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Activity of Triclopyr Herbicide Enhanced by Combination with Cobalt Chloride or Ammonium Nitrate

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Abstract. Cobalt chloride at a rate of 16 oz/acre when combined with the auxin herbicide triclopyr at 1 oz/acre synergistically enhanced the activity of the herbicide. A similar response was elicited by the addition of ammonium nitrate. Mg^{2+} or Mn^{2+} was antagonistic. The enhancement of activity by Co^{2+} or NH_4^+ appeared not to be the result of a simple enhancement of triclopyr uptake by the cations. Triclopyr uptake, as determined by gas chromatography/microwave-induced plasma/chemical reaction interface mass spectroscopy, was unaffected by the cation additions.

Key Words. Herbicide—Triclopyr—2,4-Dichlorophenoxyacetic acid—Cobalt—Ammonium nitrate—Synergism

A response of plant growth to cobalt ions was first demonstrated by Miller (1951, 1954 and reviewed by Thimann 1956), who showed that auxin-dependent growth of pea seedlings could be either stimulated or inhibited by cobalt chloride ($CoCl_2$) depending on the concentration. The dose-response relationship using stem segments cut from etiolated seedlings of soybean confirmed the interaction between cobalt and the auxin herbicide because auxin-stimulated elongation was specifically inhibited. When extended to greenhouse studies, as described in the present paper, an interaction between $CoCl_2$ and auxin herbicides was observed also. The objective of the present work was to determine if the response was the result of enhanced herbicide uptake or was intrinsic to the auxin herbicide-responsive growth mechanism.

Ammonium nitrate (NH_4NO_3) was observed by us to exert a response similar to that observed with $CoCl_2$ so that a comparison of the effects of $CoCl_2$ and NH_4NO_3 on the uptake of triclopyr ([(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid) was carried out. NH_4NO_3 was reported previously to enhance the absorption of 2,4,5-trichlorophenoxyacetic acid by tree leaves (Brady 1970) and to enhance the activity and absorption of picloram (Wilson and Nishimoto 1975a, 1975b). Although not specifically related to uptake, ammonia fertilizers have been used to decrease the antagonism between the postemergence herbicides bentazon (3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide) and sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) in mixtures (Gerwick et al. 1990, Jordan and York 1989).

In the experiments reported here, the auxin herbicide selected for detailed study was triclopyr or Garlon-4 herbicide (Dow Chemical Company). Triclopyr is a broad spectrum phenoxy type herbicide active against brush and perennial broad leaf weeds (*Herbicide Handbook*).

Materials and Methods

Measurements of Elongation Growth

Soybean (*Glycine max* Merr.) seedlings were grown for 4–5 days in darkness for use in elongation growth experiments. Hypocotyl segments were excised under subdued fluorescent light (1.5 μR day^{-1} m^{-2}), and 1-cm segments cut 5 mm below the cotyledons were used for growth experiments. Soybean hypocotyl segments were floated on 2 mL of treatment solution contained in 2-cm-diameter plastic dishes covered loosely with transparent wrap to retard evaporation. Elongation was estimated to the nearest 0.5 mm over the 18-h incubation at 25°C

Abbreviations: GC/MS, gas chromatography/mass spectrometry; GC/MIP/CRIMS, gas chromatography/microwave-induced plasma/chemical reaction interface mass spectroscopy; 2,4-D,2,4-dichlorophenoxyacetic acid.

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in darkness. Values for initial lengths were subtracted, and appropriate controls without herbicide were included in each determination. Length was determined visually to the nearest 0.5 mm using a scale graduated in 1-mm divisions.

Greenhouse Studies

Seeds of garden bean (*Phaseolus vulgaris* var.) were planted, five/pot, 1 inch deep in soil contained in 4-inch-diameter plastic pots for herbicide response and uptake studies. They were placed in a greenhouse for germination and growth. After 7–9 days, at about the time the first trifoliate leaf was expanding, the plants were treated by aerosol spray, using a compressed air-driven hand sprayer that applied a measured volume/pot to give 40 gallons/acre, with or without triclopyr, applied as ethylene glycol butyl ether (Garlon-4 herbicide) in the presence and absence of $CoCl_2$ or NH_4NO_3. Greenhouse experiments were conducted between February and April at a temperature of 25 ± 2°C and a 14-h photoperiod with illumination from 400-watt high pressure sodium GE Lucalox lamps placed 3 feet above the bench top.

