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Bio-efficacy of cyantraniliprole, a new molecule against *Scelodonta strigicollis* Motschulsky and *Spodoptera litura* Fabricius in grapes

D. S. YADAV*, A. S. KAMTE and R. S. JADHAV

National Research Centre for Grapes, Manjri, Solapur Road, Pune - 412 307, Maharashtra, India
Email: deependra81@gmail.com ; dsyadav@icar.org.in

ABSTRACT: Field experiments were conducted to evaluate the effectiveness of cyantraniliprole in comparison with recommended insecticides against flea beetle, *Scelodonta strigicollis* and caterpillar, *Spodoptera litura* in table grapes for two seasons. In addition, laboratory bioassays were also conducted to confirm the results. None of the tested doses of cyantraniliprole caused significant mortality of *S. strigicollis* in both laboratory and field experiments. However, in field experiments, the leaf damage reduction was significantly higher in vines treated with cyantraniliprole as compared to untreated check indicating antifeedant effects of cyantraniliprole. Cyantraniliprole at the rate of 80 g a.i./ha resulted in highest leaf damage reduction and was at par with cyantraniliprole at the rate of 70 g a.i./ha and thiamethoxam and spinosad during 2010 and with only spinosad during 2011. The highest population reduction of *S. strigicollis* were observed in thiamethoxam at the rate of 62.5 g a.i./ha and spinosad at the rate of 112.5 g a.i./ha which were at par with each other and superior over all the doses of cyantraniliprole. When evaluated against *S. litura*, cyantraniliprole at the rate of 0.5, 0.6 and 0.7 ml/L water resulted in 100 percent mortality at 72 hours after exposure during laboratory bioassays. In field experiments, cyantraniliprole at the rate of 70 and 80 g a.i./ha was most effective and at par with spinosad in reducing *S. litura* population.

Keywords: Cyantraniliprole, *Scelodonta strigicollis*, *Spodoptera litura*, *Vitis vinifera*

INTRODUCTION

The anthranilic diamide insecticides (Figure 1) are a new class of insecticides with a novel mode of action which target ryanodine receptors (IRAC mode of action classification, group 28) [IRAC, 2012]. This group of insecticides potently activates ryanodine receptors, releasing stored calcium from the sarco-endoplasmic reticulum leading to impaired regulation of muscle contraction (Cordova *et al.*, 2005). Further, the calcium mobilization studies using mammalian cell lines indicated that anthranilic diamides exhibited >500-fold differential selectivity toward insect, over mammalian receptors (Cordova *et al.*, 2005). Recently, two classes of synthetic chemicals have been discovered which targeted ryanodine receptors. The first class, phthalic acid diamide, produced flubendiamide and the second class, anthranilic diamides, yielded chlorantraniliprole & cyantraniliprole (Sattelle *et al.*, 2008). Chlorantraniliprole

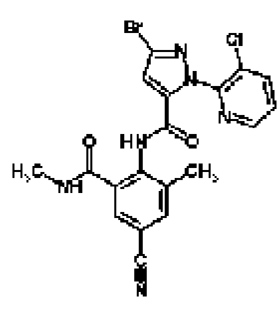
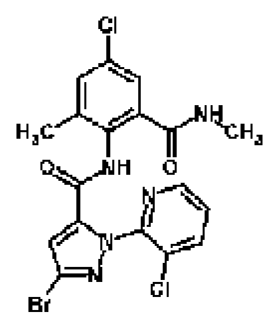
	Cyantraniliprole	**Chlorantraniliprole**
Molecular structure		
Molecular formula	($C_{19}H_{14}BrClN_6O_2$)	($C_{18}H_{14}BrCl_2N_5O_2$)
Systematic name	3-Bromo-1-(3-chloro-2-pyridinyl) -N-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-1H-pyrazole-5-carboxamide	3-Bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl) phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide

Fig.1. Chemical comparison of cyantraniliprole and chlorantraniliprole (Source: ChemSpider)

(Rynaxypyr) was the first commercialized anthranilic diamide which was registered for use in the USA in 2008 (EPA, 2008) and cyantraniliprole (Cyazypyr) is a new anthranilic diamide under development to control lepidopteron and sucking insects. Chlorantraniliprole & cyantraniliprole differ only in the 4-substituent of the anthranilic core, which are chloro and cyano, respectively; this difference was sufficient to provide broad spectrum of activity to cyantraniliprole including orders of Hemiptera and Thysanoptera, in addition to Lepidoptera (Lahm *et al.*, 2012). This chemical also exhibits antifeedant properties against thrips (Jacobson and Kennedy, 2011).

