The *Journal of Cotton Science 22:97–103 (2018)*
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ARTHROPOD MANAGEMENT

Glufosinate Ammonium Suppresses *Tetranychus urticae* in Cotton

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ABSTRACT

Twospotted spider mites, *Tetranychus urticae*, are an important agricultural pest of many field crops worldwide. Insecticides and acaricides play a primary role in controlling *T. urticae* populations on agricultural crops. Here, we used greenhouse and field applied foliar spray tests and leaf dip bioassays to examine the susceptibility of *T. urticae* to glufosinate ammonium in cotton. Leaf dip bioassay results indicated that *T. urticae* is highly susceptible to concentrations of formulated glufosinate ammonium. The LC_{50} value was determined to be 10.31 ppm. Field-applied glufosinate ammonium at 1.61 and 3.14 L ha^{-1} provided 48.9 and 80.2% control, while fenpyroximate provided 89.6% control five days after treatment in 2015. Greenhouse applications resulted in 55.4% control 14 days after treatment with 0.73 L ha^{-1}, while 1.61 L ha^{-1} resulted in 72.9% control and 3.14 L ha^{-1} resulted in 91.9% control of *T. urticae* populations. Treatment with glufosinate ammonium resulted in significant phytotoxic effects to drought-stressed cotton in the 2015 field trial. These results suggest that glufosinate ammonium may be a useful tool for integrated pest management of weeds and spider mites in cotton. Due to the high cost associated with glufosinate ammonium and possibility of phytotoxic effects under certain conditions, this herbicide it is not considered a viable treatment targeting spider mites but may prove useful for managing mites when utilized for weed management.

Twospotted spider mite (TSSM), *Tetranychus urticae* (Koch), is one of the most economically important arthropods infesting agricultural crops in the Midsouth (Smith et al. 2013). TSSM are often serious pests of corn, cotton, soybeans and grain sorghum. In 2015, infestations of TSSM in Midsouth cotton resulted in applications of acaricides on 170,109 hectares with control costs totaling $26.07 per hectare, and resulted in 6501 metric tons of cotton lint lost (Williams 2015). If not managed properly, TSSM injury can cause reductions in yield, lint quality, oil content in seeds and photosynthetic capacity of injured leaves (Wilson et al. 1991, Reddall et al. 2004).

Twospotted spider mite infestations in Louisiana's agricultural crops typically occur in fields that have late or inadequate fall and spring vegetation management, are in close proximity to tree lines or were previously treated with broad-spectrum insecticides targeted at other economically important insects. Infestations in cotton can occur from emergence until maturity (Gore et al. 2013). Control of TSSM is primarily dependent on applications of acaricides that are expensive and selective to only spider mites. Repeated use of the same modes of action lead to reduced susceptibility and resistance in the target arthropod. Therefore, an integrated approach to TSSM management in field crops helps reduce dependency on acaricides, facilitates natural enemy establishment and reduces input costs to agricultural producers.

One such approach is pre-planting and post-emergence weed management. Gotoh (1997) demonstrated that winter weed management reduced overall TSSM populations infesting pear trees. Ahn et al. (1997) demonstrated acaricidal activity of the herbicide glufosinate to populations of TSSM in apple orchards in Korea. The authors concluded that glufosinate effectively controlled all life stages of TSSM with the exception of eggs.

Glufosinate-tolerant cotton was commercially released in 2004 (Irby et al. 2013). Glufosinate-tolerant cotton was developed by Bayer CropScience and is resistant to post emergence applications glufosinate ammonium (Liberty® 280 SL, 24.5% [ai wt/v]; Bayer CropSciences, Research Triangle Park, NC). Glufosinate is a non-selective herbicide

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with activity on several grasses and broad-leaf weeds (Irby et al. 2013). Adoption of glufosinate-tolerant cotton has increased from 1.7% of United States (U.S.) cotton acres in 2009 to 5.9% of U.S. cotton acres in 2012 (USDA NASS, 2012). However, the LibertyLink cotton adoption rate has increased substantially due to the identification of glyphosate-resistant Palmer amaranth, *Amaranthus palmeri* (S. Wats), and other weeds in Midsouthern states (Irby et al. 2013). The broad-spectrum activity, as well as the ability to control glyphosate resistant weeds, has made glufosinate an important component in pre-plant vegetation management (burndown) and post-emergence weed control. Glufosinate can also be used on Dicamba tolerant cotton and Phytogen cotton varieties. In addition, Smith et al. (2013) obtained 48-80% control of TSSM populations with one application of 0.58 kg-ai/ha of glufosinate in Mississippi cotton.

