# **Tolerance of Ten Lettuce Cultivars to High Temperature Combined with NaCl during Germination**

Janice M. Coons Botany Department, Eastern Illinois University, Charleston, IL 61920

# **Robert O. Kuehl**

Statistical Support Unit, Department of Agricultural Economics, University of Arizona, Tucson, AZ 85721

# Nancy R. Simons

Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

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Abstract. Water that may contain salt often is used to cool soil to help overcome high-temperature inhibition of lettuce germination. This study was done to determine how lettuce cultivars differ in their germination response to high temperature and NaCl. Ten lettuce (*Lactuca sativa* L.) cultivars (Grand Rapids, Climax, Coolguard, Empire, Great Lakes 659-700, Mesa 659, Salinas, Vanguard 75, Red Coach 74, and Wintersupreme) were germinated at 20, 25, 30, or 35C with 0.0, -0.3, -0.6, -0.9, -1.2, or -1.5 MPa NaCl. With no NaCl, germination percentages and rates decreased significantly at 35C for all cultivars except 'Salinas', which decreased at 30C. With higher concentrations of NaCl, decreases in germination percentages and rates were observed at lower temperatures. Cultivar differences in response to temperature were present with no NaCl but became larger in the presence of NaCl. 'Great Lakes 659-700' and 'Mesa 659' were most sensitive to high temperature and salt. 'Coolguard' and 'Empire' were most tolerant to high temperature and salt, with some tolerance also present in 'Grand Rapids' and 'Vanguard 75'. Relative tolerance of cultivars to NaCl as shown by germination percentages and rates was consistent with growth of roots.

Lettuce seed germination is inhibited by soil temperatures in excess of 25 to 32C, depending on the cultivar (Borthwick and Robbins, 1928; Gray, 1975; Thompson et al., 1979). These temperatures often are exceeded when lettuce is planted during late August or September for winter production in southwestern states, such as Arizona and California (Valdes and Bradford, 1987). Germination often is poor, resulting in nonuniform stand establishment and subsequent variability in maturity at harvest (Gray, 1975; Krause, 1980; Wurr and Fellows, 1983). In an attempt to overcome this problem, growers frequently keep the soil surface moist until stands are established. Although this practice helps reduce soil temperature, salts dissolved in the irrigation water also are applied to the soil surface. Much irrigation water in southwestern states measures around 0.0 to -0.3MPa, although some measures as high as - 0.6 MPa (Dutt and McCreary, 1970). Salts in the upper few centimeters of the soil may be as high as 14 mmhos·cm<sup>-1</sup>(J. M. C., unpublished).

We found no studies in the literature considering differences in response of lettuce cultivars to combinations of high temperature and salt. Some studies dealt with response of lettuce to salt only. Lettuce is moderately sensitive to salt relative to other species (Maas and Hoffman, 1977), with some researchers reporting differences in sensitivity among cultivars (Pasternak et al., 1986; Shannon and McCreight, 1984; Shannon et al., 1983) and others reporting no differences (Ayers et al., 1951). However, seedlings frequently were started with fresh water and treated with salt solutions later. In some studies, seeds of various lettuce cultivars were imbibed in salt solutions, although only one concentration of salt was tested with no control of temperature (Shannon and McCreight, 1984; Shannon et al., 1983). Only one study was found that considered how NaCl and temperature affected lettuce during germination and early seedling growth (Odegbaro and Smith, 1969). They found that salt decreased seedling fresh weight and that high temperature accentuated this effect, but only one cultivar was tested.

Due to limited studies on the effect of high temperature and salt during lettuce germination, we determined how germination percentages and rates of 10 lettuce cultivars differ in response to high temperature and NaCl, and considered how seedlings of five of these cultivars, chosen on the basis of their salt tolerance, responded to NaCl in respect to root growth.

## **Materials and Methods**

Seeds of 'Grand Rapids', 'Climax', 'Coolguard', 'Empire', 'Great Lakes 659-700', 'Mesa 659', 'Salinas', 'Vanguard 75', 'Red Coach 74', and 'Wintersupreme' lettuce were obtained from Asgrow Seed Co. (Kalamazoo, Mich.), SunSeeds (Hollister, Calif.), and Quali-Sel Seed (Salinas, Calif.). All are crisphead types except 'Grand Rapids', which is a leaf type.