The sub-tropical viticulture system of peninsular India does not undergo winter dormancy of grapevines and vine growth remains active throughout the year. Therefore, the grapevines remain available for the pest development all the year round which leads to high pest infestation. Among various arthropod pests, flea beetle, *Scelodonta strigicollis* Motschulsky (Coleoptera: Chrysomelidae) and caterpillar, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) cause considerable damage to the grapes. In table grapes, the flea beetle damage is caused mostly by the adult beetles by scraping the sprouting buds or eating them up completely (NRCG, 2011). These damaged buds fail to sprout well. In addition, the beetles also feed on tender shoots and leaves causing substantial damage. *S. litura* mainly causes damage by defoliating the leaves thereby affecting photosynthesis. Sometimes, they can also be seen feeding on berries. There are not sufficient numbers of pesticides registered for grapes so far in India to manage these serious pests. Therefore, the present study was carried out to generate bio-efficacy data regarding minimum effective dose (FAO, 2006) for a new generation anthranilic diamide insecticide, cyantraniliprole (Cyazypyr; E.I. DuPont India Private Limited) against flea beetle and caterpillars in table grapes.

MATERIALS AND METHODS

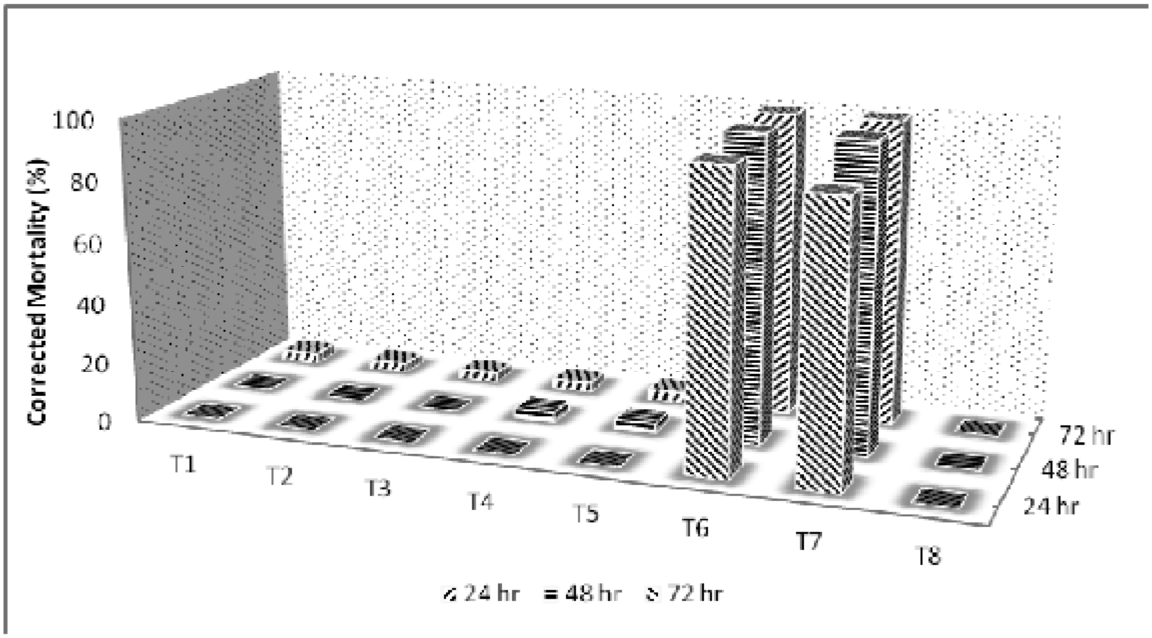
Bioassays

Laboratory bioassays were conducted during July to October, 2010. The experiments were laid out in a Completely Randomized Design (CRD) with the treatments replicated thrice. Five different doses of cyantraniliprole 10 OD (30, 40, 50, 60 and 70 mL/liter water) were evaluated with water treated control and two positive checks (recommended insecticides) thiamethoxam 25 WG (0.25 g/L water, Actara; Syngenta India Limited, Pune, India) and spinosad 45 SC (0.25 mL/liter water, Spintor, Bayer CropScience Limited, Mumbai, India). The insecticidal solutions were made in distilled water with the above mentioned doses of different treatments.

