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ECOSYSTEMS

Impact of gamma radiation dose on sterility and quality parameters of *Anastrepha fraterculus* (Diptera: Tephritidae)

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Abstract: *Anastrepha fraterculus* (Wiedemann, 1830) (Diptera: Tephritidae) is a major fruit pest, which is basicaly controlled using insecticides, which represents a risk to beneficial arthropods, human health and food contamination. The sterile insect technique (SIT) is a potential alternative tool for the management of this pest, however, only conflicting data is found regarding the optimal dose to achieve sterility. Thus, this study evaluated the effect of gamma radiation doses (0, 40, 50, 60 and 70 Gy) on male and female reproductive sterility, gonads morphometry, emergence, flight ability, and longevity under nutritional stress of *A. fraterculus*. Full female sterility was achieved at 50 Gy, while full male sterility was achieved at 70 Gy. Both ovarian and testicular sizes were affected by irradiation, while no influence was observed on the quality parameters evaluated. Our results suggest that 70 Gy applied 48 h before adult emergence can be used to sterilize *A. fraterculus* in a SIT programme.

Key words: insect quality control, pest management, South American fruit fly, Sterile Insect Technique.

INTRODUCTION

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann, 1830), is a highly polyphagous pest, considered a major fruit pest in South America, and is widely distributed throughout the tropical and subtropical regions (Allinghi et al. 2007a, Cladera et al. 2014, Martins et al. 2018). Traditionally, this species is controlled basically with organophosphate and pyrethroid insecticides applied in full coverage or as toxic baits (Martins et al. 2018). However, these insecticides are highly deleterious to natural enemies and pollinators, and represent a risk to health of agricultural workers and food contamination (Garcia et al. 2017).

The Sterile Insect Technique (SIT) is an environmentally friendly, species specific insect control method that has been used to supress and eradicate tephritid fruit flies in many regions of the world (Rull et al. 2007, Dias & Garcia 2014, Dominiak et al. 2014). SIT relies on the release of high-quality, sterilised insects and their ability to mate with the wild population and induce reproductive failure (Knipling 1955, Collins et al. 2009, Bloomfield et al. 2017).

Irradiation affects the reproductive cells during the pupae development, causing, in most species, dominant lethal mutation in sperm and ovarian atrophy (Klassen 2005). However, the irradiation dose must be sufficient to achieve an adequate level of sterility but should not impair the sexual abilities of the sterile insects, such

as flight and longevity (FAO/IAEA/USDA 2003, Krüger et al. 2018).

Tephritidae is considered a homogeneous group regarding doses to achieve sterility. The mean dose needed to sterilize tephritid flies is 65 Gy (Bakri & Hendrichs 2002); however, it is necessary to access the sterilizing dose for each species. Although sterilizing doses were tested for *A. fraterculus*, conflicting data were found. While Allinghi et al. (2007a) determined 70 Gy as the dose needed to sterilize both male and female of *A. fraterculus*, Mastrangelo et al. (2010) suggested, through a PROBIT analysis, 36.3 Gy should be the dose used to achieve 99% sterility of males and 57.3 Gy should be used to achieve the same amount of sterility in females of this species.

Besides sterility, it is important to verify the effects of the radiation on the quality of the insects. Radiation may impact somatic cells and result in abnormalities, reduction in lifespan and flight ability and even the death of the insect (Bakri et al. 2005). Negative effects of radiation on some tephritids species have been reported, such as a decrease in courtship in *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) (Lux et al. 2002), emergence and flight ability in *Anastrepha obliqua* (Macquart, 1835) (Diptera: Tephritidae) and in *Bactrocera tryoni* (Froggatt, 1897) (Diptera: Tephritidae) (Toledo et al. 2004, Dominiak et al. 2007), an increase in mating frequency in *Anastrepha ludens* (Loew, 1873) (Diptera: Tephritidae) (Rull et al. 2005) and mortality of irradiated insects in *A. obliqua* (Toledo et al. 2004). The present study aims to examine the effects of gamma radiation on reproductive sterility, gonads morphometry, flight ability and longevity under nutritional stress of *A. fraterculus*.