The increased adoption of LibertyLink cotton to combat herbicide resistant weeds and the utility of glufosinate as a non-traditional acaricide may provide agricultural producers with another option for controlling weeds and TSSM with a single application. The objectives of this study were to quantify the toxicity of glufosinate towards TSSM populations, to determine rates of glufosinate that exhibit activity towards TSSM in cotton, and to estimate efficacy from these rates relative to a commonly used acaricide.

MATERIALS AND METHODS

Foliar Efficacy of Glufosinate: All studies were performed at the Macon Ridge Research Station (LSU AgCenter), 32° 8’31.2095”N, 91°41’53.4156”W, near Winnsboro, LA (Franklin Parish) during the 2015 and 2016 growing season. The cotton variety used for both years was Stoneville 5289 GLT (Glytol Liberty Link) and was planted on 29 May in 2015 (field) and 15 September in 2016 (greenhouse). All plots consisted of four rows (centered on 1.02 m) by 13.7 m in length in the 2015 study. Plots were designated the experimental unit and treatments were arranged in a randomized complete block design with four replications. The 2016 study was conducted in a greenhouse because TSSM populations failed to colonize cotton plants during the production season. Four Stoneville 5289 GLT cotton seeds were planted in each of 80 nursery pots (0.32 m x 0.28 m) filled with growing media (Miracle Gro® Marysville, OH) for the greenhouse study. After emergence, plants were thinned to two per pot and watered as needed. All plants were kept between 26 and 30°C and a photoperiod of 16:8 (L:D). Once plants had reached eight true leaves, TSSM infested *Phaseolus lunatus* (Fordhook 242 bush lima beans) were placed in pots and allowed to naturally infest cotton plants. Plants tested were kept free of non-target arthropods, with weekly foliar applications of 0.45 kilograms per hectare acephate (Bracket 97®, 97.0%[ai wt/wt]; Winfield Solutions LLC, Shoreview, MN) for the duration of the study. Pots were placed on a level surface and oriented to simulate two rows centered on 1.02 m. Thus, each plot, consisting of four pots was considered the experimental unit and plots were arranged in a randomized complete block design and treatments were replicated four times.

The 2015 and 2016 cotton study consisted of three foliar glufosinate treatments, a standard acaricide control and a control treatment. Products used were glufosinate and fenpyroximate (Liberty® 280 SL, 24.5% [ai wt/v]; Bayer CropSciences, Research Triangle Park, NC; Portal XLO®, 5.0% [ai wt/wt]; Nichino America, Wilmington, DE). Applications were initiated once severe TSSM populations had colonized the plant. Foliar treatments were applied with a 3-liter carbon dioxide hand held sprayer calibrated to deliver 140.31 liters per hectare (L ha^{-1}) with two Teejet TX-6 hollow cone nozzles (Teejet Technologies Glendale Heights, IL). Treatments consisted of glufosinate applied at 0.73, 1.61 and 3.14 L ha^{-1}, and fenpyroximate at 1.17 L ha^{-1} for all years tested.

Leaf samples consisted of ten fully expanded leaves, randomly pulled from the top five nodes of the middle two rows in each plot (2015) or rows of pots (2016) at 0, 5 and 14 days after treatment (DAT). Samples were placed in #2 hardware paper bags (Uline, Pleasant Prairie, WI). Whole leaves were processed using a mite brushing machine, (Model 2836M, Bioquip Products, Rancho Dominguez, CA). Adult, immature and total motile mites were counted using a dissecting microscope with each ten leaf sample pooled for analysis. Total motiles were calculated by combining the mean of adult and immature mites for each sample. Mite brushing machines work by dislodging all mite life stages by passing a leaf through a set of rotating brushes. After each sample was processed, the mite brushing heads were dissembled and immersed in alcohol to prevent cross contamination of mites from consecutive samples.