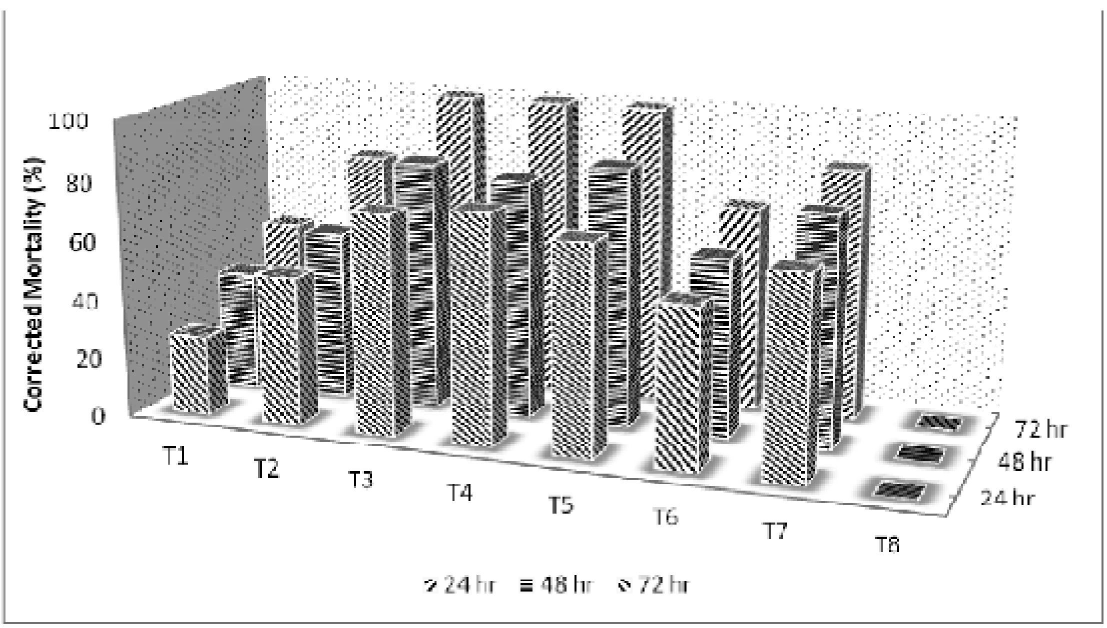
Leaf dip bioassays, modified from as described by Hill and Foster (2000), were conducted to determine the response of flea beetle and caterpillar against cyantraniliprole. Tender leaves of approximately same size were collected from never sprayed grapevine nursery plants (variety Thompson Seedless) for these studies. The leaves were dipped into the treatment solutions for 10 seconds and hung vertically to air dry for 2 hours. Control leaves were treated similarly with water. Filter paper was placed inside a glass petri dish (100mm x15 mm) and treated leaves were placed on top of the filter paper. Ten randomly selected flea beetle adults, collected from never sprayed Dog Ridge rootstock nursery plants, were released in each petri dish. In case of bioassay on caterpillar, laboratory reared 10 second instar *S. litura* larvae were placed in each petri dish. Observations were taken on live insects at 24, 48 and 72 hours after exposure and moribund insects were considered as dead. Wherever, mortality in the control was more than 20 percent, the experiment was discarded. All the experiments were repeated minimum two times and pooled data were used for statistical analysis. Corrected percentage mortality was calculated with the help of Abbott's formula (Abbott, 1925) as defined by Perry *et al.* (1998). The corrected percentage mortality values were subjected to square root transformation (x+0.5) and were analyzed using software provided by Statistical Analysis System (SAS Institute, 2011). General linear model (GLM) procedures were used to perform the analysis of variance (ANOVA) and means were separated using Tukey's honest significant difference (HSD).

