Response of *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) to Food Odor Emanating Through Consumer Packaging Films

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ABSTRACT The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), is an important pest of packaged consumer foods, yet little is known of its behavioral response to food odors. Adults and larvae are not believed to chew holes through packaging materials, but they may invade packaged food by entering through openings caused by improper sealing or handling, through holes intentionally placed in packages by the manufacturer, or through openings made by insects or other organisms. To better understand the mechanism of infestation of packaged foods by the sawtoothed grain beetle, we determined how food odor coming through the surface and through holes in consumer food packaging materials influences female sawtoothed grain beetle movement. Mated female beetles responded with an area-concentrated search to the odor of dog food emitted from 0.5-mm-diameter holes punctured in two commonly used food packaging materials (Cello and 120 AB-X). Holes emitting no food odor did not influence beetle behavior. Velocity and distance moved differed between film types, perhaps because of surface characteristics of the film influencing traction, but no influence of food odors coming through the films was detected. This study indicates the necessity for improved package designs and better sealing and handling methods to prevent flaws in packaging through which insects may enter.

KEY WORDS *Oryzaephilus surinamensis*, sawtoothed grain beetle, stored products, packaging, olfaction

MANY SPECIES OF STORED-PRODUCT insects will infest packaged foods where they have an ample supply of nourishment for themselves and their offspring and where they are protected from most chemical treatments (Highland 1984). Contaminated products can often be moved from one geographical location to another. Once these contaminated products are placed in local warehouses and retail stores, infestations can spread throughout the facility. Some products are more susceptible to insect infestation, and these products can serve as insect reservoirs, leading to the infestation of other products (Highland 1984). While food products can become infested at any point in the marketing channel, they are most likely to become infested during extended storage periods. Dry pet foods can often be a source of infestation because many pet foods are packed in multiwall paper bags that generally lack adequate seals and closures. Food may also become infested during shipment in trucks, railcars, and ships, as well as during storage at the retail level. Infestation also occurs in the home pantry, but these losses have been reduced with the increased use of resealable packages.

Stored-product insects may take advantage of any sort of opening in a packaging material to gain entry. These openings may result from the chewing of other insects or from rips, tears, or punctures from wear and tear during handling. Most infestations are the result of insects crawling through seams and closures and rarely through chewing (Mullen 1997). Many species prefer to lay eggs in tight spaces, such as those formed when multiwall paper bags or paperboard cartons are folded to create closures. These refugia provide a safe place to lay eggs and also give the newly hatched larvae an ideal location through which to invade the packages.

Olfaction is an important means by which insects identify resources on which to feed and oviposit (Bell and Cardé 1984). Various endogenous factors can influence the response of insects to food odors, including species, mating status, sex, satiation, and age. Honda et al. (1969) showed that maize weevils, *Sitophilus zeamais* Motschulsky, <10 d old, were more sensitive to volatiles from rice than were older individuals. Ohsawa et al. (1970) reported that maize weevils, *S. zeamais,* of mixed sexes held without food for 2 d had increased sensitivity to extract of rice. Species differences in response to food odors can indicate ways in which competitors partition re-

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sources. For example, *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) respond differently to the same grain-related volatiles (Phillips et al. 1993). *T. castaneum* was shown to be highly attracted to WGN (a processed consumer food product), indicating the processed and combined ingredients of this food product may yield an odor stimulus that represents an optimal food source for this species. Conversely, *S. oryzae* showed a lack of response to WGN. The greater response of *T. castaneum* to various grain oils may reflect the habitat preference for this species to develop in older and damaged grain substrates. *S. oryzae* showed a much lower response and even repellency to the same oils, indicating not all grain oils are attractive to weevils (Phillips et al. 1993).

The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), is a serious pest of consumer food products. This species is often found in packaged commodities and is presumed to enter through flaws in the packaging (Mullen and Mowery 2002). Studies showing how the sawtoothed grain beetle and other stored-product insects respond to point sources of food odor, such as those associated with flaws in consumer packaging, are limited.

