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Evaluation of *Callosobruchus maculatus* (F.) susceptibility to neem (*Azadirachta indica A*. Juss) seed oil on different cowpea (*Vigna unguiculata* (L.) (Walp.) cultivars

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Research Paper

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ABSTRACT

The susceptibility of *Callosobruchus maculatus* (F.) to cowpea grains exposed to neem (*Azadirachta indica* A. Juss.) seed oil (NSO) was evaluated. About 15 g cowpea grains each of two improved varieties (1T97K-499-35 and 189KD-288) and three local cultivars (Kanannado white, Banjara and Borno brown) were treated with freshly extracted NSO at four dosages 0.00 or untreated/control, 0.02, 0.04 and 0.08 mL. Each treatment was replicated four times and the experiment laid out in a completely randomized block design. Data obtained were subjected to the analysis of variance and significant means were separated at 5% level using the least significant difference test. The mean number of eggs laid, emerged adults, percentage grain damage, severity of grain damage, susceptibility index and developmental period of *C. maculatus* were significantly lower on all cowpea cultivar treated mainly with 0.04 and/or 0.08 mL NSO/15 g than the untreated ones. Overall, cowpea cultivar performance was in the order 189KD-288 ≥ IT97K-499-35 ≥ Kanannado white ≥ Borno brown ≥ Banjara. The use of resistant varieties should be promoted in managing *C. maculatus* devastation of stored cowpea under subsistence farming conditions. Furthermore, present information may be used as a tool in grain breeding programmes for *C. maculatus* and integrated management programmes.

Key words: *Callosobruchus maculatus, Azadirachta indica*, seed oil, cowpea cultivars, infestation, susceptibility, grain protectant.

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INTRODUCTION

The storage bruchid, *Callosobruchus maculatus* (F.), is a principal pest of stored cowpea grains throughout the tropics. Infestation by the pest initiated either in the field or storage cause severe economic losses to farmers and grain merchants. Infested cowpea pods stored for 8 months can result in up to 50% grain damage due to *C. maculatus* (Caswell, 1984). Under traditional storage conditions 100% infestation of cowpea can occur in 3-5 months (Caswell and Akibu, 1980). Attack by high density of *C. maculatus* can also limit the storage of unprotected cowpea grains in 2-4 months (Seck *et al*., 1991; Okonkwo and Okoye, 1996). Riddling with holes made by the bruchids renders cowpea grains unattractive and unpalatable, with greatly reduced market value and germinability (IITA, 1989). There is an urge, therefore, to protect cowpea grains in storage from quality and quantitative losses. Especially, in the tropics where situations of fairly high moisture content, high relative humidity and environmental temperature can easily combine in stores and provide suitable conditions for insect reproduction (Lale and Ofuya, 2001; Obeng-Ofori and Boateng, 2008). Though synthetic insecticides such as organophosphates, pyrethroids and fumigants including methyl bromide and phosphine are easily and commonly used to generally control pests on stored commodities, this strategy is usually unsustainable due to high costs of pesticides to resource poor grain handlers and periods of product unavailability in the markets (Egwuatu, 1987; Mendesil *et al*., 2007; Obeng-Ofori, 2010; Agoda *et al*., 2011; Yakubu *et al*., 2012). Further drawbacks of pesticide usage include amongst others toxic residues in food that constitute health hazard to warm-blooded animals, development of insecticide resistant insect strains and workers safety (Lale and

Ofuya, 2001; Pereira *et al*., 2009; Obeng-Ofori, 2010). This necessitates the use of alternative effective, economical and environmentally friendly control measures such as the use of resistant cultivars and botanical insecticides.

The use of resistant varieties is key for longer periods of grain storage, as well as the economical reduction and destruction of insect pests (Semple, 1992). The strategy is free of extra cost to farmers or other grain handlers, requires little knowledge to apply, and can act solely or as adjunct to other control measures (Thomas and Waage, 1995). It also reduces/eliminates the cost of insecticides, the risk of grain damage/loss to insect pest attack, and eliminates the negative effects associated with pesticides usage (Mihm, 1997).