Field tests

The field experiments were conducted during fruiting seasons of 2009-10, 2010-11 and 2011-12 in a 9-11 years old vineyard with Thompson Seedless variety on Dog Ridge rootstock. The vines were trained to 'Y' system and the location of the vineyard was at National Research Centre for Grapes, Manjri Farm, Pune, India. The experiments were laid out in a Randomized Block Design (RBD) with each treatment replicated thrice. Five different doses of cyantraniliprole 10 OD (Table 1) were evaluated with water treated control and standard checks of thiamethoxam 25 WG (62.5 g a.i./ha) and spinosad 45 SC (112.5 g a.i./ha). The fruit prunings were done during first fortnight of October during all the fruiting seasons. Separate experimental blocks were used for study on each pest. Spray volume at the rate of 1000



(a) ***S. strigicollis***



(b) ***S. litura***

(where, T1, T2, T3, T4, T5= cyantraniliprole @ 0.3, 0.4, 0.5, 0.6, 0.7 mL/L water, T6= thiamethoxam; T7=spinosad; T8= water treated control)

Figure 3. Bio-efficacy of various doses of cyantraniliprole against (a) *S. strigicollis*, and (b) *S. litura* under bioassay experiments

Table 1. Bio-efficacy of different doses of cyantraniliprole 10 OD against flea beetle and reduction in leaf damage

Treatment	Dose (g a.i. ha^{-1})	Mean number of live flea beetles per shoot						Mean percent flea beetle damage reduction on leaves per shoot on 5 DAS	
		2009-10			2010-11				
		1 DAS	3 DAS	5 DAS	1 DAS	3 DAS	5 DAS	2009-10	2010-11
Cyantraniliprole	40	19.7 a	18.0 a	17.7 a	19.7 a	19.0 a	18.7 a	15.3 d	13.4 e
Cyantraniliprole	50	20.0 a	17.7 a	17.3 a	20.0 a	19.3 a	18.3 a	20.6 cd	16.7 e
Cyantraniliprole	60	19.7 a	17.3 a	17.0 a	19.7 a	19.3 a	19.0 a	31.5 c	29.9 d
Cyantraniliprole	70	20.0 a	18.0 a	17.7 a	19.3 a	18.7 a	18.3 a	49.5 b	43.3 cd
Cyantraniliprole	80	20.0 a	17.7 a	17.3 a	20.0 a	19.3 a	19.0 a	55.3 ab	47.1 bc
Thiamethoxam	62.5	9.3 b	8.3 b	0.0 c	8.0 b	6.7 c	2.3 b	74.8 a	68.8 a
Spinosad	112.5	10.7 b	9.3 b	4.3 b	9.3 b	8.7 b	3.3 b	70.1 a	63.1 ab
Control	-	19.7 a	17.7 a	17.3 a	19.7 a	18.7 a	18.7 a	0.0 e	0.0 f
	F	64.39	30.83	111.58	63.19	103.30	249.71	31.21	26.26
	df	9, 14	9, 14	9, 14	9, 14	9, 14	9, 14	9, 14	9, 14
	P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

DAS= Days after spraying
Values were transformed to square root (X+0.5) when analyzing ANOVA
Figures in the same column with same letter (s) do not differ significantly (P > 0.05)

Table 2. Mean numbers of *S. litura* larvae on grapevine plants treated with different doses of cyantraniliprole 10 OD

