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Small-scale evaluations of select pesticides for development of management recommendations for starry stonewort (*Nitellopis obtusa*)

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ABSTRACT

Starry stonewort is a nonnative, invasive macroalgae from Europe and western Asia. Unlike many of the native green macroalgae, starry stonewort can elongate into the water column and produce dense mats. Dense growth of starry stonewort can alter aquatic community structure and interfere with recreational activities. Management of starry stonewort has been difficult and unpredictable. The current study evaluated several formulations of copper algaecides as well as some herbicides for control of starry stonewort using controlled small-scale screenings. Copper formulation did not impact efficacy on starry stonewort, and all formulations tested offered > 60% biomass reduction, depending upon treatment concentration, except for Cutrine®-Ultra during one screening. Diquat and herbicides containing diquat offered > 95% biomass reductions 4 wk after treatments. Other herbicides evaluated did not offer significant reductions in biomass. Although promising from a small-scale perspective, data from field plots and further field demonstrations are needed.

Key words: algaecide, carfentrazone-ethyl, copper, diquat, endothall, flumioxazin, herbicide.

INTRODUCTION

Starry stonewort is a nonnative, invasive macroalgae from Europe and western Asia (Blindow 1994, Kato et al. 2005). It was introduced into the United States via ballast water into the Great Lakes (Sleith et al. 2015). Since its introduction, it has spread to New York, Vermont, Pennsylvania, Michigan, Minnesota, Wisconsin, and Indiana (Kipp et al. 2017). Unlike many of the native green macroalgae, starry stonewort can elongate into the water column, and in some cases reach plant lengths of 2 m (Steudle and Zimmermann 1977). Starry stonewort is anchored to bottom sediments by rhizoids. These rhizoids are important because they often contain bulbils, the starch-containing tissues used for overwintering and perennation. When conditions are conducive for growth, bulbils sprout and grow a new thallus (Larkin et al. 2018), although new thalli can grow via fragmentation as well. Dense growth of starry stonewort can alter the community structure of aquatic habitats by extirpating native vegetation, and interfering with boating and recreation (Glisson et al. 2018). Aquatic invasive species such as starry stonewort have also resulted in declines in property values (Horsch and Lewis 2009).

Currently, much of the management of starry stonewort has been conducted using algaecides registered for use in aquatic habitats. Of these algaecides, copper products have been used most often (Glisson et al. 2018). There are several different copper formulations available, from basic copper sulfate to chelated copper formulations, which utilize a variety of chelators and copper forms to change the physical/chemical properties of the algaecides. The different physical/chemical properties of copper formulations can have an impact on biological availability, movement into algal cells, and overall efficacy (Stauber and Florence 1987, Murray-Gulde et al. 2002, Bishop and Rodgers 2011, Bishop and Rodgers 2012).

Starry stonewort is a green alga, and as such, contains chlorophyll for the purposes of undergoing photosynthesis. In addition to copper algaecides, some herbicides can have activity on starry stonewort by targeting the photosynthetic pathway. Herbicides such as diquat, carfentrazone-ethyl, and flumioxazin interrupt photosynthesis, thereby killing the target plant. Other herbicides that have been evaluated at the field scale on starry stonewort include endothall. The molecular target site of endothall in plants is serine/threonine protein phosphatases responsible for regulating an array of biochemical processes (Bajsa et al. 2012). To date, there have been limited quantitative attempts to evaluate copper algaecide formulations or herbicides as a management tool for starry stonewort (Pokrzywinski et al. 2021). If herbicides are found to be efficacious, they would offer an alternative to copper algaecides or a tank-mix partner (Pennington et al. 2001, Pokrzywinski et al. 2021).

As starry stonewort continues to spread in Minnesota and across the upper tier of the United States, it is important to develop effective management recommendations for the use of aquatic pesticides. Good management recommendations come from results obtained from studies conducted at multiple scales (Netherland and Getsinger 2018). It is advantageous to conduct small-scale trials under controlled conditions to determine an initial plant response to different aquatic pesticides prior to field evaluations. The objective of this study was to evaluate starry stonewort susceptibility to copper algaecide formulations and select fast-acting herbicides.

