Zinc and Boron Fertilization on Concentration and Uptake of Copper and Nitrogen in Corn Grain in a Calcareous Soil

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Abstract: A farm experiment was conducted to study the effect of Zn and B interaction on the concentration and total uptake of nitrogen (N) and copper (Cu) in corn grain at Fars Province of Iran. Treatments included five levels of Zn (0, 8, 16 and 24 kg Zn ha^{-1} added to the soil and Zn foliar spray with a 0.5 percent concentration) and four levels of B (0, 3, and 6 kg B ha^{-1} added to the soil and B foliar spray with a 0.3 percent concentration) in a completely randomized block design. A high Zn content in the soil helped increasing the concentration and uptake of N in the grain by B application; that is, at high levels of Zn, there was a synergism between B and N. Boron spraying helped with increasing the concentration and uptake of N in the grain by Zn application. There was a negative correlation between N and Cu concentration in the grain and a positive correlation between N and Cu uptake in the grain.

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1. Introduction

Nitrogen is present in a part of any protein compounds, any enzyme, metabolism intermediate compounds, compounds involved in production and transfer of matter and energy and even in the structure of ribonucleic acid that conducts the transfer of genetic traits. In addition to being involved in the structure of proteins, it makes up a part of the chlorophyll as well. Nitrogen is the first nutrient the deficiency of which being referred to when speaking about soils in arid and semi-arid regions. Sufficient supply of N is associated with high vegetative growth and dark green color (Tisdale et al., 1993). The N content in plant organs is next to carbon, oxygen and hydrogen (Tisdale et al., 1993). Agafone (1991) has reported that by increasing the use of N fertilizers, the plant's need to Cu will increase. Nitrogen causes an increase in uptake and concentration of Cu in the wheat the reason of which is attributed to a change in soil pH and, consequently, an increase in the solubility of Cu in the soil solution, increase in root volume and its extension, as well as the synthesis of compounds that may be carriers for Cu uptake. The plant's Cu is mostly involved in enzymatic activities. Its presence is necessary in enzymatic oxidase – catalase systems. Also, this element is involved in electron transfer reactions and is the activator of several enzymes (Tisdale et al., 1993). It is also involved in the metabolism of proteins and carbohydrates and N fixation (Pals and Benton, 1997). A correct balance between Zn and Cu concentrations plays a significant role in their uptake level. There are many reports on zinc-copper interaction in references. Copper is necessary for protein production. Therefore, adding it to soils with Cu deficiency leads into an increase in their protein content. Pals and Benton (1997) state that the Cu uptake level is lower than most micronutrients.

Based on a report by Dhillon et al. (1987), in the Zn deficiency conditions, conversion of N to protein compounds is reduced and the buildup of amino acids and amids in the plant under these conditions is an evidence for the importance of Zn in protein synthesis. According to a report made by Price et al (1972), Zn deficiency in the plant is associated with RNA and ribosome reduction the result of which is a defect in protein synthesis and consequently, build up of free amino acids in the plant. Ribosomes are located on the cell RNA and are involved in protein production. Nuttal et al. (1987) report that the joint use of B and S increases the grain protein content while joint B and N use reduces the protein content and increases the oil content. Boron is effective in the metabolism of N compounds in the plant and in its deficiency, soluble N compounds, especially the nitrates, build up in the plant (Marschner, 1995). Increase in the Zn level, affects the N uptake and production in the plant (Dhillon et al. (1987). There are many reports on the effect of N on Zn uptake (Karimian, 1995; Mishra and Singh, 1996). Gupta and Patalia (1993) reported that Zn application had no effect on N uptake if N fertilizer was not used, while with using N, application of Zn increased N uptake. They attributed the increase in N uptake to an increase in the dry weight of the airborne organs. Due to the role of Zn in RNA and protein synthesis, Zn use increases the effectiveness of N in the plant (Kitagishi et al., 1987).

Hussien and Faiyad (1996) reported that by Zn application, the plant's N concentration increased.