Aqueous solutions of NaCl were prepared to obtain osmotic potentials of 0.0, -0.3, -0.6, -0.9, -1.2, or -1.5 MPa (0.0, 3.5,7.1, 10.6, 14.2, or 17.7 g·liter<sup>-1</sup>, respectively). Osmotic potentials were checked on a vapor pressure osmometer (Wescor 5100 C, Logan, Utah).

Fifty seeds of each cultivar were placed in glass petri dishes  $(10 \times 2 \text{ cm})$ , lined with three sheets of filter paper (Whatman #1), and moistened with 5 ml of the appropriate NaCl solution. All dishes were placed inside clear plastic tubs (35 x 24 x 12 cm; Rubbermaid) to reduce water loss. No additional solution was added. Tubs were placed in growth chambers in the light for 12 hr with 60 µmol·s<sup>-1</sup>·m<sup>-2</sup> at 20, 25, 30, or 35 ± 2C, as measured with thermistors. Two petri dishes per replication were used with three replications.

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Germinated seeds were counted every day for 14 days. Seeds were considered germinated when 1 mm of radicle was visible. Germination percentages after 14 days were calculated. Germination rate was calculated as the summation of newly germinated seeds on each day divided by number of days that elapsed since onset of imbibition with seed numbers adjusted to abase of 100 (Maguire, 1962). The highest possible theoretical value using this calculation is 100, i.e., all seeds germinated on the 1st day.

Ten seeds from each of five cultivars selected on the basis of their salt tolerance in the germination experiments (sensitive— 'Great Lakes 659-700' and 'Mesa 659'; intermediate—'Climax'; tolerant—'Empire' and 'Vanguard 75') were placed in seed envelopes (Northrup King; 16.5 x 17.5 cm) that were moistened with 5 ml of the appropriate NaCl solution. The envelopes were suspended on a frame in an upright position and placed in a lighted (12 hr with 60 µmol·s<sup>-1</sup>·m<sup>-2</sup>) growth chamber held at 26  $\pm$  2C. Every other day, an additional 5 ml of the appropriate NaCl solution was added to replenish losses. Root length of each seedling was measured every other day for 14 days. Each cultivar–NaCl combination was replicated twice over time with two envelopes per replication.

For germination experiments, the experimental design was a three-factor split-plot experiment with the whole factor (temperature) arranged in a balanced incomplete-blocks design. An incomplete block consisted of one run with two growth chambers, each with one of the experimental temperatures. The subplot factors (cultivars and NaCl) were arranged randomly within each of the whole plot temperature-growth chamber units. A balanced incomplete-blocks design analysis of variance (AN-OVA) was conducted for germination percentage and rate. With the incomplete-blocks design it was necessary to calculate the least squares means adjusted for block effects (Cochran and Cox, 1957). Data on germination percentage were subjected to arcsin transformation prior to analysis. Least significant differences were calculated for mean separation at P = 0.05.

For root length experiments, the design was a randomized complete block. Data were analyzed by a two-way ANOVA with the factors being cultivar and NaCl. Means were separated by calculating least significant difference at P = 0.05.

#### Results

Germination percentage. The ANOVA table for germination percentages on arcsin-transformed data showed that all factors and all interactions of factors were significant at P = 0.01. Thus, data for various factors were considered separately (Table 1). With 0.0 MPa NaCl, no significant differences in germination percentage were observed among cultivars at 20 or 25C. At 30C, significant cultivar differences were observed, although all values were 94% or higher. These differences were even greater at 35C where none reached even 20%. Germination percentages did not decrease significantly for each cultivar until 35C, except for 'Coolguard' and 'Salinas', which had significantly lower germination at 30 than at 20C.