Botanical insecticides possess a wide spectrum of properties that act effectively against some insect pests as anti-ovipositants, anti-feedants, repellents, attractants, growth inhibitors/regulator, chemosterilants and/or toxicants (Lale, 1991; Lale and Ofuya, 2001; Ukeh, 2009; Weaver and Subramanyam, 2000; Erturk *et al*., 2004; Mordue, 2004; Negahban and Moharramipour, 2007). This option is economical, as readily available plant materials can be easily accessed, prepared and applied. Moreso, these materials are often biodegradable, and non-toxic to plants, applicators/consumers and the environment (Lale, 1991; Ukeh, 2009). Neem, *Azadirachta indica* A. Juss., products are particularly documented by several researchers to be important biopesticides that confer appreciable levels of protection to stored grains against insect pests attack and destruction (Oparaeke *et al.,* 1998; Lale and Mustapha, 2000; Maina and Lale, 2004; Ileke and Bulus, 2012; Maina *et al.,* 2012; Yahaya, 2013). Neem seed kernel oil further has several pesticidal active ingredients such as Azadirachtin, Salannin, Meliatril and Nimbin, which together are called triterpene or limnoids (Debashri and Tamal, 2012). Of which, azadirachtin alone possess insecticidal activities against more than 413 insect pests (Dimetry, 2012; Debashri and Tamal, 2012). Details of the efficaciousness of neem seed oil (NSO) against major insect pests of stored grains are however scanty. The present study was, therefore, carried out to evaluate the susceptibility of *C. maculatus* to NSO on five cowpea grain cultivars.

MATERIALS AND METHODS

Insect culture, Cowpea cultivar and Plant material

About 100 unsexed *C. maculatus* adults collected from infested grain lots were reared on 500 g clean and disinfected cowpea grains of Borno brown cultivar in a 1 L kilner jar. Two jars were prepared and covered with muslin cloth positioned with rubber band to allow aeration and to prevent escape of insects. Both jars were kept in the Entomology Laboratory, Department of Crop Protection, University of Maiduguri at temperatures between 30 and 35°C and relative humidity between 65 and 70%. Cowpea grains used were obtained in Maiduguri-Monday-Market, and then stored in a refrigerator at -4°C for one month to eliminate potential field infestation. Afterwards, the grains were kept in plastic bags for three weeks at rearing room conditions in the laboratory.

Five cowpea cultivars, two improved cowpea varieties (IT97K-499-35 and 189KD-288) and three local cultivars (Kanannado white, Banjara and Borno brown) were obtained respectively from IITA/PROSAB, Biu and Maiduguri, northeastern Nigeria. Physical observation of each cowpea cultivar was carried out to determine the testa colour. Average individual grain mass per cultivar was obtained by dividing the total weight of ten randomly selected grains by ten. Grain size or mean measurement of the diameter taken (from three positions including the middle and two different ends of the grain) using a venier caliper.

Ripe and fallen fruits of neem trees were collected at the Sanda-Kyarimi Park or Zoo in Maiduguri during November and December, 2010. The fruits were air-dried and decorticated to obtain the kernels. Neem seed oil extraction was carried out using the Soxhlet extraction process in the Chemistry Laboratory, Department of Chemistry, University of Maiduguri. Forty gram of the pulverized neem seed was weighed using a mehler balance and wrapped in a double layer of Whatman No.1 filter paper. The paper was stapled adequately at the seams to prevent any leakage. Each pack was then extracted in 200 mL of analytical grade of acetone for 1½ hours at 56°C. The freshly extracted oil was pressed out manually and then poured into a 250 mL volumetric flask and stored at room temperature. This procedure was repeated until adequate quantity of oil was obtained.

Experiment, Data collection and analysis

Fifteen gram grains of each cowpea cultivar in a 100 mL experimental glass jar was treated in four replicates with three doses (0.02, 0.04 and 0.08 mL) of freshly prepared NSO and a control or untreated grains (0.00 mL). Each dose was applied separately in 0.02 mL of analytical grade of acetone. The treated grains were then stirred with a glass rod until the acetone evaporated completely.

Control jars were treated with 0.02 mL of analytical grade of acetone only and then stirred. Each jar was infested with three pairs of newly emerged (0-1 day old) adults of *C. maculatus*, and the experiment laid out in complete randomized block design (CRBD). Oviposition lasted five days. Following which, the number of eggs laid on the grains was counted, and the contents of each experimental jar returned and kept till F_1 progeny emergence. Daily count of adult emergence was made

Table 1. Grain characteristics of five cowpea cultivars.

