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Weed Management in Fresh Market Spinach (*Spinacia oleracea*) with Phenmedipham and Cycloate

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Fresh market spinach has limited herbicides available and weed management in this crop is dependent on hand-weeding. Phenmedipham is a POST herbicide registered for use on spinach grown for processing or for seed, but not fresh market spinach. This study evaluates the potential use of phenmedipham alone and in combination with cycloate for weed control in fresh spinach production. Greenhouse and field studies were conducted in 2013 using two spinach varieties known to have low and high tolerance to phenmedipham. The greenhouse studies showed that phenmedipham at 270 and 550 g ai ha^{-1} was safe to spinach when applied at the four-leaf stage for the low- and high-tolerance varieties, respectively. Phenmedipham was evaluated alone (550 g ha^{-1}) and applied to the four-leaf stage in two varieties. Subsequently, a second experiment evaluated cycloate (1,700 g ha^{-1}) followed by (fb) phenmedipham at several rates (90, 180, and 270 g ha^{-1}). Phenmedipham alone (550 g ha^{-1}) did not result in crop injury when applied to four-leaf spinach; however, the weed control was not better than cycloate alone. When applied as a sequential treatment following cycloate, all phenmedipham rates were safe to spinach and significantly improved weed control compared to cycloate alone. Cycloate fb phenmedipham at 270 g ha^{-1} provided 87% weed control relative to cycloate alone. This level of weed control was similar to the cycloate plus hand-weeding treatment, which provided 98% control. Results here show that cycloate fb phenmedipham improves weed control compared to cycloate alone, and has the potential to reduce hand-weeding costs in the fresh spinach production.

Nomenclature: Cycloate; phenmedipham; spinach, *Spinacia oleracea* L. 'Nordic' and 'Regal' SPQOL.

Key words: Cycloate, phenmedipham, sequential application, spinach injury, spinach yield, weed control in fresh spinach.

La espinaca para el mercado fresco tiene pocos herbicidas disponibles y el manejo de malezas en este cultivo depende de la deshierba manual. Phenmedipham es un herbicida POST registrado para su uso en espinaca producida para procesamiento o para semilla, pero no para espinaca para mercado fresco. Este estudio evalúa el potencial para el uso de phenmedipham solo y en combinación con cycloate para el control de malezas en producción de espinaca fresca. Se realizaron estudios de invernadero y de campo en 2013 usando dos variedades de espinaca que se sabía que tenían tolerancias a phenmedipham baja y alta. Los estudios de invernadero mostraron que phenmedipham a 270 and 550 g ai ha^{-1} fue seguro para la espinaca cuando se aplicó en el estadio de cuatro hojas para las variedades de baja y alta tolerancia, respectivamente. Se evaluó phenmedipham solo (550 g ha^{-1}) y aplicado en el estadio de cuatro hojas en las dos variedades. Subsecuentemente, un segundo experimento evaluó cycloate (1,700 g ha^{-1}) seguido de (fb) phenmedipham a varias dosis (90, 180, and 270 g ha^{-1}). Phenmediphan solo (550 g ha^{-1}) no resultó en daño al cultivo cuando se aplicó a espinacas en el estadio de cuatro hojas. Sin embargo, el control de malezas no fue mejor que el cycloate solo. Cuando se aplicó como un tratamiento secuencial después de cycloate, todas las dosis de phenmedipham fueron seguras para la espinaca y mejoraron significativamente el control de malezas en comparación con el cycloate solo. Cycloate fb phenmedipham a 270 g ha^{-1} brindó 87% de control de malezas en relación a cycloate solo. Este nivel de control de malezas fue similar al tratamiento de cycloate más deshierba manual, el cual brindó 98% de control. Los resultados muestran que cycloate fb phenmedipham mejora el control de malezas al compararse con cycloate solo, y tiene el potencial de reducir los costos asociados a la deshierba manual en la producción de espinaca fresca.

