

## Effectiveness of GF-120 NF Naturalyte Fruit Fly Bait Spray against Different Ages of Melon Fly (Diptera: Tephritidae) Females When Applied to Border Crops of Various Widths

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**Abstract.** GF-120 NF Naturalyte Fruit Fly Bait was evaluated for its effectiveness to prevent melon fly, *Bactrocera cucurbitae* (Coquillett), females of various ages from ovipositing in cucumber patches with border crops of different widths. Cohorts of color-marked, protein-fed females, eclosed after 1, 2, or 4 weeks, were released from sites outside sorghum, (*Sudax bicolor* x *S. bicolor* var. *sudanense*) borders 1, 2, or 4 rows deep (30, 90, and 135 cm in width, respectively). Capture rates of female *B. cucurbitae* were higher for 2- and 4-week-old than for 1-week-old females. Borders sprayed with GF-120 NF Naturalyte Fruit Fly Bait were effective at preventing released sexually-mature 4-wk-old females from reaching the cucumber patches only when in association with the widest border (135 cm) treatment. Our findings suggest that for maximum effectiveness against host-seeking female *B. cucurbitae*, GF-120 NF Naturalyte Fruit Fly Bait should be applied to broader swaths of sorghum planted as a border crop.

**Key words:** *Bactrocera cucurbitae*, Tephritidae, GF-120 Fruit Fly Bait, border spray, spinosad

### Introduction

The melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), was introduced into Hawaii in 1895 (Back and Pemberton 1917) and can infest over 125 host plants, mostly in the Cucurbitaceae and Solanaceae (Christenson and Foote 1960, Weems 1964). Because *B. cucurbitae* is able to damage young fruits, flowers, stems, and petioles of host crops (Nishida 1953), it represents a major obstacle to expansion of Hawaiian agriculture due not only to direct crop loss but also to quarantine restrictions. Crop losses due to direct damage can reach 100% without insecticide control (Fabre et al. 2003).

Historically, protein baits have been used to control female populations of economically important tephritid flies, including *B. cucurbitae* (Nishida and Bess 1957, Prokopy et al. 2003, 2004). Presently, the Hawaii Area Wide Pest Management (HAWPM) program uses protein bait sprays as one component of an IPM system aimed at controlling fruit fly populations (Vargas et al. 2001, 2008, Mau et al. 2007). The application of bait sprays, male annihilation through mass trapping using male lures and sanitation comprise the three major technologies developed and implemented by the HAWPM program that small farmers and homeowners in the program have embraced.

The protein bait spray GF-120 NF Naturalyte Fruit Fly Bait (Dow AgroScience, Indianapolis, IN) is being used with great success in the HAWPM program to suppress pest fruit fly populations, including *B. cucurbitae* (Vargas et al. 2001, 2008, Mau et al. 2007). This bait contains the toxicant spinosad, an environmentally friendly alternative to malathion

(Peck and McQuate 2000, Vargas et al. 2001). Spinosad can be toxic to insects through ectodermic contact but must be ingested for maximum effectiveness (DowElanco 1994). Spinosad is derived from metabolites of the actinomycete bacterium *Saccharopolyspora spinosa* (Sparks et al. 1998) and has low mammalian and environmental toxicity (DowElanco 1994). The 24h LC50 value (95% fiducial limits) for female *B. cucurbitae* is 3.3 (3.1–3.6) mg/liter (Stark et al. 2004).

Use patterns for field applications of GF-120 NF Naturalyte Fruit Fly Bait primarily against *B. cucurbitae* (Prokopy et al. 2003, 2004) and *B. dorsalis* (Hendel) (Piñero et al. 2009) are being developed in Hawaii. Use patterns to control *B. cucurbitae* are based on the particular types of behavior exhibited by adults around agricultural fields. Melon flies tend to roost within border plants along the edge of cultivated fields, dispersing into the crop to oviposit (Nishida 1953). Bait sprays are thus most effective against melon flies when applied to border plants (Nishida and Bess 1950, 1957). In Hawaii, farmers have adopted the recommendation of planting border crops as roosting sites for *B. cucurbitae* and then treating these borders with GF-120 NF Naturalyte Fruit Fly Bait to reduce overall field damage. This bait has been shown to be an effective bait attracting and killing released 4-week-old, protein-starved female *B. cucurbitae* within 1 d of application on experimental plots having border crops with plants >36 cm wide (Prokopy et al. 2003, 2004). However, in practice there is considerable variation among farmers as to the number of rows and types of plants that are planted as border crops. Location, plant species, and density of these border plants may alter the effectiveness of protein bait sprays.