Leaf Dip Bioassay: Seven concentrations of formulated Liberty 280 SL herbicide (0, 1, 5, 10, 15, 20 and 25 ppm active ingredient) were obtained by serial dilution. Fifty-six healthy, arthropod free cotton leaves were collected from Stoneville 5289 GTL reared in the greenhouse for leaf dip assays. Collected leaves were washed with tap water and placed abaxial side up and allowed to air dry for one hour. Once all moisture was dried from leaves, eight leaves (replicates) were randomly assigned to each treatment. Leaves were arranged in a completely randomized design and each leaf was designated the experimental unit. Leaves were fully submerged in each concentration for five seconds, placed abaxial side up and allowed to air dry until all moisture has dissipated. A 2.54-cm punch was used to extract eight leaf cores for each treatment. Individual leaf cores were placed in petri dishes filled with 15 ml of agarose gel. After the cores were placed on the gel surface, ten0 female, field-collected adult TSSM were placed on each core, and each Petri dish was capped and sealed with paraffin. Sealed petri dishes were placed in a growth chamber set to 27 ºC with 75 % RH and 14:10 L:D setting. Mortality was assessed 48 hours after infestation. Mites were examined under a dissecting microscope and considered dead when they failed to respond to prodding with a fine camel hair brush.

Data Analysis: Spider mites counts from foliar tests conducted in 2015 and 2016 were subjected to a Henderson-Tilton transformation to calculate percent control taking into account the differences between the control and treatment mortality from the time of pesticide application to the time of assessment (Henderson and Tilton 1955). Mite counts were subjected to ANOVA and means were separated using an F protected LSD ($P < 0.05$) (SAS/ STAT 9.22 User's Guide, Third Edition, SAS Institute Inc, Cary, NC). Bioassay data were subjected to non-linear regression analysis with 95% confidence intervals (CI) obtained for each dosage (SigmaPlot 12 User's Guide, Systat Software, San Jose, CA).

Mite mortality at each concentration was corrected based on the control mortality using the method of Abbott (1925). The regression line was regressed through the origin. Regression analyses were tested for assumptions of linearity using the Spearman rank correlation between the absolute values of the residuals and the observed value of the dependent variable. Normality was tested using Shapiro-Wilk's test ($P < 0.05$), and outliers were detected and eliminated based on Studentized residuals, disproportional influence using Difference in Fits analysis and Leverage and Cook's distance tests.

RESULTS AND DISCUSSION

Foliar efficacy: Spider mite populations built up to damaging levels in 2015 in the field, while excessive precipitation prevented field efficacy studies in 2016. Glufosinate applied at 0.73 and 1.61 L ha^{-1} provided unsatisfactory control of TSSM relative to fenpyroximate in the 2015 field study (Table 1). Glufosinate applied at 1.61 and 3.14 L ha^{-1} provided 48.9 and 80.2% control while fenpyroximate provided 89.6% control at five DAT. At 14 DAT, glufosinate provided 3.2 to 54.2% control of TSSM, while fenpyroximate provided 69.4% control. Glufosinate applied at 3.14 L provided statistically similar control as ha^{-1} fenpyroximate at five and 14 DAT.

Significant phytotoxic effects were observed at the conclusion of this study. Glufosinate applied at 0.73 and 1.61 L ha^{-1} caused between 15 and 25 percent chlorosis and necrosis of treated plots (Figure 1). No significant differences in phytotoxicity were detected between the 0.73 and 1.61 L ha^{-1} rates. Glufosinate applied at 3.14 L ha^{-1} caused significantly more phytotoxicity than any other treatment with 50 percent of treated leaf area experiencing substantial chlorotic and necrotic injury (Figure 1). Fenpyroximate and the non-treated check exhibited almost no phytotoxic (< 5%) symptoms with no significant differences between these treatments. Visible symptoms did not appear until after the study was concluded.

