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Full Length Research Paper

Effect of wheat bran supplementation with fresh and composted agricultural wastes on the growth of Kenyan native wood ear mushrooms [*Auricularia auricula* (L. ex Hook.) Underw.]

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Nutrient supplements and agricultural wastes used for mushroom cultivation are important in improving establishment and production of mushrooms. Agricultural wastes such as sawdust, grass, sugarcane bagasse, wheat straw and maize cobs have successfully been used for the production of Kenyan wood ear mushrooms [*Auricularia auricula* (L. ex Hook.) Underw.]. However, the effect of varying concentrations of wheat bran supplements on their productivity has not been fully researched. In this study, fresh and composted agricultural wastes were supplemented with wheat bran at concentrations of 0, 5, 10 and 20%. The cultivation experiment was arranged in a completely randomized design (CRD) and replicated three times. Data was collected on days to spawn run, days to primordial initiation, primordial concentration quality and biological efficiency. The data collected was subjected to analysis of variance using SAS version 9.1. Mean separation was done using least significant difference (LSD) and effects were declared significant at 5% level.

Key words: Wood ear mushrooms, fresh and composted agricultural wastes, wheat bran, Kenya.

INTRODUCTION

Africa generates huge quantities of organic wastes annually through activities in agriculture, forestry and food processing industries. These wastes generate adverse environmental effects related to their disposal (Gateri et al., 2009). Yet, with the application of appropriate bioconversion technologies, these wastes are potentially valueble substrates for the production of mushrooms (Chang and Buswell, 2003). Although, various strategies have been developed to utilize part of the large quantities of waste lignocellulose generated annually, one of the most significant, in terms of producing a higher value product from the waste, is the cultivation of edible mush-rooms by solid-state fermentation (Chang, 2008).

Domestication of native mushrooms is highly underdeveloped in Kenya despite the high quantities of agricultural wastes which can be utilized as ingredients for cultivation (Palapala et al., 2006). Recent research on mushroom cultivation in Kenya has concentrated on *Auricularia auricula* (L. ex Hook.) commonly called wood ear mushrooms which are native to Kakamega Forest. Palapala et al. (2006) suggested their growth potential on various agricultural wastes such as maize cobs, wheat straws and sugarcane bagasse. The growing interest in wood ear mushroom cultivation in Kenya is attributed to its numerous medicinal and nutritional properties. Nutritionally, the wood ear mushrooms are rich in protein which is a diet component that is seriously insufficient in Kenya (Palapala et al., 2006). Medically, it has been found to contain polysaccharides, which stimulate the immune system in humans or cause the production of interferon and

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and interleukins that stop the proliferation of cancer cells (Stamets, 2000). They have also been found to have anti-tumor, cardiovascular, hypercholesterolemia, antiviral, antibacterial and anti-parasitic effects (Chang and Mshigeni, 2001). The antiviral and anti-cancer quality of the mushroom is crucial in Kenya, with the magnitude of HIV/AIDS pandemic and the rampant cancer cases which are the leading causes of death.

Mushroom production has previously been shown to increase remarkably through grain bran supplementation of substrates at appropriate levels (Stamets, 2000). This is because the substrates increase the nutritional content of the substrates especially balancing the carbon: nitrogen ratio. Ayodele and Akpaja (2007) reported that wheat bran supplementation of sawdust enhanced mycelia growth and sporophore yield of *Lentinus squarosulus*. Shen and Royse (2001) reported that the quality of mushrooms is highly affected by the levels of brans in the substrates and that low concentrations can improve production.

According to Chang and Miles (2004), the ability of a mushroom species to colonize a given substrate depends on the nutritional status of the substrate and the ability of the mushroom to produce lignolytic enzymes that can break down a wide range of plant matter. Narain et al. (2008) reported that mycelial growth and primordial development is dependent on the nutritional content of the substrate, especially the C : N ratio which is attained by getting the right proportions of substrate and supplement.