The objective of this study was to evaluate the effect of width of the border crop sorghum (*Sudax bicolor* x *S. bicolor* var. *sudanense*), in association with sprays of GF-120 NF Naturalyte Fruit Fly Bait on its ability to prevent color-marked *B. cucurbitae* females of various ages to penetrate the different border widths when searching for host fruit. Our experimental approach was similar to that used in a recent investigation by Prokopy et al. (2004) for comparative purposes.

## Materials and Methods

**Insect rearing.** All female *B. cucurbitae* used in experiments were F<sub>1</sub> generation that originated from wild populations. Parental flies were recovered from ground-infested papayas collected in orchards near Kapoho, HI. Melon flies were reared using the same methodologies described by Prokopy et al. (2003, 2004). All flies were supplied with water and a 3:1 mixture of sucrose and USB enzymatic yeast hydrolysate (United States Biochemical, Cleveland, OH) as a food source and water *ad libitum* until their use in a trial. Rearing was scheduled so that for each test day females of different ages (1, 2, and 4 weeks old) were available. One-week-old females were sexually immature females that show a comparatively weak response to cucumber odor (J.C. Piñero et al. unpub. data). Two week old females were females with ovaries that were already in the process of developing eggs, and four-week-old females were females with moderate to high egg load (Miller et al. 2004) and therefore they were expected to show the strongest response to the cucumber patches (see below). All experimental flies were held in a laboratory maintained at 22 ± 3°C and 60–80% RH, under a 12:12 (L:D) photoperiod.

**Experimental arena.** Large areas of mowed grass (~70 x ~170 m), located at the University of Hawaii Agricultural Experiment Station at Kainaliu, HI (Hawaii Island), were chosen for the field tests. A total of three test fields, each having two sorghum-bordered experimental plots (6 x 6 m<sup>2</sup>), were established (for details, see Prokopy et al. [2004]). All sorghum plants were cultured from seed in black plastic pots (27 cm diam. by 25 cm tall) placed outdoor fields adjacent to the test fields. Approximately 24–28 seeds were planted

per pot creating sorghum growth that was ~25 cm wide and ~150 cm tall. Within each field, the two experimental plots were identical in regards to the width of the borders, and they varied only in regards to the application of GF-120 NF Naturalyte Fruit Fly Bait (treatment versus control). The three different border widths evaluated were 30, 90, and 135 cm, and they were created by arranging 1, 2, or 4 rows of pots (15, 29, and 37 pots, respectively). The fourth side of each plot was made up of two fewer pots to allow for a ~50 cm gap at the end of the row. This setup allowed access in and out of the center of each plot. All rows were held in place using metal stakes and rope. Stakes were placed at each corner and at two equidistant points in each row. Rope was strung around each stake (~125 cm height) and used to hold the sorghum plants upright.

Each of the three border-width treatments was assigned to a particular field. These fields were rotated in a random order and in such a way that each row width was tested in every field. This was accomplished by moving the sorghum plants from field to field on the early morning of each test day. In addition, the assignment of treated (i.e., GF-120 NF Naturalyte Fruit Fly Bait) and control plots was alternated within fields to account for potential position effects.

**Experimental protocol.** On each test day and for each of the six experimental plots, three groups of color-marked females (one group per age class) were released simultaneously from each of four release sites located outside of the sorghum plots. Each release site consisted of six sorghum plants that were arranged in a tight circle surrounded by rope around a central stake, 5 m from the center of the plot (2 m outside of a row of sorghum). This dense grouping provided maximum shade and resting sites for departing flies from polyethylene containers (= release boxes) (12 cm wide x 18 cm tall x 5 cm deep) attached 80–120 cm above ground to the central stake. An 8 x 8 cm opening was cut into the lid of the release box and covered with removable netting to permit introduction of flies and their departure after release. During each trial, all release boxes containing color-marked females were setup by 0845 h.