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MATERIALS AND METHODS

Starry stonewort propagation

Starry stonewort was harvested from Lake Koronis (Stearns and Meeker counties, Minnesota) using the rake toss method in July and September 2019 and again in July 2020. Harvested biomass was placed in coolers and transported to Minnesota State University, Mankato, MN for planting. Once at the university, starry stonewort biomass was carefully separated into small clumps that would fit the diameter of a 473 ml plastic pot filled with topsoil. Sediment in each pot was amended with Osmocote® fertilizer[1] (19–6–12) at a rate of 2 g L^{-1} soil. Planted pots were placed into mesocosms in the greenhouse, which received ambient light; or into aquaria located in an indoor lab, which received artificial light on a 12:12 light:dark cycle. New starry stonewort thalli began to grow from the clumps within 1 to 2 wk, and became anchored in the sediment by 3 wk. All water in the greenhouse mesocosms and lab aquaria is from a potable source and therefore was treated using sodium thiosulfate after filling to neutralize chlorine. The water coming into the facility has a circumneutral pH, and the temperature in the aquaria was held at 27 C ± 0.2 SE by using an automated heating/cooling system. The temperature was held at 27 C because starry stonewort exhibits a late-season phenological pattern when water temperature is highest in a lake (Glisson et al. 2022). Compressed air and air stones were used to add CO_2 and some disturbance to the water in each mesocosm and aquarium. Two layers of mesh window screen were placed over each mesocosm or aquarium to reduce light intensity and promote starry stonewort growth. Low light conditions seemed to favor starry stonewort growth, and using fresh mass worked better, was faster, and was more reliable than using bulbils to propagate starry stonewort for research purposes.

Algaecide screening

The algaecide screening study was conducted in 30, 55-L aquaria under lab conditions as previously described, from December 2019 through January 2020. Starry stonewort was taken from culture populations in the lab or greenhouse. Three pots of starry stonewort were placed into each aquarium. After the pots were placed in the aquaria, two layers of mesh window screen were installed over each aquarium to reduce light availability. Compressed air was supplied to each aquarium using vinyl tubing and an air stone. All water quality and growth parameters were like the conditions described in the propagation methodology. Starry stonewort was allowed to acclimate to the smaller aquaria for 2 wk, after which one pot from each tank was removed for pretreatment biomass harvesting. Starry stonewort was anchored to the sediment in each pot; the aboveground biomass was clipped at the sediment surface and placed in individually labeled paper bags for drying. In these short-duration, small-scale trials, bulbil production was minimal, or absent, and only aboveground biomass was used to assess efficacy. All biomass was dried at 48 C for at least 48 h then weighed to determine biomass (g dry weight [DW] pot^{-1}).

Table 1. Algaecide formulations and percent copper equivalent of selected formulations to screen against starry stonewort. Target copper concentrations were based on elemental copper in each product.

Formulation	Target Concentration (mg L^{-1})	% Copper	Trade Name
Copper ethanolamine complex	1.0	9	Cutrine®-Plus[2]
	0.5		
	1.0	9.1	Captain®[2]
	0.5		
Emulsified copper ethanolamine complex	1.0	9	Cutrine®-Ultra[2]
	1.0	9.1	Captain® XTR[2]
Copper ethylenediamine complex	1.0	8	Harpoon®[2]
	1.0	8	Komeen®[2]
	1.0	8	Current®[3]

After the pretreatment harvest, the water column (52 L) in each aquarium was treated via submersed injection with a concentrated aqueous solution of the pesticides at rates outlined in Table 1. The pesticide solution was injected into the top 8 cm of the water column. All algaecide applications targeted a concentration of 1 mg L^{-1} of elemental copper (maximum label rate) using an 8-h exposure time. Cutrine-Plus and Captain were also evaluated at 0.5 mg L^{-1} of elemental copper. After 8 h, each aquarium was drained and refilled with fresh water. All treatments and the nontreated reference were replicated in three aquaria. At 4 wk after treatment (WAT), all green starry stonewort thalli (biomass) were harvested by clipping at the sediment surface. All chlorotic or necrotic biomass was excluded from the biomass samples. Samples were placed in labeled paper bags and dried in a forced air oven at 48 C for at least 48 h. Biomass was weighed to determine posttreatment biomass (g DW pot^{-1}). Biomass data from the two pots harvested from each aquarium were averaged within aquarium (treatment) and the averaged biomass data from the aquaria were subjected to a one-way analysis of variance (ANOVA)[6]; treatment means were separated using a Tukey's HSD test. All analyses were conducted at $\alpha \leq 0.05$ significance level.