Cultivar	Type	Testa colour	Grain mass (g)	Grain size (mm)	Moisture content (%)
IT97K-499-35	Improved	White	0.17	0.78	12.7
189KD-288	Improved	White	0.29	0.77	11.5
Kanannado white	Local	White	0.29	0.89	11.0
Banjara	Local	Brown	0.27	0.91	9.70
Borno brown	Local	White	0.38	0.80	10.2

Table 2. Simple correlation matrix between different parameters.

	1	2	3	4	5	6
No. eggs laid	-					
No. adults emerged	0.617*	-				
% seed damage	0.661*	0.943*	-			
Severity of seed damage	0.494	0.651*	0.668*	-		
Susceptibility index	0.654*	0.551	0.702*	0.599	-	
Developmental period	-0.111	-0.138	-0.106	0.544	0.077	-

* = Significant at P<0.05; 1 = No. eggs laid, 2 = No. adults emerged, 3 = % seed damage, 4 = Severity of seed damage, 5 = Susceptibility index, 6 = Developmental period.

per replicate, and the total number of F_1 adults recorded. Grain damage (holed by *C. maculatus*) was expressed as a proportion of the total number of grains sampled. Severity of grain damage was obtained by dividing the number of emergence holes by the number of damaged seeds. The median development period was calculated as the time (days) from the middle of the oviposition period to the emergence of 50% of the F_1 adults. The index of susceptibility was calculated using the formula (Dobie and Kilminster, 1977):

Susceptibility index = 100 x [loge (total number of F_1 adults emerged) / (median development time)]

Data obtained were subjected to one-way analysis of variance (ANOVA). Significantly different means at P<0.05 were separated using the least significant difference (LSD) (Gomez and Gomez, 1984). Correlation matrix was performed between all the parameters assessed.

RESULTS

The physical characteristics of five cowpea cultivars are provided in Table 1. The grains were either white or brown in tester color. Grain mass and size respectively ranged from 0.17 on the variety IT97K-499-35 to 0.38 on Borno brown cultivar and 0.77 on the variety 189KD-288 to 0.91 on Banjara cultivar. Moisture content was observed to fall between 9.7 on Banjara cultivar and 12.7 on the variety IT97K-499-35.There was positive and fairly strong correlation between the number of eggs laid and the number of adults emerged, percentage grain damage and susceptibility index (Table 2). Correlation was also positive and fairly strong between the number of emerged adult insects and the severity of grain damage, as well as between percentage grain damage and the severity of grain damage or susceptibility index. Correlation between the number of emerged adult insects and severity of grain damage was however strongly positive.

The mean number of eggs laid by *C. maculatus* was lowest (0) on cowpea grains of Kanannado white treated with freshly extracted NSO at 0.08 mL/15 g grain and highest (231) on untreated grains of Borno brown (Table 3).

The mean number of *C. maculatus* adults emerged (Table 4), percentage grain damage (Table 5), severity of grain damage (Table 6), susceptibility index of cowpea cultivar (Table 7) and developmental period of *C. maculatus* (Table 8) fell between zero on 189KD-288, Kanannado white and Banjara treated with freshly extracted NSO at 0.08 ml/15 g grain and 177/100/3/22/23 on untreated grains of mainly Borno brown.

Compared to untreated cowpea grains, the number of eggs laid, emerged *C. maculatus* adults and percentage grain damage were significantly lower on all cowpea cultivars treated with freshly extracted NSO at 0.02, 0.04 and 0.08 mL/15 g grain (Tables 3, 4 and 5). Compared to untreated cowpea grains, the mean severity of grain damage was significantly lower on only two cowpea cultivars, Kanannado white and Borno brown, treated with freshly extracted NSO at all test concentrations, 0.02, 0.04 and 0.08 mL/15 g grain (Table 6).

Table 3. Mean number of eggs laid by *C. maculatus* on cowpea grains of five cultivars treated with NSO at three different dosages and a control.