Four 50 x 50 cm black plastic trays were placed on the ground 1 m from the center of each plot in each cardinal direction (north, east, south or west). Three cucumbers (*Cucumis sativus* L.), a favored host of melon flies (Piñero et al. 2006), were placed on top of each tray and simulated a patch of an attractive crop. All cucumbers were purchased from a local grocery store the night before the field tests and were hand washed with tap water before placement within plots. Each cucumber was cut laterally every 0.5 h to ensure emission of fresh volatiles. Cucumbers were cut at 0830 h (30 min before flies were released) and every 30 min thereafter until 1630 h.

On each test day, one of the two plots per field was sprayed with GF-120 NF Naturalyte Fruit Fly Bait using a hand-pumped backpack sprayer (Professional Back Pack Sprayer: S. P. Systems, Santa Monica, CA) using the same methodology described by Prokopy et al. (2004). These methods produce droplet sizes and coverage recommended by the manufacturer (DowElanco 1994). All sorghum plants were drenched with water each day after field trials to remove residual protein bait from the leaves.

Three trials were conducted with separation of at least 5 days to allow non-captured previously-released flies to emigrate out of the test plots. On each test day, equal numbers (20–25) of color-marked females (see below) from each age group were released simultaneously from each of the four sides of each of six experimental plots (1,920 females were released per day; 5,760 females were released in all).

**Marking and censusing of flies.** One day before conducting a trial test females were marked on the pronotum with a dot of a distinctive color (Gloss Enamel, Tester Corp., Rockford, IL) and held in release boxes overnight. Depending on fly availability, each box received 20–25 marked females of a particular age (either 1, 2, or 4 weeks old) and food

(sucrose + enzymatic yeast hydrolysate) and water. Different two-color combinations were used to identify the age of fly and the location of their release. Each fly was observed after marking to ensure her continued flight capacity before being placed in the release box. Censusing of flies arriving at the cucumbers was done from 0900 h and every 30 min. thereafter until 1700 h. Females found on the cucumbers were collected and held in the plastic cups with a cotton wick soaked in a sugar/water solution. The number of flies dead was recorded 8 and 24 h after they were placed inside the cups.

**Statistical Analysis.** Because a preliminary Analysis of Variance (ANOVA) revealed a significant effect of female age on the outcome, then three separate 2-way ANOVAS (one for each of the three female ages: 1, 2, and 4 weeks) were conducted to test the specific effects of "border width" and "bait treatment." Means were compared, whenever appropriate, using the Fisher-protected least significant differences test with  $\alpha = 0.05$ . Data, being proportions, were arcsin-transformed before analysis. A Wilcoxon signed rank test was performed to compare mortality for females captured in sprayed versus control plots after 8h and 24 h. All statistical analyses were conducted using SAS statistical program, version 8 (SAS Institute 1999) and significance was determined at a 95% confidence level.

## Results

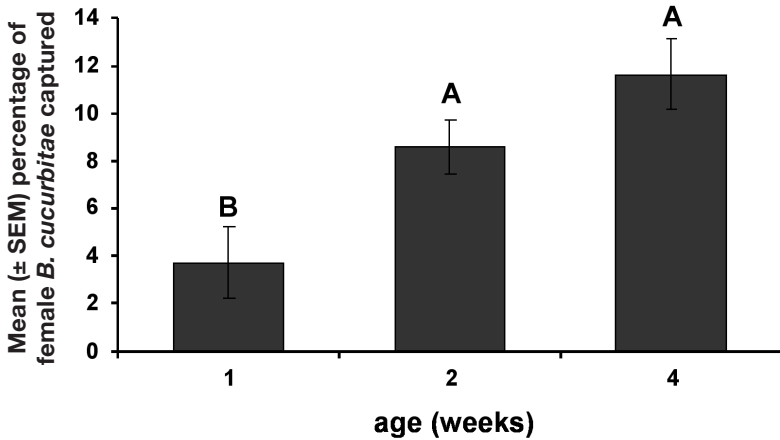
Release rates of color-marked females (successful escape from release boxes) were high, with only 2.6% of the total remaining in the release boxes. In addition, 90% of the number of females released was captured in the same fields they were released in indicating little between-field movement. A significant effect of the age of the females on their ability to penetrate the border and arrive at cucumber patches was recorded (ANOVA:  $F = 15.97$ ,  $df = 2$ ,  $P < 0.001$ ), with 1-week-old flies being recovered at a significantly lower rate than 2- and 4-week-old flies (Fig. 1).

