Paclobutrazol Residues in Recirculated Water in Commercial Greenhouses

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ABSTRACT. Reusing irrigation water has technical, environmental, and financial benefits. However, risks are also associated with the accumulation of agrochemicals, in addition to ions, plant and food safety pathogens, and biofilm organisms. In this project, we measured the concentration of paclobutrazol (a persistent and widely used plant growth regulator) in recirculated water in greenhouses producing ornamental plants in containers. Solutions were collected from catchment tanks at nine commercial greenhouses across seven states in the United States in Spring and Fall 2014. Paclobutrazol was detected in all samples, with differences observed by season, greenhouse operation, paclobutrazol application method, and irrigation method. Across operations, the residual concentration of paclobutrazol was higher in spring for most greenhouses (ranging from 0 to 1100 $\mu g \cdot L^{-1}$) compared with the fall (ranging from 0 to 8 $\mu g \cdot L^{-1}$). The spray-drench application method resulted in the highest residual concentrations (up to 35 $\mu g \cdot L^{-1}$), followed by substrate drench (up to 26 $\mu g \cdot L^{-1}$) and foliage spray (concentrations under 3 $\mu g \cdot L^{-1}$). Residual concentrations were higher with overhead irrigation (up to 35 $\mu g \cdot L^{-1}$) compared with subirrigation systems (up to 15 $\mu g \cdot L^{-1}$). Our results indicate that paclobutrazol is likely to be a growth retardant risk in greenhouse operations recirculating water. A clear understanding of the risks associated with recirculated water intends to support the development and implementation of risk management strategies to ensure and promote safe use of recirculated water in greenhouses. Overall, the most effective preventative strategy is to ensure the use of the minimum amount of the a.i. necessary per unit of space and time.

Reusing irrigation water has technical, environmental, and financial benefits. However, risks are also associated with the accumulation of agrochemicals, plant and food safety pathogens, and biofilm organisms, particles, and ions that cause clogging of irrigation systems (Ferrarezi et al. 2015; Stewart-Wade 2011; van Os 1999). In this project, we measured the residual concentration of paclobutrazol (PBZ) [(±)-(R*,R*)-β-(4-chlorophenyl) methyl-α-(1,1-dimethylenthyl)-1H-1,2,4-triazole-1-ethanol)], a persistent and widely used plant growth regulator, in recirculated water in greenhouses in the United States producing ornamental plants in containers.

Closed-irrigation systems (where water is captured and reused) use less fresh water for irrigation and result in reduced nutrient runoff into the environment compared with open drain-to-waste systems (Grewal et al. 2011). Horticultural operations with expensive or scarce water sources obtain financial benefits in reusing water by saving on the cost of source water and fertilizers, which tend to be higher than the cost of treating water (DeVincentis et al. 2015; Pitton et al. 2018; Raudales et al. 2017). Subirrigation is an irrigation system used in greenhouses where water or nutrient solutions are delivered to the containers from below and solutions then move upward through the substrate via capillary action (Ferrarezi et al. 2015; van Os 1999). The unabsorbed solution is captured and stored for further reuse, reportedly reducing water use by up to 56% (Dumroese et al. 2006).

Paclobutrazol is a plant growth retardant commonly used in the ornamental greenhouse industry since the 1980s (Goulston and Shearing 1985), with a half-life of 164 d in water (Massachusetts Department of Agricultural Resources 2012). Recommended label rates for ornamental plants range between 1 and 10 $mg \cdot L^{-1}$ paclobutrazol for substrate drench application and 5 to 100 $mg \cdot L^{-1}$ for foliar sprays. Million et al. (2002) evaluated subirrigation as a method to apply plant growth regulators (PGR) to ornamental bedding plants and concluded that a 30% plant growth suppression could be achieved by applying paclobutrazol via subirrigation with a single application of 0.1 to 2.4 $mg \cdot L^{-1}$ or a continuous application of 0.005 to 0.4 $mg \cdot L^{-1}$, with the specific rate depending on the crop. Wax Begonia (Semperflorens-Cultorum Group) was the most sensitive of the crops tested, with 30% less biomass than untreated plants resulting from a single application of 0.1 $mg \cdot L^{-1}$ or continuously at 0.005 $mg \cdot L^{-1}$ paclobutrazol. Grant et al. (2018a) reported residual concentrations of up to 72.5 $\mu g \cdot L^{-1}$ (0.0725 $mg \cdot L^{-1}$) paclobutrazol in recirculated water in a commercial greenhouse. The extended half-life of paclobutrazol in water increases the risk of it persisting and accumulating in recycled irrigation water, resulting in the potential to negatively influence the growth of subsequent crops irrigated with low concentrations of residual paclobutrazol.

