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Clopyralid Tolerance in Strawberry and Feasibility of Early Applications in Florida

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Abstract

Broadleaf species escape current integrated weed management strategies in strawberry [*Fragaria* × *ananassa* (Weston) Duchesne ex Rozier (pro sp.) [*chiloensis* × *virginiana*]] production. Clopyralid is a registered POST control option, but current application timings provide suppression of only some species. Earlier clopyralid application timings may increase spray coverage to weeds at the planting hole, but strawberry plant tolerance to applications shortly after transplant is unknown. The objectives of the study were to determine the degree of clopyralid tolerance when applied to mature strawberry plants according to current management strategies, whether clopyralid absorption and translocation were involved in the tolerance response demonstrated by mature strawberry plants, and whether clopyralid could be safely applied to immature strawberry plants shortly after transplant. Clopyralid caused no damage when applied to mature strawberry plants and did not affect crop height, number of crowns, flowers, immature berries, or yield. Maximal strawberry absorption of radiolabeled clopyralid was 82% of the recovered radioactivity and reached peak (90%) absorption at 15 h. Maximal total translocation of radioactivity from the treated leaf was 17% and reached peak translocation at 52 h. Translocation was primarily to the new leaves and reproductive structures. In the early-application experiment, damage induced by clopyralid for all application timings reached 0 by 8 wk after treatment. Across all timings, maximal damage at 140 g ha^{-1} was 17% when applied 14 d after transplant (DATr) and 56% at 28 g ha^{-1} when applied at 21 DATr. Clopyralid dose did not affect the number of crowns, aboveground biomass, or yield. There was some stunting in plant height (3%) by the high labeled dose of clopyralid. Labeled dose clopyralid applications appear safe for application timings closer to strawberry transplant, though considerations of leaf cupping should be taken under consideration for label changes.

Introduction

Strawberries [*Fragaria* × *ananassa* (Weston) Duchesne ex Rozier (pro sp.) [*chiloensis* × *virginiana*]] are a valuable Florida horticultural crop, with a production value of $306.5 million in 2014 (USDA 2015). Florida strawberry production uses a raised-bed, fumigated, drip-irrigated, plasticulture system, typically initiated in August (Dittmar et al. 2017; Whitaker et al. 2017). Current weed management practices use plastic mulch, fumigants, and PRE herbicides to control weeds within the bed (Dittmar et al. 2017).

Strawberry plants are transplanted in mid-October in central Florida (Whitaker et al. 2017). Broadleaf weeds escape control during crop establishment, likely emerging from the planting hole after fumigants dissipate and PRE herbicide efficacy diminishes. Two of the most common and problematic broadleaf species in Florida strawberry fields are black medic (*Medicago lupulina* L.) and Carolina geranium (*Geranium carolinianum* L.) (Webster 2014).

Clopyralid is a viable POST broadleaf herbicide, but labeled doses only provide suppression (Anonymous 2011). Clopyralid is typically applied in January (~100 d after transplant [DATr]) when *M. lupulina* is flowering (Sharpe et al. 2018b). Clopyralid labeled doses provide adequate control of *M. lupulina* when directly applied to plants when small (0.5- to 1-cm stem length), but efficacy diminishes with size (Sharpe et al. 2016). Crop shielding exacerbates the loss of control with clopyralid (Sharpe et al. 2018c). Doubling the application volume doubled coverage at the planting hole (Sharpe et al. 2018c) and is within the labeled application limits (Anonymous 2011) but does not account for size-based *M. lupulina* clopyralid tolerance (Sharpe et al. 2016).

Clopyralid application earlier in the production cycle may control *M. lupulina* and addresses all limitations with current control practices. *Medicago lupulina* emerged between 861 and 1,416 growing degree days posttransplant (GDD) (45 to 78 DATr) (Sharpe 2017).

Ideal spray timing for an early-emerging population was between 890 and 1,152 GDD (46 and 60 DATr) (Sharpe et al. 2018b). These timings are much earlier than current clopyralid application practices. The degree and nature of whole-plant, mature strawberry tolerance to clopyralid require further study as does crop safety of immature strawberry plants (with respect to clopyralid application).

Clopyralid is the only POST broadleaf herbicide currently registered for use over the top of the strawberry crop. Clopyralid generally does not reduce yield in matted-row (Figueroa and Doohan 2006) or plasticulture production (Boyd and Dittmar 2015; McMurray et al. 1996). How clopyralid injures strawberry plants has been variable and typically associated with the vegetative portion of the plant body, including slight reductions in plant height (McMurray et al. 1996), reductions in groundcover (Figueroa and Doohan 2006), reductions in leaf number (Boyd and Dittmar 2015), leaf malformations (Boyd and Dittmar 2015; Clay and Andrews 1984), and a reduction in the number of crowns (Clay and Andrews 1984). Previous studies did not examine the effect of clopyralid on all plant components, but strawberry plants do tolerate clopyralid, and any damage observed in the vegetative portion of the plant body does not affect the reproductive output (Boyd and Dittmar 2015).

The mechanism for clopyralid tolerance in strawberry is unknown, but may be related to absorption or translocation. Clopyralid absorption and translocation has been studied in many susceptible members of Asteraceae (Anonymous 2010), including yellow starthistle (*Centaurea solstitialis* L.) (Valenzuela-Valenzuela et al. 2001), Canada thistle [*Cirsium arvense* (L.) Scop.] (Bukun et al. 2009; Devine and Vanden Born 1985; Turnbull and Stephenson 1985), perennial sowthistle (*Sonchus arvensis* L.) (Devine and Vanden Born 1985; Zollinger et al. 1992), and common sunflower (*Helianthus annuus* L.) (Hall and Vanden Born 1988). Relevant studies on naturally tolerant species are less common but include rapeseed (*Brassica napus* L.), in which clopyralid is registered for use (Anonymous 2010). Rapeseed absorption of radiolabeled clopyralid was >97% within 24 h and translocated 65% of radioactivity by 144 h after treatment (HAT) (Hall and Vanden Born 1988). Hemp dogbane (*Apocynum cannabinum* L.) is a tolerant weed species that absorbed 47% of applied radiolabeled clopyralid by 72 HAT and translocated 47% by 72 HAT when the herbicide was applied to the vegetative stage (Orfanedes et al. 1993). When the herbicide was applied to an early reproductive stage, *A. cannabinum* absorbed 56% by 144 HAT and translocated 75% by 144 HAT (Orfanedes et al. 1993). Tolerant tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] treated with aminocyclopyrachlor, another pyridine carboxylic acid herbicide, absorbed 68% of applied radiation at 48 HAT and translocated 34% at 96 HAT (Lewis et al. 2013). The objectives of this study were to test clopyralid safety across a wide range of application doses on mature strawberry plants according to the current management strategy timing, determine the role of clopyralid absorption and translocation in mature strawberry plant tolerance, and test crop safety of clopyralid applications on immature strawberry plants shortly after transplant.