For 1-week-old females a significant effect of the application of GF-120 NF Naturalyte Fruit Fly Bait treatment (ANOVA  $F = 7.397$ ;  $df = 1, 12$ ;  $P = 0.019$ ) on the capture rates of female *B. cucurbitae* in the cucumber patches was recorded. For this age group, the effect of border width and the interaction term were non-significant (ANOVA  $F = 0.326$ ;  $df = 2, 12$ ;  $P = 0.728$ , and  $F = 0.636$ ;  $df = 2, 12$ ;  $P = 0.546$ , respectively) (Fig. 2A).

For 2-week-old females the effect of the application of GF-120 NF Naturalyte Fruit Fly Bait treatment was highly significant (ANOVA  $F = 20.274$ ;  $df = 1, 12$ ;  $P < 0.001$ ), whereas the effect of border width (ANOVA  $F = 2.189$ ;  $df = 2, 12$ ;  $P = 0.155$ ) and the interaction term were non-significant (ANOVA  $F = 0.073$ ;  $df = 2, 12$ ;  $P = 0.930$ ) (Fig. 2B)

For 4-week-old females there was a significant effect of both bait application (ANOVA  $F = 12.208$ ;  $df = 1, 12$ ;  $P = 0.004$ ) and border width (ANOVA  $F = 3.656$ ;  $df = 2, 12$ ;  $P < 0.05$ ). GF-120 NF Naturalyte Fruit Fly Bait was very effective in preventing 4-week-old females from reaching the cucumber patches compared to control plots only when the plots had either 4 (maximum border width tested) or 2 rows. For plots that had only one row of sorghum plants the proportions of mature females that reached the cucumber patches were statistically similar in plots sprayed with GF-120 NF Naturalyte Fruit Fly Bait and in control plots (Fig. 2C). The interaction term was non-significant (ANOVA  $F = 0.180$ ;  $df = 2, 12$ ;  $P = 0.837$ ).

For mortality data, significantly ( $P < 0.05$ ) more females died within 8 h of capture from plots treated with GF-120 NF Naturalyte Fruit Fly Bait than those recovered from control plots (Fig. 3). The same trend was observed for the 24 h mortality but the significance level was not reached ( $P > 0.05$ ).