Growers do not apply paclobutrazol directly in subirrigation systems, but they do apply it frequently during the peak production season as a drench (high volume to substrate only), foliar spray (low volume to foliage), or medium volume spray-drench combination. Considering the extended half-life of paclobutrazol in water, its ability to adsorb to surfaces and desorb when the surfaces are rewet, the low dose required to reduce plant growth significantly, and how water moves into the substrate when subirrigating, there is a critical need to identify whether residual concentrations of paclobutrazol are a

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common risk in recirculated water resulting in the need for remediation or preventative practices. This research aimed to measure the residual concentration of paclobutrazol in recirculated water in commercial greenhouses producing bedding plants that capture and reuse water.

Materials and methods

Greenhouse operations. Nutrient solutions and water samples ("samples") were collected from commercial greenhouses across seven states in the United States in 2014. Eight or five greenhouses were sampled in spring (weeks 14–18) and fall (weeks 33–37), respectively. Nonprobability sampling was not implemented, as random operation selection was not possible because we had limited operations that met the eligibility criteria. Operations were eligible to participate if, during the sampling season, they 1) were producing containerized-ornamental crops in greenhouses; 2) were applying paclobutrazol as a plant growth regulator on a regular basis; 3) were capturing solutions ("runoff") from subirrigation in tanks or catchment basins ("ponds"); 4) had the ability to sample runoff at a return tank, drain to a pond, or the pond itself; and 5) agreed to collect samples for this project.

Nine commercial greenhouses met the aforementioned eligibility criteria for participating in the research (Table 1). The operations, located in Connecticut, Florida, Michigan, New Hampshire, New Jersey, North Carolina, and Ohio (all in the United States), were dedicated to the greenhouse production of containerized ornamental crops for wholesale with target markets or businesses such as retail growers, garden retailers, and professional landscapers. Four greenhouses participated in both seasons, four only in spring, and one only in fall. During the fall, the target dose of paclobutrazol that each operation intended to achieve and the dose that was applied according to the brand of product used was recorded by each grower. In the spring, two operations primarily applied paclobutrazol via foliar spray, one via spray-drench, and five via drenches (Table 1). In the fall, all four reporting operations applied via drench.

Sampling sites. Each greenhouse chose a single sampling point to be sampled once per week for 3 weeks, with four replicates per week for a total of 12 samples per location per season. Sampling points included either return pipes, drains, or ponds that collected solutions from areas that had been irrigated and contained crops that received paclobutrazol sprays, spray-drenches, or drenches. For some greenhouses, the application or irrigation methods were not constant over the 3 weeks or the two seasons (for greenhouses that participated in fall and spring). Growers were provided with a sampling kit to guarantee a consistent quality of samples. The sampling kit included a written sampling protocol, a form to complete with each sampling, disposable gloves, four plastic sampling bottles (~240 mL), four sample labels, a water-resistant marker, paper towels, and packaging materials (plastic bags, a cold pack, tape, and bubble wrap). Growers collected four 240-mL samples per week, refrigerated the samples until they were ready to ship, and then shipped all samples with a frozen cold pack to the US Department of Agriculture, Agricultural Research Service

Table 1. Season, area treated, application method, and irrigation characteristics of nine commercial greenhouses that applied paclobutrazol to ornamental crops for growth control and captured and reuse irrigation water. Paclobutrazol *applied* levels are self-reported numbers and *measured* levels were residual levels quantified in the solutions captured in water that was going to be recirculated.