Experiments with $MgCl_2$ and $MnCl_2$ were carried out in a manner identical to those with $CoCl_2$ and NH_4No_3.

All experiments were replicated minimally in triplicate (see tables and figures). Significance was determined using Student's two-tailed *t*-test.

Measurement of Stem Curvature

Curvature of the stem away from the treated leaves is a sensitive criterion to quantitate response to auxin herbicides. Stem curvature of bean plants treated with herbicide in the greenhouse was measured by a modification of the procedure of Day (1952) between 24 and 36 h after treatment using a protractor. Curvature was expressed in degrees of curvature from the vertical. Values from each of the five plants in each pot were averaged for each replicate.

Determination of Triclopyr Uptake

For uptake studies, triclopyr was applied to the plants by aerosol spray at a rate of approximately 1 oz/acre (1 × rate) or 10 oz/acre (10 × rate) in the presence of absence of 5 oz/acre $CoCl_2$ or NH_4NO_3. After a 1-h drying time, the leaves were removed, washed thoroughly with water to remove surface herbicide, and quick-frozen in liquid nitrogen. The leaves were then lyophilized to total dryness and stored in sealed, light-protected bags before analysis. Treatments were repeated three times.

Freeze-dried leaf material (500 mg) was ground using a mortar and pestle. The organic compounds were extracted with three 10-mL aliquots of acetone, and the solvent was decanted after settling of the insoluble residue. This solvent phase was then dried under a stream of nitrogen. Samples for normal GC/MS and GC/MIP/CRIMS were derivatized (methylated) as follows. The dried samples were suspended in 10 mL of methanol, and one drop of concentrated sulfuric acid was added to the solution. This solution was heated gently for 20 min and stirred for a few minutes. The mixture was dried under a stream of nitrogen gas and then suspended in 10 mL of hexane. The water-soluble components of this mixture were extracted by partitioning with three 10-mL aliquots of water. Using a direct insertion probe, the water-extracted solution was tested mass spectrometrically for chlorine-containing compounds. Only a trace of nonmethylated triclopyr was observed, indicating that most of the triclopyr had been derivatized. The hexane solution was first dried under a stream of nitrogen gas and then dissolved in 1 mL of hexane. This solution was used for the selective detection of triclopyr in soybean leaves. A 1-μL solution of the derivatized triclopyr in hexane was injected into the gas chromatograph using a split mode of injection, with a split ratio of approximately 1:10 for *p*-dichlorobenzene.

For analysis, a 1-μL solution of the derivatized triclopyr in hexane was injected into the GC/MIP/CRIMS. This technique, which involves post-column reactions (a reaction interface), included a low pressure microwave-induced helium plasma (Matovsek et al. 1994) to which a reaction gas was added. Effluents of a chromatographic column which entered this reaction interface were converted into small stable neutrals. The mass spectra of these neutrals identified and quantified the elements of interest, i.e. chlorine. Once the retention times of the chlorinated peaks were obtained, their full mass spectra were acquired by repeating the experiment with the MIP off. SO_2 was used as a reaction gas in this study. In the SO_2-containing plasma, HCl (*m/z* 36) was the most abundant ion that was completely selective for chlorine-containing compounds, and CO_2 (*m/z* 44) was the nonselective ion chromatogram for all carbon-containing compounds. Therefore, to quantitate the uptake, two masses were acquired: *m/z* 36 for chlorine-containing compounds and *m/z* 44 for nonselective carbon-containing compounds. When the MIP was off, the mass spectra obtained were consistent with the butoxyethyl ester of triclopyr (Garlon-4) (Morré and Moini 1992, 1993).

To determine uptake, three different organic extracts at two different concentrations (1× and 10×) were analyzed. One was from plants treated with triclopyr only. Another was from plants treated with a mixture of triclopyr and $CoCl_2$. The last was from plants treated with triclopyr and NH_4NO_3. The relative error of the detection method was about ±5%.