At – 0.3 MPa NaCl, significant cultivar differences still were not present for germination percentages at 20C, but were at 25C (Table 1). At 35C, germination percentage dropped to near zero for all cultivars with no significant differences among them. Several more cultivars showed significant decreases in germination percentages at 30C relative to 20C, i.e., 'Climax', 'Coolguard', 'Great Lakes 659-700', 'Mesa 659', 'Salinas', 'Red Coach 74', and 'Wintersupreme'. Thus, 'Grand Rapids', 'Empire', and 'Vanguard 75' were most tolerant to the higher tem-

Fable 1. Least squar	es estimates of ge	ermination perc	centages after 14
days for 10 lettuc	e cultivars at fou	r temperatures	with five NaCl
concentrations. <sup>2,y</sup>			

	Temperature (°C)					
Cultivar	20	25	30	35		
		0.0 MPa NaCl				
Grand Rapids	100 a	97 a	94 c	9 b		
Climax	100 a	99 a	97 abc	0 c		
Coolguard	100 a	98 a	95 c	10 ab		
Empire	100 a	98 a	97 abc	19 a		
Great Lakes 659-700	100 a	99 a	97 abc	0 c		
Mesa 659	100 a	98 a	96 bc	1 c		
Salinas	100 a	99 a	95 c	0 c		
Vanguard 75	100 a	99 a	99 ab	1 c		
Red Coach 74	100 a	99 a	98 abc	18 a		
Wintersupreme	100 a	100 a	99 a	9 b		
		-0.3 MPa NaCl				
Grand Rapids	99 a	98 ab	95 ab	1 a		
Climax	99 a	99 a	91 bc	0 a		
Coolguard	100 a	97 ab	95 ab	3 a		
Empire	100 a	98 ab	96 ab	1 a		
Great Lakes 659-700	100 a	99 ab	71 e	0 a		
Mesa 659	98 a	96 b	80 de	0 a		
Salinas	100 a	98 ab	85 cd	0 a		
Vanguard 75	100 a	99 a	96 a	0 a		
Red Coach 74	100 a	100 a	96 ab	0 a		
Wintersupreme	100 a	99 ab	86 cd	0 a		
~		-0.6 MPa NaCl				
Grand Rapids	98 ab	95 b	40 c	0 a		
Climax	100 ab	99 a	36 c	0 a		
Coolguard	99 ab	97 ab	90 a	0 a		
Empire	100 ab	98 ab	79 b	1 a		
Great Lakes 659-700	976	98 ab	2 f	0 a		
Mesa 659	97 ab	98 ab	6 e	0 a		
Salinas	99 ab	97 ab	24 d	0 a		
Vanguard 75	100 ab	99 a	69 c	0 a		
Red Coach 74	100 a	99 a	49 c	0 a		
Wintersupreme	100 a	96 ab	19 d	0 a		
Crond Donida	07 .h	-0.9 MPa NaCl				
Grand Rapids	97 ab	89 DC		Ua		
Contained	99 a 08 sh	70 d	00	0 a		
Empire	98 ab	97a	23 a	1 a		
Great Lakes 650 700	99 a 07 sh	82 CU	0 /	0 a		
Meso 650	97 au 02 h	53 1	04	0 a		
Solinos	95.0	55 e		0 a		
Jannuard 75	99 a 100 o	95 ab	00	0 a		
Pad Coach 74	100 a	9/a 92 ad	3 UC	0 a		
Wintersunreme	100 a	03 CU 22 f		0 a		
wintersupreme	92.0	251		0 a		
Grand Ranids	75 bc	-1.2 M	Pa Naci Na	0 a		
Climax	75 00 24 e	3 de	0 2	0 2		
Coolguard	77 ah	25 9	0.2	0 a		
Empire	74 hc	6 cd	0 a	0 a		
Great Lakes 659-700	30	1 de	0.2	0 a		
Mesa 659	25 3 a		0.2	0 a		
Salinas	65 6	2 de	0 a	0 a		
Vanguard 75	85 a	21 ah	0 2	0 a		
Red Coach 74	48 d	1.6	0 2	0-a		
Wintersupreme	8 f	<u> </u>	0 2	0.9		

Least squares means adjusted for block effects; standard error for difference among temperatures within one cultivar and NaCl concentration is 4.7; standard error for difference among cultivars within one temperature and NaCl concentration is 4.6; mean separation within each column for a given NaCl concentration based on least significant difference at P = 0.05 on arcsin-transformed data. None of the seeds germinated at -1.5 MPa NaCl. peratures with no significant decrease in germination percentages until 35C.

At -0.6 MPa NaCl, cultivar differences in germination percentages were apparent at 20, 25, and 30C (Table 1), and were greatest at 30C. All cultivars showed a significant decrease in germination percentage at 30C relative to 20C, and it was at or near 0% at 35C for all.