Materials and Methods

Clopyralid Dose Response

The experiment was conducted at the Gulf Coast Research and Education Center (GCREC) in Balm, FL (27.76 °N, 82.22 °W), and was repeated over two production cycles. Plants were grown using the raised-bed plasticulture system common in Florida strawberry production. Soil type was a Zolfo fine sand (sandy, siliceous, hyperthermic Oxyaquic Alorthods; USDA 2015). Raised beds were formed with 1.2-m centers, 30.5-cm heights, and a bed-top width of 66 cm. Trials were fumigated with 213 kg of 1,3-dichloropropene ha^{-1} + 117 kg chloropicrin ha^{-1} (Telone C-35, Dow AgroSciences LLC, Indianapolis IN) applied with a dual-shank applicator. A single drip tape (Eurodrip USA, Madera, CA) was inserted in the center of the bed, with a flow rate of 2.06 L ha^{-1} and emitters every 0.3 m. Raised beds were covered with very impermeable film plastic mulch (Berry Plastics, Evansville, IN). Strawberry plants (‘Strawberry Festival’) were transplanted in two rows per bed on October 9, 2014, in year 1 and October 8, 2015, in year 2. Plant spacing was 38 cm. Plants were fertilized and irrigated according to standard practices in Florida. Berry harvest began in late December and ended in early March each year.

The experimental design was a randomized complete block with four blocks. Plot size was 6.1 by 0.7 m within a single bed, with a 1.5-m buffer. The main factor was clopyralid dose (Stinger®, 359 g ae L^{-1}, Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN) at eight doses: 0, 35, 70, 140, 280, 560, 1,120, and 2,240 g ae ha^{-1}. Plants were sprayed with clopyralid on January 19, 2015 (102 DATr) in year 1 and January 14, 2016 (98 DATr) in year 2. Herbicide applications were made with a handheld CO_2-pressurized sprayer (Bellspray, , Opelousas, LA) at 276 kPa using a single-nozzle boom installed with a TeeJet® 8002EVS nozzle (TeeJet Technologies, Wheaton, IL). The application volume was 281 L ha^{-1}.

Response variables included damage ratings, plant heights, plant part counts, biomass, and yield. The damage scale varies from traditional scales, where 100% damage indicates plant death. Instead, the damage scale accounts for the natural strawberry tolerance to clopyralid. Damage was rated on a percent scale based on strawberry leaf malformations (epinasty, cupping, twisting, and curling) across the whole plot, where 100% injury indicated that 100% of the strawberry plants were demonstrating typical auxinic symptomology. Damage ratings were taken at 1, 2, 3, 4, and 6 wk after treatment (WAT). For plant heights and plant part counts, 5 plants plot^{-1} were marked before the application of the experimental treatments and repeatedly measured throughout the experiment. Plant heights were measured with a ruler, from the base of the strawberry crown on top of the bed to the top of the plant canopy. Plant heights and plant parts were measured and counted at 1, 2, 3, and 6 WAT. Plant part counts included the number of leaves, flowers, and immature berries. Plant parts were counted in situ with efforts to minimize physical damage to plant organs and growth habit. Fruit was harvested by hand, biweekly, until the end of the growing season. Both the weight and the number of harvested berries were measured. The number of plants per plot was counted, and yield was expressed as yield per plant. The end-of-season biomass was taken via destructive harvest, with plants dried and then weighed. Plants were divided into above- and belowground portions, excluding berries, by cutting through the crown at the soil line.

Data were subjected to ANOVA using PROC MIXED in SAS v. 9.4 (SAS Institute, Cary, NC). Treatment differences were compared by Tukey’s honest significant difference (HSD) means comparison in the LSMEANS statement ($\alpha = 0.05$). Repeated measures were accounted for using the REPEATED statement. Both trial run and block were considered random effects, and trial runs were analyzed together. Model assumptions of constant variance and normality were checked. If in violation, data were transformed using a root transformation for biological parameters and a sine function for damage, and the back-transformed data are presented. Nonlinear regression was performed using SigmaPlot v. 12.5 (Systat Software, San Jose, CA).

Uptake and Translocation

Strawberry transplants were planted on October 9, 2015, in 9-cm-diameter square pots filled with Fafard® Super-Fine Germination Mix (Sun Gro® Horticulture, Agawam, MA) and fertilized with 3 g pot^{-1} of Plantacote® Pluss 14-9-15 controlled release fertilizer (Plantacote, Amsterdam-Zuidoost, Netherlands) in a greenhouse at the GCREC in Balm, FL. Plants were provided moisture through drip irrigation.

The experimental design was a randomized complete block with four blocks, and the trial was repeated. The main factor was time after radiolabeled clopyralid treatment with seven timings: 0, 6, 12, 24, 48, 96, and 192 HAT. The response variable was the amount of radioactivity recovered in each plant part and leaf wash, expressed as a percentage of the total recovered.