MATERIALS AND METHODS

Rearing technique

The laboratory colony of *A. fraterculus* was kept in climate-controlled rooms, with temperature of 25±1°C, 70±10% relative humidity and 12h photophase. Flies were obtained from infested peaches (*Prunus persica* L.) collected in Pelotas, Rio Grande do Sul, Brazil (31.461792 S, 52.524371 W), in the spring of 2016. Adults were kept in plastic cages (570 × 385 × 371mm) (l by w by h) and provided with a solid diet based on sugar (União®, São Paulo, SP, Brazil), wheat germ (Walmon®, São Paulo, SP, Brazil) and brewer's yeast (Bionis® YE MF and NS; Biorigin, Lençóis Paulistas, SP, Brazil) (3:1:1) (Nunes et al. 2013) and a water soaked cotton clump in a Petri dish (55mm) served as water source. Mangoes (*Mangifera indica* L.) fruits were exposed to the flies and served as oviposition substrate and for larval development, as described by Dias et al. (2017). Pupae used in the experiments were from the 4^{th} to 7^{th} generation of the laboratory rearing.

Irradiation procedure

Approximately 250 pupae (48 hours before emergence), per dose and per block, were placed in 50 mm Petri dishes, sealed with plastic film, and irradiated using a cobalt-60 source (Eldorado 78, Atomic Energy of Canada Ltd Chalk-River, Canada). Irradiation was performed at ambient temperature at different target doses (40, 50, 60 and 70 Gy), calibrated following Krüger et al. (2018). In addition, a control Petri dish (0 Gy) was prepared, but it was not exposed to irradiation. A total of four irradiation events occurred between May 2017 and September 2017, and each event was considered as one block during which the following bioassays were performed.

Reproductive sterility

Following irradiation, pupae were placed into plastic cups (700 mL), and allowed to freely emerge at 25±1°C, 70±10% relative humidity and 12h photophase. Within 2 days after emergence, 8 males and 8 females of each dose treatment were placed into plastic cages (220 × 140 × 120mm). For each dose treatment, three cages were set up for both combinations: irradiated males and unirradiated females and unirradiated males and irradiated females. All of the unirradiated adults were sourced from plastic cups that stayed in the laboratory. The cages were provided with water-soaked cotton and the solid food described above. When the adults were 15 days old, a mango fruit was placed inside each cage, and the flies were allowed to oviposit for three days. After the period of exposition, the mangoes were kept in a 2L plastic container, on a layer of vermiculite, for larval development. The plastic container was covered with voil. After 15 days, the number of larvae and pupae were assessed to estimate the sterility.

Ovary and testes morphometry

After the mango exposition for reproductive sterility assessment, 15 males and 15 females from each dose and each block were killed in 70% ethanol. The reproductive system was extracted from the abdomen under a stereomicroscope, following the dissection procedure indicated by Chou et al. (2012). The following biometric parameters were recorded for ovaries: length, from the anterior end of the germarium to the calyx area and width, taken from the anterior end of the vitellarium. In addition, we calculated the ovarian index, by multiplying ovary length by ovary width, as suggested by Chou et al. (2012). Similarly, the testicular biometric parameters recorded were: length, from the apical region to the vas deferens, and width, taken from the spermatid region. We also calculated the testicular index, by multiplying testes length by testes width.

Flight ability

Three subsamples of 30 pupae from each dose treatment and the control were placed over moist black cloth on 90mm Petri dishes. Black 100mm tall tubes (94mm inner diameter) with a fine coat of unscented talcum powder in the interior (to prevent flies from walking out) were placed over the Petri dishes. A 15mm width of talcum powder was wiped off the base of each tube to provide newly emerged flies an additional surface to rest. The three tubes containing the subsamples of each treatment were placed into mesh cages (350 × 280 × 280mm). In the top of each cage, six yellow stick cards (90 × 100mm) were hung to trap fliers and prevent flies returning into the tubes. Once emergence was completed, individual flies were classified following the FAO/IAEA/USDA (2003) manual, as: 1) fliers if they successfully escape the tube, 2) not emerged if still inside an unopen pupal case, 3) partly emerged if they failed to emerge completely from the pupal case, 4) deformed if they had completely shed the pupal case but had damaged wings, and 5) not fliers if they had completely shed the pupal cases, and had morphologically normal wings, but failed to escape the tube. Calculations from Collins et al. (2008) were used to assess percentage of emergence, percentage of fliers and rate of fliers (the percentage of fliers corrected by emergence). All flies that emerged (fliers and not fliers) were sexed to identify any effects of irradiation treatment on sex ratio.