Cultivar differences in germination percentage with -0.9 MPa NaCl were apparent at 20, 25, and 30C (Table 1). However, significant decreases now found were between 20 and 25C for 'Grand Rapids', 'Climax', 'Empire', 'Great Lakes 659-700', 'Mesa 659', 'Red Coach 74', and 'Wintersupreme'. Germination was absent or minimal at 35C for all cultivars.

With -1.2 MPa NaCl, cultivars differed in germination percentages only at 20 and 25C (Table 1); none germinated at 30 or 35C. Significant decreases were noted for all cultivars held at 25C relative to those held at 20C, except for 'Great Lakes 659-700' and 'Mesa 659' because germination percentages of these cultivars already were very low at 20C.

At -1.5 MPa NaCl, none of the cultivars germinated at 25, 30, or 35C (data not shown). At 20C, germination percentages were between 1% and 5%, with those of 'Empire' being significantly higher than for 'Climax', 'Great Lakes 659-700', 'Mesa 659', 'Salinas', and 'Wintersupreme' while the remaining four cultivars were intermediate. Only for 'Empire' was germination percentage significantly higher at 20C than at higher temperatures (i.e., 30 and 35 C).

*Germination rate. The* ANOVA tablehfor germination rates showed that all factors and all interactions of factors were significant at P = 0.01: Thus, data for the different factors were considered separately (Table 2). With no NaCl, germination rates were similar for all cultivars at 20 or 25C, although they did respond differently at 30 and 35C. Rates were significantly lower at 35C relative to 20C for all cultivars except 'Salinas', which was lower at 30C.

With – 0.3 MPa NaCl, cultivar differences now were found at 20 and 25C, although at 35C all cultivars failed to germinate (Table 2). All cultivars but 'Coolguard' and 'Empire' now showed decreases in germination rates at 30C relative to 20C. For 'Climax' and ' Wintersupreme', rates were higher at 25 than at 20C.

At – 0.6 MPa NaCl, cultivar differences were noted within each temperature except at 35C, where none germinated (Table 2). Significant decreases were noted at 30C relative to 20C for rates of each cultivar except 'Coolguard', for which germination rates did not decrease until 35C. The germination rate of 'Great Lakes 659-700' actually decreased significantly at 25C and again at 30C.

At -0.9 MPa NaCl, differences among cultivars within each temperature were found only at 20 and 25C, with rates  $\leq 5$  at 30 and 35C (Table 2). Significant decreases were noted for rates of some cultivars at 25C relative to 20C, i.e., 'Grand Rapids', 'Climax', and 'Empire'.

With – 1.2 MPa NaCl, no cultivar differences in rates were present at 25, 30, or 35C, but all were low (Table 2). At 20C, the rate for 'Empire' was significantly higher than the rates for 'Great Lakes 659-700', 'Mesa 659', or 'Wintersupreme'. Rates were significantly higher at 20 than 35C for 'Grand Rapids', 'Coolguard', and 'Vanguard 75'. For 'Empire', rates at 20C were higher than at all other temperatures.

At -1.5 MPa NaCl, rates were  $\leq 1$  for all cultivars and temperatures (data not shown).

*Root length.* The effects of salt and cultivar were significant at P = 0.05, although the interaction was not significant. With

Table 2.	Least squares estimates of germination rate of 10 lettuce cultivars
at four	emperatures with five NaCl concentrations for 14 days <sup>2</sup> , <sup>y</sup> , <sup>x</sup> .