The experiment began on December 13, 2015, and the trial was repeated on January 8, 2016. Plants were assigned to blocks by size, and blocks were then arranged from the front to the back of the designated greenhouse space. The methods of Nandula and Vencill (2015) for absorption and translocation experimentation were followed. Commercial-grade clopyralid was applied at 240 g ha^{-1} using a spray chamber (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN). Plants were air-dried and then treated with ^{14}C-radiolabeled clopyralid (Dow AgroSciences) on the youngest fully expanded leaf at 11.77 kBq plant^{-1}. Leaves transition from sink to source when they are 30% to 60% expanded (Turgeon 1989), so it was assumed all leaves were transitioned to source functionality for photoassimilate export. Radiolabeled clopyralid was applied by depositing five, 1-µl droplets on the selected leaf. Plants were harvested at 0, 6, 12, 24, 48, 96, and 192 HAT. No surfactant was used for the commercial or radiolabeled application due to clopyralid label recommendations for strawberry.

On each harvest date, the treated leaf was washed with five 1-ml rinses of deionized water, and the rinsate was collected. The plants were then pressed and dried at 70 C for 3 d. Plant parts were divided into categories: the treated leaf, crowns, leaves older than the treated leaf, leaves younger than the treated leaf, roots, and reproductive organs (primarily inflorescence). Leaves older and younger than the treated leaf were categorized based on petiole attachment to the crown, size, and degree of leaf expansion. Each plant part category was weighed and then ground in a Thomas Wiley® Mini-Mill Cutting Mill (Thomas Scientific, Swedesboro, NJ). A 0.2-g subsample was oxidized using a biological oxidizer (OX-500, R.J Harvey Instrument, Tappan, NY) for 2 min and bubbled into a scintillation vial filled with R.J. Harvey Carbon-14 Cocktail (OX-161, R.J. Harvey Instrument) to capture the carbon dioxide. A 1-ml aliquot of the leaf wash was selected and filled with the ^{14}C cocktail. Radioactivity from the leaf wash and plant parts was counted using liquid-scintillation spectroscopy (Beckman LS 6000 Scintillation Counter, Beckman Coulter, Brea, CA). Absorption was calculated as the total radioactivity found within all plant parts, expressed as a percentage of the total radioactivity recovered per plant. Total translocation was calculated as the total radioactivity counted within all plant parts but the treated leaf, expressed as a percentage of the total recovered per plant.

Absorption and translocation data were fit with an asymptotic regression function (ARF), which has desirable and meaningful attributes for modeling absorption (Kniss et al. 2011). The model was parameterized as:

$$\text{Absorption} = A_{\max} \times \left\{1 - \exp\left[(\log 0.1) \times \left(\frac{t}{t_{90}}\right)\right]\right\} \qquad [1]$$

where $A_{\max}$ is the upper limit or percent maximum absorbed dose, t is the time after application, and t_{90} is the time taken to reach peak (90%) absorption (Kniss et al. 2011). Model goodness of fit was examined using the coefficient of determination (R^2) and the adjusted coefficient of determination (R$^2_{\text{adj}}$):

$$\mathrm{R}^2 = 1 - \frac{\sum\left(y_{\text{obs}} - y_{\text{pred}}\right)^2}{\sum\left(y_{\text{obs}} - \bar{y}\right)^2} \qquad [2]$$

and

$$\mathrm{R}^2_{\text{adj}} = 1 - \frac{n\left(1 - \mathrm{R}^2\right)}{n - p} \qquad [3]$$

where y_{obs} and y_{pred} are the observed and predicted values of the model respectively, n is the number of observations, $\bar{y}$ is the overall mean, and p the number of model parameters within the model (White et al. 2012). Data were fit to the model using nonlinear regression in SigmaPlot.

Early Clopyralid Application

Field trials were conducted at the GCREC in Balm, FL. Strawberries were grown as per the clopyralid dose–response study outlined earlier. Strawberry transplants ('Strawberry Festival') were planted on October 8, 2015.

The experimental design was a three by two supplemented factorial arranged as a randomized complete block with four blocks, and the trial was repeated in two locations within the same field. The main factors were clopyralid application date and clopyralid dose. Application dates were: 14, 21, or 28 DATr. Clopyralid doses were: 140 and 280 g ha^{-1}. Clopyralid was applied as previously described in the clopyralid dose–response study. Application dates were: October 22, 2015; October 29, 2015; and November 4, 2015. The experimental design was supplemented with two nontreated control plots per block. The application volume was 187 L ha^{-1}.

Response variables included strawberry shoot damage, plant heights, aboveground biomass, and strawberry yield. Damage was taken at 1, 2, 4, and 8 WAT. Damage was rated on a percent scale based on whole-plot strawberry leaf malformations (epinasty, cupping, twisting, and curling). Plant heights were taken at 4 and 8 WAT, as previously described, with five subsamples per plot. Plant height measurements were staggered over time due to varying application timings and were expressed as a percentage of the control within each block during that timing. Aboveground biomass dry weight was measured as previously described, with five subsamples per plot, on March 11, 2016. Strawberry yield was measured as previously described. The number of plants per plot was counted, and yield was adjusted to yield per plant.

Data were subjected to ANOVA using the PROC MIXED in SAS. Treatment differences were compared by Tukey's HSD specified in the LSMEANS statement. Repeated measures were specified using the REPEATED statement. Both trial run and block were considered random effects, and trial runs were analyzed together. Control plots were removed from the analysis for damage and height data, and the model was analyzed as a full factorial. Model assumptions of constant variance and normality were verified.

Results and Discussion

Clopyralid Dose Response

There was no interaction between clopyralid dose and measurement timing on the number of leaves ($P = 1.0$), flowers ($P = 1.0$), immature berries ($P = 0.97$), the total number of reproductive organs ($P = 1.0$), or plant heights ($P = 0.80$). Clopyralid doses between 35 and 2,400 g ha^{-1} did not reduce growth rates or development over time. Measurement timing influenced the number of leaves ($P < 0.0001$), flowers ($P < 0.0001$), immature berries ($P < 0.0001$), total reproductive organs, ($P < 0.0001$), and plant heights ($P < 0.0001$). The number of flowers, immature fruit, and total reproductive organs increased over time, while shoot height decreased (Table 1). Results were expected given the typical increased growth and reproductive output of plants over time.