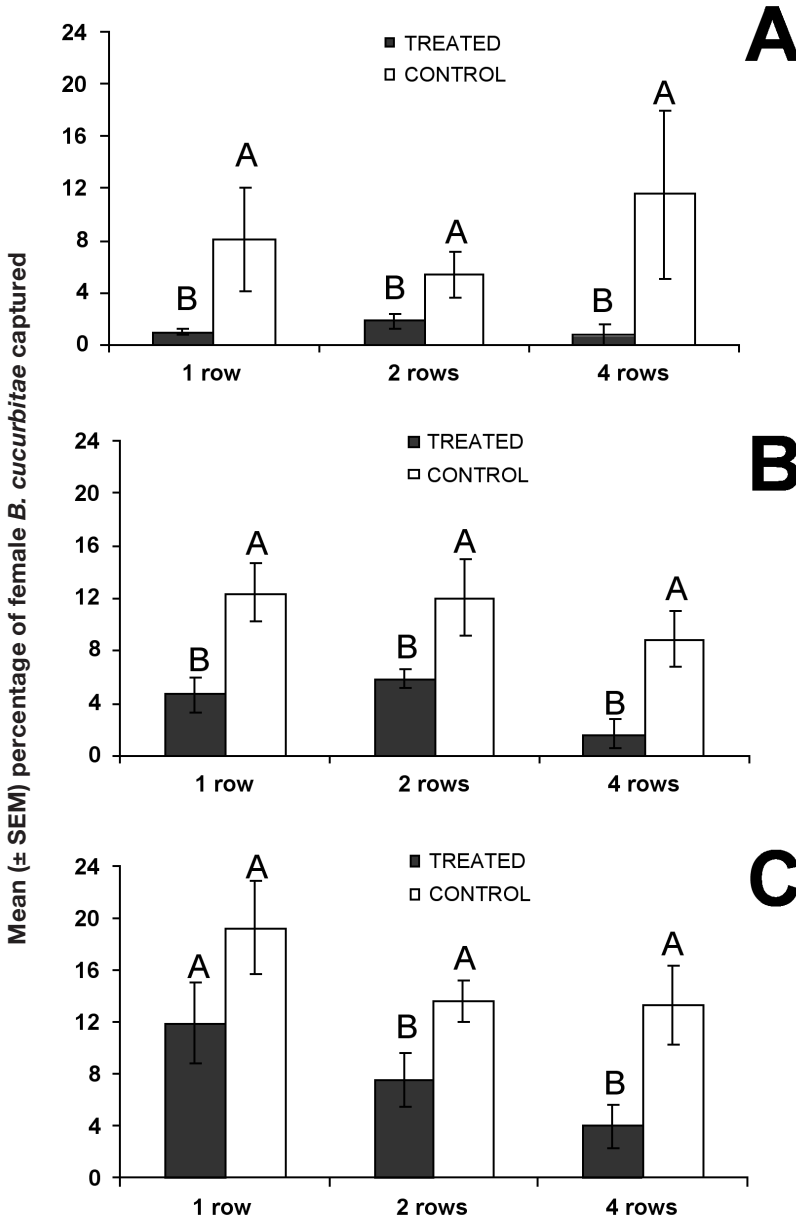


**Figure 1.** Mean ( $\pm$  SEM) percentage of female *B. cucurbitae* of various ages (1, 2, and 4 weeks post eclosion) captured in cucumber patches surrounded by treated or control sorghum borders. Data are presented combining sprayed and unsprayed plots and for the three border widths across all census periods from 0900 to 1700 h. Bars with different letters indicate significant differences between treatments according to ANOVA, followed by Fisher-protected LSD tests at  $\alpha = 0.05$ .

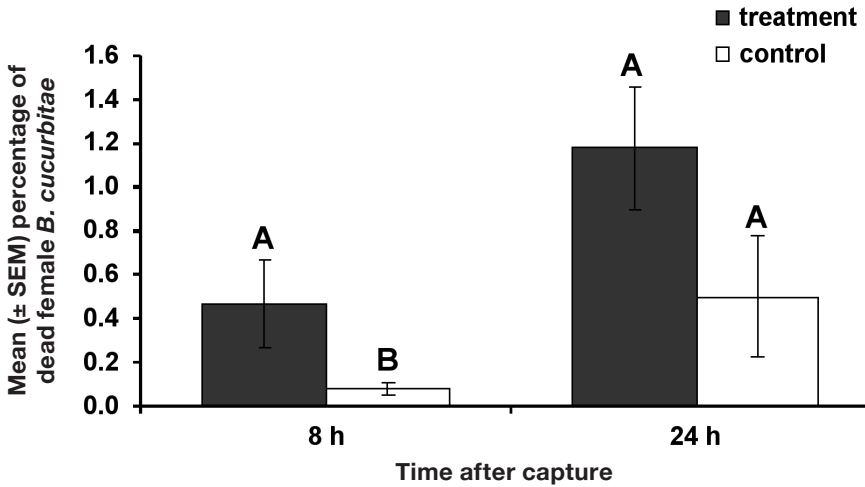
### Discussion

Our results indicate that 2- and 4-week-old females were captured at higher rates on the cucumbers than 1-week-old females and that cohorts of color-marked, sexually mature (4 weeks old) female *B. cucurbitae* released from outside borders sprayed with GF-120 NF Naturalyte Fruit Fly Bait were less likely to reach cucumber patches in plots with wide (135 cm) borders than in similar border-width unsprayed plots. Plots with the narrowest border (30 cm), sprayed or unsprayed, did not prevent sexually mature females from accessing the cucumber patches. For very young (1 week old) females and for females that were in the process of developing eggs (2 weeks old) the effect of border width was non-significant.