Herbicide screening

The screening was conducted in a similar manner as the algaecide screening from January through February 2020. Herbicides were selected based on mode of action; those that are generally fast-acting, and those that can be efficacious under reduced exposure time scenarios (Table 2). The maximum label rates were chosen for flumioxazin, carfentrazone-ethyl, diquat, and the endothall + diquat combinations because these herbicides have not been extensively screened to date. Additionally, the half-maximum label rate of diquat was included for comparison purposes. Both salts of endothall have been evaluated under field conditions. The rates chosen were based on potential fish impacts ((mono (N,N-dimethylalkylamine) salt)), and economics relative to amount of herbicide needed (dipotassium salt). All herbicide treatments were made via submersed injection using a concentrated aqueous solution of the herbicides listed in Table 2. The pesticide solution

Table 2. Herbicide formulations, active ingredient, and mode of action for select herbicides to screen against starry stonewort.

Active Ingredient	Target Concentration ($mg\ L^{-1}$)	Mode of Action	Trade Name
Dipotassium salt of endothall	3.0	Inhibition of serine/threonine protein phosphatase	Aquathol®-K[3]
Mono (N,N-dimethylalkylamine) salt of endothall	0.30	Inhibition of serine/threonine protein phosphatase	Hydrothol®[3] 191
Flumioxazin	0.40	Inhibition of protoporphyrinogen oxidase	Clipper® SC[4]
Carfentrazone-ethyl	0.20	Inhibition of protoporphyrinogen oxidase	Stingray®[2]
Diquat	0.19	Inhibition of Photosystem I	Reward®[5]
	0.37		
Endothall + Diquat	1.8 + 0.36	Multiple	Aquastrike®[3]

was injected into the top 8 cm of the water column. After 12 h, each aquarium was drained and refilled with fresh water to remove herbicide residues. Each herbicide treatment and a nontreated reference were replicated in three aquaria. At 4 WAT, all green starry stonewort thalli (biomass) were harvested by clipping at the sediment surface. All chlorotic or necrotic biomass was excluded from the biomass samples. Samples were placed in labeled paper bags and dried in a forced air oven at 48 C for at least 48 h. Biomass was weighed to determine posttreatment biomass (g DW pot^{-1}). Biomass data from the two pots harvested from each aquarium were averaged within aquarium (treatment) and the averaged biomass data from the aquaria were subjected to a one-way ANOVA; treatment means were separated using a Tukey's HSD test. All analyses were conducted at $\alpha \leq 0.05$ significance level.

Repeated screening

A follow-up trial was conducted from August through September 2020 using similar methods from the previously described screenings. All treatments were made as a submersed injection using a concentration aqueous solution of the pesticides and rates outlined in Table 3. The pesticide solution was injected into the top 8 cm of the water column. Pesticide selection was based on the better-performing treatments from previous screenings and included the maximum labeled rates for Cutrine-Plus, Cutrine Ultra, Captain, Captain XTR, Current, Harpoon, Komeen, diquat, and endothall + diquat. All treatments including a nontreated reference were replicated in three aquaria. All algaecide treatments were made using an 8 h exposure time.

Table 3. Pesticide formulation and active ingredient for select herbicides to screen against starry stonewort and select nontarget aquatic plants. Target copper concentrations were based on elemental copper in each product.