Clopyralid dose did not affect crop damage ($P = 0.77$) or plant height ($P = 0.15$) but did affect leaf number ($P = 0.0009$). The maximal leaf number was at 560 g ha^{-1}, which was higher than the 35 or 1,120 g ha^{-1} doses (Figure 1). Even so, no clopyralid treatments differed from the controls (Figure 1). Clopyralid-induced strawberry leaf number reductions are due in part to cultivar selection and application timing (Boyd and Dittmar 2015; Hunnicutt et al. 2013a).

Plant height results were inconsistent with previous findings, in which higher doses of clopyralid induced 6% damage, which was attributed to reductions in plant heights (McMurray et al. 1996). Growth cessations, such as reductions in leaf number or plant height, are symptomatological of the lethal action of auxinic herbicides (Grossman 2010). This is often not observed in strawberry plants due to natural tolerance, but clopyralid damage may manifest due cultivar differences and application timing (Boyd and Dittmar 2015; Hunnicutt et al. 2013a, 2013b).

Clopyralid applications across a range of doses (35 to 2,400 g ha^{-1}) did not affect the number of crowns per plant ($P = 0.76$) (3 plant^{-1}), shoot dry weight ($P = 0.0797$) (45.5 g plant^{-1}), or root dry weight ($P = 0.726$) (17.0 g plant^{-1}). The influence of clopyralid on season-long strawberry plant biomass accumulation has not been previously reported. The crown is a compressed stem that gives rise to inflorescence, leaves, and auxiliary buds at the nodes (Darnell 2003). The effect of clopyralid on the crown has not been examined in a plasticulture system. Results were consistent with previous findings for two application timings in the maiden year for a matted-row system (Clay and Andrews 1984). A third timing had reduced the crown number by 0.3 plant^{-1} (Clay and Andrews 1984).

Table 1. Effect of time after clopyralid treatment on plant heights and reproductive parameters of plasticulture-grown strawberry plants at Balm, FL, in 2016.[a]

Time after treatment	Leaves	Height	Flowers	Immature berries	Reproductive organs
weeks	no. plant^{-1}	cm plant^{-1}	——————no. plant^{-1}——————————		
1	28 c	21 a	3 c	5 b	8 c
2	30 bc	20 b	4 c	4 b	8 c
3	32 b	19 c	6 b	4 b	11 b
6	35 a	18 d	10 a	12 a	22 a

[a] Data presented are least-square estimates and are averaged across all clopyralid treatments. Different lowercase letters indicate a significant difference using Tukey's honest significant difference means test ($\alpha = 0.05$).

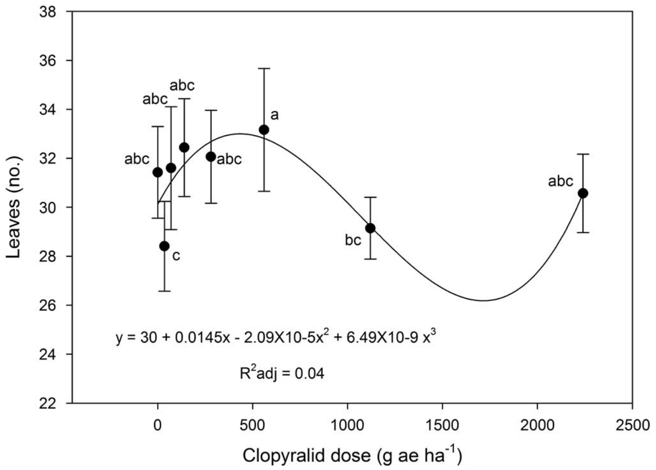

Figure 1. Effect of clopyralid dose on the number of strawberry leaves produced within a plasticulture setting in Balm, FL. Data were pooled over the 2015 and 2016 harvest seasons and averaged across all timing measurements. Different letters between means indicate a significant difference using Tukey's honest significant difference ($\alpha = 0.05$). Error bars represent the standard error of the mean.

Clopyralid applications had no effect on flower number ($P = 0.99$), immature berry number ($P = 0.63$), total reproductive organs ($P = 0.92$), total yield by weight ($P = 0.491$) (216 g plant^{-1}), and berry number ($P = 0.66$) (10 plant^{-1}). The safety of clopyralid on flower number and yield was consistent with previous findings (Boyd and Dittmar 2015; Figueroa and Doohan 2006; McMurray et al. 1996). The influence of clopyralid on the immature fruit number and the total reproductive load on the plant has not been previously reported. Current results demonstrate the safety of clopyralid on both parameters. Clopyralid is safe to apply across a wide range of doses (35 to 2,240 g ha^{-1}) when the plants are fully mature with no effect on the production, development, and harvest of reproductive organs of the strawberry plant.

This study was the first to conduct a thorough examination on the effect of a wide range of clopyralid doses (35 to 2,400 g ha^{-1}) on the mature strawberry plant body. No negative effects of clopyralid dose were found on the number of strawberry crowns and leaves, the vegetative biomass both above- and belowground, the number of reproductive organs, or the reproductive output over time. Strawberry is naturally tolerant to clopyralid across a wide range of doses (35 to 2,250 g ha^{-1}) and generally only demonstrates clopyralid symptomology with leaf curling and malformations. Such symptomology is typical of an alternative pathway induced by auxinic herbicides (Grossman 2010). Strawberry plants may experience a mild growth cessation in response to clopyralid for both plant height and the number of leaves (Boyd and Dittmar 2015; McMurray et al. 1996), but inconsistencies in strawberry tolerance are infrequent.

Clopyralid Absorption

The total radioactivity recovered was 91% and 106% for trial run 1 and 2, respectively. Maximal strawberry absorption (A_{max}) was 84% and reached peak absorbance (t_{90}) in 15 h (Figure 2). Water was used to rinse the unabsorbed clopyralid from the leaf, so it was assumed that the cuticle was intact, and cuticular absorption was counted with the treated leaf.