The mass spectrometer was a Finnigan 4023 with a Finnigan 4500 ion source. Operating conditions of the mass spectrometer were as follows: source temperature, 150°C; electron impact ionization in the positive mode, 70 eV; electron energy with an emission current of 0.56 mA. The mass spectrometer was controlled by an INCOS data acquisition interface, software version 4.07.82, and a Nova 3 computer.

Results

The elongation of 1-cm segments of soybean hypocotyl stimulated by triclopyr was inhibited by $CoCl_2$ (Fig. 1). Not only was elongation inhibited by $CoCl_2$, but over the range 0.1–1 M, the triclopyr-induced growth was specifically inhibited. Results with the auxin herbicide, 2,4-D, were similar (Fig. 2). The $CoCl_2$ inhibited the 2,4-D-stimulated component of growth more strongly than did triclopyr with much less of an effect on the growth in the absence of 2,4-D.

When treated with 1 oz/acre triclopyr in the presence or absence of 10 oz/acre $CoCl_2$, plants of garden beans showed a synergistic interaction with the $CoCl_2$ in terms of an enhanced herbicidal response (Table 1). $CoCl_2$ by itself was largely without effect. In a series of experiments not illustrated, the optimum concentration of triclopyr to induce the bending response and to result in death of the plants in 1–2 weeks was determined to be 1 oz/acre. Triclopyr at 2 oz/acre or higher tended to kill the bean plants too rapidly to utilize the greenhouse assay as a means to test for synergism between the triclopyr and the additives. Triclopyr at 0.5 oz/acre produced only a

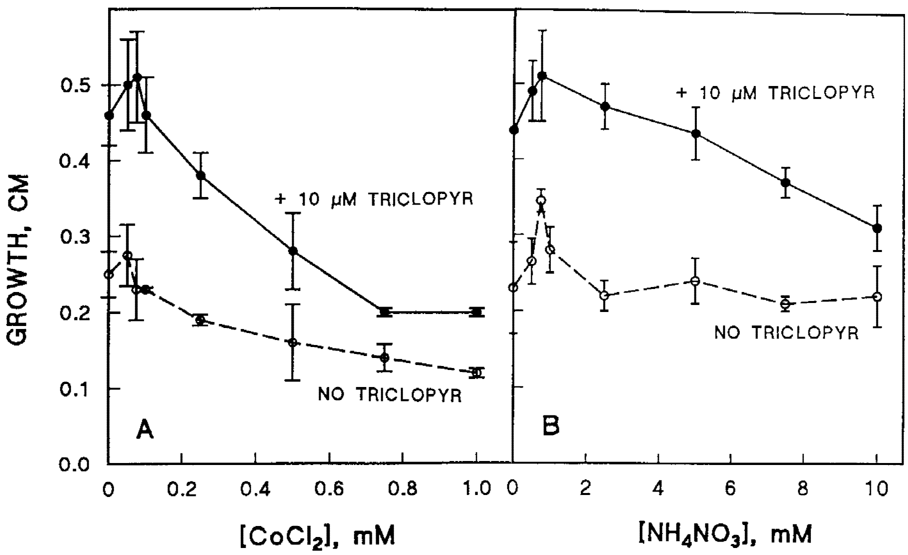

Fig. 1. Inhibition of elongation of 1-cm-long segments of the elongating zone of dark-grown seedlings of soybean by increasing concentrations of $CoCl_2$ (*A*) or NH_4NO_3 (*B*) over 18 h of growth in the presence or absence of 10 μM triclopyr.