Gupta et al. (1986) observed that with the use of 2.5 mg Zn/kg soil, the N uptake by corn increased from 53.4 mg in the control to 206.2 mg in the fertilized treatment. Many authors have reported the effect of Zn on the increase in N uptake by the plant (Salam and Subramanian, 1988; Sahu et al., 1996). Latife (1983) reported that by using zinc sulfate, the plant height, ear length and diameter, the number of grains per ear, the weight of 1000 grains, grain yield and the N uptake significantly increased. Kumar et al. (1981) stated that adding Zn to the soil reduced Fe, Mn and Cu concentration and increased Zn concentration in the plant. Many reports are made by authors on zinc–copper interaction (Cayton et al. 1983). Some authors reported that Zn use increased plant growth and reduction of Cu concentration in corn and cereals. Some authors attribute the antagonism of Cu and Zn uptake to having common uptake sites on the root surface and some have attributed the same to their antagonism in transfer of one another from the root to airborne organs (Cayton et al. 1983; Mesquita, 2000). Parker et al. (1992) showed that Zn application increased Mn concentration in the plant but had no effect on Fe and Cu concentrations. Gupta (1993) has reported an interaction between N and B.

The grain being rich in nutrients, including N and Cu plays an important role in human nutrition. The grain being rich in such elements is also an evidence for improve of the harvest, qualitatively and quantitatively. Therefore, by studying Zn and B interaction in the grain, we can find its indirect effects that arte improvement of the harvest, qualitatively and quantitatively, while enriching the grain as well. Also, it has been shown that if we use grains rich in these elements as seeds, we can improve the harvest, qualitatively and quantitatively.

The objective of the study was to evaluate the concentration and uptake of N and Cu in corn grain as affected by Zinc sulfate and Boric acid application.

2. Materials and Methods

A field experiment was conducted at the farm of Aref in Abadeh Tashk, Fars province of Iran, on the corn (*Zea mays* L.), cultivar "Single Cross 401" during 2009 cropping season. The site is located 200 km northeast of Shiraz, with latitude 29° 43' 44'' N and longitude 53° 52' 07" E and 1580 m altitude. Before implementing the project sampling from the soil (0-30 cm depths) was made in order to select a zone in which the available amount of Zn and B was low (less than 1 mg kg^{-1} extracted by methods DTPA and hot water, respectively). This soil had a loam texture, pH of 8.2, 0.59 % organic matter, 229 mg kg^{-1} available K, 12.1 mg kg^{-1} available P, DTPA extractable Fe, Mn, Zn and Cu concentration were 1.65, 8.14, 0.32 and 0.62 mg kg^{-1} and available B with hot water extractable was 0.78 mg kg^{-1}.

This experiment included 20 treatments and 3 replications in the form of completely randomized block design and factorial that combinations of five levels of Zn (0, 8, 16 and 24 kg Zn ha^{-1} added to the soil, and Zn solution spray with a 0.5 percent concentration) and four levels of B (0, 3, and 6 kg B ha^{-1} added to the soil, and B solution spray with a 0.3 percent concentration). Due to a high pH and the high calcium content of the soil in question, a high level of Zn was used. Nitrogen, P and K used at 180, 70 and 75 kg ha^{-1} according to the recommendation, from sources of urea (with 46% N), triple super phosphate (with 46% P_2O_5) and potassium sulfate (with 50% K_2O), respectively, were added to all treatments (plots). Half of the urea was used when planting and the remainder at two different times: at vegetative growth and when the corn ears were formed. Zinc and B, from zinc sulfate and boric acid sources, respectively, were used by two methods, adding to the soil and spraying. Addition to the soil was made at the time of plantation and the sprayings were made at 0.5 % zinc sulfate and 0.3 % boric acid two times: one at vegetative growth stage and the other after corn ears formation. The Zn and B were both applied to the leaves with uniform coverage at a volume solution of 2500 L/ha using a knapsack sprayer. Each experimental plot was 8 m length and 3 m width, had 5 beds and 4 rows, equally spaced, and seeds 20 cm apart on the rows.

