

Sebastian Gustavo Zuil\*, Sonia Beatriz Canavelli and  
Laura Addy Orduna

# Oil-con hybrids as a potential tool to prevent eared dove damage to sunflower crops

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**Abstract:** Eared doves (*Zenaida auriculata*) cause significant economic damage to mature sunflower in Argentina. Empirical evidences indicate that some sunflower hybrids (e.g. confectionary and stripped oilseed) might be less susceptible to bird damage than others (e.g. black oilseed). However, these less susceptible hybrids could imply a trade-off between damage reduction and oil content on the seeds. In this work, we investigated the potential of a new oil-con hybrid types (OC: oilseed × confectionary cross) as a tool to prevent eared dove damage to sunflower crops. We compared the performance (damage values), morphological characteristics and nutritive quality of this hybrid compared to other three standard sunflower hybrids (BO: black oilseed, SO: striped oilseed, and CON: confectionary), both in the field and in cages. In both tests, doves significantly selected BO and SO hybrids over CON and OC hybrids. The OC hybrid was not selected, despite its high nutritional value. Results suggest that achene size could be an important morphological trait influenced eared dove selection of hybrids, independently of other seed characteristics. Based on these results, the OC hybrid tested in this study seems to be a promising tool for preventing eared dove damage to sunflower in Argentina.

**Keywords:** bird-resistant hybrids; confectionary; eared doves; feeding preferences; oilcon.

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\***Corresponding author: Sebastian Gustavo Zuil**, Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Rafaela (Argentina), Ruta 34 km 227 C.C. 2300 Rafaela, Argentina, E-mail: [zuil.sebastian@inta.gob.ar](mailto:zuil.sebastian@inta.gob.ar). <https://orcid.org/0000-0002-3244-5990>

**Sonia Beatriz Canavelli and Laura Addy Orduna**, Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Paraná, Paraná, Argentina, E-mail: [canavelli.sonia@inta.gob.ar](mailto:canavelli.sonia@inta.gob.ar) (S.B. Canavelli), [addyorduna.laura@inta.gob.ar](mailto:addyorduna.laura@inta.gob.ar) (L. Addy Orduna). <https://orcid.org/0000-0003-4642-395X> (S.B. Canavelli). <https://orcid.org/0000-0002-2895-686X> (L. Addy Orduna)

## Introduction

Bird damage to sunflower (*Helianthus annuus* L.) is a widespread challenge for farmers and wildlife managers all around the world (Linz and Hanzel 1997; Linz et al. 2015). Wild birds, including blackbirds (Icteridae) in the United States; parrots (Psittacidae) in Australia and the Indian subcontinent and doves (Columbidae) and sparrows (Emberizidae, Passeridae) in Europe and Africa are mentioned among the main culprits of sunflower damage (Linz et al. 2015). In South America, bird damage to sunflower occurs at both emergence and maturity in countries such as Argentina, Uruguay, and Brazil (Bruggers et al. 1998; Linz and Hanzel 1997; Linz et al. 2015; Rodriguez et al. 1995, among others). Pigeons (*Patagioenas maculosa* and *P. picazuro*) usually are related to emergence damage, while monk parakeets (*Myiopsitta monachus*) and principally eared doves (*Zenaida auriculata*) are related to damage at maturity (Bernardos and Farrel 2013; Bruggers et al. 1998; Canavelli et al. 2008; Linz and Hanzel 1997; Rodriguez and Zaccagnini 1998; Silva and Guadagnin 2018; Zaccagnini 1985). Generally, parakeets' damage starts with the still immature heads, destroying the edges. Instead, eared doves usually damage the mature heads only extracting the achenes, without destroying it (Canavelli 2010).

Managers and farmers have developed and tested multiple management strategies to prevent and decrease bird damage to sunflower. Such methods and tools include cultural practices (e.g. habitat management and decoy crops); frightening mechanical devices and chemical agents; and bird population reduction (see Linz and Hanzel 1997; Linz et al. 2015; Linz and Klug 2017 for comprehensive reviews). Also have been tested chemical desiccation and repellents to prevent or reduce damage to mature sunflower, with unpredictable results (Castaño 2018; Radić 2006; Rodriguez et al. 1995; Szemruch et al. 2019).