Active Ingredient	Target Concentration ($mg\ L^{-1}$)	Trade Name
Copper ethanolamine complex	1.0	Cutrine-Plus
	1.0	Captain
Emulsified copper ethanolamine complex	1.0	Cutrine-Ultra
	1.0	Captain XTR
Copper ethylenediamine complex	1.0	Harpoon
	1.0	Komeen
	1.0	Current
Diquat	0.37	Reward
Endothall + Diquat	1.8 + 0.36	Aquastrike

After 8 h, each aquarium was drained and refilled with fresh water. All herbicide treatments were made using a 12-h exposure time. After 12 h, all water in each aquarium was drained and refilled with fresh water. At 4 WAT, all green starry stonewort thalli (biomass) were harvested by clipping at the sediment surface. All chlorotic or necrotic biomass was excluded from the biomass samples. Samples were placed in labeled paper bags and dried in a forced air oven at 48 C for at least 48 h. Biomass data from the two pots harvested from each aquarium were averaged within aquarium (treatment) and the averaged biomass data from the aquaria were subjected to a one-way ANOVA; treatment means were separated using a Tukey's HSD test. All analyses were conducted at $\alpha \leq 0.05$ significance level.

RESULTS AND DISCUSSION

Algaecide screening

Starry stonewort was actively growing throughout the study because biomass increased 43% over the course of the screening. Biomass achieved during this study would be representative of an early season infestation and probably is the best-case scenario for the use of copper algaecides. All copper algaecides evaluated during this screening reduced ($P = 0.04$) starry stonewort biomass when compared to nontreated reference plants, with the exception of Cutrine-Ultra (Figure 1). However, the lack of significant reduction is somewhat surprising because this product is being used effectively in operational management programs in other states. There were no rate effects observed in this study, in that the 0.5 mg L^{-1} concentration performed similarly to the 1.0 mg L^{-1} concentration for both Cutrine-Plus and Captain formulations. Biomass levels seen under field conditions tend to be much higher (> 4,000 g DW m^2) than what is seen in small-scale trials (Glisson et al. 2018). Copper efficacy is density-dependent and is influenced by biomass or cell densities during the time of application. The lower biomass in this study was conducive to using a lower concentration of copper algaecide (Figure 1). However, scaling this rate to field populations would be more difficult and would likely result in a treatment failure due to higher biomass and effects of bulk water exchange at the point of application (dilution and off-target movement). Although using lower algaecide rates is appealing from an environmental perspective, more studies are needed to determine biomass effects on algaecide uptake and efficacy before implementing reduced concentrations in the field.

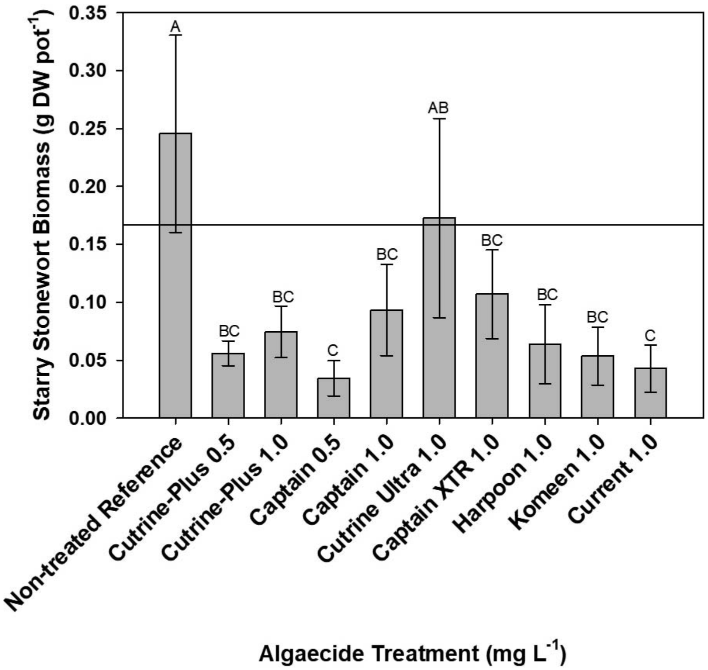

Figure 1. Mean (± 1 SE) starry stonewort biomass 4 wk after treatment with select copper algaecides. Target copper concentrations were calculated based on the elemental copper content in each product. Bars sharing the same letter are not different according to a Tukey's HSD test. All analyses were conducted at $\alpha \leq 0.05$ significance level. The solid horizontal line represents pretreatment biomass.