Fresh market spinach is an important vegetable crop in many parts of the world, and is one of the most nutritious vegetables consumed in the United States (Morelock and Correll 2008). In the past two decades, the demand for fresh spinach has significantly increased, resulting in a 200% increase in

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production (Correll et al. 2011). In 2012, the U.S. fresh spinach crop was produced on 15,000 ha at a value of $224 million (USDA 2013). Fresh spinach is produced mainly in California, Arizona, New Jersey, and Texas. California leads production with 161,551 metric tons or 60% of the nation's total production by weight on 8,500 ha. In California, fresh spinach is produced in different seasons and districts: (1) all year in the Salinas Valley and on the central coast; (2) fall and winter in the Central Valley; and (3) during winter in the deserts of Southern California and the Imperial Valley.

There are few herbicides registered for use in fresh spinach production (Fennimore and Doohan 2008). In California, cycloate applied PRE is the primary herbicide used in fresh spinach production on about 50% of the fields (CADPR 2013; USDA 2013). Because fresh spinach is an intensively cultivated crop, fields usually have low weed densities. Growers apply cycloate alone at 1,700 kg ha^{-1} where only limited hand-weeding is needed. However, under high weed pressure, cycloate alone provides limited weed control and often results in partial control of typical broadleaf weeds (Fennimore et al. 2001; Smith et al. 2013). To achieve commercially acceptable weed control, spinach producers have to hand-weed intensively to produce a crop. Hand-weeding is usually carried out prior to harvesting when weeds are already large, and consequently, it is a slow and expensive practice (Takele 2013). Buyers have no tolerance for spinach contaminated with weeds, so growers have no choice but to hand-weed rather than risk rejection of their crop (Smith et al. 2013).

Phenmedipham is a POST herbicide for grasses and broadleaf weeds registered for use on spinach grown for processing or seed, but not for use in fresh spinach production (Smith et al. 2013; Wallace et al. 2007). Phenmedipham may cause temporary injury (e.g., growth retardation, chlorosis, leaf margin– and tip-burn) and because fresh spinach is a short-season crop (30 d in midseason), there is little time for recovery (Agrian 2013a). Phenmedipham can be applied when spinach has four to six true leaves at rates ranging from 550 to 1,100 g ai ha^{-1}. It can also be applied as a split application when the spinach has two true leaves at rates from 460 to 550 g ha^{-1}, fb a second application 4 to 6 d later (Wallace and Petty 2007). For sugar beet (*Beta vulgaris* L.), the recommended application rate is 270 g ha^{-1} for two or three sequential applications. For optimal results phenmedipham is usually applied in a tank mix (Abdollahi and Ghadiri 2004; Dale et al. 2006). We are not aware of data describing the use of phenmedipham on fresh spinach, including the rates and timing that may be safe. Also we are not aware of any attempts to test sequential applications of cycloate and phenmedipham in fresh spinach production. Therefore, the objectives of this study were (1) to determine by a greenhouse study safe phenmedipham application rates and timing for fresh market spinach and (2) to evaluate by a field study the possible use of phenmedipham for fresh market spinach as a single application or a sequential application following cycloate.

Materials and Methods

Two spinach varieties were used for the greenhouse and field studies: 'Nordic' and 'Regal'. These varieties were chosen because in earlier trials they exhibited different levels of tolerance for phenmedipham: Regal had high tolerance and Nordic had low tolerance (R. Lati, unpublished data).

Greenhouse Study. Experiments were conducted to develop preliminary data for objective 1, regarding the spinach response to phenmedipham rates at several growth stages. Pots (8 cm diam) were filled with sandy loam soil (2.1% organic matter and pH 7.0) and seeded with each of the two spinach varieties separately. Phenmedipham was applied to two-leaf spinach at 270 g ha^{-1}, and to four-leaf spinach at 270, 550, and 1,100 g ha^{-1}. Treatments were applied with a CO_2-pressurized backpack sprayer equipped with 8002VS flat fan nozzles (Tee Jet Technologies, Wheaton, IL) calibrated to deliver 337 L ha^{-1} at 290 kPa. Ten days after the four-leaf application all plants were harvested and their dry weights were determined after drying at 80 C for 6 d. The experiment was conducted twice with a complete randomized design and all treatments were replicated five times. Plant dry weights were subjected to ANOVA, and mean separation was performed using Fisher's protected LSD ($\alpha \leq 0.05$). One-way ANOVA was conducted to determine the effects of phenmedipham on the spinach dry weight using PROC GLM. A *t* test was used to compare spinach biomass following the phenmedipham applications. The four-leaf applica-

Table 1. Critical dates in the field experiments. Planting, phenmedipham application, crop injury estimates, weed density measurement, and yield evaluation dates, and days after phenmedipham treatment (in parentheses). All experiments were conducted on 2013.