Treatment	Dose (g a.i. ha^{-1})	Number of live caterpillars per plant							
		2009-10				2010-11			
		Pre-count	3 DAS	7 DAS	10 DAS	Pre-count	3 DAS	7 DAS	10 DAS
Cyantraniliprole	30	14.7	10.3 ab	9.3 a	8.0 a	-	-	-	-
Cyantraniliprole	40	15.0	10.3 ab	9.0 a	7.7 a	14.3	13.7 a	12.3a	9.0 a
Cyantraniliprole	50	13.3	9.0 ab	8.3 ab	6.7 ab	14.0	13.0 a	11.3 a	8.3 a
Cyantraniliprole	60	14.0	8.0 b	7.3 ab	6.7 ab	16.3	15.3 a	12.0 a	8.7 a
Cyantraniliprole	70	13.7	5.3 c	4.7 c	4.3 c	17.3	8.7 b	5.3 b	4.0 b
Cyantraniliprole	80	-	-	-	-	15.0	8.3 b	5.0 b	3.7 b
Thiamethoxam	62.5	14.7	10.3 ab	8.3 ab	7.3 a	15.0	13.3 a	11.0 a	7.7 a
Spinosad	112.5	14.3	8.0 b	6.3 bc	5.0 bc	14.0	8.0 b	4.3 b	3.0 b
Control	-	13.0	11.0 a	9.3 a	8.7 a	14.3	13.3 a	11.0 a	9.0 a
	F	1.72	4.02	3.80	3.60	2.06	10.05	15.98	16.99
	df	9, 14	9, 14	9, 14	9, 14	9, 14	9, 14	9, 14	9, 14
	P	0.1749*	0.0101	0.0128	0.0160	0.1094*	0.0001	< 0.0001	< 0.0001

DAS= Days after spraying
* = Non-significant (P > 0.05)
Values were transformed to square root when analyzing ANOVA
Figures in the same column with same letter (s) do not differ significantly (P > 0.05)

liter water per hectare was used. The sprayings were done using knapsack sprayer (Inter 16 Green, Goizper S. Coop., Antzuola) fitted with hollow cone nozzle.

For field studies on *S. strigicollis*, single treatment application was made at 20 days after fruit pruning when 8-10 and 5-7 leaf stage of the vines was reached during 2010 and 2011, respectively. Never sprayed 24 plants were selected for the experiment. Above mentioned doses of the treatments were sprayed on respective plants. After an hour of spraying, one shoot was selected randomly from each plant and all flea beetle damaged leaves were removed from the shoot. Each shoot was covered with insect breeding net cage (AC-186, Amar Chand and Company, Ambala Cantt, Haryana, India). Twenty flea beetle adults, collected from never sprayed Dog Ridge rootstock nursery plants, were released in each cage. Observations on flea beetle mortality per shoot were taken after 1, 3 and 5 days after treatment application. On 5th day, observations on flea beetle leaf damage were also taken. The characteristic linear and rectangular shaped holes on the leaves were taken as infestation symptom to identify damaged leaves by this pest. *S. litura* larvae were not evenly distributed in the experimental plot during the trial period during both 2009-10 and 2010-11 seasons. Therefore, laboratory reared 20 second instar larvae of *S. litura* were released on each caterpillar free never sprayed plant before treatment application at 40-50 days after fruit pruning during both seasons. The test insect chosen for bio-efficacy studies was *S. litura* because this was the most frequently observed species of caterpillars in the vineyards. *S. litura* egg masses were collected from vineyards, reared on fresh grape leaves in the laboratory and 20 second instar larvae of *S. litura* were released at the rate of five larvae per shoot. These were allowed to adapt in field for 3 days and pre-count of number of caterpillars per plant were taken before single application of treatments on 3rd day. Observations on number of live caterpillars per plant were taken after 3, 7 & 10 days of treatment application also.

The visual count data of field tests were subjected to square root transformation and analyzed using software provided by Statistical Analysis System (SAS Institute 2011). GLM procedures were used to perform the analysis of variance and significant means were separated using HSD.

RESULTS AND DISCUSSION

Bioassays

When exposed to flea beetle adults, even the highest dose of the cyantraniliprole (0.7 mL/L) could not cause significant mortality even after 72 hours of exposure ($F = 35.90$; df = 7, 16; $P < 0.0001$) (Fig. 3 a). The significant mortality was observed in positive checks thiamethoxam and spinosad which were at par with each other and superior over all other treatments after 24 hours of exposure ($F = 1141.14$; df = 7, 16; $P < 0.0001$). Both of these treatments caused 100 percent mortality of flea beetles after 48 hours of treatment application ($F = 99.01$; df = 7, 16; $P < 0.0001$). Against second larval instar of *S. litura*, after 24 hours after exposure ($F = 105.34$; df = 7, 16; $P < 0.0001$) highest mortality of 76.67 percent was observed in cyantraniliprole (0.60 mL/L) which was at par with its other doses of 0.5 & 0.7 mL/L (Fig. 4) and spinosad which caused 73.33, 70.0 and 66.67 mortality, respectively. In all the treatments, mortality increased at 48 hours ($F = 432.98$; df = 7, 16; $P < 0.0001$) and 72 hours after exposure ($F = 406.33$; df = 7, 16; $P < 0.0001$). Further, 100 percent mortality was achieved in three doses of cyantraniliprole (0.5, 0.6 and 0.7 mL/L) after 72 hours of treatment application which were superior over spinosad and thiamethoxam. As evident in laboratory bioassays, cyantraniliprole showed better potential for the management of caterpillars, as even lower dose of 0.5 mL/L was most efficient and at par with higher doses. The toxicity of this chemical to Lepidoptera is becoming well established (Rhainds and Sadif, 2009; Sial *et al.*, 2010). The laboratory results also showed that the mortality of *S. litura* was higher at 72 hours after exposure than 24 hours. It indicated that to get visual results after spraying of this chemical three days waiting is required to take decision on next spraying as effective immediate knockdown is not seen with this chemical.