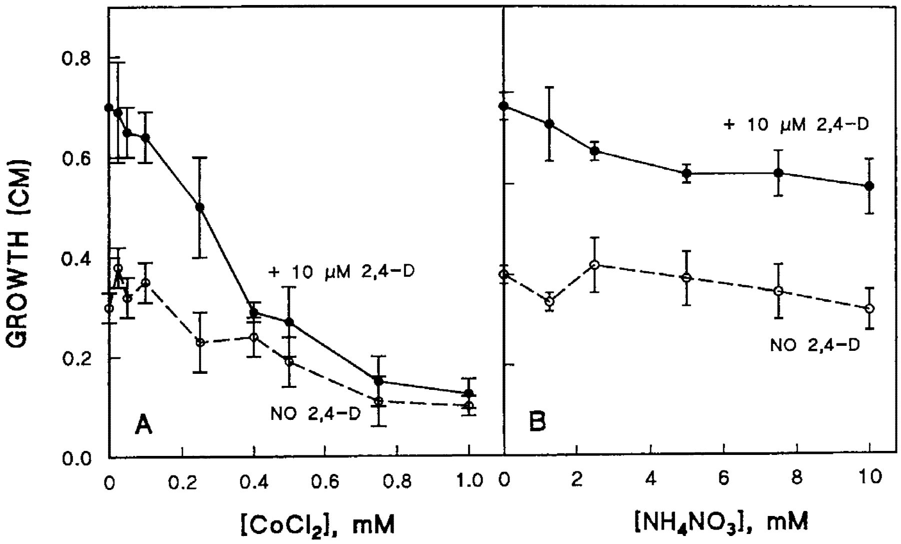

Fig. 2. Inhibition of elongation of 1-cm-long segments of the elongating zone of dark-grown seedlings of soybean by increasing concentrations of $CoCl_2$ (*A*) or NH_4NO_3 (*B*) over 18 h of growth in the presence or absence of 10 μM 2,4-D.

weak and variable herbicidal response. Therefore, for the greenhouse studies, the rate of triclopyr was fixed at 1 oz/acre.

In the presence of 1 oz/acre triclopyr, the concentration of $CoCl_2$ was varied over the range 0–10 oz/acre. A near optimum curvature response was observed at about 5 oz/acre (Figs. 3 and 4). Because of the enhanced stem curvature, the plants treated with triclopyr plus $CoCl_2$ appear shorter. When quantitated and analyzed statistically, a highly significant ($p < 0.01$) enhanced curvature response appears at 5 oz/acre $CoCl_2$ (Fig. 4).

The effect of $CoCl_2$ was also evident in the herbicidal activity with the death of the plant as an end point. After several days, plants treated with triclopyr plus $CoCl_2$ were all dead, whereas plants treated with triclopyr alone were still alive. An enhancement of herbicidal activity similar to that observed with $CoCl_2$ was observed with NH_4NO_3 plus triclopyr (Table 1).

The enhanced herbicidal effectiveness of the triclopyr was not a nonspecific cation effect. Magnesium and manganese were antagonistic (Table 2). The herbicide response with calcium was not enhanced significantly compared with no salt (Table 2).

When investigated in the stem elongation assay, the effect of NH_4NO_3 in inhibiting triclopyr-induced growth (Fig. 1*B*) was similar to the effect of $CoCl_2$ (compare with Fig. 1*A*), although approximately tenfold higher amounts of NH_4NO_3 were required compared with $CoCl_2$ to elicit a similar response (Fig. 1*B*). In studies carried out in parallel with 2,4-D, however, the NH_4NO_3

Table 1. Response of garden bean in the greenhouse to triclopyr in the presence or absence of $CoCl_2$ or NH_4NO_3. 1 oz/acre = 57 μg/4-inch diameter pot. 10 oz/acre = 568 μg/4-inch diameter pot. Results are from six treatment groups ± S.D. Evaluations were 3–5 days after treatment. Results are the averages of two groups of three treated plants each ± mean average deviations. Means not followed by the same letter are significantly different ($p < 0.05$).

Triclopyr	$CoCl_2$	NH_4NO_3	Stem curvature (°)
Series I			
None	None		0 ± 0a
None	10 oz/acre		0 ± 0a
1 oz/acre	None		65 ± 12b
1 oz/acre	10 oz/acre		102 ± 11c
Series II			
None	None	None	0 ± 0a
None	5 oz/acre	None	0 ± 0a
None	None	5 oz/acre	0 ± 0a
None	None	20 oz/acre	0 ± 0a
1 oz/acre	None	None	67 ± 15b
1 oz/acre	5 oz/acre	None	95 ± 5c
1 oz/acre	None	5 oz/acre	104 ± 5c
1 oz/acre	None	20 oz/acre	117 ± 4d