Experiment	Planting	Phenmedipham application	Crop injury estimates	Weed densities measurements	Yield evaluations
1	July 30	August 15	August 21 (7)	August 29 (14)	September 6 (22)
2	July 30	August 15	August 21 (7)	August 29 (14)	September 6 (22)
3	September 9	September 26	October 3 (7)	October 17 (21)	October 17 (21)
4	September 20	October 21	October 28 (7)	November 12 (22)	November 12 (22)

tions and the control data were subjected to regression analysis using PROC REG. Statistical analyses and regression were conducted using SAS (version 9.3, SAS Institute Inc., Cary, NC).

Field Studies. Field experiments were conducted to address objective 2. Table 1 lists the critical dates for the field experiments, including planting, phenmedipham application, crop injury evaluation, weed density evaluation, and harvesting dates. Experiments were carried out during 2013 at the Hartnell field station near Salinas, CA, in an Antioch sandy loam soil, fine, smectitic, thermic Typic Natrixeralf (53% sand, 32% silt, and 15% clay) with a pH of 7.0 and organic matter content of 2.1%. All plantings were grown on 1-m-wide by 6-m-long raised beds with two seed lines per bed, 30 cm apart, one planted to Nordic and the other to Regal. A tractor-mounted planter (Stanhay Webb Ltd., Grantham, U.K.) was used for planting, and overhead sprinkler irrigation and other common spinach cultural practices were used (LeStrange et al. 2013). Herbicides were applied to the bed top with a CO_2-pressurized backpack sprayer as described in the previous section. Hand-weeded checks were maintained to estimate the level of yield loss due to weeds and herbicide treatment. About 2 to 3 wk after seeding, cultivation was performed using a standard cultivator equipped with knives similar to the implement described in Fennimore et al. (2010), which left an approximately 10-cm-wide strip of the intra row weeds for the weed density evaluations. The trials were repeated twice (experiment 1 was repeated by experiment 2 and experiment 3 was repeated by experiment 4). Each experiment was arranged in a randomized complete block design with four replications. High and low temperatures and radiation values were recorded using the California Irrigation Management Information System weather station network (using data from the weather station nearest the test site) to detect any interactions between environmental conditions and crop injury.

In the first set of field experiments (experiments 1 and 2), phenmedipham was evaluated alone, and in the second set (experiments 3 and 4), phenmedipham was applied as a sequential treatment following cycloate. In the first set of experiments, phenmedipham was applied alone to four-leaf spinach at 550 g ha^{-1}. As a standard, cycloate alone was applied PRE at 3,400 g ha^{-1}. Cycloate was sprinkler-activated according to the product label the same day as application (Agrian 2013b). Crop injury estimates were recorded on a scale of 0 (no injury) to 10 (plant death). Weed densities were measured in a sample area of 3,900 cm^2 in the center of the plot. Spinach fresh biomass was evaluated by harvesting a 3-m section sample area from each seed line.

In the second set of experiments, cycloate was applied PRE at 1,700 g ha^{-1}, fb phenmedipham POST on four-leaf spinach at 90, 180, and 270 g ha^{-1}. In these experiments the PRE application of cycloate fb hand-weeding was the commercial standard and cycloate alone was the non weeded reference. Crop injury estimates and yield biomass were recorded as described above. Weed control was evaluated by harvesting aboveground biomass within the 3,900-cm^2 sample area from each plot. In cases where there was no experiment by treatment interaction, data were combined; otherwise data from each experiment were analyzed separately. There was no variety effect on weed control so weed density and biomass were averaged over varieties. Injury estimates, weed density, weed biomass, and yield data were subjected to ANOVA using PROC GLM in SAS and means were separated by Fisher's protected LSD at $\alpha \leq 0.05$.