Cultivar	NSO dosage (mL/15 g grain)				Mean
	0	0.02	0.04	0.08	
1T97K-499-35	127.00	45.33	35.33	15.33	55.75
189KD-288	43.00	32.00	36.00	7.67	29.67
Kanannado white	127.33	12.00	7.00	0.00	36.58
Banjara	75.33	45.67	24.67	5.67	37.84
Borno brown	231.00	142.33	140.33	63.00	144.17
Mean	150.92	69.33	60.83	22.92	

SED = 9.201, LSD (0.05) = 18.600 (Cultivar); SED = 8.230, LSD (0.05) = 16.633 (Dosage of NSO); SED =18.403, LSD (0.05) = 37.193 (Interaction).

Table 4. Mean number of *C. maculatus* adults that emerged from cowpea grains of five cultivars treated with NSO at three different dosages and a control.

Cultivar	NSO dosage (mL/15 g grain)				Mean
	0	0.02	0.04	0.08	
1T97K-499-35	77.67	6.00	5.67	1.67	22.75
189KD-288	77.67	20.67	16.00	0.00	28.59
Kanannado white	51.33	2.00	1.33	0.00	13.67
Banjara	18.23	3.33	1.33	0.00	5.72
Borno brown	176.67	21.33	15.00	7.33	55.08
Mean	100.39	13.33	9.83	2.25	

SED = 8.838, LSD (0.05) = 17.862 (Cultivar); SED = 7.905, LSD (0.05) = 15.976 (Dosage of NSO); SED = 17.676, LSD (0.05) = 35.724 (Interaction).

Table 5. Mean percentage damage caused by *C. maculatus* to five cultivars treated with NSO at three different dosages and a control.

Cultivar	NSO dosage (mL/15 g grain)				Mean
	0	0.02	0.04	0.08	
1T97K-499-35	75.83	6.80	5.53	1.83	22.50
189KD-288	75.03	23.67	17.20	0.00	28.98
Kanannado white	57.73	2.53	1.70	0.00	15.49
Banjara	27.23	5.30	2.00	0.00	8.63
Borno brown	99.53	27.77	19.73	9.17	39.05
Mean	83.84	16.52	11.54	2.75	

SED = 5.066, LSD (0.05) = 10.238 (Cultivar); SED = 4.531, LSD (0.05) = 9.157 (Dosage of NSO); SED = 10.131, LSD (0.05) = 20.476 (Interaction).

Compared to untreated cowpea grains, the mean susceptibility index of cowpea cultivars and developmental period of *C. maculatus* were significantly lower on all cowpea cultivars treated with at least two dosages, 0.02 and 0.04 or 0.04 and 0.08 mL/15 g grain of freshly extracted NSO (Tables 7 and 8).

Except for the mean number of eggs laid where non-significant difference was observed at all test concentrations (Table 3), the mean number of emerged *C. maculatus* adults, percentage grain damage, severity of grain damage, susceptibility index and developmental period of *C. maculatus* were lower and not-significantly different amongst all five cowpea cultivars treated with freshly extracted NSO at particularly 0.08/0.04 mL/15 g grain (Tables 4, 5, 6, 7, and 8).

Significant interaction between cowpea cultivar and NSO dosage exists only for severity of seed damage, but not for the number of eggs laid, the number of adult insects emerged, percentage grain damage, susceptibility index and developmental period (Tables 3, 4, 5, 6, 7, and 8). Comparison amongst all five cowpea cultivars showed that (Table 9), whereas the number of eggs laid and severity of grain damage were significantly higher on grains of both Banjara and Borno brown cultivars,

Table 6. Mean severity of seed damage caused by *C. maculatus* to five cultivars treated with NSO at three different dosages and a control.

Cultivar	NSO dosage (mL/15 g grain)				Mean
	0	0.02	0.04	0.08	
1T97K-499-35	1.13	1.07	1.07	1.00	1.07
189KD-288	1.30	1.13	1.10	0.00	0.88
Kanannado white	1.23	0.67	1.00	0.00	0.73
Banjara	1.03	1.00	0.67	0.00	0.68
Borno brown	2.57	1.07	1.07	1.07	1.45
Mean	1.82	1.24	1.23	0.52	

SED = 0.130; LSD (0.05) = 0.263 (Cultivar); SED = 0.116; LSD (0.05) = 0.235 (Dosage of NSO); SED = 0.260; LSD (0.05) = 0.526 (Interaction).