Field Studies

During both season studies during 2010 and 2011 (Table 1), none of the doses of cyantraniliprole were able to provide reduction in flea beetle population and were at par with control during all observations. However, thiamethoxam and spinosad were able to provide reduction in flea beetle population even after one day after spraying. During two observations, 5 DAS during 2010 and 3 DAS during 2011, thiamethoxam was statistically superior over spinosad and for remaining observations both were at par with each other in reducing flea beetle adult population. However, despite non-significant results of cyantraniliprole in reducing the flea beetle population, the leaf damage due to flea beetle was significantly lower in all the cyantraniliprole treatments during both the seasons. The percentage leaf damage reduction was highest in cyantraniliprole at the rate of 70 and 80 g a.i./

ha during both the seasons. The leaf damage reduction due to cyantraniliprole at the rate of 80 g a.i./ha was statistically at par with thiamethoxam and spinosad during 2010 and with spinosad during 2011 season. The toxicity of cyantraniliprole against Coleoptera is less documented which is in conformity with the present findings where cyantraniliprole was not able to cause mortality of flea beetle. However, it was able to reduce leaf damage indicating antifeedant effects against flea beetle. In conformity, cyantraniliprole was also found to have antifeedant properties (Gonzales-Coloma *et al.*, 1999 and Jacobson and Kennedy, 2011) against a number of insects.

During first season study (Table 2), reduction in *S. litura* larval population was statistically evident after 3 days after spraying in treatments cyantraniliprole (70 g) and spinosad. The pest population in cyantraniliprole (30, 40, 50 & 60 g) and thiamethoxam did not differ significantly from each other and water treated control. Same trend continued upto 7 and 10 days after spraying where *S. litura* larval population in treatments, *viz.*, cyantraniliprole (70 g) and spinosad were statistically superior over control. The pest population in cyantraniliprole (30, 40, 50 & 60 g) and thiamethoxam did not differ significantly from each other and control. During second season (Table 4), reductions in *S. litura* larval population were statistically evident after 3 days after spraying in treatments cyantraniliprole (70 & 80 g) and spinosad and were at par with each other. The pest population in cyantraniliprole (40, 50 & 60 g) and thiamethoxam did not differ significantly from each other and control. Same trend continued upto 7 and 10 days after spraying where *S. litura* larvae population in treatments, *viz.*, cyantraniliprole (70 & 80 g) and spinosad were statistically superior over control and at par with each other. The pest population in cyantraniliprole (40, 50 & 60 g) and thiamethoxam did not differ significantly from each other and control.

Findings in both laboratory and field studies suggest that cyantraniliprole was effective in reducing leaf damage by flea beetle and reducing caterpillar population. Cyantraniliprole (0.7 mL/L or 70 g a.i. ha^{-1}) was the minimum effective dose in field experiments and thus can be used for field applications. Presently, to manage *S. litura* and flea beetle each only two insecticides are recommended for use in grapes in India (NRCG, 2012). It may lead to repeated use of these chemicals which may result into insecticide resistance and residue problem. The inclusion of cyantraniliprole in the pest management programme in grapes will increase the choice of insecticides. Further, cyantraniliprole has completely different mode of action, by acting on ryanodine receptors, thus, it also has ability to support insecticide resistance management programme.

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