Wheat bran is an industrial by-product rich in nutrients which can be used to improve mushroom production in Kenya. Wheat bran has been previously used as an additive to the base ingredient to improve mushroom productivity (Oei, 2005; Stamets, 2000). This is because it contains nitrogen which is a crucial requirement in the development of most mushrooms. Although Onyango et al. (2011) reported a better effect of wheat bran in comparison with rice bran on wood ear mushroom growth, no work has been done on the effect of varying concentrations of wheat bran supplements on the spawn run, primordia emergence, quality and quantity of wood ear mushrooms. Since nutritional requirements of mushrooms differ, this study evaluated specific supplementation protocols for the Kenyan native wood ear mushrooms cultivated with composted and fresh agricultural substrates.

MATERIALS AND METHODS

Substrate and supplement preparation

Agricultural wastes, namely sugarcane bagasse (*Saccharum officinarum*), maize cobs (*Zea mays*), wheat straw (*Triticum aestivum*) and grass straw *(Cenchrus ciliaris)* were used in this study. Sugar cane bagasse was obtained from West Kenya Sugar Factory in Kakamega County, while maize cobs, wheat and grass straws were obtained from farms within Kakamega, Eldoret and

Kisumu Counties, respectively. Wheat bran was obtained from Eldoret Unga Feeds Company in Uasin Gishu County. Fresh substrates were prepared as previously described by Onyango et al. (2011). They were separately dried for 4 h under the sun and cut into small pieces (<4 cm) using a sharp knife. The cut substrates were soaked in water for 24 h and the surplus water was drained off. Individual substrates were laid in piles on the floor of the cultivation room and left exposed to free aeration. The substrates were then weighed using an electric balance to obtain 100, 95, 90 and 80 g. They were supplemented with wheat bran at varying concentrations by combining with 0, 5, 10 and 20 g, respectively to give the required supplement concentrations of 0, 5, 10 and 20%. For every 1 kg of the mixture, 30 g of $CaCO_3$ was added and thoroughly mixed by hand. Water was sprinkled and a squeeze test was done by squeezing the mixture between the hands. The fresh substrate-supplement combination was considered ready for pasteurizing when no drops of water were obtained from the squeeze. Where there was excess water, sun drying was done followed by a squeeze test till the required moisture level was obtained.

Composted substrates were prepared using modified methods of Sinden and Hauser (1980). On day 1, the substrates were soaked in excess water and left for 3 days. On the fourth day, the substrates were drained of excess water and arranged in a pile, then compressed manually using wooden plank. The first turn was done on day 5 using a forked rake and 30 g of $CaCO_3$ for every 1 kg of mixture was added as the pile was turned. The wheat bran supplements were also added at appropriate proportions as described above. The second turn was done on day 7, while water was sprinkled until the substrates were completely wet. On day 9, the third turn was done and a little water was sprinkled as needed after performing a squeeze test. Where there was excess water, sun drying was done followed by a squeeze test till the required moisture level was obtained. The compost was then ready for pasteurizing and inoculation.

Cultivation of wood ear mushrooms

Wood ear mushroom cultivation was done at Masinde Muliro University of Science and Technology in Kakamega County. The cultivation procedure was conducted according to the methods of Oei (2005). Fresh and composted substrates were divided into lots of 1 kg each and packed into heat resistant polypropylene bags with a diameter of 12 cm and a length of 20 cm. The open ends of the bags were tightly tied using sterile cotton strings and autoclaved at 121°C for 1 h. The substrate bags were cooled to room temperature for 30 min and inoculated using grain spawns obtained from mycelia cultured from a single strain of wood ear mushrooms. Grain spawn was prepared using the standard methods (Oei, 2005). The inoculated substrates were labeled and kept in total darkness in enclosed cabinets for 14 to 25 days to allow complete colonization of the substrates. Upon completion of spawn run, two holes 10 mm diameter were made on each bag.

After the substrate bags were fully colonized by mycelia, they were slit using a sharp razor at the sides, while the tops were completely opened. Similar agronomic conditions were provided for each of the treatments to determine variations due to the effect of wheat bran supplement combinations. Substrate temperatures were lowered to the fruiting range of 18 to 23°C by submerging the sealed substrates in cold-water refrigerator for 10 min. Air temperatures were lowered using two electric fans during the day to maintain low CO_2 level (<1200 ppm). At night, the windows were left open to lower the room temperature. Humidity was maintained at between 90 nad 95% through constant flooding of the floor with sterile water and spraying each bag of substrate with 1 L of water twice a day. The room was lighted on a 12-h on/off cycle using two fluorescent bulbs of 100 W.