Results and Discussion

Greenhouse Study. As there was no experiment by treatment interaction, data from both experiments

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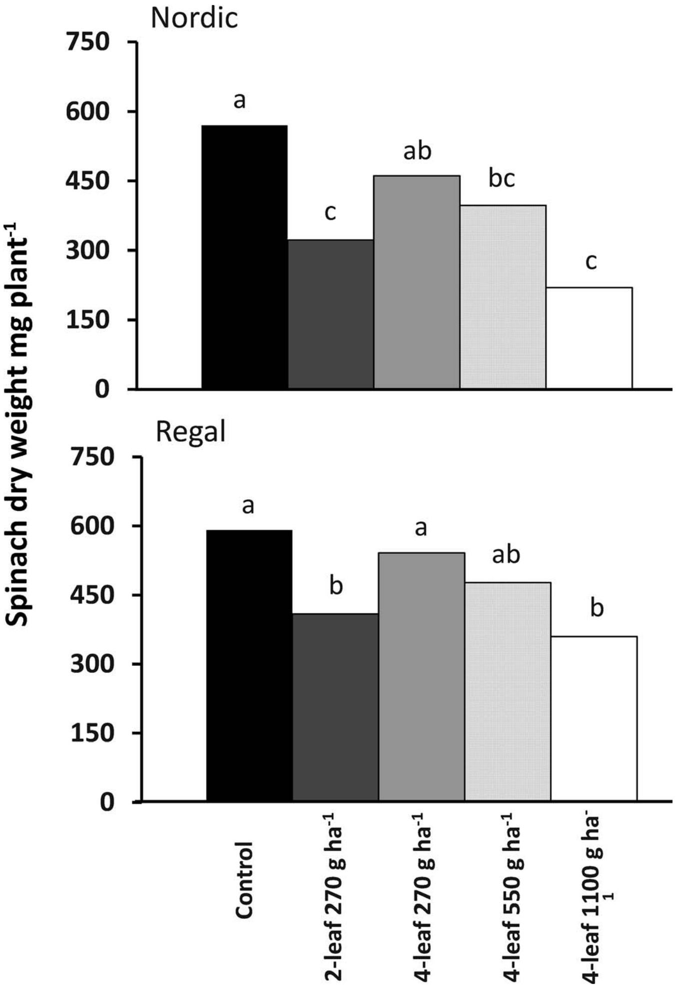

Figure 1. Dry weight of the 'Regal' and 'Nordic' plants following phenmedipham application at the two- or four-leaf stage in the greenhouse study. Means with the same letter are not significantly different according to Fisher's protected LSD at $\alpha \leq 0.05$.

were combined. Phenmedipham application timing and rate had a significant impact on the spinach dry weight. After application of phenmedipham at 270 g ha^{-1} at the two-leaf and four-leaf stages the spinach dry weight for the Regal was 409 and 541 mg, respectively ($P < 0.0001$), and for Nordic, 323 and 451 mg, respectively ($P < 0.0001$) (Figure 1). These results show that fresh spinach was more sensitive to phenmedipham application at the two-leaf stage than at the four-leaf stage. Spinach was probably too sensitive at the two-leaf stage to recover from herbicide injury, and due to its short growing season even a low phenmedipham rate (270 g ha^{-1}) resulted in dry weight reduction.

Phenmedipham at 1,100 g ha^{-1} applied at the four-leaf stage injured both varieties, and their dry weight values were 39 and 61% lower than the control for Regal and Nordic, respectively (Figure 1). Phenmedipham at 550 g ha^{-1} at the four-leaf stage reduced only the Nordic dry weight, and the 270 g ha^{-1} rate did not reduce dry weight for either variety. The linear regression ($y = ax + b$) yielded different a parameters (the slope parameter) for the Regal and Nordic, –0.21 and –0.3 respectively, demonstrating differing phenmedipham tolerance between these varieties (Table 2).These results show that fresh spinach was affected by phenmedipham application rate, and the 1,100 g ha^{-1} rate would not be recommended on four-leaf spinach. Similar results were also obtained by Wallace et al. (2007), who observed yield reduction and crop injury in processing spinach caused by phenmedipham at 1,100 g ha^{-1}. The greenhouse study provided preliminary information on the possible phenmedipham treatments for fresh spinach production in terms of crop safety. Data showed that phenmedipham should not be applied to spinach before the

Table 2. The effect of phenmedipham rate on dry weight in 'Nordic' and 'Regal' spinach. Coefficients of determination (R^2) and parameter estimations (value, degrees of freedom [df], standard error [SE], t value [t], probability value [P], and 95% confidence limit [95% CL]) of the linear regression ($y = ax + b$) between applications rates at the four-leaf stage and their dry weight.