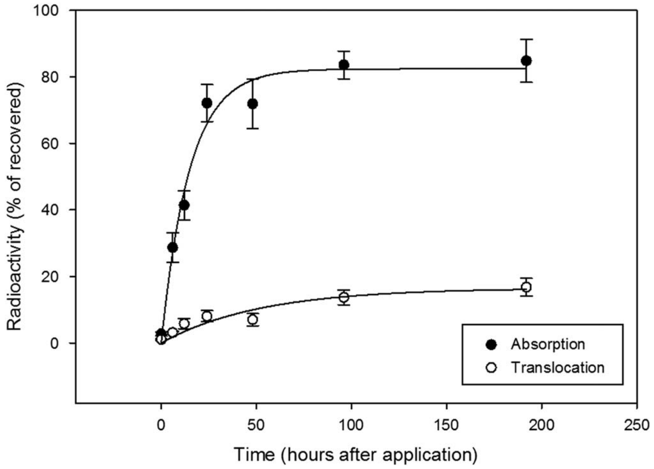

Figure 2. Radiolabeled clopyralid absorption and translocation into strawberry. Radioactivity expressed as a percentage of the total applied. Error bars are the standard error of the mean. The model for clopyralid absorption ($R^2_{adj} = 0.79$) was: $\text{Absorption} = 82 \times \left\{1 - \exp\left[(\log 0.1) \times \left(\frac{t}{15}\right)\right]\right\}$, while the model for clopyralid translocation from the treated leaf ($R^2_{adj} = 0.50$) was: $\text{Translocation} = 17 \times \left\{1 - \exp\left[(\log 0.1) \times \left(\frac{t}{51}\right)\right]\right\}$.

Considering other tolerant species, total clopyralid absorption for strawberry was less then rapeseed (>97% at 24 HAT) and greater than *A. cannabinum* when applied at the vegetative (47% of applied radiation at 72 HAT) and early reproductive stages (56% of applied radiation at 144 HAT) (Hall and Vanden Born 1988; Orfanedes et al. 1993). Species differences may be due to cuticle structure, as clopyralid is a lipophilic, ionic herbicide and is absorbed in foliage by nonfacilitated diffusion (Sterling 1994). Given the high degree of radioactivity recovered within the plant (84%), strawberry tolerance is not due to absorption.

Total Translocation

Clopyralid translocation out of the strawberry treated leaf reached a maximum of 17% of recovered radiation by 51 HAT (Figure 2), approximately 14% of absorbed radiation. This was dramatically lower than results for rapeseed (65% of recovered radiation at 144 HAT) (Hall and Vanden Born 1988) and *A. cannabinum* for applications to the vegetative (81% of absorbed radiation at 72 HAT) and early reproductive stages (76% of absorbed radiation at 144 HAT). Limited translocation has been demonstrated in *L. arundinaceum* with aminocyclopyrachlor, wherein 37% of the recovered radioactivity was translocated by 96 HAT, and the parent molecule remained intact with no degradation (Lewis et al. 2013). Carboxylic acid herbicides such as clopyralid and aminocyclopyrachlor are known to remain biologically active in decaying tissue that was exposed to the herbicide (Anonymous 2010).

Strawberry tolerance to clopyralid is due, in part, to limited translocation. Why strawberry plants demonstrate limited clopyralid translocation is still unknown but may be due to several factors and requires further study. Movement into the endoplasmic reticulum may occur due to its role in auxin binding protein 1 storage (Henderson et al. 1997). Clopyralid interaction with strawberry auxin influx and efflux proteins may be limiting (Hosek et al. 2012). Clopyralid may be limited by strawberry plant phloem loading and unloading (Devine and Hall 1990). The auxin receptor AFB5, which is a site of action for carboxylic acids but not aryloxyacetates or benzoates, may be missing or mutated in strawberry plants (Walsh et al. 2006). Tolerance may also be linked to the strawberry industry's reliance on propagating physiologically mature daughter plants for transplants, as differential tolerance to 2,4-D based on physiological maturity has been demonstrated (Pazmiño et al. 2011). Strawberry leaves exposed to clopyralid generally only experience slight growth malformation such as cupping (Boyd and Dittmar 2015), which may be due to an alternate pathway in the action of auxinic herbicides that stimulates cell expansion (Grossman 2010), and subsequent metabolism may prevent the slower phytotoxic pathway, though further study is required.

Translocation to Plant Parts

Within the strawberry plant, the organs that contained the most radioactivity were the leaves younger than the treated leaf ($A_{\max} = 6\%$) and the inflorescence ($A_{\max} = 4\%$) (Figures 3 and 4). Time to reach peak translocation was 18 and 66 h for the younger leaves and inflorescence, respectively. This was expected, as the younger leaves and inflorescence represent strong sinks for resources and thus dictate the direction of phloem bulk flow and subsequent long-distance transport of radioactivity.

Due to the low degree of translocation and high variability, ARF functions were not able to be fit to the data for the crowns, roots, and older leaves. At 196 HAT, 4% of the recovered radioactivity was found within the older leaves (Figure 3). This was similar to the amount found within the reproductive structures, although the reproductive structures represented far less biomass than the older leaves (unpublished data). Only 2% of recovered radioactivity was within the crowns at 196 HAT (Figure 4). With very low translocation to the crown, the reproductive potential and new growth would likely be unaffected, especially given the overall time frame for translocation (Figure 2). Only 1% of radioactivity was recovered within the roots at 196 HAT (Figure 4). With minimal translocation to the roots, nutrient uptake was also likely unaffected. This was consistent with previous findings that clopyralid does not consistently suppress vegetative or reproductive growth and yield (Boyd and Dittmar 2015).