Several parameters were averaged from three replicates per treatment to test for the suitability of fresh and composted substrates for cultivation of wood ear mushrooms. Two flushes were collected during the cropping period and mature basidiomata were collected at the same time each day, counted and weighed. For all

replicates, the duration for complete substrate colonization and appearance of primordia was also recorded. In summary, the parameters evaluated included: Spawn run duration, time (in days) the mycelia took to completely ramify the substrates; Duration to pinning, time (in days) that elapsed between the day of completion of spawn run and the day of first pinhead formation; Yield, fresh weight measured in grams for all mature fruiting bodies collected from each bag; Fruit body quality, evaluated on a scale of 1 to 4 using the scale given is shown in Table 1.

Data analysis

Data collected on quantitative growth characters were subjected to analysis of variance (ANOVA) at 5% level of significance using the SAS version 9.1 (SAS Institute, 2005). Least significant difference and standard error margin were calculated to compare the means of spawn run duration, duration to primordia initiation, average fresh weight of mushrooms and fruit body quality.

RESULTS

Spawn run duration

Mycelia growth rates in days were assessed on composted and fresh substrates supplemented at 0, 5, 10 and 20% wheat bran. Results show that the substrate used and the wheat bran concentration had a pronounced effect on the wood ear mushroom mycelia growth rates as shown in Table 2. This demonstrated that mycelia ramification of the substrates responded differently to different supplements levels during the spawn.

The shortest ($p \leq 0.05$) duration to spawn run of 4 days was recorded in composted maize cob substrate at 10% supplement concentration and the longest duration of 13 days was seen in grass straw substrate at 0% supplement concentration. All substrates without wheat supplement (0%) had a higher number of days than those without wheat bran since it took 9, 8 and 7½ days to complete spawn run on fresh bagasse, wheat straw and maize cobs, respectively. On the other hand, when the same substrates were composted and supplemented at 10% with wheat bran, shorter days of 6, 5 and 4 days were recorded, respectively. It was clear that 10% supplement gave averagely the shortest duration to complete spawn run (7 days) on all the substrates.

Time to primordia initiation

Significantly different periods were detected in the number of days it took to produce mushroom pinheads on each substrate (Table 3). There was also a strong influence of different wheat bran supplement concentrations on the duration to primordia initiation. Composted maize cobs at 10% wheat bran supplement concentration gave the least duration to start of pin-head formation of 8 days. This differed significantly ($p \leq 0.05$) with the values obtained from the other substrates at varying supplementation levels. Fresh grass straw at 0% supplement concentration had the longest time of 13 days to form primordia. It clearly showed that 10% wheat bran supplement had the best influence on all the substrates followed by 5 and the 20%. On composted wheat straw, sugarcane bagasse and grass straw all supplemented with 10% wheat bran, the pinheads took 12, 17 and 19 days, respectively to emerge, while it lasted for 13, 18 and 20 days when the respective substrates were supplemented with 20% wheat bran.

In the case where there was no supplementation, pinheads took significantly ($p \leq 0.05$) the longest duration to form. Notably, wheat bran supplement had a better effect reducing the duration to pinhead emergence in all the substrates. At the same time, there occurred differences between composted and fresh substrates with composted substrates mostly showing faster earliness. Composted maize cobs, wheat straw, bagasse and grass straw had 11, 13, 17 and 21 days, respectively as compared to the 12, 14, 18 and 22 days recorded for the same substrates when fresh. The significantly shortest ($p \leq 0.05$) duration occurred on composted maize cobs, wheat straw, sugar cane bagasse and grass straw in that order. These values were numerically better than those observed in the same substrates when fresh.

Fruit body quality

Fruit body quality of wood ear mushrooms measured on a scale of 1 to 4 revealed significant differences depending on the supplement concentration of the substrates used (Table 4). The most pronounced effect was on composted maize cobs at 10% wheat bran supplement level which produced significantly ($p \leq 0.05$) the highest quality mushrooms of 4.0 as compared to a reduced 3.8 when the same substrate (and supplement level) was used when fresh. A similar trend was seen in all cases with composted substrates producing higher quality mush-rooms than the fresh substrates even at similar supplement levels.