Among cultural practices, the development of bird-resistant crops is usually an attractive alternative to prevent bird damage, due to its non-lethal characteristic and, consequently, high societal support (Conover 2001). Since birds can be attracted to sunflower crops by several proteins and essential amino acids necessary for bird development and reproduction (Besser 1978), chemical characteristics of the achenes can be modified to prevent damage. In this sense, oil content, as well as other achene chemicals (such as anthocyanins), had been explored as a method to prevent blackbird damage to sunflower (Mason et al. 1989). In addition to chemical characteristics, physical characteristics of the achenes and/or phenotypic characteristics of sunflower plants also have been explored to make them less attractive to birds (Linz et al. 2015). For example, plant height, concave head shape, mean head-to-stem distance, head position, lower achene density, and achene cover, have been previously explored as a plant

breeding measure to discourage bird feeding in sunflower fields (Khaleghizadeh 2011; Linz and Hanzel 1997; Mah et al. 1990; Parfitt 1984; Parfitt and Fox 1986; Seiler and Rogers 1987; Yasumoto et al. 2012).

In spite of previous information suggesting the importance of sunflower hybrids to manage bird damage worldwide (Bullard 1988; Dedio 1995; Rauf et al. 2008; Seiler and Rogers 1987), limited research has been conducted to explore the potential of bird-resistant sunflower to prevent damage by eared doves in South America (Zuil and Colombo 2012). Empirical evidence would indicate that hybrids that combine small size with high nutritional value would be selected by eared doves, as is the case of black oilseed hybrid (BO) (Castaño 2018; Zuil and Colombo 2012). For this reason, striped oilseed hybrids (SO), with visual differences of outer hull and oil percentage of the achene, are recommended by field technicians and frequently used by farmers as an alternative to BO for reduce eared dove damage in sunflower fields. Furthermore, when possible, field technicians also recommend confectionary hybrids (CON) with large achenes, based on empirical evidence that eared doves avoid their consumption, even when they have a very limited and restricted market. Therefore, hybrids that combine relatively large seed size, outer hull similar to SO, and high nutritional quality of achenes, such as oilseed  $\times$  confectionary hybrid types (OC), could be promising options to reduce eared doves' damage to sunflower.

In this work, we first evaluated damage by eared doves to four types of sunflower hybrids (BO, SO, OC and CON) in field growing conditions and reliably determined their morphological and nutritional characteristics, in order to relate them with eared doves' damage. Based on the results obtained in the field and the morphological and nutritional characteristics of the hybrids, we carried out two-choice feeding tests in individual cages, in order to relate and confirm the damage patterns observed in the field to the hybrids characteristics. In this way, we expect to establish a promising path for sunflower breeding programs to develop novel hybrids less attractive to eared doves and, consequently, less prone to damage.

## Materials and methods

### Eared dove selection in field conditions

We evaluated eared dove selection for sunflower achene hybrids in field conditions during the 2016–2017 growing season from experimental field of the Instituto Nacional de Tecnología Agropecuaria (INTA) Reconquista Experimental Station (29° 11' S; 59° 52' W). We sowed the hybrid achenes on August 14, 2016 (a standard planting date for this region), with a mean plant density of 4.5 plants  $\text{m}^{-2}$ . Soil at the Experimental Station is Aquic Argiudol (USDA Soil Taxonomy), characterized by 1.83% of

organic matter; pH of 6.2; 14.3 mg kg<sup>-1</sup> of available phosphorus; and 19.9 mg kg<sup>-1</sup> of N-NO<sub>3</sub> (based on ad-hoc soil analysis). Livestock and crop production dominate the landscape, with an interspersed of pasture, cultivated land, shrub communities, streams and rivers.

We tested the following sunflower hybrids: 1) BO (SYN 3825 and SYN 3970, Syngenta, length 9.0 ± 0.4 mm and width 4.9 ± 0.1 mm), 2) SO (DK 4045, Syngenta, length 10.6 ± 0.2 mm and width 5.1 ± 0.1 mm), 3) OC (two OC experimental hybrids, length 14.4 ± 0.2 mm and width 7.1 ± 0.2 mm), and 4) CON (Puma, Jaguar and NTC hybrids, length 15.9 ± 0.2 mm and width 7.7 ± 0.3 mm). The experimental OC hybrids were similar in agronomics behavior but different genetic background (Zuil et al. 2016). Each genotype was sown in plots. Eight plots were sown with BO hybrids, 4 with SO hybrid, 8 with OC hybrids and 12 with CON hybrids. Each plot consisted of four, 5 m long rows of a single genotype. The inter-row spacing was 0.52 m and inter-plant spacing was 0.30 m. We sowed three seeds of each genotype per hill by hand, and thinned to one plant per hill 15 days after sowing. Pests, diseases and weeds were adequately controlled by recommended cultural and chemical methods (Díaz-Zorita and Duarte 2003). From flowering to physiological maturity, we covered heads of the plots with a net in order to prevent any previous damage from bird or some other pests. After physiological maturity, net was removed and the head were exposed to natural eared doves' consumption. We registered plant phenology according to Schneiter and Miller (1981), establishing R9 on each plot when 95% of the plants reached stage physiological maturity. We measured damage incidence in the plots as the relationship between numbers of heads damaged per plot and total heads per plots (see Eq. (1) below), and visually estimated damage severity (achene removal) as mean proportion of sunflower head with achenes consumed by eared doves (method adapted from Dolbeer 1975). We were able to attribute the damage mainly to eared doves due to its characteristics (loss of achenes in advanced physiological maturity without breakage of the edge of the sunflower head). We conducted both measures of damage (incidence and severity) 15 and 30 days after physiological maturity (DAPM).