The sawtoothed grain beetle is unable to attack foods such as sound grain, but it can attack damaged grain and other processed foods (Fraenkel and Blewett 1943). Although adults and larvae are not generally believed to create openings in packaging materials, the sawtoothed grain beetle may exploit existing openings such as those made by insects, improper seals, or mishandling of packages during the packaging and shipping processes. Invasion of packaging by sawtoothed grain beetle is limited by the size of the flaws. Adults fit through holes >0.71 mm diameter (Cline and Highland 1981), but neonate larvae (average head capsule width 0.3 ± 0.01 mm) are able to enter packages through extremely small openings (Mowery et al. 2002). Thus, females laying eggs near package flaws yield offspring able to exploit a wider range of packages.

Food and beverage packaging comprises over $70 billion of the packaging market in the United States and more than $200 billion worldwide annually (Wilkinson 1998). Considering that insect infestation of stored consumer food products is of such importance to the industry (Mullen and Mowery 2000), disproportionately little information is available on the mechanisms by which insects, especially the sawtoothed grain beetle, enter packaged goods. The objective of this study was to determine how female sawtoothed grain beetle movement is influenced by food odors that escape through artificial flaws (holes) and through the surface of packaging films.

Materials and Methods

Sawtoothed grain beetles were obtained from colonies maintained for over 15 yr at the USDA Grain Marketing & Production Research Center in Manhattan, KS (Kansas State University, Department of Entomology, voucher specimen number 132). The insects were maintained in climate-controlled incubators at a temperature of 27 ± 2°C and 50 ± 5% RH and reared on a diet consisting of rolled oats, flour, and brewer's yeast.

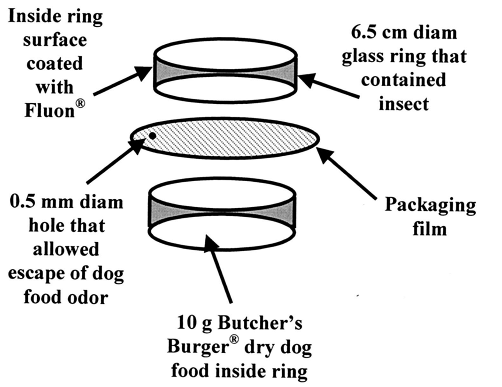

Fig. 1. Construction of the arena used to assess sawtoothed grain beetle responses to dog food odor on two different consumer food packaging films (120 AB-X and Cello).

Insect movement was observed on the packaging films within an arena consisting of a single sheet of packaging film sandwiched between two 6.5 cm diameter by 2.5 cm high glass rings (Fig. 1). The inside surface of the top ring was coated with Fluon (Northern Products, Woonsocket, RI) to prevent insect escape. Three parameters were tested: packaging film type, presence/absence of food, and presence/absence of a hole in the film. Two packaging films were tested: 120 AB-X (acrylic on both surfaces, 0.03 mm thick), which is considered to be a good odor barrier, and Cello (0.02 mm thick), which is considered to be a poor odor barrier (Anon. 1995). Both packaging films are manufactured by Exxon Mobil Chemical (Stratford, CT). In the food treatments, the bottom ring contained ≈10 g of Butcher's Burger dry dog food (Ralston Purina Co., St. Louis, MO). In the treatments requiring a hole in the packaging film, a 0.5 ± 0.01-mm-diameter hole was made using a heated insect pin. This hole size allowed for the escape of food odor but prevented adult beetles from passing through the hole. The four treatment combinations for each packaging film type were hole and food, no hole and food, hole and no food, and no hole and no food (H/F, NH/F, H/NF, and NH/NF, respectively).

Beetle movement was recorded using a black and white video camera (Panasonic Digital WV-BP330, Matsushita Communication Industrial, Osaka, Japan) in a 76.2 by 63.5 by 71.2 cm Plexiglas (ATOFINA Chemicals, Philadelphia, PA) observation chamber to avoid excessive air movement within the testing area. The sides of the chamber were covered in cardboard to lower light intensity. Tests were run at 22.6 ± 0.01°C, 43.6 ± 0.2% RH, and light intensity of 18.4 ± 0.1 lumens (HOBO data logger, BoxCar Pro; Onset Computer Corporation, Bourne, MA, 1997–1998).