Overally, grass straw and bagasse marginally affected fruit body quality when compared with maize cobs and wheat straw. Very low quality mushrooms at rate of 1.0 were observed on non supplemented fresh grass straw. Such a low rate was also observed on sugar cane bagasse supplemented with 5% wheat straw. On the other hand, 10% supplement level on maize cobs and wheat straw produced superior quality mushrooms ranging between 3.3 and 4.0 when composted and 2.1 and 3.8 when fresh. Comparatively, 10% wheat bran supplement had a better effect on the quality of mushrooms obtained from all the substrates than when the substrates were supplemented at 20%.

Effect of composted and non-composted substrates on fresh weight

The nature of agricultural waste and the concentration of wheat bran used affected crop productivity considerably

Table 1. Descriptors for fruit body quality.

Descriptor Name	Shape	Fruit body diameter	Texture
Descriptor state	Cup-shaped/discoid	Very small (<10 mm)	Soft
	Lobed	Small (11-20 mm)	Rubbery
	Flattened/appressed	Large (21-40 mm)	Leathery
	Ear shaped	Very large (>40mm)	Gelatinous

Table 2. Spawn run duration in different concentrations of wheat bran supplements.

Wheat bran supplement concentration (%)	Agricultural substrate								Mean
	Bagasse		Wheat straw		Grass straw		Maize cob		
	FR	COM	FR	COM	FR	COM	FR	COM	
0	9.0	8.6	8.2	7.6	13.5	13.0	7.5	7.1	9.3
5	8.8	8.2	7.4	7.2	13.0	12.4	6.3	6.0	8.7
10	8.1	6.1	6.9	5.2	10.3	10.6	5.0	3.9	7.0
20	7.9	7.1	6.3	6.9	11.9	11.5	6.4	6.3	8.0
Mean	8.5	7.5	7.5	6.7	12.4	11.9	6.4	5.8	8.3
$LSD_{(5\%)}$				0.41					
SEM				1.57					
CV (%)				5.61					

FR = Fresh substrates, COM = composted substrates.

Table 3. Time to primordia initiation on varying levels of wheat bran supplements.

Wheat bran supplement concentration (%)	Agricultural substrate								Mean
	Bagasse		Wheat straw		Grass straw		Maize cob		
	FR	COM	FR	COM	FR	COM	FR	COM	
0	18.5	18.2	15.0	14.8	23.0	22.8	12.8	12.2	17.2
5	17.5	16.8	14.6	13.6	22.5	21.3	11.3	10.6	16.0
10	16.4	16.0	12.3	11.7	20.5	19.2	10.3	8.4	14.3
20	18.0	17.7	14.6	13.2	21.6	20.4	12.0	11.4	16.1
Mean	17.6	17.2	14.1	13.3	22.0	20.9	11.6	10.6	15.9
$LSD_{(5\%)}$				0.52					
SEM				2.31					
CV (%)				4.01					

FR = Fresh substrates, COM = composted substrates.

as shown in Table 5. In general, composted substrates supplemented with 10% wheat bran supported higher yields in all the substrates, while the 0, 5 and 20% fresh substrates supplemented were less efficient with regards to productivity. The heaviest mushrooms were collected from composted maize cobs at 10% wheat bran level recording significantly ($p \leq 0.05$) the highest fresh weight of 282 g. This was followed by fresh maize cobs at the same supplement level giving a fresh weight of 230 g. In many cases, absence of supplementation (0%) lowered fresh weight with the least being 78 g recorded for fresh grass straw.

For instance, fresh weight reduced in non supplemented composted maize cobs, grass straw, wheat straw and bagasse, each recording 198, 82, 92 and 100 g, respectively, while the same substrates when fresh gave 178, 78, 84 and 92 g. A marked result from this study was the better output of mushrooms when a supplement level of 10% was used as compared to 20 and 5% wheat bran concentrations.

DISCUSSION

This study has shown that wood ear mushrooms can successfully be cultivated on locally available substrates at

Table 4. Quality of mushrooms (scale 1 to 4) on different concentrations of wheat bran.