Season	Greenhouse code	Production area (ft^2)[i]	Predominant application method	Water treatment	Paclobutrazol		Irrigation (%)[iv]		
					Applied ($mg \cdot L^{-1}$)[ii]	Measured ($\mu g \cdot L^{-1}$)[iii]	Subirrigation	Overhead	Drip
Spring	A	4,260	Spray	Copper ionization and pool filter	ND[v]	0.6–9.0	74	26	0
	B	232,000	Drench	Paper and pool filters	ND	7.4–10.9	40	45	15
	C	275,000	Spray-drench	Cloth filter paper	ND	0.6–814.6	13	65	22
	D	10,375	Drench	Ozone	ND	0.0–15.2	20	80	0
	E	1,000,000	Drench	Ionized glass filter and copper ionization	ND	3.4–13.3	100	0	0
	F	993,60	Drench	Cascading filter with coarse metal mesh	ND	0.4–74.0	25	75	0
	G	1,500,000	Drench	Sodium hypochlorite and sand filter	ND	5.2–1077.2	0	100	0
	H	348,481	Spray	Chlorine pump	ND	0.0–1.5	100	0	0
Fall	C	500,000	Drench	Bag and carbon filters	1–5	0.0–2.7	0	75	25
	D	20,500	Spray	Ozone	14	0.0–0.2	50	50	0
	E	4,791,600	Drench	Ozone, microfilter, and copper ionization	2	0.4–6.1	90	10	0
	G	740,520	Drench	Sand filters, chlorine, sulfuric acid	2	0.3–8.0	0	100	0
	I	1,103,667	ND	Chlorine and micron filter system	ND	0.0–3.0	30	70	0

[i] 1 square foot (ft^2) = 0.0929 m^2.
[ii] 1 $mg \cdot L^{-1}$ = 1 ppm.
[iii] 1 $\mu g \cdot L^{-1}$ = 1 ppb.
[iv] The proportion of usage in comparison with all methods employed. Subirrigation, overhead, and drip irrigation add up to 100%.
[v] ND indicates that no data are available because the question was not asked in the Spring sampling season or was not answered in the survey.

(USDA-ARS) Application Technology Research Unit laboratory in Wooster, OH, USA, within the same week. After receipt at USDA-ARS, samples were stored at 4 °C until processing. All growers used the same containers and packing, provided by the research team.

Quantifying paclobutrazol. Paclobutrazol was quantified using a gas chromatograph (GC, model 7890B; Agilent Technologies, Santa Clara, CA, USA) equipped with a mass spectrometer (MS, model 5977A; Waters Corp., Santa Clara, CA, USA) as described by Altland et al. (2015). Equipment was calibrated using the external standard method with serial dilutions of a paclobutrazol standard in acetonitrile (MeCN) ranging from 0.5 to 5.0 mg·L^{-1}.

Statistical analysis. All statistical analyses were conducted using the RStudio statistical software version 2023.06.0 (Posit, Boston, MA, USA). The main packages used were 'agricolae' (de Mendiburu 2023), 'ggplot2' (Wickham 2023), 'Rmisc' (Hope 2022), and 'ggpubr' (Kassambara 2023). For each greenhouse, a descriptive statistical analysis was performed. The applied concentration reported on the survey and measured residual paclobutrazol data were subjected to the Shapiro–Wilk test for normality of variance, showing a nonnormal distribution. Therefore, the nonparametric Kruskal–Wallis test was adopted to compare the levels between seasons, greenhouses, paclobutrazol application methods, and irrigation methods. The levels compared were fall and spring for the season; spray, drench, and spray-drench for the paclobutrazol application method; overhead and subirrigation for the irrigation method; and the nine commercial operations for the greenhouse factor. For the application and irrigation methods, missing information for some greenhouses was removed from the database, and not considered in the analysis. The Dunn's multiple comparisons post hoc test with Bonferroni correction was used following a significant ($P < 0.05$) Kruskal–Wallis test. Additionally, a Pearson statistical correlation analysis ($P < 0.05$) was performed to analyze the correlation between reported application and measured residual concentrations of paclobutrazol.

Results

Paclobutrazol applied dose. Operations reported applying between 1 and 14 mg·L^{-1} paclobutrazol (Table 1), which was within product label rates for annuals of 1 to 200 mg·L^{-1} for foliar application and 0.01 to 8.0 mg·L^{-1} for drench application. Spray applications had higher paclobutrazol concentrations compared with drench applications (consistent with the product label). The applied spray application was 14 mg·L^{-1}, whereas drench applications ranged from 1 to 5 mg·L^{-1} (Table 1). There was a significant negative correlation (r = –0.36, $P = 0.007$) between the reported applied concentration of paclobutrazol and the residual measured concentration in the irrigation water in the fall.