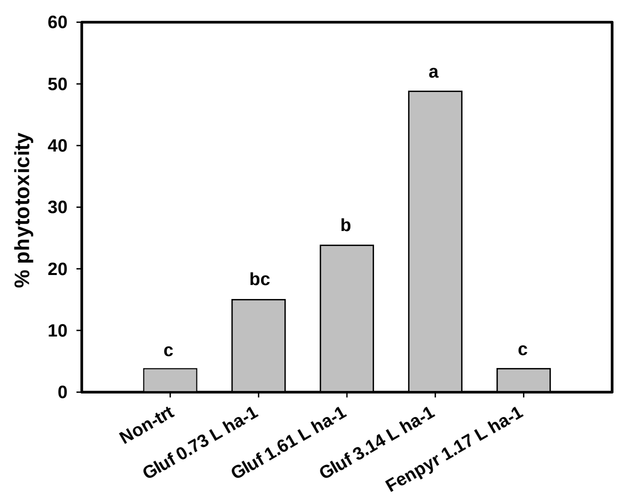

Figure 1. Phytotoxicity ratings of foliar applications of glufosinate to cotton in 2015. Bars containing a common letter are not statistically different based on ANOVA and an F protected LSD ($P \leq 0.05$). $F (4, 19) = 25.06$ ($P < 0.0001$).

Table 1. Efficacy of glufosinate ammonium and fenpyroximate to field populations of TSSM at 5 and 14 days after treatment (DAT)

Treatment	L ha^{-1}	Number of TSSM per 10 leaves			
		13 Aug (pre-treatment)			
		adult	immature	motile	% chk^z
Non-treated	—	85.6 ± 14.6	136.7 ± 31.6	232.3 ± 69.7	—
glufosinate	0.73	43.3 ± 8.3	27.2 ± 8.3	74.8 ± 12.3	—
glufosinate	1.61	98.6 ± 56.8	36.1 ± 10.3	135.4 ± 68.8	—
glufosinate	3.14	64.7 ± 17.0	56.2 ± 12.4	127.6 ± 25.8	—
fenpyroximate	1.71	42.3 ± 7.1	83.8 ± 20.1	129.9 ± 54.1	—
		18 Aug (5 DAT)			
		adult	immature	motile	% chk^z
Non-treated	—	328.4 ± 142.1 a	213.6 ± 70.4 a	552.4 ± 229.1 a	0.0 c
glufosinate	0.73	40.4 ± 11.9 b	33.5 ± 15.2 bc	76.8 ± 30.7 bc	45.6 ± 20.1 c
glufosinate	1.61	61.0 ± 31.8 b	91.5 ± 35.7 ab	153.1 ± 73.8 b	48.9 ± 30.3 bc
glufosinate	3.14	19.4 ± 12.5 b	18.1 ± 4.5 c	41.3 ± 16.7 c	80.2 ± 11.1 ab
fenpyroximate	1.71	16.5 ± 4.5 b	13.4 ± 3.3c	31.5 ± 13.1 c	89.6 ± 1.2 a
		27 Aug (14 DAT)			
		adult	immature	motile	% chk^z
Non-treated	—	461.3 ± 104.1 a	753.3 ± 350.8 a	1249.7 ± 449.5 a	0.0c
glufosinate	0.73	272.5 ± 65.6 ab	282.1 ± 63.9 ab	561.1± 119.8 ab	3.19 ± 0.2 c
glufosinate	1.61	177.8 ± 42.9 bc	232.8 ± 102.7 b	414.6 ± 144.1 b	33.5 ± 13.5 b
glufosinate	3.14	101.5 ± 37.1 c	125.3 ± 38.3 bc	244.6 ± 44.4 bc	54.2 ± 13.9 ab
fenpyroximate	1.71	99.3 ± 27.2 c	65.8 ± 26.1 c	165.7 ± 53.1 c	69.4 ± 9.8 a

Means in the same column with different letters are statistically different based on ANOVA and a protected LSD (P ≤ 0.05).

^{z} Percent of non-treated control (Henderon-Tilton) of foliar applications on TSSM populations.

For the 2016 greenhouse study, glufosinate effectively controlled TSSM at all rates tested. Five DAT, only the 3.14 L ha^{-1} rate reduced TSSM populations equal to fenpyroximate (Table 2). Glufosinate applied at 0.73 L ha^{-1} resulted in 55.4% control 14 DAT while 1.61 L ha^{-1} resulted in 72.9% control, and 3.14 L ha^{-1} resulted in 91.9% percent control of TSSM populations. Overall, fenpyroximate provided the maximum observed control and did not induce phytotoxicity in the 2015 field trial.