Longevity under nutritional stress

From each treatment group, 24 pupae were placed into separate wells of 24-well microplates, covered and allowed to emerge. No food or water

was provided. The microplates were checked for mortality three times each day (0900, 1300, 1700 h) as indicated by Collins et al. (2009). Date and time of emergence and death of each adult in each cell was recorded

Statistical analysis

Hartley and Shapiro-Wilk tests were applied, respectively, in order to verify the assumptions of homoscedasticity and normality of residues for flight ability, longevity, ovary and testicles morphometry data. Data from testicles morphometry and male sterility were root-squared transformed. Posteriorly, all the data was submitted to analysis of variance. If significant ($P \leq 0.05$) differences were detected, results were analysed using exponential or polynomial function, where "y" is the observed variable, "y0" correspond to the maximum or minimum level of the observed variable, "a" is the maximum estimated value for the observed variable, "b" is the slope and "x" is the irradiation dose. For female sterility, absence of larvae collected in several irradiated groups restricted the possible statistical approaches. As the main concern of a SIT program is achieving sterility above 99.5% (FAO/IAEA/USDA 2003), we combined all results across blocks for each treatment.

RESULTS

Reproductive sterility

Irradiation dose affected male sterility ($F_{4,52}$=291.04; P<0.0001). The number of larvae obtained in mango fruits presented a decrease in function of dose increase (F=1095.45; df=4; P<0.0001; Figure 1). From the 0 Gy male × unirradiated female (male control) treatment, we collected 1219 larvae. From the 40 Gy irradiated male × unirradiated female treatment, 46 larvae were collected. From the 50Gy irradiated male × unirradiated female treatment, 42 larvae. From the 60 Gy irradiated male × unirradiated females treatment we collected 13 larvae, and from the treatment where 70 Gy irradiated male were coupled with unirradiated females, we did not collected any larvae.

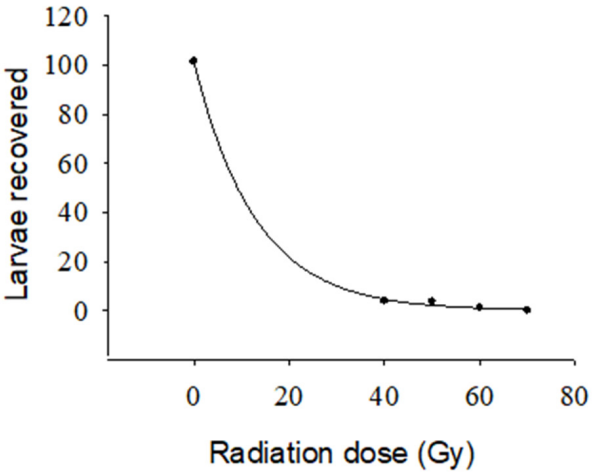

Figure 1. Number of larvae recovered from fertile female and irradiated males crosses of *A. fraterculus* at different gamma radiation doses.

Irradiation dose also affected female sterility ($F_{4,52}$=163,63; P<0.0001). From the unirradiated male × 0 Gy female (female control) treatment, we collected 1074 larvae. From the unirradiated male × 40 Gy irradiated female treatment three larvae were collected, from one single mango. Females irradiated at 50, 60 and 70 Gy did not lay any eggs.

Ovary and testes morphometry

Irradiation dose affected all the parameters evaluated for ovaries (length: $F_{4,551}$=156.88; P<0.0001; width: $F_{4,551}$=61.22; P<0.0001 and index: $F_{4,551}$=432.01; P<0.0001). These parameters presented an exponential decay in function of irradiation dose increase (length: F= 3070.62; df= 4; P<0.0001; width: F= 3953.93; df= 4; P<0.0001 and index: F= 4491.29; df= 4; P<0.0001; Figure 2).