$$\text{Damage Incidence} = (\text{NDH}/\text{NTH}) * 100 \quad (\text{Equation 1})$$

NDH = Number of heads damaged per plot.

NTH = Number of total heads per plot.

To determine the average size of the achenes, we randomly selected a sample of 50 achenes collected from three-marked plant in physiological maturity and covered with a pollination bag at grain filling stage to avoid bird damage during the field test. We selected achenes located between ring 4 and ring 19 of the three sunflower head (Izquierdo et al. 2008) and measured length (L), width (W) and thickness (T) to 0.01 mm for each individual achene using a micrometer. We then removed achene coats with tweezers and calculated the hull percentage to total achene weight (HP) and hull to embryo ratio (H:E) (Santalla and Mascheroni 2003; De Figueiredo et al. 2011). We measured oil percentage of whole achene with Nuclear Magnetic Resonance (NMR) equipment (Spinlok, Córdoba, Argentina).

In cage conditions, we conducted a two-choice feeding tests using 24 healthy non-sexed adult eared doves kept in captivity at the Parana Experimental Station communal aviary (31° 50' 53" S, 60° 32' 19" W). Doves were captured with bait traps and held in outdoor group-holding cages (2 m width × 9 m length × 2.5 m height) for at least one month before the test. Then, they were moved to indoor individual cages (0.50 m width × 0.50 m length × 0.50 m height) for the test trial. Maintenance food consisted on a commercial seed mix (wheat, corn, sorghum, millet, and rape seeds) for doves, sold by a commercial store in Paraná city ("Forrajería Laurita", Paraná, Entre Ríos, Argentina). Bird housing and general care on the aviary followed published guidelines regarding the use of wild birds in research (Fair et al. 2010).

## Eared dove preferences in cage conditions

Based on the morphological and nutritional characteristics of each hybrid and the results from the field trials, we tested the preference of hybrid types by eared doves in individual cages. For this purpose, we offered seeds of the BO hybrid (with smaller achenes, higher nutritional value and higher damage in the field) and one of the other three hybrid types (i.e., BO vs. CON, OC, and SO), in paired conditions (two-choice tests). Additionally, we tested the preference of OC versus SO hybrids, given the color of the outer hull and oil percentage of the achene of both hybrids were similar, but not the achene size (OC achene size > SO). In this exploratory study, we did not test preference of OC versus CON, because CON hybrids have very limited use in the field due to limited commercialization, compared to BO and SO (Feoli and Ingaramo 2015). However, we recognize this evaluation will be necessary to have a more complete spectrum of eared dove preferences for sunflower hybrids and to determine physical and chemical characteristics of sunflower seeds influencing preferences by eared doves in a more conclusive way.

Eared doves were weighed ( $\pm 0.1$  g) and randomly assigned to one of the four groups of two-choice tests ( $n = 6$  doves group<sup>-1</sup>), considering a homogeneous distribution of weight on each group (i.e., eared doves with low and high weights were distributed equally in each group). The test extended for 19 consecutive days. During the first 7 days, we offered water, grit, and maintenance food *ad libitum* to each eared dove. Maintenance food was offered in two separate cups, one on each side of the cage. For the next 7 days, we offered an equal mix in both cups of all sunflower seeds used in the trial (BO, CON, OC, and SO) *ad libitum* for 5 h day<sup>-1</sup>, to accustom birds to test seeds. Finally, we tested the preference of eared doves for sunflower achenes during the next five consecutive days, offering the test seeds (8 g per cup of each kind of achene separately) for 5 h (between 08:45 and 13:45 h). Each day, we cleaned the cage floor and replaced maintenance food with test achenes, randomly assigning sunflower achene type to each cup. At the end of the 5 h, we collected achenes remaining in the feeding cup and on the floor, taking care to keep each achene hybrid separate, and we replaced them with maintenance food. We also measured the humidity factor by weighing cups of seeds (2 for each hybrid) not offered to doves at the start and the end of the 5 h. Finally, we estimated the daily consumption of each hybrid by calculating the difference between offered and remaining seeds, including the humidity factor (Addy Orduna et al. 2010). At the end of the fifth test day, we weighed eared doves ( $\pm 0.1$  g) to determine the final weight.