During analysis using EthoVision video tracking, motion analysis, and behavioral recognition system

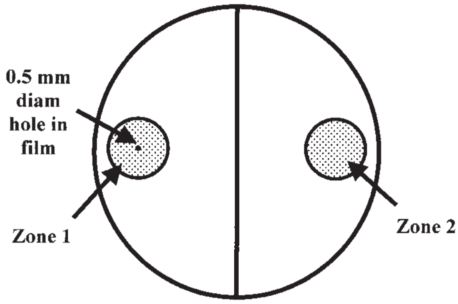

Fig. 2. Top view of the designated zones and halves of the surface of plastic food packaging films on which sawtoothed grain beetle response to dog food odor was assessed. A 0.5-mm-diameter hole was used in some treatments to allow dog food odor into the arena, but insects were unable to fit through the hole.

(Noldus Information Technology, Sterling, VA), the video images of the arenas were divided into the following zones to facilitate the analysis of the movement paths of the beetles. Two round zones, 1 cm in diameter and 3 mm from the edge, were defined on opposite sides of the arena (Fig. 2). In the treatments requiring a hole in the packaging film, the hole was located in the center of the first zone (zone 1). The other zone, containing no hole, was designated zone 2. The circular arena was also divided in half in such a way that there was an arena half with zone 1 and a half with zone 2.

One 1-wk-old female sawtoothed grain beetle was placed using a brush onto the surface of the film in the center of arena, and its behavior was recorded for 5 min after a 5-min acclimation period. The beetles were held without food and water for 2 d before testing. Each combination of film type, food, and hole was replicated 19 times, and a different individual was used for each replicate (152 individuals total). The observations were blocked through time, with all four treatments randomized within each block. Arena orientation was also varied to account for possible environmental differences in the testing arena. The location of zone 1 was positioned at North, East, South, or West (orientations 1, 2, 3, and 4). Five replications were performed at orientations 1, 2, and 4, and four at orientation 3.

Average movement parameters were calculated for the treatments to determine differences in beetle response to food odor and to the presence of holes. Mean time (s) spent and distance (cm) moved were determined for zone 1, the arena half containing zone 1, zone 2, and the arena half containing zone 2, respectively. Sinuosity (degrees/cm$^{0.5}$) is the amount of random turning associated with a given path length. This was determined for the two halves and for the whole arena, but not for zones 1 and 2 because of their small size. Distance moved, velocity (cm/s), and sinuosity were determined for beetle movement over the entire surface of each individual packaging film to assess how beetles responded to diffuse food odor when no holes were present.

Table 1. Influence of hole and food on time (s) spent and distance moved (cm) by female sawtoothed grain beetles in 1-cm zones in a 6.5-cm-diameter arena on one of two film types (120 AB-X and Cello)

Treatment[a]	Time (s)			
	Film type			
	120 AB-X		Cello	
	Zone 1[b]	Zone 2[c]	Zone 1	Zone 2
H/F	45.2 ± 9.8	4.4 ± 0.7	30.8 ± 5.9	5.0 ± 1.0
H/NF	7.9 ± 1.7	6.6 ± 0.9	6.9 ± 1.1	4.6 ± 0.6
NH/F	6.6 ± 2.4	4.2 ± 0.9	5.1 ± 0.8	5.5 ± 0.8
NH/NF	7.4 ± 1.3	6.6 ± 1.8	7.5 ± 1.2	5.1 ± 0.7
	Distance moved (cm)			
	Film type			
	120 AB-X		Cello	
	Zone 1	Zone 2	Zone 1	Zone 2
H/F	8.4 ± 1.1	1.6 ± 0.3	6.7 ± 0.9	1.7 ± 0.3
H/NF	2.3 ± 0.4	2.1 ± 0.3	2.7 ± 0.3	2.0 ± 0.3
NH/F	1.8 ± 0.5	1.5 ± 0.3	2.3 ± 0.3	2.4 ± 0.3
NH/NF	2.2 ± 0.3	1.6 ± 0.3	2.7 ± 0.4	1.3 ± 0.3

[a] H, hole; NH, no hole; F, food; NF, no food.
[b] Zone 1 contained a 0.5-mm-diameter hole in the treatments with hole.
[c] Zone 2 was the control zone with no hole.