Paclobutrazol residual levels in irrigation water by season and greenhouse operation. The paclobutrazol concentrations measured in the irrigation water varied by season ($P < 0.001$) and greenhouse ($P < 0.001$) (Fig. 1). Paclobutrazol detected in the recirculated water was higher and had a wider range of variation in spring compared with fall (Fig. 1A). In the spring, the median level of paclobutrazol concentration measured in the water was 8.9 µg·L^{-1}, but it varied widely between 0 and 1100 µg·L^{-1}. In the fall, all samples were less than 8 µg·L^{-1} paclobutrazol with a median level of 0.48 µg·L^{-1}. In terms of individual operations (Fig. 1B), the residual concentration of paclobutrazol in the recirculated water for most commercial operations varied between 0 and 20 µg·L^{-1}, with two greenhouses (F and G) having values of up to 60 µg·L^{-1} and some outliers close to 1000 µg·L^{-1} (greenhouses C and G).

Influence of application and irrigation methods on paclobutrazol residual concentrations. Paclobutrazol residual concentration in the irrigation water varied significantly by PGR application method ($P < 0.001$) and type of irrigation ($P = 0.002$) (Fig. 2). The residual concentrations of paclobutrazol were the highest with spray-drench with values up to 35 µg·L^{-1}; however this application method was only applied in the spring by greenhouse operation C. Drench applications had values up to 26 µg·L^{-1} and outliers above 1000 µg·L^{-1} (Fig. 2).

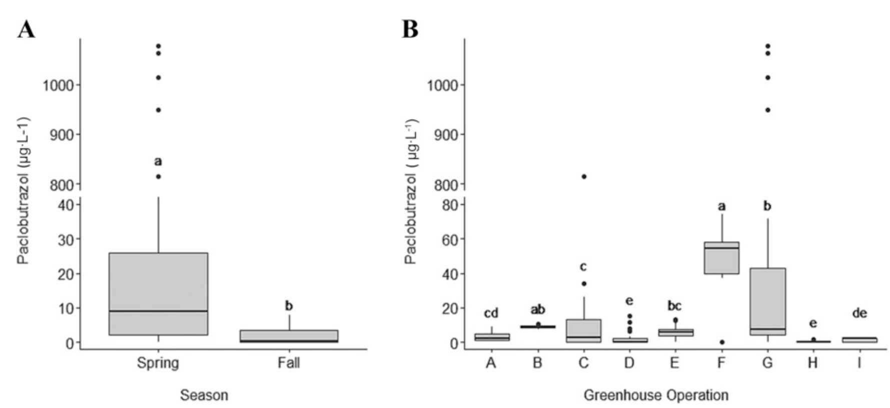

Fig. 1. Residual concentrations of paclobutrazol (µg·L^{-1}) in recirculated water in two seasons, spring and fall (A) and nine commercial greenhouses (B). Commercial greenhouses are described with letters from A to I (Table 1). Each boxplot represents the distribution of each variable and replicates (n = 12 for greenhouses A, B, F, H, and I that participated in a single season, n = 24 for greenhouses C, D, E, and G that participated in both seasons, n = 96 for spring, and n = 60 for fall), the inner line shows the median, the ends of the box represent the first and third quartiles, and the dots represent outliers. Boxplots (medians) with the same letters are not significantly different according to the Kruskal–Wallis rank-sum test and the Dunn's post hoc analysis at $P \leq 0.05$. 1 µg·L^{-1} = 1 ppb.