Wheat bran supplement concentration (%)	Agricultural substrate								Mean
	Bagasse		Wheat straw		Grass straw		Maize cob		
	FR	COM	FR	COM	FR	COM	FR	COM	
0	1.1	1.4	1.3	2.3	1.0	1.0	2.0	2.8	1.6
5	1.3	1.9	1.8	3.2	1.0	1.2	2.6	3.2	2.0
10	2.1	2.5	2.1	3.3	1.3	2.0	3.8	4.0	2.6
20	1.6	1.8	3.1	3.5	1.1	1.8	3.1	3.7	2.4
Mean	1.5	1.9	2.1	3.1	1.1	1.5	2.9	3.4	2.1
$LSD_{(5\%)}$					0.16				
SEM					0.04				
CV (%)					5.23				

FR = Fresh substrates, COM = composted substrates.

Table 5. Effect of wheat bran supplements on yield (fresh weight/bag in grams).

Wheat bran supplement concentration (%)	Agricultural substrate								Mean
	Bagasse		Wheat straw		Grass straw		Maize cob		
	FR	COM	FR	COM	FR	COM	FR	COM	
0	92.4	100.2	83.9	92.4	78.4	81.7	177.8	198.2	113.1
5	106.5	123.4	156.5	226.5	92.2	96.2	198.2	259.4	157.4
10	143.7	153.5	168.2	223.7	128.4	141.8	230.0	281.8	183.9
20	125.0	134.8	136.6	146.9	116.9	129.7	198.2	274.1	153.1
Mean	116.9	128.0	136.3	172.4	104.0	112.4	201.1	253.4	
$LSD_{(5\%)}$					16.69				
SEM					3.31				
CV (%)					12.46				

FR = Fresh substrates, COM = composted substrates.

varying concentrations of wheat bran supplement. Generally, there occurred better productivity on all the substrates supplemented at 10% with wheat bran as compared to those supplemented at 5, 20 or those that were not supplemented. It also emerged that composted substrates gave better performance on all the attributes evaluated than when the substrates were fresh. Comparison between the individual substrates showed that maize cobs and wheat bran were superior to sugar cane bagasse and grass straw in terms of overall productivity of wood ear mushrooms.

In the first experiment, mycelia ramification of the substrates was evaluated to investigate the influence of substrate type, supplement concentration as well as effect of pretreatment (composting or fresh) on spawn run duration. Consistently, faster growths (fewer days) were revealed on composted wastes than on the fresh wastes. This may be attributed to the activity of microorganisms which may have degraded the lignocelluloses present in the substrate during composting into simpler carbon compounds which could easily be accessed by the growing mushroom mycelia. Chang (2006) attributed differential mycelia growth rates in mushrooms to requirements for higher concentrations of simpler carbohydrates such as cellulose than lignin. Scrase (1996) reported successful use of composted substrates for *Pleurotus* spp. and *Volvariella volvacea*. In a previous study, composting of substrates causes breakdown of complex lignocellulosic compounds that increases the concentration of simpler carbohydrates (Otieno, 2011). This may have provided an energy rich condition for the mycelia leading to a faster growth. In addition, Phillippoussis et al. (2001) attributed fast mycelia growth to the C : N ratio. It is plausible to state that the wheat bran level of 10% provided a more appropriate ratio which caused faster mycelia growth. The results reported in this study regarding the substrates used confirm the conclusions previously drawn that maize cobs, wheat straw, bagasse and grass straw are sequentially suitable for cultivation of the Kenyan native wood ear mushroom.

Pinhead formation was found to vary between the various substrates and supplement levels used. This agrees with the findings of Kimenju et al. (2009) that the duration taken by mycelia to start pinning depends on the type of substrate and supplement level used. Faster pinning oc-

curred in composted substrates supplemented with 10% wheat bran than in the fresh ones. According to Philippoussis et al. (2001), duration to pinning of mushrooms is highly dependent on free circulation of moisture and air in the substrate. Another factor that influences rapid pinning is the availability of free simple carbohydrates in the substrate (Chang, 2006).

Previous analysis of the substrates used in this study showed that all composted substrates had higher moisture and cellulose content than the fresh ones. It is possible to argue that fast mycelia growth in composted substrates was due to the high moisture content which may have been conducive for the mycelia. At the same time, the composting process may have initiated microbial breakdown of the cellulosic matter in the substrates releasing them faster for mycelia utilization. Therefore, composted maize cobs may have produced better results due to their ability to retain moisture in addition to having more cellulose matter.