In summary, strawberry absorption reached a maximum of 82% and reached peak absorption at 15 HAT. Only 16.5% of the recovered radiation was translocated out of the strawberry treated leaf and reached peak translocation at 52 HAT. Translocation out

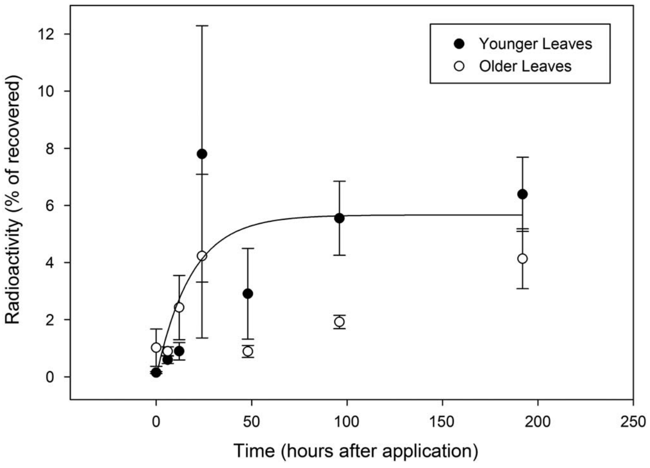

Figure 3. Translocation over time of radiolabeled clopyralid based on radioactivity found in the strawberry leaves. Error bars are the standard error of the mean. The model for leaves younger than the treated leaf (R^2_{adj}=0.11) was: $\text{Translocation} = 6 \times \left\{1 - \exp\left[(\log 0.1) \times \left(\frac{t}{18}\right)\right]\right\}$.

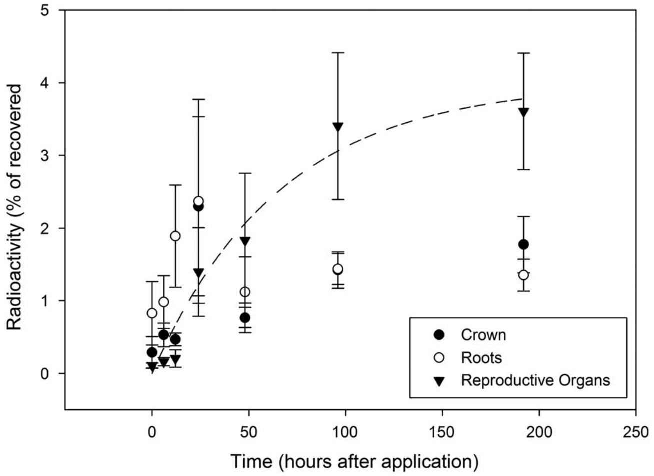

Figure 4. Translocation over time of radiolabeled clopyralid based on radioactivity found in strawberry reproductive organs, crowns, and roots. Error bars are the standard error of the mean. The model for translocation to the reproductive organs in strawberry ($R^2_{adj}=0.40$) was: $\text{Translocation} = 4.0 \times \{1 - \exp[(\log 0.1) \times (\frac{t}{66})]\}$.

of the strawberry treated leaf appeared preferential toward dominant sinks such as leaves younger than the treated leaf (6%) and reproductive organs (4%) but also translocated to the older leaves, crowns, and roots. Limited herbicide translocation and dispersal throughout the plant factors into the mechanism of strawberry tolerance to clopyralid but does not entirely explain it. Further study is required to determine the involvement of auxin-related reception and metabolism.

Early Clopyralid Application

There was a three-way interaction between clopyralid dose, application timing, and the measurement date ($P < 0.0001$) on clopyralid damage to strawberry plants. Clopyralid damage primarily manifested as leaf cupping. When applied at 14 DATr, clopyralid doses of 140 and 280 g ha^{-1} induced a maximum of 4% and 15% leaf cupping, respectively (Table 2). Repressed translocation from limited water availability during root hair reestablishment may affect clopyralid safety on strawberry plants at 14 DATr. Strawberry plant root hair reestablishment has taken 3 to 4 wk in a growth chamber (Borkowska 2001). Alternatively, the limited clopyralid translocation demonstrated in mature strawberries (Figure 2) may provide safety in immature strawberry plants, though further study is required.

Table 2. Induced damage to plasticulture-grown strawberry plants by clopyralid when applied in combinations of two doses and three timings in Balm, FL, in 2016.[a,b]

Clopyralid dose	Application timing	Damage[c]			
		1 WAT	2 WAT	4 WAT	8 WAT
g ae ha^{-1}	DATr	------------% of control--------			
140	14	4 ef	0 f	0 f	0 f
140	21	17 de	7 ef	3 ef	0 f
140	28	11 def	6 ef	0 f	0 f
280	14	10 def	15 def	11 def	0 f
280	21	37 bc	56 a	24 cd	0 f
280	28	46 ab	36 bc	8 ef	0 f

[a] Abbreviations: DATr, days after planting of the strawberry transplants; WAT, weeks after clopyralid treatment.

[b] Data were averaged across both trial runs and back-transformed means are presented.

[c] Damage was determined on a percentage scale of the degree of leaf cupping and malformation of leaves per experimental unit (whole plot). Different lowercase letters indicate a significant difference using Tukey's honest significant difference means test ($\alpha = 0.05$).

Leaf cupping induced by 140 g ha^{-1} reached a maximum of 17% at 1 WAT when applied at 21 DATr (Table 2). Leaf cupping induced by 280 g ha^{-1} reached a maximum of 56% at 2 WAT when applied at 21 DATr (Table 2). The degree of clopyralid-induced leaf cupping at 21 DATr was higher than previous studies on mature strawberry plants in Florida (~40%) (Boyd and Dittmar 2015) and North Carolina plasticulture (0% malformations) (McMurray et al. 1996). By 8 WAT, there was no leaf cupping (Table 2). Such recovery was consistent with previous results with leaf malformations (Boyd and Dittmar 2015). The 140 g ha^{-1} dose was safe across all application timings examined, while the 280 g ha^{-1} induced substantial leaf cupping post-14 DATr.

Strawberry plant height was affected by clopyralid dose ($P = 0.027$) but not application timing ($P = 0.55$) or the measurement timing ($P = 0.22$). There was slight stunting for the 280 g ha^{-1} dose (97.2% of the nontreated control) compared with 140 g ha^{-1} (100.0%). Results were consistent with previous research on mature strawberry plants (McMurray et al. 1996), although inconsistent with findings in the clopyralid dose–response study. Size-based tolerance to clopyralid has been demonstrated in *M. lupulina* (Sharpe et al. 2016) and is possibly a result of the sheer degree of tissue available to alter the auxin stream (Zazimalova et al. 2014), though further study is required.