Analysis of the grain and soil was carried out using common lab procedures. Phosphorous in soil was measured by Olsen method, available K by 1 M NH_4OAc extraction method and potassium assessment in the extract by flame photometer, organic carbon by the Walkley and Black method. Available Fe, Zn, Mn and Cu in the soil were first extracted by DTPA and then were read by atomic absorption setup. The soil available B was extracted by hot water and then was measured by spectrophotometer by azomethine-H colorimetric method. For N determination, dried grains were digested with 2 N HCl and were analyzed by micro-Kjeldahl method (Bremner and Mulvaney, 1982). Digestion method by dry burning was used to measure Cu and then they were measured by atomic absorption setup. Statistical analysis of data was made using SAS software with Duncan test.

3. Results and Discussion

3.1. Soil analysis before culture

The results of soil analysis before culture are summarized in Table 1. The P, Zn, B, Cu and Fe in the soil were low but soil available K and Mn were higher than the critical level. Karimian and Ghanbari

(1990) reported the critical P level by Olsen method in calcareous soils to be 18 mg kg^{-1}. Sims and Johnson (1991), reported the critical limits of soil Fe, Zn, Mn and Cu by the DTPA extraction method and B by the hot water method to be 2.5-5. 0.2-2, 1-5, 0.1-2.5 and 0.1-2 mg kg^{-1}, respectively. Agrawala (1992) reported that the critical level of Fe, Zn, Mn and Cu in the soil by the DTPA extraction were 2.5, 0.8, 5.5 and 0.75 mg kg^{-1} soil, respectively.

Table 1. Soil mechanical and chemical analysis

Soil properties	Values
Depth of soil(cm)	0 -30
Soil texture	Loam
pH	8.2
EC (ds m^{-1})	2.41
Organic matter (%)	0.59
Nutrients (mg kg^{-1})	
P	12.1
K	229
Fe	1.65
Mn	8.14
Zn	0.32
Cu	0.62
B	0.78

3.2. Nitrogen concentration in the grain

The effects of Zn and B on the grain N concentration were insignificant at a 5% level (table 2). The study of the effect of Zn and B interaction on the grain N concentration showed that B use only at 24 kg ha^{-1} Zn level increased the N concentration in the grain. Application of 3 kg ha^{-1} B at 24 kg ha^{-1} Zn increased the grain N concentration from 1.5 to 1.8 percent (20% increase as compared with no B use at this Zn level) while other B levels showed no significant effect. At other Zn levels, application of B had no effect on grain N concentration. Zinc spraying use only at the B solution spraying level increased grain N concentration from 1.55 to 1.99 percent, showing a 28.38 percent increase as compared with the no Zn use level. The lowest and the highest leaf N concentration, 1.5 and 1.99%, were observed at using 24 kg ha^{-1} Zn and joint Zn and B spraying levels, respectively. Except for these two treatments, other treatments showed no significant difference from the control.

3.3. Nitrogen uptake by the grain

The main effect of Zn on N uptake by the grain (kg ha^{-1}) was significant at 5% level (table 3). The lowest mean N uptake by the grain at 125.99 kg ha^{-1} was seen at no Zn level. With applying 16 and 24 kg ha^{-1} Zn, N uptake by the grain increased from 125.99 at zero Zn level to 155.92 and 148.57 kg ha^{-1}, respectively (23.76 and 17.92 percent increase, respectively), but no significant difference was seen between these two Zn levels. Zinc spraying increased grain N uptake to 155.79 kg ha^{-1}, showing a 23.65% increase relative to zero Zn level; but there was no significant difference between the Zn spraying and applying Zn to the soil in that regard. The highest N uptake by the grain, which is 155.92 kg ha^{-1}, was seen at 16 kg ha^{-1} Zn level.

The effect of applying different B levels on N uptake by the grain was significant at 5% level. The lowest grain N uptake, 129.07 kg ha^{-1}, was seen at zero B level. Boron use at all levels (applying to the soil and spraying) increased grain N uptake relative to zero B use. The use of 3 and 6 kg ha^{-1} B increased grain N uptake from 129.07 at zero B level to 150.78 and 148.56 kg ha^{-1}, respectively (16.82 and 15.1 % increase, in that order); but there was no significant difference between these two B levels in that regard. Boron spraying, too, increased grain N uptake from 129.07 to 155.26 kg ha^{-1}, a 20% increase relative to zero B level; but no significant difference was seen between Zn spraying and its addition to the soil in that regard. The highest grain N concentration was due to B spraying.