Table 7. Mean susceptibility index of five cowpea cultivars to *C. maculatus* infestation when treated with NSO at three different dosages and a control.

Cultivar	NSO dosage (mL/15 g grain)				Mean
	0	0.02	0.04	0.08	
1T97K-499-35	16.13	6.57	6.00	1.37	7.52
189KD-288	15.73	11.13	9.23	0.00	9.02
Kanannado white	11.77	2.67	0.53	0.00	3.74
Banjara	7.37	4.43	0.90	0.00	3.18
Borno brown	22.37	11.73	12.30	6.30	13.18
Mean	18.34	9.13	7.24	1.92	

SED = 1.809, LSD (0.05) = 3.656 (Cultivar); SED = 1.618, LSD (0.05) = 3.270 (Dosage of NSO); SED = 3.618, LSD (0.05) = 7.312 (Interaction).

Table 8. Mean developmental period of *C. maculatus* on cowpea grains of five cultivars treated with NSO at three different dosages and a control.

Cultivar	NSO dosage (mL/15 g grain)				Mean
	0	0.02	0.04	0.08	
1T97K-499-35	21.00	14.33	14.33	18.33	17.00
189KD-288	21.67	16.67	14.67	0.00	13.25
Kanannado white	21.33	10.67	14.67	0.00	11.67
Banjara	22.67	21.67	11.67	0.00	14.00
Borno brown	21.67	13.67	11.67	15.00	15.50
Mean	27.09	19.25	16.75	8.33	

SED = 2.100, LSD (0.05) = 4.244 (Cultivar); SED = 1.878, LSD (0.05) = 3.796 (Dosage of NSO); SED = 4.199, LSD (0.05) = 8.487 (Interaction).

the number of adults emerged, percentage grain damage and susceptibility index were significantly higher on grains of only Banjara cultivar. The developmental period of *C. maculatus* was however not significantly different on the grains of all cowpea cultivars.

DISCUSSION

Results comparing untreated grains and those treated with freshly extracted NSO indicate that *C. maculatus* is highly susceptible to this botanical insecticide especially when applied at moderate to high rates. The mean number of bruchid eggs laid, adult bruchids emerged, percentage grain damage, severity of grain damage, susceptibility index of cultivars and developmental period of *C. maculatus* were much lower on cowpea grains treated with NSO especially at higher dosages, 0.04 and/or 0.08 mL/15 g.

These imply that NSO application appreciably confers protection to storage cowpea against *C. maculatus* attack, and thus, can valuably preserve the grains in good condition for planting and domestic consumption as well as postponement of sales to achieve better pricing. Our results concur with those of Maina and Lale (2004), who observed on four (Borno brown, Kanannado,

Table 9. Comparison of six parameters separately across five different cowpea cultivars treated with NSO.

Cultivar	No. eggs laid	No. adults emerged	Percentage grain damage	Severity of grain damage	Susceptibility index	Developmental period
IT97K-499-35	35.50	33.75	21.27	1.66	4.60	22.50
189KD-288	21.50	7.25	7.37	0.65	2.22	11.25
Kanannado white	40.25	22.25	15.21	1.75	3.30	22.50
Banjara	126.25	101.25	60.27	2.35	14.29	11.00
Borno brown	99.50	40.75	22.35	2.57	5.57	22.50
SED	13.73	30.12	11.82	0.56	2.41	6.43
LSD (0.05)	28.14	61.52	24.15	1.14	4.92	13.25