There was no effect of clopyralid dose ($P = 0.108$) or application timing ($P = 0.137$) on the strawberry plant aboveground biomass (43.9 g plant^{-1}). Results were consistent with the dose–response study. Neither clopyralid dose ($P = 0.20$) nor application timing ($P = 0.50$) affected the number of crowns (3 plant^{-1}). Results were consistent with the dose–response study and two timings by Clay and Andrews (1984), but contrasted with results from a third application timing in a matted-row system (0.3 plant^{-1}). With only slight stunting in plant heights and no difference in aboveground biomass or the crown number, clopyralid applications shortly after transplant appear safe on the season-long development of the vegetative plant body.

For berry weight, there was an interaction between the clopyralid dose and the date of harvest ($P = 0.016$). This was likely due to the natural variability of the yield over time and the small amount of harvest in the growing season. There was no indication of a delayed harvest induced by clopyralid application, with no differences between treatments within each timing, including controls (unpublished data). There was no interaction for the number of berries per plant between the treatments and the date of harvest ($P = 0.20$), but there was an effect of treatment ($P = 0.017$). Delayed harvest due to clopyralid typically has not occurred when applied to fully developed strawberries at labeled doses in annual plasticulture (Boyd and Dittmar 2015; McMurray et al. 1996) or matted-row systems (Figueroa and Doohan 2006).

There was no effect of application timing or dose on the berry number ($P = 0.0543$) (5 berries plant^{-1}) or the total harvested weight of the berries ($P = 0.056$) (83.6 g plant^{-1}). Results were consistent with previous literature for clopyralid applications to fully mature strawberry in annual plasticulture (Boyd and Dittmar 2015; McMurray et al. 1996) and matted-row systems (Figueroa and Doohan 2006). There was no increase in yield with the high labeled dose compared with controls, which was contrary to previous findings in matted-row systems (Figueroa and Doohan 2006).

Clopyralid applied at 140 g ha^{-1} to strawberry plants at 14 to 28 DATr induced a maximum of 17% damage (Table 2). This dose and timing did not reduce plant heights, aboveground biomass, number of crowns, or yield. Clopyralid applied at 280 g ha^{-1} at 14 to 28 DATr induced maximal damage of 56%, with recovery by 8 WAT (Table 2). The 280 g ha^{-1} dose induced 3% stunting compared with controls. Although there were no further reductions in biomass or yield, the damage may be too severe for producer recommendation. Clopyralid applied to strawberry from 14 to 21 DATr at 140 g ha^{-1} represents a safe application timing and dose to increase spray penetration to gain control of problematic weeds when they are of an ideal size. Results for earlier applications are promising due to the potential for reduced strawberry canopy shielding, increased coverage to *M. lupulina*, overcoming limited clopyralid translocation out of treated *M. lupulina* branches, and targeting a susceptible *M. lupulina* size (Sharpe 2017; Sharpe et al. 2016, 2018b, 2018c). *Medicago lupulina* emergence began at 45 DATr (861 GDD) and reached 90% peak emergence at 78 DATr (1,416 GDD) (Sharpe 2017). Warmer temperatures both pre- and post-clopyralid application have increased strawberry leaf cupping in growth rooms, though they did not affect growth parameters (Sharpe et al. 2018a). Further research is required to determine whether clopyralid can control *M. lupulina* at this timing at doses safe for strawberry in the field.

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References

Anonymous (2010) Stinger® herbicide specimen label. Indianapolis, IN: Dow AgroSciences LLC

Anonymous (2011) Stinger® supplemental labeling for annual strawberry in Florida. Indianapolis, IN: Dow AgroSciences LLC

Borkowska B (2001) Morphological and physiological characteristics of micropropagated strawberry plants rooted in vitro or ex vitro. Sci Hortic (Amst) 89:195–206

Boyd NS, Dittmar PJ (2015) Impact of application time and clopyralid rate on strawberry growth and yield. Weed Technol 29:821–826

Bukun B, Gaines TA, Nissen SJ, Westra P, Brunk G, Shaner DL, Sleugh BB, Peterson VF (2009) Aminopyralid and clopyralid absorption and translocation in Canada thistle (*Circium arvense*). Weed Sci 57:10–15

Clay DV, Andrews L (1984) The tolerance of strawberries to clopyralid: effect of crop age, herbicide dose and application date. Asp Appl Biol 8:151–158

Darnell RL (2003) Strawberry growth and development. Pages 3–10 *in* Childers NF, ed. The Strawberry: A Book for Growers, Others. Gainesville, FL: Dr. Norman F. Childers Publications

Devine MD, Hall LM (1990) Implications of sucrose transport mechanisms for the translocation of herbicides. Weed Sci 38:299–304

Devine MD, Vanden Born WH (1985) Absorption, translocation, and foliar activity of clopyralid and chlorosulfuron in Canada thistle (*Cirsium arvense*) and perennial sowthistle. Weed Sci 33:524–530

Dittmar PJ, Dufault NS, Noling JW, Stansly P, Boyd N, Paret ML, Webb SE (2017) Integrated Pest Management. Pages 19–29 *in* Vallad G, Smith H, Freeman J, Dittmar P, eds. Vegetable Production Handbook of Florida 2017–2018. Gainesville, FL: University of Florida

Figueroa RA, Doohan DJ (2006) Selectivity and efficacy of clopyralid on strawberry (*Fragaria* × *ananassa*). Weed Technol 20:101–103

Grossman K (2010) Auxin herbicides: current status of mechanism and mode of action. Pest Manag Sci 66:113–120

Hall JC, Vanden Born WH (1988) The absence of a role of absorption, translocation, or metabolism in the selectivity of picloram and clopyralid in two plant species. Weed Sci 36:9–14