Irradiation also had an effect on the testicular parameters (length: $F_{4,566}$=41.76; $P \leq 0.0001$; width: $F_{4,566}$=213.25; $P \leq 0.0001$ and index:

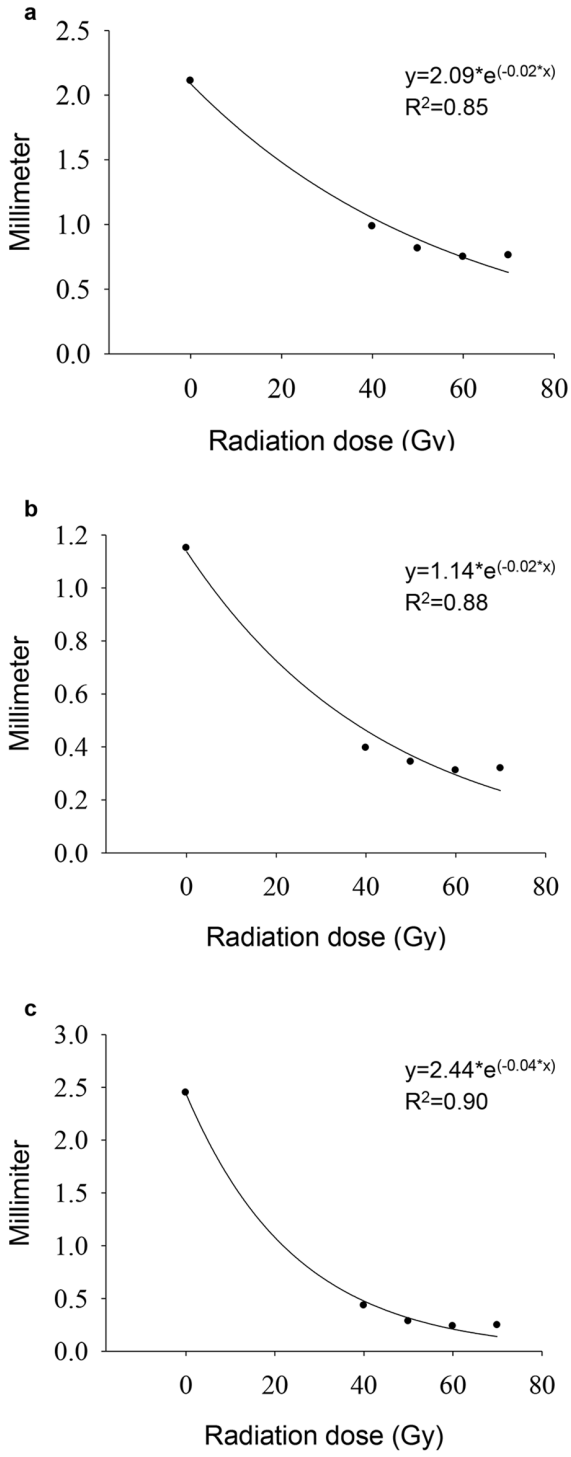

Figure 2. Ovary length (a), ovary width (b) and ovary index (c) of females of *A. fraterculus* irradiated at different doses.

$F_{4,566}$=198.18; P≤0.0001). The parameters decrease in function of irradiation dose increase (length: F= 42.01; df= 4; P=0.0233; width: F= 172.37; df= 4; P=0.0058 and index: F= 114.48; df= 4; P=0.0087) (Figure 3).

Flight ability

There was no evidence of irradiation dose effect on percentage of emergence ($F_{4,52}$=0.61; P=0.6544; average(±sd) =95.72±3.90), percentage of fliers ($F_{4,52}$=0.77; P=0.55; average(±sd) =60.61±19.11) or rate of fliers ($F_{4,52}$=0.58; P=0.6744; average(±sd) =63.40±20.14). There was also no evidence that the treatments influenced the sex ratio ($F_{4,12}$=0.51; P=0.7317).

Longevity under nutritional stress

Longevity in hours, as a continuous outcome, was not affected by irradiation dose ($F_{4,444}$=1.88; P=0.1125). The average (±sd) longevity of flies was 104.39±23.81 h.