Studying the effect of Zn and B interaction on grain N uptake showed that B use at highest Zn level (24 kg ha^{-1} Zn), increased grain N uptake but at other Zn levels, it showed no significant effect on the N uptake. At 24 kg ha^{-1} Zn level, the use of 3 kg ha^{-1} and B spraying significantly increasing grain N uptake from 115.65 kg ha^{-1} to 165.99 and 174.53 kg ha^{-1}, respectively (43.52 and 50.91 percent increase relative to zero B use), but the use of 6 kg ha^{-1} B had no significant effect.

Zinc use at B spraying level, significantly increased grain N uptake but at other B levels, had no significant effect on N uptake. At B spraying level, application of 16 and 24 kg ha^{-1} Zn, increased grain N uptake from 116.43 to 171.9 and 174.53 kg ha^{-1}, respectively (47.64 and 49.9 percent increase, respectively). Zinc spraying at B spraying level, too, increased grain N uptake to 169.38 kg ha^{-1} (45.48% increase) but no significant difference was seen with the case in which Zn was directly applied to the soil.

The lowest N uptake by the grain, 108.83 kg ha^{-1}, was seen in the case where no Zn and no B was used (the control). The highest N uptake by the grain, 174.9 kg ha^{-1}, was seen when 6 kg ha^{-1} B + 16 kg ha^{-1} Zn was used, leading to 60.71% increase relative to the control.

Table 2. The effect of Zn and B on the N concentration (%) by the grain*

B ($kg\ ha^{-1}$)	Zn (kg ha-1)					
	0	8	16	24	Foliar Spray	Mean
0	1.62 bcd	1.6 bcd	1.69 bcd	1.5 d	1.75 abcd	1.63 a
3	1.76 abcd	1.63 bcd	1.65 bcd	1.8 abc	1.67 bcd	1.72 a
6	1.83 ab	1.64 bcd	1.81 abc	1.69 bcd	1.71 bcd	1.74 a
Foliar Spray	1.55 cd	1.66 bcd	1.76 abcd	1.7 bcd	1.99 a	1.73 a
Mean	1.69 ab	1.63 b	1.73 ab	1.67 ab	1.78 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

Table 3. The effect of Zn and B on the N uptake ($kg\ ha^{-1}$) by the grain*

B ($kg\ ha^{-1}$)	Zn ($kg\ ha^{-1}$)					
	0	8	16	24	Foliar Spray	Mean
0	108.83 b	138.69 ab	128.51 ab	115.65 b	153.68 ab	129.07 b
3	145.2 ab	148.07 ab	148.37 ab	165.99 a	146.28 ab	150.78 a
6	133.5 ab	142.45 ab	174.9 a	138.09 ab	153.84 ab	148.56 a
Foliar Spray	116.43 b	144.08 ab	171.9 a	174.53 a	169.38 a	155.26 a
Mean	125.99 b	143.32 ab	155.92 a	148.57 a	155.79 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

3.4. Copper concentration in the grain

The main effect of Zn and B on the grain Cu concentration was not significant at a 5% level (table 4). In the study of the effect of Zn and B interaction on the grain N concentration, it was observed that at 8 $kg\ ha^{-1}$ Zn, application of B only in spray form reduced grain Cu concentration from 4.67 to 2.67 mg kg^{-1} (42.82% reduction) but at other Zn levels, B use had no significant effect on the grain Cu concentration. Zinc application had no effect on Cu concentration in the grain at any B levels.

No treatment showed a significant difference from the control. The highest and the lowest grain Cu concentration, 2.67 and 4.67 mg/ha, showed 19.82% reduction and 40.42% increase as compared with 3.33 mg kg^{-1} of the control.

3.5. Copper uptake by the grain

The main effect of Zn and B on the grain Cu uptake was not significant at a 5% level but the effect of Zn and B interaction was significant at 1% level (table 5). Boron application in the form of spraying at 8 $kg\ ha^{-1}$ Zn level reduced Cu uptake by the grain from 39.6 to 23.13 g/ha (41.59% reduction) but application of B directly to the soil had no significant effect. At a high Zn level (24 $kg\ ha^{-1}$ Zn), only B spraying increased Cu uptake by the grain from 26.43 g/ha to 44.57 g/ha (68.63% increase) but at other Zn levels, B application showed no significant effect on uptake. Probably a high soil Zn content (24 $kg\ ha^{-1}$ Zn) reduced B toxicity and, consequently, an increase in Cu uptake by the grain with B application; but a low Zn level (8 $kg\ ha^{-1}$ Zn) was not able to reduce the toxicity and, consequently, the Cu uptake by the grain with B application was reduced.