Henderson J, Bauly JM, Ashford DA, Oliver SC, Hawes CR, Lazarus CM, Venis MA, Napier RM (1997) Retention of maize auxin-binding protein in the endoplasmic reticulum: quantifying escape and the role of auxin. Planta 202:313–323

Hosek P, Kubes M, Lankova M, Dobrev PI, Klima P, Kohoutova M, Petrasek J, Hoyerova K, Jirina M, Zazimalova E (2012) Auxin transport at cellular level: new insights supported by mathematical modelling. J Exp Bot 63:3815–3828

Hunnicutt CJ, MacRae AW, Dittmar PJ, Noling JW, Ferrell JA, Alves C, Jacoby TP (2013a) Annual strawberry response to clopyralid applied during fruiting. Weed Technol 27:573–579

Hunnicutt CJ, MacRae AW, Whitaker VM (2013b) Response of four strawberry cultivars to clopyralid applied during fruiting stage. HortScience 23:301–305

Kniss AR, Vassios JD, Nissen SJ, Ritz C (2011) Nonlinear regression analysis of herbicide absorption studies. Weed Sci 59:601–610

Lewis DF, Roten RL, Everman WJ, Gannon TW, Richardson RJ, Yelverton FH (2013) Absorption, translocation, and metabolism of aminocyclopyrachlor in tall fescue (*Lolium arundinaceum*). Weed Sci 61:348–352

McMurray GL, Monks DW, Leidy RB (1996) Clopyralid use in strawberries (*Fragaria* × *ananassa* Duch.) grown on plastic mulch. Weed Sci 44:350–354

Nandula VK, Vencill WK (2015) Herbicide absorption and translocation in plants with radioisotopes. Weed Sci 63:140–151

Orfanedes MS, Wax LM, Liebl RA (1993) Absence of a role for absorption, translocation, and metabolism in differential sensitivity of hemp dogbane (*Apocynum cannabinum*) to two pyridine herbicides. Weed Sci 41:1–6

Pazmiño DM, Rodriguez-Serrano M, Romero-Puertas MC, Archilla-Ruiz A, Del Rio LA, Sandlio LM (2011) Differential response of young and adult leaves to herbicide 2,4-dichlorophenoxyacetic acid in pea plants: role of reactive oxygen species. Plant Cell Environ 34:1874–1889

Sharpe SM (2017) Use of Clopyralid to Control Black Medic (*Medicago lupulina*) in Florida Strawberry (*Fragaria* ×*ananassa*) Production. Ph.D dissertation. Gainesville, FL: University of Florida. 130 p

Sharpe SM, Boyd NS, Dittmar PJ (2016) Clopyralid dose response for two black medic (*Medicago lupulina*) growth stages. Weed Technol 30:717–724

Sharpe SM, Boyd NS, Dittmar PJ, MacDonald GE, Darnell RL (2018a) Effect of temperature on clopyralid safety in strawberry. Weed Technol. doi: 10.1017/wet.2017.119

Sharpe SM, Boyd NS, Dittmar PJ, MacDonald GE, Darnell RL, Ferrell JA (2018b) Control recommendations for black medic (*Medicago lupulina*) based on growth and development in competition with strawberry. Weed Sci 66:226–233

Sharpe SM, Boyd NS, Dittmar PJ, MacDonald GE, Darnell RL, Ferrell JA (2018c) Spray penetration into a strawberry canopy as affected by canopy structure, nozzle type, and application volume. Weed Technol 32:80–84

Sterling TM (1994) Mechanisms of herbicide absorption across plant membranes and accumulation in plant cells. Weed Sci 42:263–276

Turgeon R (1989) The sink-source transition in leaves. Annu Rev Plant Phys Plant Mol Biol 40:119–138

Turnbull GC, Stephenson GR (1985) Translocation of clopyralid and 2,4-D in Canada thistle (*Cirsium arvense*). Weed Sci 33:143–147

[USDA] U.S. Department of Agriculture (2015) National Agricultural Statistics Service: Data_and_Statistics. http://www.nass.usda.gov/Data_and_Statistics. Accessed: June 19, 2015

[USDA] U.S. Department of Agriculture (2017) Natural Resources Conservation Service: Web Soil Survey. https://websoilsurvey.sc.egov.usda.gov. Accessed: October 2, 2017

Valenzuela-Valenzuela JM, Lownds NK, Sterling TM (2001) Clopyralid uptake, translocation, metabolism, and ethylene induction in picloram-resistant yellow starthistle (*Centaurea solstitialis* L.). Pest Biochem Physiol 71:11–19

Walsh TA, Neal R, Merlo AO, Honma M, Hicks GN, Wolff K, Matsumura W, Davies JP (2006) Mutations in an auxin receptor homolog AFB5 and in SGT1b confer resistance to synthetic picolinate auxins and not to 2,4-dichlorophenoxyacetic acid or indole-3-acetic acid in *Arabidopsis*. Plant Phys 142:542–552

Webster TM (2014) Weed survey—southern states 2014. Vegetable, fruit and nut crop subsection. Page 288 *in* Proceedings of the Southern Weed Science Society 67th Annual Meeting. Birmingham, AL: Southern Weed Science Society

Whitaker VM, Boyd NS, Peres NA, Noling JW, Renkema J (2017) Strawberry production. Pages 293–312 *in* Vallad G, Smith H, Freeman J, Dittmar P, eds. Vegetable Production Handbook of Florida 2017–2018. Gainesville, FL: University of Florida

White SN, Boyd NS, Van Acker RC (2012) Growing degree-day models for predicting lowbush blueberry (*Vaccinium angustifolium* Ait.) ramet emergence, tip dieback, and flowering in Nova Scotia, Canada. HortScience 47:1014–1021

Zazimalova E, Petrasek J, Benkova E (2014) Auxin and Its Role in Plant Development. Heidelberg, Germany: Springer-Verlag. 444 p

Zollinger RK, Penner D, Kells JJ (1992) Absorption, translocation and foliar activity of clopyralid and tribenuron in perennial sowthistle (*Sonchus arvensis*). Weed Sci 40:528–533