DISCUSSION

A sharp decrease in larval recovery was observed as male irradiation dose increase, and while there was a reduction of approximately 96.23% of larvae recovery from the 40 Gy irradiated males x unirradiated females treatments, no larvae were recovered at 70 Gy, showing complete male sterility. Allinghi et al. (2007a) observed similar results when testing effects of irradiation dose on an Argentinean laboratory population of *A. fraterculus*, and suggested 70 Gy as the dose to be used in SIT programmes. In fact, other species of this genus are also sterilized using less than 100 Gy. The dose used to sterilize *Anastrepha serpentina* (Wiedemann, 1830) (Diptera: Tephritidae) and *A. ludens* males is 80 Gy (Rull et al. 1996, Landeta-Escamilla et al. 2016), for *Anastrepha suspensa* (Loew, 1862) (Diptera: Tephritidae) males the dose

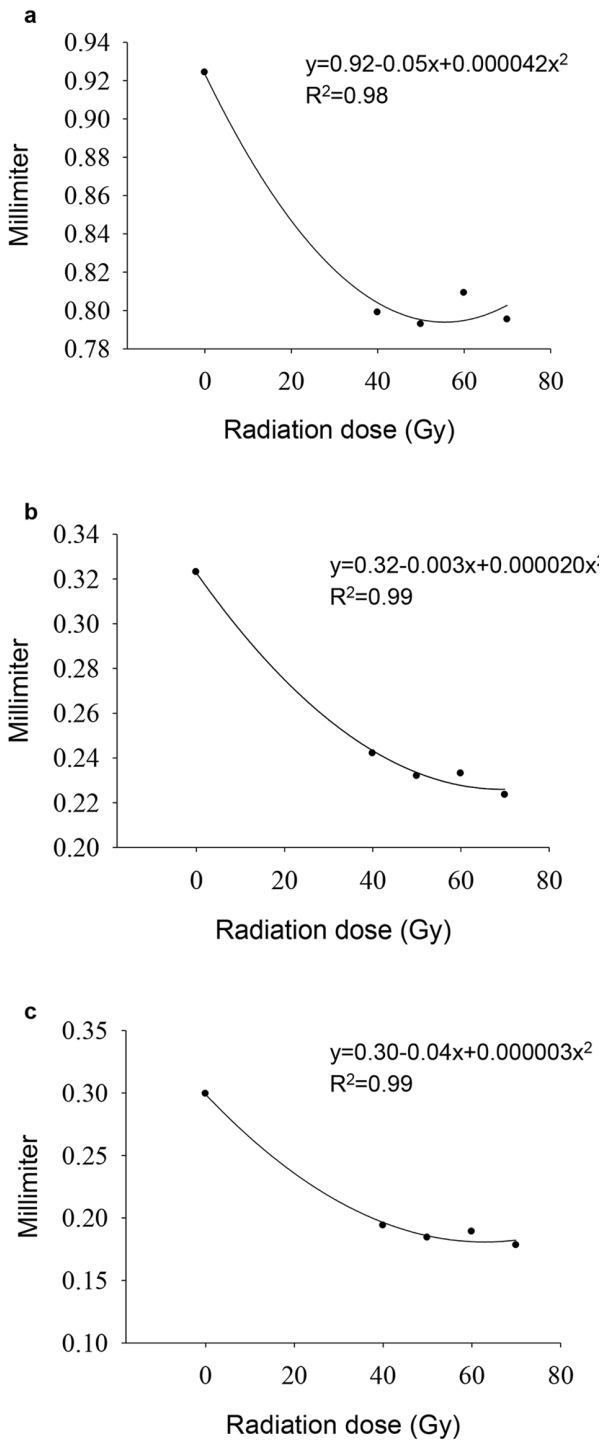

Figure 3. Testicles length (a), testicles width (b) and testicles index (c) of males of *A. fraterculus* irradiated at different doses.

needed is 50 Gy (Walder & Calkins 1993) and for *A. obliqua*, 40 Gy is sufficient to result in 99.5% male sterility (Toledo et al. 2004).

Difference in radiosensitivity between males and females is expected due to the stage of development of the gametes when pupae are irradiated (Carpenter et al. 2005). In our experiments females were more sensitive to irradiation than males, becoming fully sterile at 50 Gy. Larvae were recovered from one single repetition from 40 Gy irradiated females, showing that although sterility is high, it is not complete at this dose. Allinghi et al. (2007b) also observed a reduced number of fertile eggs laid by females irradiated at 40 Gy. For other species of *Anastrepha*, lower doses are needed to completely sterilize females when compared to males. The dose used to sterilize *A. ludens* females is 40 Gy (Rull et al. 2007), for *A. obliqua* females is 20 Gy (Toledo et al. 2004), and for *A. suspensa* females is 25 Gy (Walder & Calkins 1993). It is extremely important to completely sterilize a female before releasing them in the ambient, since residual fertility can contribute in progeny to the next generation of the target population (Robinson 2002).