Data were analyzed using three-factor analysis of variance (ANOVA) (SAS Institute 2000). Data were checked for violations of ANOVA assumptions using UNIVARIATE procedure (SAS Institute 2000) before analysis. Problems with normality in zone 1 and zone 2 data sets were corrected using square-root transformation. Paired t-tests for means were performed using Statistix7 software (Analytical Software, Tallahassee, FL.). Data are presented as means ± SEM.

Results

Female beetles spent more time around the zone with the hole (zone 1) when food was present than when food was not present (Table 1). When comparing among treatments, there was a significant difference in the time spent in zone 1 ($F = 17.10$; df = 7, 144; $P < 0.0001$). Hole ($F = 44.69$; df = 1; $P < 0.0001$), food ($F = 25.80$; df = 1; $P < 0.0001$), and hole/food interaction ($F = 45.62$; df = 1; $P < 0.0001$) were the only significant variables. For both film types with a hole present, females spent more time in zone 1 when food was present than when no food was present (Cello: $F = 23.90$; df = 1, 36; $P < 0.0001$ and 120 AB-X: $F = 23.63$; df = 1, 36; $P < 0.0001$). The effect of hole was not significant when no food was present (Cello: $F = 2.48$; df = 1, 36; $P = 0.1239$ and 120 AB-X: $F = 0.91$; df = 1, 36; $P = 0.3458$). There were no significant differences among treatments in the time spent by females in zone 2 ($F = 0.88$; df = 7, 144; $P = 0.5232$). Within the same arena, there were differences in average time (s) spent by female beetles in the zone with the hole (zone 1) compared with the zone without the hole (zone 2) when food was present (paired two-tailed t-test, 120 AB-X: $t = 4.0$, df = 18, $P = 0.0008$; Cello: $t = 4.4$, df = 18, $P = 0.0004$), but no differences when no

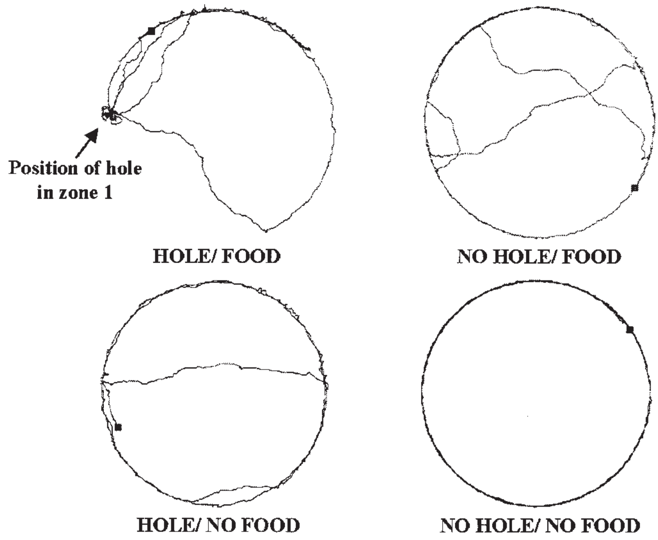

Fig. 3. Representative EthoVision tracks showing sawtoothed grain beetle movement paths in four treatment combinations. Movement was recorded for 5 min. As needed, a 0.5-mm-diameter hole was punctured through the plastic food packaging material as indicated by the arrow. The beetle in the hole/food treatment combination spent 546 of 1,500 total data points in zone 1. Squares indicate the locations of the beetle at the end of the observations.

food was present (120 AB-X: $t = 1.0$, df = 18, $P = 0.3$; Cello: $t = 1.8$, df = 18, $P = 0.08$).