A remarkable finding in this study was the long duration it took for primordia to emerge in both non supplemented substrates and those supplemented at 20%, while those supplemented at 5% gave average rates of pinhead formation. In all the substrates tested, 10% supplementation with wheat bran shortened the time to pinning unlike in a previous study by Nshemereirwe (2004) where a similar quantity of wheat bran reportedly caused contamination of the substrates. This showed that pinhead formation required an average amount of wheat bran supplement. It appears reasonable to assume that 20% supplement level increased the nitrogen component of the substrates beyond what was necessary for the mushrooms, while 5% was lower than the requirements for this particular fungus. At the same time, absence of supplements (0%) reduced nitrogen content to levels that were unbearable for induction of sporophores. Iqbal et al. (2005) and Chang (2008) reported the need for average amounts of supplements since the nitrogen component is usually required in moderate quantities by most mushrooms including the wood ears.

On crop production characters including fruit body quality, supplemented substrates at 10% presented significantly higher basidiomata size and shape in comparison to those supplemented at 20%, 5% and the non supplemented ones. This indicated that fruit body quality was significantly affected by the different substrates and supplement levels. Shashireka et al. (2005) proposed that substrates with high nutrient bases produce higher quality mushrooms as compared to substrates deficient of important components such as carbon and nitrogen. Previous studies by the same authors revealed a high concentration of lignin and cellulose in addition to crude proteins in all the substrates used. However, available nitrogen was found to be low and supplementation with wheat bran was necessary. Therefore, 10% wheat bran consistently gave high qualities due to the presence of appropriate levels of nitrogen compounds.

Another important finding of this study is that better quality mushrooms were obtained from composted substrates as compared to the fresh substrates. Philippousis et al. (2001) reported that high cellulase activity in mushrooms is increased in composted substrates and this shows better utilization of carbohydrates, leading to production of high quality mushrooms. Non composted substrates may have produced mushrooms of less quality due to reduction in cellulase activity. Even though large sized fruit bodies were considered to be of good quality and were rated high, Shen and Royse (2001) commented that this is a lower quality since such fruit bodies tend to break during packaging, thereby reducing their quality. This might be improved by lowering the quantity of the wheat bran supplement in the substrates, which would slightly reduce the size of the fruit bodies while improving their durability (Shen and Royse, 2001).

Yield was evaluated as the total fresh weight of all the mushrooms collected from each bag. Higher fresh weights were recorded from mushrooms collected from maize cobs substrate supplemented with wheat bran at 10%. Results obtained during mycelia growth showed that this combination had the fastest rate of mycelia development. According to Stamets (2000), good primary growth of mushrooms with thick and vibrant mycelia usually results in production of large and well developed sporophores. The consistent better performance of mushrooms on composted maize cobs could be attributed to faster and more vibrant primary growth at the mycelia stage. This may have been translated to better uptake of nutrients for sporophore formation, leading to higher fresh weights. The converse was true for mushrooms cultivated on grass straw and sugarcane bagasse which had poor mycelia growth that resulted in smaller fruiting bodies leading to low fresh weights. In addition, influence of wheat bran concentrations was clearly observed with higher fresh weights noted in 10% wheat bran supplement. This may be attributed to enhanced performance of mycelia due to availability of several amino acids, protease as well as transaminase enzyme activities on wheat bran (Shashireka et al., 2005).

Conclusion

Organic supplements with good nitrogen sources play an important role in improving the production of mushrooms. In this study, wood ear mushrooms that are native to Kenya were shown to improve in productivity when cultivated on agricultural wastes supplemented with wheat bran. Composted maize cobs and wheat straw supplemented with 10% wheat bran produced the best results and were recommended for wood ear mushroom production. High wheat bran levels (20%) or absence of supplementation was found to have a negative effect on all the production values that were examined.

Therefore, the immense amounts of agricultural wastes generated from agriculture, food processing industries

and forestry in Kenya have a great potential to promote the livelihoods of the rural populace through the production of mushrooms. This can constitute a cost-effective means of supplementing the food nutrition by supplying the much needed protein; alleviating the suffering caused by certain kinds of illnesses through medicinal mushrooms and reducing environmental pollution as well as amending the soils through mushroom mycelia activities.

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