Peugeot and TVx 3236) different cowpea cultivars that NSO applied at 0.08 mL/20 g considerably reduced egg laying by *C. maculates.* In addition, they found that at 0.16 mL NSO/20 g, no *C. maculatus* adult progeny emerged. Similar to the present results, Yahaya (2013) demonstrated that neem seed kernel oil applied straight or in mixture with cotton, groundnut, castor and desert dates at between 0.5 and 4.0 mL/50 g cowpea grains reduced the number of eggs laid by individual *C. maculatus* females. Effects of the oil on female fecundity was further observed to be indirectly proportional to the treatment dosage tested, such that when maintained on cowpea grains treated with the highest oil dosage, no eggs were laid by the bruchids. *Azadirachta indica* oil applied at 8 mL/kg seeds of cowpea and bambara groundnut, not only reduced the oviposition of *C. maculatus*, but also killed emerged larvae and the insecticidal activity persisted for more than 90 days on cowpea and 180 days on bambara groundnut (Pereira, 1983). Interestingly, however, neem seed kernel oil at low to moderate concentrations of between 0.001 and 10% w/w have been found to significantly reduce egg laying in *Sitotroga cerealella* Oliver on wheat grains as effectively as a 5% malathion dust treatment (Verma *et al.*, 1985). Likewise, the same oil was greatly responsible for reduced feeding, oviposition, moulting and increased mortality of larval instars (4^{th}, 5^{th} and 6^{th}) of the tree locust, *Anacridium melanorhodon melanorhodon* Walker, on the gum arabic producing tree (*Acacia senegal* L. Willd.) (El atta *et al.*, 2011). Even the powder of neem kernel when mixed with wheat seed at a proportion of 1-2 to 100 (w/w) parts has been found to satisfactorily protect wheat seeds against infestation by *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Trogoderma granarium* Everts for 270, 320, and 380 days, respectively (Jotwani and Sircar, 1965). Also, Ileke and Oni (2011) reported that the powder of *A. indica* together with *Alstonia boonei* at 2.5, 5.0, 12.5 and 25.5% (w/w) concentrations conferred better protection to wheat grains over *Garcina kola* and *Moringa oleifera* by exerting high mortality rates within 24 to 96 hours post-treatment, and in addition significantly reduced percentage grain damage and weight loss after adult emergence by the first filial generation.

Present results also showed that treating cowpea grains with NSO significantly reduced their susceptibility to *C. maculatus* in storage through reduced bruchid egg laying and progeny emergence. Considering the mean values of all test parameters separately across screened cultivars, the performance of cowpea cultivars seemed to be in the order of 189KD-288 ≥ IT97K-499-35 ≥ Kanannado white ≥ Borno brown ≥ Banjara. Both improved cowpea varieties, 189KD-288 and IT97K-499-35, screened demonstrated high tolerance to *C. maculatus* infestation and damage than the three local cowpea cultivars, Kanannado white, Borno brown and Banjara. Almost similar results were reported in maize by Zakka *et al.* (2013) who observed that all three screened local cultivars (Akparike, Bende and Ogbia muno) commonly cultivated in the Niger Delta supported higher *Sitophilus zeamais* (Motschulsky) adult progeny than 14 improved varieties (ACR.97 TZL COMP.1-W, ACR.8328 BNC7, TZL COMP.4C2, OBA SUPER 1 and 2, SINE 9449-SR, IWD SYN C3F2, TZL COMP.1SYN STR-Y, TZSR White and Yellow; 95TZEE-W, MASYN VAR-3 F2, ADV.NCRE-STR and BG 97 TZE COMP.3XL). Although better performance of the new improved maize varieties was attributed to greater seed hardness and thicker seed coats, such conclusions could not be made in this study as such traits were not assessed. Moreover, grain cultivar susceptibility could be influenced by grain particle size, testa thickness and other non-physical traits such as chemical inhibitors, mostly protein / phenolics and total carbohydrates content (Lale and Yusuf, 2001; Dasbak *et al*., 2009; Astuti *et al*., 2013; Zakka *et al*., 2013; Nwankwo *et al*., 2014). Though cowpea cultivars are primarily developed for higher crop yields under field conditions, the two varieties screened in this study have promising good storage qualities, particularly against *C. maculatus* infestation and damage. Breeders can further identify all the physical and biochemical traits that enable some local cowpea cultivars like Kanannado white or Borno brown that store better than their counterpart(s) like Banjara, for incorporation into breeding programmes against *C. maculatus* infestation to help reduce the high grain losses during storage under subsistence farming conditions in Africa. Resistant cowpea varieties should aid keep grains in storage for longer periods of time without suffering economic damage or the use of pesticides and their harmful consequences. Being cheap and effective, the strategy should be readily adopted by grain handlers solely or in combination with other strategies as found appropriate to protect stored cowpea from destruction by *C. maculatus*.

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