The distance moved by females in the zone with the hole also differed among the treatments (Table 1; ANOVA: $F = 16.95$; df = 7, 144; $P < 0.0001$). Representative movement paths in the different treatments are presented in Fig. 3. Hole ($F = 42.43$; df = 1; $P < 0.0001$), food ($F = 30.43$; df = 1; $P < 0.0001$), and hole/food interaction ($F = 41.23$; df = 1; $P < 0.0001$) were the only significant variables. For both film types with a hole, females moved further in zone 1 when food was present than when no food was present (Cello: $F = 17.82$; df = 1, 36; $P = 0.0002$ and 120 AB-X: $F = 26.82$; df = 1, 36; $P < 0.0001$). The effect of hole was not significant when no food was present (Cello: $F = 0.82$; df = 1, 36; $P = 0.3720$ and 120 AB-X: $F = 0.31$; df = 1, 36; $P = 0.5829$). There were no significant differences among treatments in the distance moved in zone 2 ($F = 1.24$; df = 7, 144; $P = 0.2854$). Within the same arena, there were differences in distance moved by female beetles in the zone with the hole (zone 1) compared with the zone without the hole (zone 2) when food was present (paired two-tailed t-test, 120 AB-X: $t = 5.3$, df = 18, $P = 0.00001$; Cello: $t = 5.6$, df = 18, $P = 0.00001$), but no differences when no food was present (120 AB-X: $t = 0.5$, df = 18, $P = 0.6$; Cello: $t = 1.9$, df = 18, $P = 0.07$).

The presence of food odor emanating from a hole influenced behavior in the immediate vicinity of the hole but did not measurably influence the behavior of female beetles within the half of the arena containing the hole (Table 2). There were no differences among treatments in time spent by beetles (ANOVA: $F = 1.34$; df = 7, 144; $P = 0.2336$) or in the sinuosity of their movement ($F = 0.89$; df = 7, 144; $P = 0.5168$) in the arena half with zone 1. There was also no difference in behavior among treatments for the control half of the arena without the hole ($F = 1.16$; df = 7, 144; $P = 0.3273$ for ANOVA of time spent in arena half and $F = 1.44$; df = 7, 144; $P = 0.1950$ for ANOVA of sinuosity in arena half). Within an arena, there was no difference in path sinuosity between the half of the arena containing the hole and the half without a hole for both film types when food was present (120 AB-X: $t =$

Table 2. Influence of a hole and food on time (s) spent and sinuosity of movement (degrees/cm$^{0.5}$) by female sawtoothed grain beetles in two halves of a 6.5-cm-diameter arena on one of two film types (120 AB-X and Cello)

Treatment[a]	Time (s)			
	Film type			
	120 AB-X		Cello	
	Zone 1 half[b]	Zone 2 half[c]	Zone 1 half	Zone 2 half
H/F	170.7 ± 11.9	129.4 ± 11.9	158.0 ± 8.0	142.1 ± 8.0
H/NF	134.8 ± 9.3	161.5 ± 9.6	151.5 ± 5.2	148.5 ± 5.2
NH/F	148.1 ± 12.4	151.9 ± 12.4	146.0 ± 7.3	154.0 ± 7.3
NH/NF	140.9 ± 13.4	157.7 ± 13.4	142.5 ± 6.1	157.6 ± 6.1
	Sinuosity (degrees/cm$^{0.5}$)			
	Film type			
	120 AB-X		Cello	
	Zone 1 half	Zone 2 half	Zone 1 half	Zone 2 half
H/F	30.1 ± 7.7	21.6 ± 6.7	27.4 ± 7.1	43.7 ± 7.8
H/NF	26.0 ± 7.2	30.9 ± 7.8	29.3 ± 7.4	30.9 ± 6.9
NH/F	26.1 ± 7.6	20.3 ± 7.1	30.3 ± 5.9	30.3 ± 6.8
NH/NF	14.2 ± 5.0	21.6 ± 6.8	37.7 ± 7.4	38.7 ± 6.7

[a] H, hole; NH, no hole; F, food; NF, no food.
[b] Zone 1 half contained a 0.5-mm-diameter hole in the treatments with hole.
[c] Zone 2 half was the control half with no hole.

1.3, df = 18, $P = 0.2$; Cello: $t = -2.0$, df = 18, $P = 0.06$) and when food was not present (120 AB-X: $t = -0.6$, df = 18, $P = 0.6$; Cello: $t = -0.2$, df = 18, $P = 0.9$).