Leaf dip bioassay: Leaf dip bioassay results indicated that TSSM were highly susceptible to concentrations of formulated glufosinate. The LC_{50} value was determined to be 10.3 ppm with 95% CI determined to be (6.0 – 15.8) (Figure 2). Non-linear regression analysis indicated a significant dose mortality relationship ($P < 0.0001$, $R^2 = 0.48$).

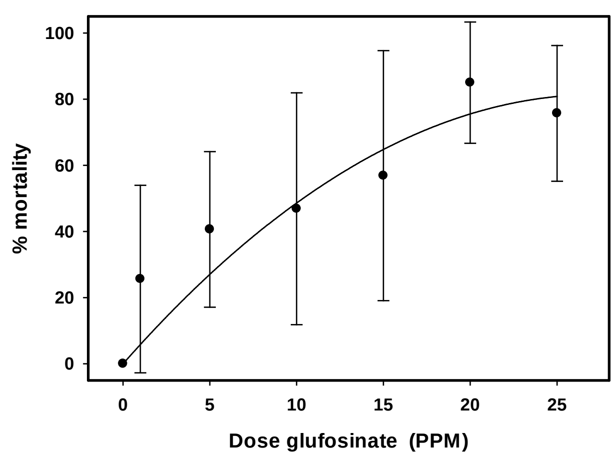

Figure 2. Dose response curve of twospotted spider mite to glufosinate ammonium in leaf dip bioassays. $y = 23.31 + 4.34x – 0.078x^2$. $R^2 = 0.48$, $F (2, 54) = 49.65$, ($P < 0.0001$).

Table 2. Efficacy of glufosinate ammonium and fenpyroximate to greenhouse populations of TSSM at 5 and 14 days after treatment (DAT)

Treatment	L ha^{-1}	Number of TSSM per 10 leaves			
		7 Dec (pre-treatment)			
		adult	immature	motile	% chk^{z}
Non-treated	—	38.1 ± 19.9	51.8 ± 18.7	89.8 ± 32.7	—
glufosinate	0.73	41.3 ± 18.9	23.5 ± 5.2	64.8 ± 16.2	—
glufosinate	1.61	53.1 ± 17.6	49.8 ± 15.6	102.8 ± 20.7	—
glufosinate	3.14	79.3 ± 8.6	58.1 ± 15.1	137.3 ± 17.4	—
fenpyroximate	1.71	46.2 ± 18.6	56.3 ± 17.7	102.1 ± 36.1	—
		12 Dec (5 DAT)			
		adult	immature	motile	% chk^{z}
Non-treated	—	45.1 ± 19.7	61.8 ± 19.5a	106.8 ± 33.9a	0.0c
glufosinate	0.73	25.1 ± 12.5	11.5 ± 6.3b	36.5 ± 12.4b	55.5 ± 12.4b
glufosinate	1.61	33.5 ± 16.3	17.8 ± 3.9b	51.3 ± 18.5b	57.4 ± 16.4b
glufosinate	3.14	16.3 ± 8.3	12.8 ± 3.1b	29.1 ± 8.9b	80.6 ± 8.1ab
fenpyroximate	1.71	6.8 ± 2.4	5.5 ± 1.9b	12.3 ± 4.1b	89.4 ± 0.9a
		21 Dec (14 DAT)			
		adult	immature	motile	% chk^{z}
Non-treated	—	54.8 ± 21.1	79.5 ± 17.3a	134.3 ± 32.6a	0.0d
glufosinate	0.73	26.3 ± 13.5	19.8 ± 7.4b	46.0 ± 14.2b	55.4 ± 13.5c
glufosinate	1.61	25.3 ± 14.5	12.8 ± 3.1b	38.0 ± 15.4b	72.9 ± 13.4bc
glufosinate	3.14	8.3 ± 5.1	6.3 ± 1.3b	14.5 ± 6.3b	91.9 ± 5.1ab
fenpyroximate	1.71	2.3 ± 0.9	2.0 ± 0.9 b	4.3 ± 1.8b	96.9 ± 0.7a

Means in the same column with different letters are statistically different based on ANOVA and a protected LSD ($P \leq 0.05$).

^{z} Percent of non-treated control (Henderon-Tilton) of foliar applications on TSSM populations.