		Parameter estimates													
		a[a]							b[b]						
	R^2	Value	df	SE	t	P	95% CL		Value	df	SE	t	P	95% CL	
'Nordic'	0.99	−0.30	1	0.01	−17.9	0.0031	−0.38	−0.23	560	1	10	51	0.0004	514	607
'Regal'	0.99	−0.21	1	0.004	−46.3	0.0005	−0.22	−0.2	593	1	2	206	< 0.0001	581	606

[a] The slope parameter.
[b] The y-intercept.

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Table 3. Spinach injury estimates, yields, and weed densities resulting from cycloate PRE and phenmedipham POST in experiments 1 and 2.

Treatment	Rate	Injury estimates[a,b]		Yield[a]		Weed density[a]
		Regal	Nordic	Regal	Nordic	
	g ai ha^{-1}			—gram m^{-2}—		No. m^{-2}
Hand-weeding	0	0 b	0 c	2,621 a	2,238 a	0 c
Cycloate PRE	3,400	1.3 a	1.5 a	1,918 a	1,391 b	442 b
Phenmedipham POST	550	0.2 b	0.6 b	2,171 a	1,582 b	809 a

[a] Means with the same letter within columns are not significantly different according to Fisher's protected LSD at $\alpha < 0.05$.

[b] Injury estimates were taken on a scale of 0 to 10, with 0 = no injury and 10 = dead plants. A treatment with ≤ 2 injury estimate on spinach was considered safe.

four-leaf stage, and based on the evaluated treatments, rates should not be higher than 550 g ha^{-1}.

Phenmedipham Alone. Phenmedipham applied at 550 g ha^{-1} caused slight injury to spinach, and injury estimates were 0.2 and 0.6 for the Regal and Nordic, respectively (Table 3). Yields in Regal were similar among the hand-weeded control, and the cycloate and phenmedipham treatments (Table 3). In Nordic, spinach yields were similar when treated with cycloate and phenmedipham, but were less than the hand-weed control. These results show that phenmedipham at 550 g ha^{-1} applied at the four-leaf stage was safer to spinach than cycloate PRE.

Most of the weeds in these experiments were burning nettle (*Urtica urens* L.)

(66%) and common purslane (*Portulaca oleracea* L.) (28%). Phenmedipham applied alone at the four-leaf stage did not provide satisfactory weed control and weed densities in the phenmedipham-treated plots were approximately twice that of the cycloate-treated plots, 809 and 442 weeds m^{-2}, respectively (Table 3). These results show that phenmedipham alone at 550 g ha^{-1} was not adequate for fresh spinach with high weed pressure. Limited weed control obtained with phenmedipham was also reported by Norsworthy and Smith (2005) who used the same phenmedipham rate in mustard greens (*Brassica juncea* L.), kale (*Brassica oleracea* var. *acephala*), and collard (*Brassica oleracea* L. var. *acephala*). Applying phenmedipham at the four-leaf stage was likely too late and the weeds were too large to be controlled by this herbicide. Cycloate also provided only partial weed control with more than 400 weeds m^{-2} (Table 3). We concluded that phenmedipham alone does not offer any advantage over cycloate alone.

Sequential Application of Cycloate and Phenmedipham. The reference treatment in these experiments was cycloate at 1,700 g ha^{-1} without hand-

Table 4. Spinach injury and yield in experiment 3 and 4, resulting from cycloate PRE followed by (fb) phenmedipham POST at 7 and 21 d after spraying, respectively.