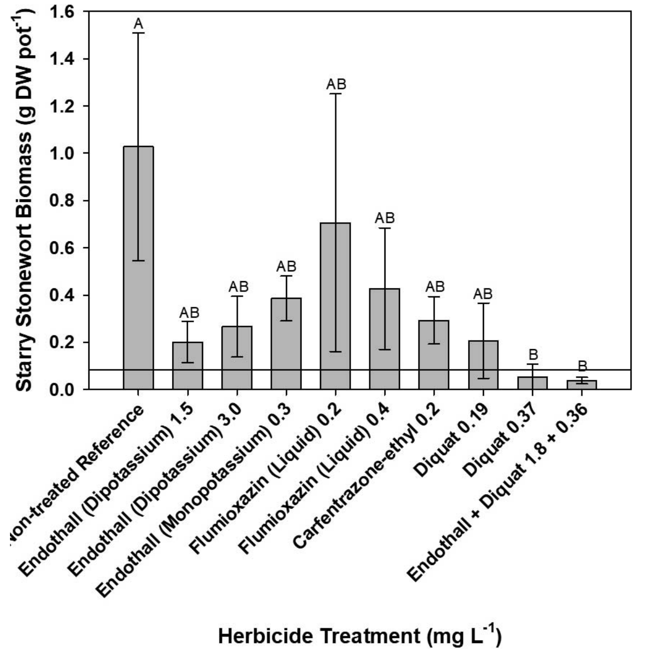

Figure 2. Mean (± 1 SE) starry stonewort biomass 4 wk after treatment with select herbicides. Bars sharing the same letter are not different according to a Tukey's HSD test. All analyses were conducted at $\alpha \leq 0.05$ significance level. The solid horizontal line represents pretreatment biomass.

Although biomass reductions were observed, no formulation completely controlled starry stonewort after 4 wk. Regrowth was evident in all treatments as early as 2 wk after treatment; however, one copper application was enough to reduce biomass below pretreatment levels for at least 4 wk, which would offer nuisance relief from starry stonewort. Copper algaecides have been utilized in Lake Koronis, MN to manage starry stonewort since 2016 (Glisson et al. 2018). Copper algaecides were successful in reducing starry stonewort biomass in the year of treatment, but no effect on bulbil densities was observed (Glisson et al. 2018). Field demonstrations in small plots are needed to further test the efficacy of these products in-lake under differing water exchange scenarios, to determine effective application timing, and refine integrated approaches as described by Glisson et al. (2018).

Herbicide screening

Starry stonewort biomass was greater during this screening than during the algaecide screening because of plants growing longer in the culture population. Potted starry stonewort was transferred from the culture tanks into the treatment aquaria directly. Unlike vascular macrophytes, which can be clipped to a desired height or thinned to a desired biomass, starry stonewort does not regrow in a similar manner or as rapidly. In fact, under field conditions harvesting was a very effective management technique for starry stonewort (Glisson et al. 2018). Therefore, to minimize the effect of clipping or thinning to standardize biomass across trials prior to pesticide applications, each trial was conducted on actively growing starry stonewort obtained from the culture population.

Starry stonewort was actively growing throughout the trial;biomass increased 93% when biomass from nontreated reference plants were compared to pretreatment biomass. At 4 wk after treatment, only diquat at the 0.37 mg L^{-1} concentration, and formulations containing diquat, reduced ($P = 0.02$) starry stonewort biomass when compared to nontreated reference plants (Figure 2). All other herbicide treatments did not result in significant biomass reductions when compared to reference plants. The 0.37 mg L^{-1} concentration of diquat is the maximum label rate for this product and it resulted in a 96% reduction in biomass. The endothall + diquat formulation resulted in a 97% reduction in biomass. All other herbicides tested offered greater than 68% biomass reduction, with the exception of flumioxazin applied at 0.2 mg L^{-1}.