Film type influenced the movement behavior of the beetles over the whole arena, regardless of the presence or absence of either food or hole. There was a difference in total distance moved within the arena among the treatments ($F = 3.06$; df = 7, 144; $P = 0.0050$), but film type was the only significant variable ($F = 18.72$; df = 1; $P < 0.0001$). Beetles moved further on Cello (104.4 ± 3.9 cm, $n = 76$) than on 120 AB-X film (81.9 ± 3.4 cm, $n = 76$). There was also a difference in average velocity over the whole arena among the treatments ($F = 3.07$; df = 7, 144; $P = 0.0049$), but again film type was the only significant variable ($F = 18.70$; df = 1; $P < 0.0001$). Beetles moved faster on Cello (0.349 ± 0.013 cm/s, $n = 76$) than on 120 AB-X film (0.274 ± 0.011 cm/s, $n = 76$). However, there was no difference among the treatments in path sinuosity ($F = 0.97$; df = 7, 144; $P = 0.4581$).

Discussion

The sawtoothed grain beetles switched from a ranging search to an area-concentrated search (Bell 1991) when they encountered the odor of the dog food emanating through holes in the packaging materials tested (as measured by an increase in time and distance traveled in zone with hole), indicating that the beetles can detect food volatiles seeping through even small openings in packaging materials. This type of area concentrated search is common in many animals. For example, the movement paths of the house fly, *Musca domestica* L., and the fruit fly, *Drosophila melanogaster* Meigen, switch from ranging search to localized search after being fed a sucrose solution (Bell 1985). For stored-product insects that exploit packaged food, this type of behavioral response could facilitate locating routes of entry into packages or locations at which to lay eggs.

Barrer and Jay (1979) determined that the odor of kibbled wheat emanating from holes attracted gravid almond moths, *Cadra* (*Ephestia*) *cautella* (Walker), and females laid eggs near the holes. The sawtoothed grain beetle has been observed to lay eggs around and through flaws in packaging when flaws were too small for adults to enter (Mowery et al. 2002). In the absence of a hole and food odor, females mainly walked in a circular pattern around the arena edges, rarely diverting from this path. This behavior indicates that females would be more likely to encounter holes along the edges and folds that occur in packaging. The influence of hole location on beetle behavior is an area that needs further research.

The influence of food odors emanating through the hole seems to be a fairly localized arrestment response. When the amount of time spent by beetles in the arena halves with and without holes was compared, no statistical difference was observed. Females walked toward the hole and spent time in its vicinity, but the beetles soon walked away and continued the pattern of movement around arena edges. This response suggests that giving-up time may be involved. According to Bell (1991), when resource-specific cues stimulate an insect to perform a local search, animals eventually habituate to the stimuli and leave the patch in the absence of positive reinforcement. Another possible explanation is that the strength of the stimulus was not sufficient to trigger longer stops by beetles. This idea may be supported by behavior observed in the predatory mite, *Neoseiulus fallacis* (Garman). The mite will follow an arena edge at all temperatures and prey densities. The mites perform this behavior to the exclusion of other behavioral types such as a random walk-type searching and resting when prey egg density is <4 eggs per cm^2 (Berry and Holtzer 1990). When prey density was raised, the other behaviors were observed.

Although Cello and 120 AB-X films have different odor barrier properties, food was not a significant variable when comparing behaviors across the whole arena. That beetles responded to food odors coming through holes, but not through the film itself, suggests that insufficient levels of volatile cues were coming through the two film types and/or that any differences between the two film types were insufficient to trigger a measurable behavioral response. Additional studies are needed with a wider range of film types to determine if the sawtoothed grain beetle responds behaviorally to food odors coming through packaging films. The packaging film itself does seem to influence movement. Increased beetle velocity and distance moved on Cello may be because of a difference in surface texture between the films. Distance moved and average velocity are correlated traits and together suggest that beetles had better traction on Cello film compared with 120 AB-X.

The low tolerance for insect infestation of stored products makes effective insect-resistant packaging vital. Over the next 5 yr, the most advanced segment of the food industry will be "functional foods" (packaged processed foods). This segment of the food market is expected to grow 11.5% (Kindle 2001). Because of this, the need for improved insect-resistant packaging is likely to become increasingly important. Our understanding of how stored-product insects find and infest packages is poor. The results presented here provide insight into the infestation behavior of one of the most destructive stored-product pests. It will serve to aid in the development of improved packaging methods and materials based on the ability of the adult sawtoothed grain beetle to locate flaws in packaging materials through which to enter packages themselves or to inject eggs. Investigations into the infestation process of this and other stored-product insect species will help in the development of food packages with good insect resistance qualities.

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