Zinc use in cases where B was not applied directly to the soil (zero and B spraying levels) increased Cu uptake by the grain but in cases where it was applied directly to the soil (3 and 6 $kg\ ha^{-1}$ B), it had no significant effect on Cu uptake by the grain. At zero B level, only application of 8 $kg\ ha^{-1}$ Zn significantly increased Cu uptake by the grain from 22.53 to 39.6 g/ha (75.76% increase). The use of 24 $kg\ ha^{-1}$ Zn at B spraying level increased Cu uptake by the grain from 25.17 to 44.57 g/ha (77.07% increase) but other Zn levels had no significant effect. Probably due to a Zn and B antagonism, B application prevented from Zn use affecting Cu uptake by the grain.

Table 4. The effect of Zn and B on the Cu concentration (%) by the grain*

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	8	16	24	Foliar Spray	Mean
0	3.33 abcd	4.67 a	4 abc	3.33 ab	3 bc	3.67 a
3	4 abc	4.33 ab	4 abc	2.67 c	3.67 abc	3.73 a
6	3.67 abc	3.33 abc	2.67 c	3 bc	3.67 abc	3.27 a
Foliar Spray	3.33 abc	2.67 c	3.33 abc	4.33 ab	3 c	3.33 a
Mean	3.58 a	3.75 a	3.5 a	3.33 a	3.33 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

Table 5. The effect of Zn and B on the Cu uptake (kg ha^{-1}) by the grain*

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	8	16	24	Foliar Spray	Mean
0	22.53 e	39.6 ab	30.5 bcde	26.43 cde	26.4 cde	29.09 a
3	32.93 abcde	38.57 abc	36.3 abcd	24.93 de	31.8 bcde	32.91 a
6	26.1 cde	29.13 bcde	25.9 de	24.43 de	33.17 abcde	27.75 a
Foliar Spray	25.17 de	23.13 e	32.6 abcde	44.57 a	24.23 de	29.94 a
Mean	26.68 a	32.61 a	31.33 a	30.09 a	28.9 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

3.6. Correlation between the concentration and total uptake of Cu and N in grain with other variables

Concentration and uptake and other variables, correlation coefficients (R) and (R^2) between different variables were computed using the Pearson method and equations relating to each variable were derived using the step-by-step method. The symbols * and ** in equations denote significance at 5 percent level $(\alpha = 0.05)$ and 1 percent level $(\alpha = 0.01)$ respectively.

3.6.1. Nitrogen concentration in the grain

The grain N content showed a positive correlation with the leaf K content (R= 0.38), Fe (R= 0.32), Zn (R= 0.31) and B (R= 0.47*), the grain B content (R= 0.43), the uptake of N (R= 0.67**) and B (R= 0.42) by the grain, the percentage of grain in the ear (R= 0.40) and grain protein content (R= 0.99**) and a negative correlation with the leaf N content (R= -0.38) and P (R= -0.33), the grain Cu content (R= -0.32), the ear length (R= -0.33), the ear diameter (R= -0.40) and 1000-grain weight (R= -0.38). The equations of which were:

1) NG = - 0.00144 + 0.176 P R = 1**

2) NG = - 0.00186 + 0.176 P – 0.00000945 BUG R = 1**

3) NG = 0.00408 + 0.176 P – 0.0000143 BUG + 0.0000496 NGL R = 1**

NG, P, BUG and NGL are N grain content (%), grain protein content (%), B uptake by the grain (g ha^{-1}) and number of grains in the ear length, respectively.