			(0.0)	
		Temperat	ure (°C)	
Cultivar	20	25	30	35
		0.0 MPa	ı NaCl	
Grand Rapids	94 a	93 a	91 ab	2 b
Climax	95 a	98 a	95 a	0 b
Coolguard	97 a	96 a	93 a	бab
Empire	95 a	95 a	94 a	13 a
Great Lakes 659-700	98 a	98 a	91 ab	0 b
Mesa 659	94 a	98 a	93 a	0 b
Salinas	97 a	98 a	82 Ь	0 b
Vanguard 75	99 a	97 a	96 a	0 b
Red Coach 74	100 a	98 a	98 a	14 a
Wintersupreme	92 a	100 a	99 a	6 ab
		-0.3 MF	a NaCl	
Grand Ravids	85 b	94 ab	74 b	0 a
Climax	68 c	93 ab	62 cd	0 a
Coolguard	94 ab	93 abc	90 a	0 a
Empire	93 ab	96 ab	87 a	0 a
Great Lakes 659-700	69 c	70 d	23 f	0 a
Mesa 659	70 c	65 d	43 e	0.a
Salinas	88 ab	87 bc	54 d	0 2
Vanguard 75	96 a	96 ab	67 bc	0 a
Red Coach 74	93 ah	100 a	70 bc	0.2
Wintersupreme	· 72 c	83 c	54 d	0 u 0 a
		0.5 U	Da NaCl	οu
Grand Rapids	50 hcd	57 b	18 cd	Ωa
Climax	46 cd	51 bc	8 def	0.2
Coolguard	65 a	70 a	63 a	0.2
Empire	66 a	67 a	35 h	0 a
Great Lakes 650-700	00 a 44 de	28 d	55 U 1 F	0 a
Meso 650	47 de	20 U 22 d	1 I 2 f	
Selines	42 UC	33 u	31 7 of	U a
Vanguard 75	45 Cue	40 C	25 0	0 a
Pad Coach 74	54 DC	50 D	25 C	0 a
Winterounreme	36 a	32.00	15 cue	U a
wintersupreme	50 E	52 U		0 a
Grand Banida	22 h	-0.9 MF	a NaCi	0.0
Climox	32 U 19 cde	22 a 7 o	1 0	U a
Coolguard	19 Cut 28 ba	26 0	1 a	0 a
Empire	20 00	20 a	5 a 2 -	0 a
Creat Labor (50,700	42 a	20 ab	2 a	0 a
Great Lakes 639-700	14 de	4 C	1 a	Ua
Mesa 039	14 de	5 C	1 a	Ua
Salinas	22 bcd	20 ab		Ua
vanguard 75	20 DC	29 a	2 a	Ua
Red Coach 74	19 cde	11 bc	1 a	0 a
Wintersupreme	12 e	3 c	1 a	0 a
a	-1.2 MPa NaCl			
Grand Rapids	10 ab	2 a	1 a	0 a
Climax	3 ab	3 a	1 a	0 a
Coolguard	9 ab	4 a	la	Ua
Empire	12 a	2 a	La	0 a
Great Lakes 659-700	1 b	0 a	1 a	0 a
Mesa 659	I b	0 a	1 a	0 a
Salinas	7 ab	1 a	1 a	0 a
Vanguard 75	10 ab	2 a	1 a	0 a
Red Coach 74	5 ab	3 a	1 a	0 a
Wintersupreme	1 b	1 a	1 a	0 a

'Germination rate: summation of newly germinated seeds on each day divided by number of days that elapsed since onset of imbibition.

<sup>2</sup>Least squares means adjusted for block effects; standard error for difference among temperatures within one cultivar and NaCl concentration is 5.1; standard error for difference among cultivars within one temperature and NaCl concentration is 4.9; mean separation within each column for a given NaCl concentration based on least significant difference at P = 0.05. <sup>5</sup>None of the seeds germinated at -1.5 MPa NaCl. 0.0 MPa NaCl, 'Empire' and 'Great Lakes 659-700' had significantly longer roots than other cultivars (Table 3). At - 0.3 MPa NaCl, root lengths for 'Empire' and 'Great Lakes 659-700' still were similar but only those of 'Empire' were longer than those of all other cultivars. With - 0.6 and - 0:9 MPa NaCl, 'Great Lakes 659-700' and 'Mesa 659' had no root growth and 'Climax' nearly none. At - 1.2 MPa NaCl, only 'Empire' still grew roots. No root growth occurred for any cultivars at - 1.5 MPa NaCl. Thus, sensitivity to NaCl based on root length was greatest for 'Great Lakes 659-700' and 'Mesa 659', intermediate for 'Climax', and least for 'Empire' and 'Vanguard 75'. For 'Empire' and 'Vanguard 75', roots were longer at - 0.3 MPa than at 0.0 MPa.

