplanting; fb, followed by; ME, microencapsulated; NTC, nontreated control;

POST-T, 1-day post-transplant application; POST, 14-days post-transplant application; PRE-T, pre-transplant application.

^bInjury primarily consisted of crop stunting with some minimal leaf burn

^cMain effect means within a column followed by the same letters are not significantly different from each other according to Tukey's honest significance test ($P \le 0.05$).