3.6.2. Nitrogen uptake by the grain

There was a positive correlation between N uptake by the grain and the leaf Zn content (0.41), the grain N content (0.67**), P content (R= 0.33), Mn content (0.38) and B content (0.53*), the uptake of P

(0.79**), K (0.78**), Fe (0.31), Mn (0.63**), Zn (0.56**), Cu (0.31) and B (0.76**), ear weight (0.68**),), grain weigh in the ear (0.69**), total grain yield (0.88**), the number of grains in the ear length (0.58**), the number of grains across the ear diameter (0.31), grain protein content (0.67**), and a negative correlation with leaf Mn content (-0.32) and Cu content (-0.33). The equations of which were:

1) NUG = -22.417 + 0.0197 TGY R = 0.882**

2) NUG = -145.955 + 0.017 TGY + 86.073 NG R^2 = 0.998**

3) NUG = -146.408 + 0.0171 TGY + 83.816 NG + 4.847 CuS R^2 = 0.999**

4) NUG = -139.577 + 0.0172 TGY + 81.666 NG + 6.573 CuS – 0.000374 DM R^2 = 0.999**

NUG, TGY, NG, CuS and DM denote N uptake by the grain (kg ha^{-1}), total grain yield (kg ha^{-1}), grain N content (%), soil Cu content after harvest (mg kg^{-1}) and dry matter (kg ha^{-1}), respectively.

3.6.3. Copper concentration in the grain

The grain Cu content showed a positive correlation with the leaf P content (R = 0.39), Cu uptake by the grain (R = 0.85**), and a negative correlation with the percentage of grain in the ear (R = -0.30) and grain protein content (R = -0.32). The equations of which were:

1) CuG = 1.063 + 0.0814 CuUG R = 0.854**

2) CuG = 3.624 + 0.114 CuUG – 0.000412 TGY R^2 = 0.988**

3) CuG = 3.089 + 0.117 CuUG – 0.000436 TGY + 0.0667 P R^2 = 0.991**

4) CuG = 3.193 + 0.116 CuUG – 0.000403 TGY + 0.0726 P – 0.00198 GW R^2 = 0.993**

CuG, CuUG, TGY, P and GW are grain Cu content (mg kg^{-1}), Cu uptake by the grain (g ha^{-1}), total grain yield (kg ha^{-1}), grain protein content (%) and grain weight in the ear (g), respectively.

3.6.4. Copper uptake by the grain

There was a positive correlation between Cu uptake by the grain and with the leaf P content (R = 0.46*), the grain Cu content (R = 0.85**), the uptake of N (R = 0.31), P (R = 0.36), Mn (R = 0.41), and Zn (R = 0.39) by the grain, ear weight (R = 0.33), the total grain yield (R = 0.55**), the number of grains along the ear (R = 0.44**), the number of grains across the ear diameter (R = 0.43), and a negative correlation with leaf Fe content (R =-0.53) and grain K content (R = -0.40). The equations of which were:

1) CuUG = -1.421 + 8.955 CuG R = 0.854**

2) CuUG = -31.551 + 8.695 CuG + 0.00363 TGY R^2 = 0.992**

3) CuUG = -25.995 + 8.484 CuG + 0.00374TGY – 0.596 P R^2 = 0.995**

4) CuUG = -27.144 + 8.553 CuG + 0.00348 TGY – 0.642 P + 0.167 GW R^2 = 0.996**

CuUG, CuG, TGY, P and GW are Cu uptake by the grain (g ha^{-1}), grain Cu content (mg kg^{-1}), total grain yield (kg ha^{-1}), grain protein content (%) and grain weight in the ear (g), respectively.

4. Conclusion

Application of Zn to the soil and spraying it increased N uptake in the grain. The least and the highest N uptake in the grain at 125.99 and 155.92 kg ha^{-1}, were seen at no Zn level and 8 kg ha^{-1} Zn level, respectively. Also application of B at all levels increased N uptake in the grain. The least and the highest N uptake in the grain at 129.07 and 155.26 kg ha^{-1}, were seen at no B level and B spraying level, respectively. Boron spraying at 8 kg ha^{-1} Zn level decreased Cu concentration and uptake in the grain; but at 24 kg ha^{-1} Zn level, increased Cu uptake in the grain. Zinc application had no effect on Cu concentration in the grain at any B levels. Application of 8 kg ha^{-1} Zn at zero B level, and 24 kg ha^{-1} Zn at B spraying level, increased Cu uptake in the grain.

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