#### Discussion

Cultivar differences in germination were observed in response to high temperatures. With no NaCl. differences between sensitive and tolerant cultivars were not as clear as they were with NaCl. 'Coolguard', 'Empire', 'Red Coach 74', and to some extent 'Wintersupreme' were tolerant to high temperature relative to 'Climax', 'Great Lakes 659-700', 'Mesa 659', 'Salinas', and 'Vanguard 75'. However, these groupings narrowed considerably as NaCl was added. With high temperature and NaCl, 'Coolguard' and 'Empire' were clearly the most tolerant cultivars, and to some extent 'Grand Rapids' and 'Vanguard 75' were tolerant. The most sensitive cultivars with high temperature and NaCl clearly were 'Great Lakes 659-700' and 'Mesa 659'. Other studies comparing lettuce cultivars for sensitivity to salt used many cultivars other than those in the present study, although a few were the same. 'Climax' and 'Salinas' had similar salt tolerance (Pasternak et al., 1986), whereas 'Climax' had greater salt tolerance than 'Grand Rapids' (Shannon and McCreight, 1984). These discrepancies from findings in the present study may be related to their evaluation of salt tolerance by fresh weight at later stages or because greenhouse and field conditions provided less temperature control than our tests.

The maximum temperature for germination of lettuce varied depending upon cultivars and NaCl concentration. With no NaCl, high temperature effects first were observed at 35C for all cultivars except 'Salinas', which showed significantly lower germination percentages and rates at 30 than at 20C. The temperatures where germination decreased significantly were similar to those reported for crisphead lettuce types in other studies (Gray, 1975), although 'Grand Rapids' is the only cultivar tested in the present and in previous studies (Borthwick and Robbins, 1928; Thompson et al., 1979). With the addition of NaCl, the maximum germination temperatures were lower, as indicated by germination percentages and rates.

Table 3. Root lengths (centimeters) of five cultivars of lettuce seedlings held 14 days at 26C with five NaCl concentrations.<sup>+</sup>

Cultivar	NaCl concn (MPa) <sup>y</sup>				
	0.0	-0.3	-0.6	-0.9	-1.2
Empire	6.8 a	7.6 a	3.4 a	1.2 ab	1.0 a
Vanguard 75	5.5 b	6.6 bc	2.2 b	1.4 a	0.0 b
Climax	5.7 b	5.9 c	2.5 b	0.5 bc	0.0 Ь
Great Lakes 659-700	7.4 a	7.1 ab	0.0 c	0.0 c	0.0 b
Mesa 659	5.8 b	6.5 bc	0.0 c	0.0 c	0.0 b

<sup>a</sup>None of the cultivars produced roots at – 1.5 MPa NaCl.

<sup>s</sup>Mean separation within each column based on least significant difference at P = 0.05; standard error for difference among NaCl concentrations within a cultivar is 0.38; standard error for difference between cultivars within a NaCl concentration is 0.35. The experiment on root lengths with a subset of cultivars confirmed our germination findings of relative tolerance to NaCl in some cultivars. For 'Great Lakes 659-700' and 'Mesa 659', which were rated sensitive at germination, the roots did not grow at all with NaCl less than or equal to -0.6 MPa. This lack of root growth would prevent establishment of seedlings. 'Climax' was intermediate in tolerance to salt in both germination and root length studies. 'Empire' and 'Vanguard 75' clearly were tolerant relative to the other cultivars in root length studies where root growth in length actually was stimulated at -0.3 MPa NaCl relative to no NaCl. In the germination studies, "Empire' clearly was tolerant, and 'Vanguard 75' was tolerant to some extent. The tolerant cultivars would produce more vigorous roots than sensitive ones in the presence of NaCl, and thus establish a better stand.

From a "grower standpoint, if soil temperatures are near or above 35C all day, germination, problems may develop for the cultivars tested (mostly crisphead types) even if no salts (i.e., NaCl) were present in the soil or irrigation water. However, the presence of NaCl would accentuate this high temperature problem. If NaCl is present, extremely sensitive cultivars such as 'Great Lakes 659-700' and 'Mesa 659' should be avoided. Cultivars that are more tolerant to high temperature and NaCl included 'Coolguard', 'Empire', and to some extent 'Grand Rapids' and 'Vanguard 75'. Consideration should be given to the concentration of salt both in the irrigation water and in the upper few centimeters of soil where lettuce seeds are placed.

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