In males, gamma irradiation is capable of causing damage on spermatogenesis, leading to dominant lethal mutations in spermatids and spermatozoids, resulting in sterility, and sometimes, smaller testicles. Testicles of irradiated males were smaller in about 13.52% in length and 28.02% in width, when compared to testicles of unirradiated males. Our results differ from those found by Bartolucci et al. (2008), where no differences on length and width were observed between testicles of irradiated and unirradiated *A. fraterculus*. However, reduction on biometric parameters of male gonads of irradiated insects were already reported in *C. capitata* (Abdel-Malek et al. 1975) and *Bactrocera zonata* (Saunders, 1842) (Diptera: Tephritidae) (Shehata et al. 2006). Despite the effects of sterility

on testicles size, a previous study reported that sterile *A. fraterculus* males showed similar results as their wild counterparts regarding capacity of female remating inhibition, refractory period and amount of sperm transferred (Abraham et al. 2013). Female sterility is caused by the ovarian atrophy. The reduction is size were observed for *A. fraterculus* in all doses applied, where the ovaries dissected from irradiated flies were, on average, 60.84% smaller in length and 70.24% in width. The atrophy is caused by the interference of the radiation on cell division in the female reproductive system during its development in the pupal phase (Walder & Calkins 1992). Ovarian atrophy results in the lack of egg production, which was reported in other irradiated tephritid females (Walder & Calkins 1992, Toledo et al. 2004, Allinghi et al. 2007a, Bartolucci et al. 2008, Collins et al. 2009, Rull et al. 2014). The inability of an irradiated female to lay eggs is favorable do SIT implementation, since released females would not oviposit into the fruits (Allinghi et al. 2007a). Moreover, the variation in size allows the differentiation between ovaries of irradiated and wild females (Bartolucci et al. 2008). This characteristic can be helpful to recognize sterile insects released in the field, since once they are trapped, it is possible to differentiate them, mainly to evaluate their dispersion and density in relation to the wild population. However, this technique should be used with caution, since young flies cannot be easily distinguished based on ovary size (Mastrangelo et al. 2018). Radiation has mutagenic properties that can cause somatic damage to sterile flies, thus, quantifying the negative effects of dose on quality of irradiated insects is essential to determine the optimal irradiation dose. In our study, the quality parameters evaluated were not affected by irradiation dose. Our results are similar with data observed for *B. tryoni* (Collins et al. 2009, Bloomfield et al. 2017), but in contrast to *A. obliqua* (Toledo et al. 2004), *A. ludens* (Rull et al. 2005, Rull et al. 2007), *B. zonata* (Mahmoud & Barta 2011) and *C. capitata* (Lux et al. 2002, Guerfali et al. 2011). In both this study and that of Collins et al. (2009), pupae and flies from all treatments were kept under identical conditions, separating treatments and control only during the irradiation, aiming to consider specifically the effects of irradiation on quality parameters. The late pupae stage presents a smaller number of mitotic cells, resulting in less somatic damage due to radiation, and better sterile insect quality (Allinghi et al. 2007a, Paithankar et al. 2017). Thus, the lack of detrimental effects of irradiation on quality control shows that irradiation applied to *A. fraterculus* mature pupae is adequate, since metamorphosis is almost complete.

The implementation of a SIT programme requires an optimal condition for pupal irradiation, to avoid undesirable side effects on physiology and behavior of sterile insects, which can reduce their competitiveness. The results obtained in this study support the use of SIT as a control strategy for *A. fraterculus*. A dose of 70 Gy applied 48 h before emergence not only induced male and female sterility but also did not impair sterile insect's quality. Currently, there is no active SIT programme for *A. fraterculus,* yet the release of sterile and competitive could lead to a reduction in pest population, and it is not expected to conflict with other control methods nor rendering any adverse impact on agroecosystem.

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