We estimated absolute and relative daily achene intake ( $\pm 0.1$  g) of each eared dove for each test day. Absolute achene-intake for each hybrid was estimated based on the weight of the offered achenes minus the weight of the remaining achenes, using the Eq. (2).

$$Ca = (W0 \pm H) - Wr \quad (\text{Equation 2})$$

W0 = weight of sunflower achenes offered in each cup at the beginning of the test ( $8.0 \pm 0.1$  g).

H = weight variation on “control cups”, i.e., the initial achene weight ( $8 \text{ g} \pm 0.1 \text{ g}$ ) minus the weight at the end of 5 h of treatment exposure.

Wr = weight ( $\pm 0.1$  g) of uneaten/remaining sunflower achenes at the end of the test period (5 h).

We did not have expended hulls included in the weight measurements because eared doves' swallowed whole achenes and grind them in the gizzard (as dove species do with the seeds, Gill 2001). In order to standardize and compare daily achene intake for each hybrid (based on weight measurements) on a standard basis, we estimated relative daily achene intake (Cr, Eq. (3)) as the proportion of achene intake of each hybrid (Ca) related to the total achene intake of the individual eared dove each day (Tc):

$$\begin{aligned} Cr \text{ hybrid 1} &= Ca \text{ hybrid 1} / Tc && (\text{Equation 3}) \\ Tc &= Ca \text{ hybrid 1} + Ca \text{ hybrid 2} \end{aligned}$$

## Statistical analyses

In order to determine eared dove's selection of sunflower hybrids in field conditions, we analyzed achene shape (length, thickness, width, grain weight, hull proportion, H:E) and grain quality (oil percentage, protein percentage, oil yield and protein yield). We used damage incidence (number of damaged plants) and severity (proportion of removed achenes per head) as a proxy for type of hybrid selection by eared doves. We tested for normality assumptions using Q-Q plots and for homoscedasticity comparing predicted values by residuals. All field data met the assumptions for General Linear Mix Model. We considered type of hybrid and days after physiological maturity (DAPM) as fixed effects. Genotype and plot were considered random effects. We determined statistically significant differences among hybrids means using Least Significant Difference (LSD)'s multiple-range tests ( $p < 0.05$ ).

For the two-choice tests in individual cage conditions, we compared mean Absolute Achene Intake (Ca) and Relative Achene Intake (Cr) of each hybrid for the total test period using Kruskal-Wallis. To assess preference of hybrids and potential changes over the days, we analyzed the 5-day treatment period in a repeated measures ANOVA with the relative daily achene intake (Cr) of each hybrid as dependent variable, the type of hybrid as inter-subject factor, and day and type of hybrid  $\times$  day as intra-subject factors. Additionally, we used repeated measure ANOVA tests to determine if the total achene intake (Tc) and the body mass (g) of eared doves changed during the 5-day test period. In each case, we considered Tc or body mass as the dependent variable, the group as the inter-subject factor, and the day and group  $\times$  day as intra-subject factors. In all cases, we verified the assumptions of normality and homoscedasticity in the residues of the model using Q-Q plots and comparing the predicted with observed values. We used Infostat (Di Rienzo et al. 2017) to perform statistical analyses and SigmaPlot (SigmaPlot 8.0, SPSS Inc., Chicago, IL) to graph the relationships between variables.

## Results

### Eared dove selection of sunflower hybrid types in field conditions

The average time from sowing to flowering of sunflower hybrids was 84 days (range = 81–88) with no significant difference among hybrids (General Linear Models,  $p > 0.0894$ ). However, days to physiological maturity was significantly different among hybrids (BO = 129, SO = 142, OC = 123 and CON = 125 days;  $F = 125$ ,  $DF = 31$ ,  $p < 0.001$ ). Mean length, width; thickness of achenes and hull thickness significantly differed among hybrids (Table 1). The CON hybrids were 80% longer and 64% wider than BO hybrids had the heavier achenes ( $p \leq 0.0001$ , Table 1). The achene size of OC hybrids was similar in thickness and width but 25% shorter compared to CON hybrids. The proportion of hull to seed ( $p = 0.0018$ ) and the hull:embryo ratio (H:E,  $p = 0.0017$ ) were also significantly different between the CON hybrids and all other hybrids (BO, SO, and OC) with the biggest proportion in the CON hybrids (Table 1).