In a previous study, Prokopy et al. (2004) documented that GF-120 NF Naturalyte Fruit Fly Bait applied to a single row of potted non-host sorghum plants that surrounded a patch of cucumbers on all four sides was not effective at preventing released sexually mature female *B. cucurbitae* from penetrating into cucumber patches and that it was even less effective when applied to single rows of potted sorghum plants that bordered a patch of cucumbers on two sides (east, west) only. In the present study we determined that only when comparatively wide borders (135 cm) were sprayed, GF-120 NF Naturalyte Fruit Fly Bait was effective at preventing sexually mature females, which are actively seeking for hosts for oviposition, from reaching the cucumber patches. We conclude from these results that comparatively broad swaths of sorghum sprayed with GF-120 NF Naturalyte Fruit Fly Bait are more effective at preventing sexually mature female *B. cucurbitae* from accessing cucumbers than narrower swaths. The more abundant foliage provided by comparatively thick borders provides greater shelter for foraging females, and with potentially less host odor permeating through the sorghum, borders with at least four rows of sorghum may increase the likelihood of locally encountering protein droplets.



**Figure 2.** Effect of application of GF-120 NF Naturalyte Fruit Fly Bait and border width (30, 90, and 135 cm, created by 1, 2 and 4 rows of abutting pots of sorghum, respectively) on the numbers (mean percentage  $\pm$  SEM) of released female *B. cucurbitae* captured in cucumber patches according to female age: (A) 1 week, (B) 2 weeks, and (C) 4 weeks old. Data were combined across all census periods from 0900 to 1700 h. Bars with different letters indicate significant differences between treatments according to ANOVA and Fisher-protected LSD tests at  $\alpha = 0.05$ .



**Figure 3.** Mortality rates (mean percentage  $\pm$  SEM) of female *B. cucurbitae* captured in sprayed and unsprayed cucumber patches at 8 and 24 h post collection. All females recovered during field trials ( $n = 600$ ) were kept individually in cups with sugar and water. For each time period, bars with different letters indicate significant differences between treatments according to a Wilcoxon signed rank test at  $\alpha = 0.05$ .

Physiological state has been shown to influence significantly the type of response that female fruit flies will exhibit toward protein baits. For example, for both *B. dorsalis* (Cornelius et al. 2000) and *B. cucurbitae* (Miller et al. 2004), protein-fed females have been found to respond more to fruit odors than to protein odors (including GF-120 NF Naturalyte Fruit Fly Bait), while protein-deprived *B. cucurbitae* females have been reported to be equally attracted to protein and fruit odors. Because in this study all females evaluated were protein-fed then better results would have been expected had protein-starved females been evaluated. As expected, the effect of GF-120 NF Naturalyte Fruit Fly Bait application was greatest for youngest females, as evidenced by the very low capture rates in the cucumber patches, compared to unsprayed plots. The effect of age has also been investigated by J.C. Piñero et al. (unpub. data), who documented that nearly 100% of released 1-week-old female *B. cucurbitae* in field cages respond to GF-120 NF Naturalyte Fruit Fly Bait within 20 min.

As stated above, GF-120 NF Naturalyte Fruit Fly Bait does not have high contact toxicity (in comparison to traditional organophosphate insecticides) and must be ingested for maximum effect (DowElanco 1994). Exposure to this bait resulted in higher mortality rates of *B. cucurbitae* females within 8 h compared to non-exposed flies, a result that indicates that females captured had indeed fed on the GF-120 NF Naturalyte Fruit Fly Bait sprayed onto the sorghum before reaching the cucumbers. Further studies should address the question of whether females that have fed on GF-120 NF Naturalyte Fruit Fly Bait are able to lay any eggs on the host fruit in significant numbers before dying.

Successful suppression of *B. cucurbitae* populations in an area could be achieved through a combination of pest management practices including applications of GF-120 NF Naturalyte Fruit Fly Bait spray, sanitation, and male annihilation technique (Mau et al. 2007, Vargas et

al. 2008). Integrated pest management approaches, like those being conducted in Hawaii and Guatemala, also combine releases of sterile insects (SIT) and/or fruit fly parasitoids with bait spray applications. During area-wide programs, protein baits are commonly applied at comparatively short intervals, usually weekly (Roessler 1989, Piñero et al. 2009). For *B. cucurbitae*, our data and previous reports (Prokopy et al. 2003, 2004) support the recommendation of establishing preferred roosting hosts as crop borders (McQuate and Vargas 2007) to improve suppression of fruit flies by providing sites for bait spray applications. As shown in the present study, key to the success of these border plants is the thickness of the foliage, particularly against sexually mature, host-seeking females.

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