Results from this small-scale screening show promise in further developing use patterns of herbicides for use in starry stonewort management programs. Diquat should be further tested as a stand-alone application under field conditions. If it offers acceptable control of starry stonewort at larger spatial scales, it could be used in rotation with copper algaecides in order to minimize reliance on one formulation (i.e., copper) as part of a pesticide stewardship program. The other herbicides tested might be evaluated in combination with copper, or one another, to assess interaction affects. The combination of two products might give better results than either applied alone. Or the combination might allow for lower use rates to be used

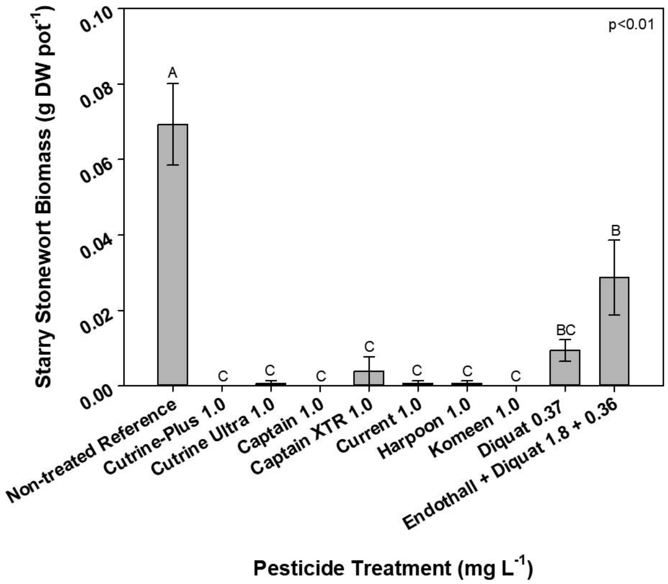

Figure 3. Mean (± 1 SE) starry stonewort biomass 4 wk after treatment with select copper algaecides and herbicides. Target copper concentrations were calculated based on the elemental copper content in each product. Bars sharing the same letter are not different according to a Tukey's HSD test. All analyses were conducted at $\alpha \leq 0.05$ significance level.

than if each component was applied alone (Madsen et al. 2010, Madsen et al. 2015).

Repeated screening

Starry stonewort biomass during this screening was lower than in the previous two screenings because of the new culture plants being established in July 2020; therefore, the starry stonewort in culture was less dense. At the conclusion of 4 wk, all treatments reduced starry stonewort biomass when compared to nontreated reference plants (Figure 3). Biomass reductions ranged from 60% to 100% depending upon the product used. Cutrine-Plus, Captian, and Komeen applied at 1.0 mg L^{-1} resulted in 100% control of starry stonewort. Treatments of Cutrine-Ultra, Captain XTR, Harpoon, Current, and diquat all resulted in similar control to the three above-mentioned products. Applications of endothall + diquat resulted in a significant reduction in biomass when compared to nontreated reference plants; however, its use did not result in the same level of control as the copper algaecides. The repeated trial confirmed efficacy observed in the previous algaecide and herbicide screening trials (even though biomass levels were not similar); and offers confidence in the efficacy of copper-based algaecides and diquat for control of starry stonewort.

When developing management recommendations or use patterns for pesticides, these small-scale screening studies are vital to quickly identify formulations and rates that might be efficacious at larger scales, while ruling out noneffective products. Results from these studies can be use used to prioritize field-scale research and accelerate the development of operational management recommendations. Based on results from the current studies, copper algaecides are the most effective, and most reliable pesticide option. Current operational management of starry stonewort should continue to utilize copper-based products and expand in-lake testing of different formulations. It is recommended that diquat be evaluated in small plots within lakes that have starry stonewort infestations. Positive results in small plots would corroborate results from this study and offer data to support wider adoption of this herbicide for starry stonewort control. Copper + herbicide combinations should be tested in both small-scale and in-lake plots to fully evaluate interaction effects (Pokrzywinski et al. 2021). Furthermore, additional integrated approaches should be evaluated to better pair management techniques to maximize efficacy (Glisson et al. 2018).

SOURCES OF MATERIALS

[1]Osmocote 19-6-12 fertilizer, Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd., Marysville, OH 43041.

[2]Cutrine-Plus®, Captain®, Cutrine®-Ultra, Captain® XTR, Harpoon®, Komeen®, Stingray®; SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

[3]Current®, Aquathol®-K, Hydrothol® 191, Aquastrike®; United Phosphorus, Inc., 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406.

[4]Clipper® SC; Nufarm Americas Inc., 11901 South Austin Avenue, Alsip, IL 60803.

[5]Reward®; Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419.

[6]Statistix 10 software, Analytical Software, 2105 Miller Landing Rd, Tallahassee, FL 32312.

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