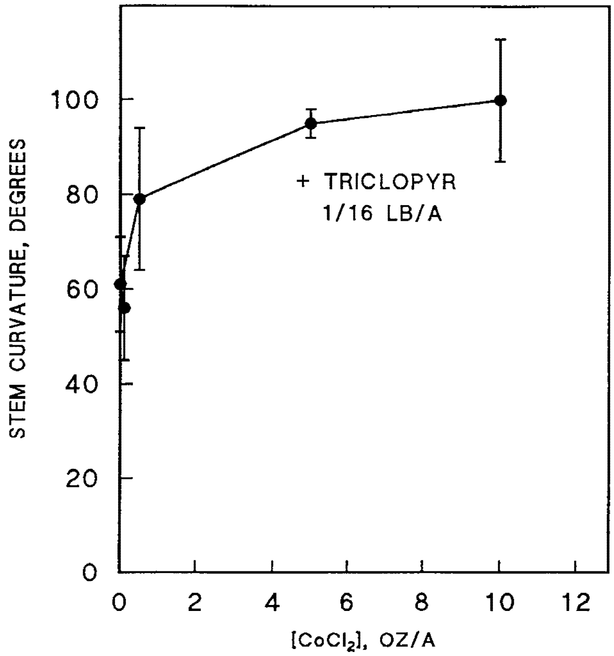

Fig. 4. Curvature of garden bean in response to 1 oz/acre triclopyr as a measure of herbicidal response to increasing amounts of $CoCl_2$. Plants grown in 4-in-diameter pots in the greenhouse were sprayed as the first trifoliate leaf was beginning to expand. Curvature was measured 2 days after treatment.

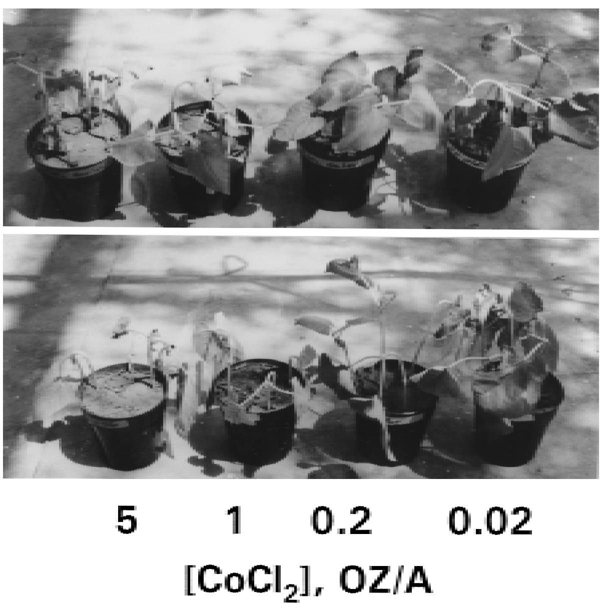

Fig. 3. Replicate treatments of plants of garden bean comparing 1 oz/acre triclopyr with (from *left* to *right*) decreasing amounts of $CoCl_2$. Plants were grown in 4-in-diameter pots in the greenhouse and were sprayed as the first trifoliate leaf began to expand. The plants were photographed 6 days after spraying.

Table 2. Response of garden bean in the greenhouse to triclopyr in the presence or absence of $MgCl_2$ or $CaCl_2$. Results are from three treatment groups ± S.D. deviations. Evaluations were 3–5 days after treatment. Abbreviations are as in Table 1.

Triclopyr	Salt	Stem curvature, (°)
None	None	0 ± 0
1 oz/acre	None	70 ± 10
1 oz/acre	$MgCl_2$, 10 oz/acre	15 ± 8
1 oz/acre	$MnCl_2$, 10 oz/acre	30 ± 6
1 oz/acre	$CaCl_2$, 10 oz/acre	85 ± 11

was largely without effect on the 2,4-D-induced increment of growth (Fig. 2*B*).

Within the margin of error of the determinations (±5%), neither the $CoCl_2$ nor the NH_4NO_3 appeared to influence the uptake of triclopyr by the bean plants (Table 3). Enhanced triclopyr uptake also was not evident when the experiment was repeated with a tenfold higher amount of triclopyr. Under these conditions, uptake appeared to be even reduced by the $CoCl_2$ rather than enhanced (Table 3).