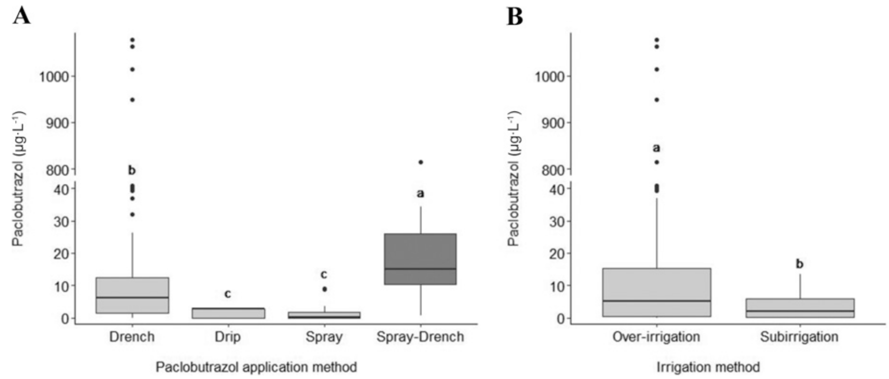

Fig. 2. Residual concentration of paclobutrazol in recirculated water by application (A) and irrigation method (B) used by the nine commercial greenhouse operations. Each boxplot represents the replicates (n = 96 for drench, n = 4 for drip, n = 28 for spray, n = 12 for spray-drench, n = 104 for over-irrigation, and n = 48 for subirrigation), the inner line shows the median, the ends of the box represent the first and third quartiles, and the dots represent outliers. Boxplots (medians) with the same letters are not significantly different according to the Kruskal–Wallis rank-sum test and the Dunn's post hoc analysis at $P \leq 0.05$. 1 $\mu g \cdot L^{-1}$ = 1 ppb.

The mean and median for spray applications showed a residual concentration lower than 3 $\mu g \cdot L^{-1}$ (Fig. 2A). Regarding the irrigation method, the residual concentrations of paclobutrazol were higher when the greenhouses used overhead irrigation (0 to 40 $\mu g \cdot L^{-1}$, without considering outliers) compared with subirrigation (0 to 15 $\mu g \cdot L^{-1}$) (Fig. 2B). This trend may suggest that overhead systems resulted in nontarget losses associated with the water falling between the containers followed by flushing of nonabsorbed paclobutrazol into the captured water. Additionally, subirrigation systems tend to have lower leaching fractions than overhead watering, which could result in lower residual concentrations.

Discussion

Our study demonstrates that greenhouse operations that capture and reuse irrigation water are at risk of phytotoxicity or growth retardation on susceptible crops caused by the residual effect of paclobutrazol. We found paclobutrazol concentrations up to 1000 $\mu g \cdot L^{-1}$ in the catchment tanks or ponds of the ornamental greenhouse operations sampled during the spring (Fig. 1). These values are significantly higher than those reported by Grant et al. (2018a), who observed a maximum concentration of 72.5 $\mu g \cdot L^{-1}$ paclobutrazol in catchment ponds of a commercial operation. Our data show that biologically active concentrations of paclobutrazol are commonly found in recirculated solutions in greenhouses.

Our results point to the importance of implementing safe water management frameworks, similar to those used in drinking water (World Health Organization 2017), to reduce risks associated with water use and recirculation. First, the threshold targets at which paclobutrazol in solution can be a risk to crops should be based on label rates and research-based recommendations (Million et al. 2002). Once these targets are defined by crop, it would be beneficial to implement a system assessment that determines changes throughout the system (WHO 2017). Application method and seasonality is another important component to best management practices to prevent contamination of irrigation water by paclobutrazol. In this study, we observed higher concentrations in the spring, and with spray-drench and drench applications compared with spray applications. To assess the risk of paclobutrazol contamination from recirculated water, the following factors must be considered: applied concentration, volume, frequency, and canopy capture (the percent of applied solution that enters the pot rather than as overspray onto the greenhouse bench or floor).