Treatment	Rate	Injury estimates[a,b]		Yield[a]	
		Regal	Nordic	Regal	Nordic
	g ai ha^{-1}			—gram m^{-2}—	
Cycloate[c]	1,700	0 a	0 b	652 b	372 b
Cycloate fb hand weeding[d]	1,700	0 a	0 b	813 a	555 a
Cycloate fb phenmedipham	1,700 fb 90	0.2 a	0.1 b	720 a	465 ab
Cycloate fb phenmedipham	1,700 fb 180	0 a	0.1 b	773 a	476 ab
Cycloate fb phenmedipham	1,700 fb 270	0.1 a	0.5 a	735 a	504 a

[a] Means with the same letter within columns are not significantly different according to Fisher's protected LSD at $\alpha \leq 0.05$.

[b] Injury estimates were taken on a scale of 0 to 10, with 0 = no injury and 10 = dead plants. A treatment with ≤ 2 injury estimate on spinach was considered safe.

[c] This treatment was used as a nonweeded reference.

[d] This treatment was used as a commercial standard.

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Table 5. The effects of cycloate PRE followed by (fb) phenmedipham POST on weed biomass and compared to cycloate in experiments 3 and 4.

Treatment	Rate	Weed biomass[a]			
		Experiment 3		Experiment 4	
	g ai ha^{-1}	g m^{-2}	Reduction (%)[b]	g m^{-2}	Reduction (%)[b]
Cycloate[c]	1,700	0.78 a	0	1.88 a	0
Cycloate fb hand weeding[d]	1,700 fb 0	0.01 c	98 a	0.02 c	99 a
Cycloate fb phenmedipham	1,700 fb 90	0.37 b	52 b	0.48 b	73 c
Cycloate fb phenmedipham	1,700 fb 180	0.26 bc	66 b	0.35 bc	75 c
Cycloate fb phenmedipham	1,700 fb 270	0.08 bc	87 a	0.17 bc	88 b

[a] Means with the same letter within columns are not significantly different according to Fisher's protected LSD at $\alpha \leq 0.05$.

[b] Reduction compared to cycloate.

[c] This treatment was used as a nonweeded reference.

[d] This treatment was used as a commercial standard.

weeding and cycloate fb hand-weeding was the commercial standard. Burning nettle was the primary weed in these studies (99%).

All cycloate (PRE) treatments fb phenmedipham (POST) were safe for spinach and resulted in injury of 0.5 or less (Table 4). Correspondingly, spinach fresh weight from all cycloate fb phenmedipham treatments was similar to the commercial standard (cycloate and hand-weeding), showing the safety of these treatments (Table 4). Compared to the first experiments (experiments 1 and 2), lower biomass values were observed. The shorter day length and lower radiation values (approximately 80 W m^{-2} in experiments 3 and 4, compared to approximately 120 W m^{-2} in experiments 1 and 2) resulted in slower plant growth.

Weed control was significantly improved by all cycloate fb phenmedipham treatments compared to cycloate alone. Phenmedipham at 270 g ha^{-1} in experiment 3 reduced the weed biomass from 0.78 to 0.08 g m^{-2} (87% control; Table 5). The sequential use of herbicides with two different modes of action expanded the weed control spectrum to include burning nettle and common purslane, which were not controlled by cycloate alone (Table 5). The main purpose of using a POST herbicide in fresh spinach production is to reduce hand-weeding costs (Fennimore et al. 2001). Applying cycloate PRE either controlled or stunted weeds, which allowed time for the POST application of phenmedipham at the four-leaf spinach stage to control remaining weeds. Although the degree of control was not sufficient for commercial growers, the improved weed control provided by cycloate fb phenmedipham may lower weeding costs.

On the practical level, the phenmedipham label requires a 21-d period between application and harvest for processing uses (Agrian 2013a). This sequential application will be useful for the October to March plantings when the spinach growing season is longer and there is adequate time (at least 21 d) from phenmedipham application at the four-leaf stage to harvest (R. Smith, personal communication). In this study, two seed lines were used, resulting in low spinach density and this represents a worst-case scenario since commercial high-density spinach plantings go from emergence to full canopy in about a week. Using a higher number of seed lines (which is typical for commercial plots in this area) would likely result in better weed control due to higher crop densities and more favorable competitive conditions. Further studies must be done to determine the effectiveness of this management in commercial fields and to examine wider combinations of rates of phenmedipham and cycloate. However, this study shows the potential of phenmedipham to become a valuable tool for fresh spinach production, where a sequential application after cycloate is recommended.

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