Discussion

Based on information from in vitro growth assays, the use of divalent and monovalent cations Co^{2+} and NH_4^+, antagonists of Mg^{2+} and Mn^{2+}, were found to enhance the herbicidal activity of the triclopyr.

This paper concerns the basis for the stimulation. Two possible explanations were considered. The first would

Table 3. Uptake of triclopyr (1 oz/acre) after 1 h into primary leaves of bean as measured by the relative intensity of the triclopyr (ratio of triclopyr signal to the signal of an internal dichlorobenzene standard) of leaf extracts of weighed amounts of bean leaves dried and acetone extracted and response to either 5 lb/acre $CoCl_2$ or NH_4NO_3. Results are averages of triplicate determinations. The experiment was repeated three times and with a tenfold higher amount of triclopyr with consistent results (relative intensity of 1.8 with triclopyr alone and a relative intensity of 1.2 with $CoCl_2$).

Treatment	Relative intensity
Triclopyr alone	0.209 ± 0.013
Triclopyr + $CoCl_2$	0.233 ± 0.014
Triclopyr + NH_4NO_3	0.197 ± 0.008

be an interaction with the herbicide response mechanism which would result in enhancement of activity. This possibility was investigated using in vitro growth assays with excised, herbicide-responsive elongating stem sections. The second explanation would be through enhanced uptake of the triclopyr (Gerwick et al. 1990). To determine the latter, the newly developed technique GC/MIP/CRIMS (Moini and Abramson, 1991), previously applied to the selective detection of chlorine-containing compounds in complex mixtures, was used (Morré and Moini 1992).

With $CoCl_2$, the response of the elongation growth of 1-cm stem segments of etiolated seedlings of soybean was similar for both triclopyr and 2,4-D. The growth response of both herbicides was inhibited by the $CoCl_2$ to approximately the same extent with very similar dose-response characteristics (compare Figs. 1 and 2). However, with the NH_4NO_3, an effect was seen with the triclopyr but not with 2,4-D.

It is difficult to relate inhibition of elongating stem sections to herbicidal activity. However, in the herbicidal range of concentrations, both 2,4-D and picloram do inhibit rather than promote elongation growth (Morré and Key 1967). Thus, it may be that substances such as $CoCl_2$ and/or NH_4NO_3 which augment the inhibition of auxin growth might also augment the herbicidal activity.

Whatever the mechanism, the observed interaction does appear to translate into the field. We have observed enhanced herbicidal activity of 2 lb/acre triclopyr from the inclusion in the spray mixture of 0.1–1 lb/acre of either $CoCl_2$ or 1–10 lb/acre NH_4NO_3. The mixture has shown enhanced activity both for the control of woody vegetation and for control of herbaceous (especially perennial) weeds. The observation that $CoCl_2$ was ten times more effective than NH_4NO_3 in the laboratory was also experienced in the field. However, both were effective in enhancing triclopyr activity at 1 lb/acre. The action of 2,4-D also appears to be augmented by $CoCl_2$, but an interaction between the NH_4NO_3 and 2,4-D in the field has been difficult to ascertain just as with the elongation growth experiments.

The rates of 1 oz/acre triclopyr used to treat bean plants were below the normal label rates 4–16 1 oz/acre, but garden beans are very susceptible to killing by triclopyr, and the rate used was selected to be non-lethal.

Although we do not rule out completely an effect of the $CoCl_2$ or NH_4NO_3 on herbicide absorption as part of the mechanism of enhancement of activity, we were unable to demonstrate such an effect by the sensitive methods employed in our study. Additionally, the pattern of inhibition of elongation growth demonstrates a very fundamental interaction between triclopyr and both $CoCl_2$ and NH_4NO_3 and between $CoCl_2$ and 2,4-D which correlates with the observed enhancement of herbicidal activity of triclopyr by $CoCl_2$ and NH_4NO_3 and of 2,4-D by $CoCl_2$ but not by NH_4NO_3. Although such a correlation may, indeed, be fortuitous, it still demonstrates a plant response to $CoCl_2$ and/or NH_4NO_3 in combination with herbicides not readily attributed solely to enhanced absorption.

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