Because of the diversity of crops grown by the sample population (well over 100 cultivars during the spring), we were not able to gain specific data on application volume and concentration in this population. However, recommended application loads (concentration × volume) for a given area (Table 2) indicates that residual levels and volumes of water in this project exceed the biologically active application load that can impact crop growth and quality through all irrigation methods. For spray and spray-drench, application concentrations are usually 5 to 100 $mg \cdot L^{-1}$, and drenches are between 1 and 10 $mg \cdot L^{-1}$, although some growers will use lower (microdrenches at $\leq$0.125 $mg \cdot L^{-1}$; Lopez 2021) or higher (for less sensitive crops; Whipker 2023) concentrations. Spray and spray-drench applications are normally made on a per unit area basis, with 1 gal/200 ft^2 for sprays and 50% higher for spray-drenches to ensure dripping of the foliar spray into the substrate surface layer. In contrast, drenches are applied on a volume-per-pot basis (4 fl oz per 6-inch pot, with less or more volume for smaller or larger containers, respectively). Plant spacing therefore impacts the amount (mg) of a.i. applied per container, and per unit area (Table 2). Unit area is particularly important because the greenhouse area is the equivalent of the "catchment basin" for surface runoff of paclobutrazol. Spray-drenches apply more milligrams per unit area than foliar sprays because increased volume is applied with the spray-drench method. With high plant density, drenches apply more milligrams per unit area than the other two methods. If small plants are grown at low density, which is likely to occur when plants are young, then canopy capture is reduced, and the risk of overspray is increased. In addition, loss of paclobutrazol from the target container can occur if leaching occurs during the application or subsequent irrigations (Ochoa et al. 2009). For example, from Table 2, a spray-drench applied at high concentration to plants with low density and only 25% canopy capture would result in a high level of

Table 2. Applied load of paclobutrazol based on typical concentrations for applied concentration at high plant density [four 6-inch (15.2 cm) pots per square foot] or low plant density (one 6-inch pot per square foot).

Application Method Concentration	Typical Applied Concentration ($mg \cdot L^{-1}$)[i]		Typical application volume	Load per unit	High plant density		Low plant density	
	Low	High			Low	High	Low	High
Spray	5	100	1 gal/200 ft^2	mg/pot[ii]	0.02	0.47	0.09	1.89
				mg/200 ft^2[ii]	19	379	19	379
Spray-drench	5	100	1.5 gal/200 ft^2	mg/pot	0.04	0.71	0.14	2.84
				mg/200 ft^2	28	568	28	568
Drench	1	10	4 fl oz/6-inch pot[iv]	mg/pot	0.12	1.18	0.12	1.18
				mg/200 ft^2	95	946	24	237

Adapted from Whipker (2023).
[i] 1 $mg \cdot L^{-1}$ = 1 ppm.
[ii] 1 mg = 3.5274×10^{-5} oz.
[iii] 1 mg/200 ft^2 = 0.5382 $g \cdot ha^{-1}$ = 0.0077 oz/acre.
[iv] 1 pot/ft^2 = 10.7639 pots/m^2.

nontarget application, with 75% * 568 mg/200 ft^2 = 426 mg/200 ft^2 of overspray per application. Frequency of application also varies because drenches have longer growth retarding activity than foliar sprays (Desta and Amare 2021). Overall, the best preventative practice would be to ensure that the a.i. is applied directly into the substrate (drenches) or foliage (spray or spray-drench), without overspray or leaching onto the greenhouse bench or floor, at the minimum mg of a.i. necessary per unit space and time.

In commercial operations, another preventative management strategy that growers should implement is monitoring of paclobutrazol residual concentration in irrigation water. Altland et al. (2015) recommends a water sample of 100 to 200 mL be collected in a new plastic bottle and shipped as soon as possible so that samples are analyzed within 30 d from the time of collection. Cold temperature during sample storage and shipping is not necessary, although it is recommended that safeguards are used to ensure temperatures do not exceed 20 °C.

This assessment also emphasizes the importance of treatment of recirculated irrigation water before reuse to remain below the desired maximum threshold target. In commercial greenhouse operations, granular activated carbon has been observed as an effective and inexpensive solution to remove paclobutrazol from recirculated nutrient solutions (Grant et al. 2018b). Finally, growers should have maintenance and surveillance plans, as well as documentation, to ensure the efficacy of removal. The wide range in residual concentrations observed across the operations sampled points to the importance of testing recirculated nutrient solutions for risk factors, such as paclobutrazol. For example, operation F had very high paclobutrazol concentrations, presenting a high risk of paclobutrazol accumulation that could lead to crop damage in sensitive species (Table 1). Considering that paclobutrazol has a half-life of ~6 months in water, it can potentially build up over time in recirculating water systems and impact future crop cycles.

In summary, a clear understanding of the risks associated with recirculated water, including contamination with persistent agrochemicals such as paclobutrazol, is needed to support the development and implementation of risk management strategies to ensure and promote safe use of recirculated water in greenhouses.

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