The use of glufosinate on damaging populations of TSSM provided control comparable to a standard acaricide when used at the maximum label rate in the field. Dose mortality bioassays indicated that TSSM were highly susceptible to glufosinate and appropriate field use rates may provide an added acaricidal benefit to pre-plant weed management or post emergence use during the recommended label use window. Glufosinate application coverage is important for not only weed but for TSSM control;, the use of a leaf dip bioassay ensures adequate coverage over the entire leaf surface. It is important to note that use of glufosinate at the stage .conducted in the 2015 experiment would be considered an off-label application.

Glufosinate requires a 70-day pre-harvest interval (PHI) which allows for foliar applications to made in the early squaring to first bloom period. Furthermore, the cost associated with the use of this herbicide is not considered a viable treatment targeting spider mites. Glufosinate, formulated as Liberty 280 SL herbicide, would cost producers \$54 per hectare when applied at 0.73 L ha^{-1} and \$106 per hectare when applied at 3.14 L ha^{-1} while fenpyroximate formulated as Portal XLO costs \$30 per hectare. Dedicated acaricides such as fenpyroximate are significantly less expensive (\$22.00 – 30.00 per hectare), have shorter PHI's and cause very little phytotoxicity when used appropriately. The cotton utilized for this test was experiencing severe drought stress and coupled with advanced maturity, may explain in the abnormal levels of phytotoxicity observed.

The use of glufosinate as an alternative form of mite control may be a highly effective tool for managing TSSM populations resistant to traditional acaricides. Ahn et al. (1997) demonstrated efficacy of glufosinate to TSSM field populations highly resistant to various acaricides. Thus, the acaricidal mode of action of glufosinate may be different from that of known compounds, although the exact mechanism remains unknown. Furthermore, Ahn et al. (1997) also demonstrated a positive temperature coefficient for glufosinate (10 to 32ºC) on TSSM mortality when applied by the mite dipping method. Glufosinate toxicity was shown to increase 17 and 20 times that at 10ºC when temperatures were elevated to 25 and 32ºC (Ahn et al. 1997). This may help elucidate a possible mechanism of action of glufosinate but may also have other implications for mite control as well. The use of glufosinate as a pre-plant herbicide may impart only partial acaricidal benefits if the weather is cool. Louisiana has an average spring temperature of 19ºC while the average summer temperature is 27ºC (NCDC 2015). Spring pre-plant herbicide applications are made while squaring and bloom applications are often made during the summer. Applications of glufosinate during spring months may only suppress TSSM populations while applications made during summer months may provide better control of TSSM populations.

Glufosinate does not exhibit any repellency that may cause mite movement to non-affected weeds or refuges where further feeding and reproduction would result in outbreaks. Further, glufosinate is relatively non-toxic to non-target arthropods, including beneficial insects and mites (Ahn et al. 2001). Ahn et al. (2001) found that glufosinate applied at 540 ppm, field applied rate for weed control in apples, was non-toxic to eggs of *Amblyseius womersleyi* (Schicha), *Phytoseiulus persimilis* (Athias-Henriot), and *T. urticae*, but acutely toxic to TSSM nymphs and adults. Experiments with *Chrysopa pallens* (Rambur) demonstrated little or no harm to larvae and pupae, while mortality of *Orius strigicollis* (Poppius) was determined to be 71.2% to eggs, 65.0% to nymphs and 57.7% to adults at 540 ppm. Overall, glufosinate is less toxic to beneficial insects with the exception of the predatory mite *P. persimilis* (Ahn et al. 2001).

In conclusion, glufosinate may be a key component of integrated pest management for TSSM control in cotton. The use of glufosinate as a resistance management tool, for glyphosate resistant weeds such as palmer amaranth, coupled with the acaricidal benefits demonstrated in this study, may give producers an effective option in controlling weeds as well as populations of spider mites in cotton. Further investigations are needed to determine the effects of glufosinate on TSSM populations or other mite pests in soybeans and corn and to determine this compound's mode of action.

ACKNOWLEDGEMENTS

Special thanks to the technicians and student workers at the Macon Ridge Research Station and Kyle Fontenot with Bayer Crop Science for donating cotton seed. This study was funded by grants from Cotton Incorporated Louisiana State Support Committee.

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