**Table 1:** Sunflower shape achene characteristics (length, thickness, width, whole grain weight, hull proportion and hull: embryo ratio [Hi:E]) and grain quality in oil, protein, oil yield, protein yield of sunflower black oilseeds (BO), stripped oilseeds (SO), oilseed × confectionary (OC) and confectionary (CON) hybrids.

Length (mm)	Thickness (mm)	Width (mm)	Whole Grain Weight (mg)	Hull Proportion (%)	Hi:E Ratio	Oil (%)	Protein (%)	Oil yield (mg grain <sup>-1</sup> )	Protein yield (mg grain <sup>-1</sup> )	
BO	9.5 ± 0.3 c	3.1 ± 0.1	5.1 ± 0.1 b	45 ± 1.6 c	31 ± 2.9 b	0.46 ± 0.07 b	53 ± 0.7 a	13 ± 0.4 b	23 ± 1.1 b	6 ± 0.4 c
SO	10.5 ± 0.3 c	4.5 ± 0.3	2.8 ± 0.3 c	45 ± 2.9 c	35 ± 1.9 b	0.55 ± 0.05 b	47 ± 0.6 b	12 ± 0.6 bc	21 ± 1.3 b	6 ± 0.5 c
OC	13.7 ± 0.4 b	3.7 ± 0.4	7.4 ± 0.8 a	68 ± 3.0 b	30 ± 0.4 b	0.42 ± 0.01 b	45 ± 0.9 b	15 ± 0.4 a	30 ± 1.0 a	10 ± 0.4 b
CON	17.1 ± 0.7 a	3.8 ± 0.3	8.4 ± 0.8 a	107 ± 1.7 a	46 ± 2.5 a	0.87 ± 0.08 a	29 ± 2.1 c	11 ± 0.6 c	31 ± 1.3 a	12 ± 0.5 a
F	36.4	1.9	10.51	31.3	46.1	10.55	164.3	19.9	27.5	98.1
p-Value	<0.0001	NS	0.0007	<0.0001	<0.0001	0.0017	<0.0001	0.0001	<0.0001	<0.0001

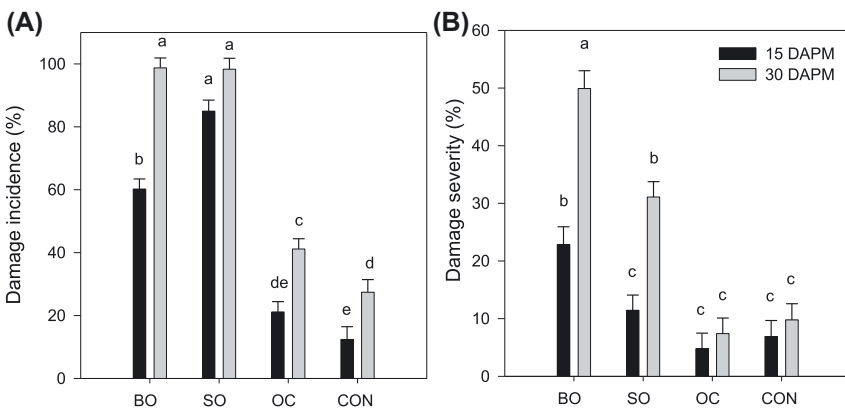
Mean ± standard errors. Different letters represent statistical differences among types of hybrids.

Although CON achenes were larger compared to BO achenes, CON had less oil content (Table 1). In addition, the OC hybrids had similar oil concentration to SO (Table 1). The achene protein concentrations were statistically different among sunflower hybrids, were BO hybrids had the highest protein percentage (13%) and the CON the lowest (11%, Table 1).

The incidence and severity of damage at 15 days after plant maturity (DFPM) at the field scale showed a statistically significant difference in sunflower selection by eared doves ( $R^2 = 0.73$  and  $0.84$  for incidence and severity, respectively;  $p \leq 0.0001$ , Figure 1). The incidence and severity of damage at 30 DFPM was higher than at 15 DFPM (67 and 47%, respectively). Damage incidence was higher in BO and SO hybrids than OC and CON hybrids (Figure 1A). We observed a similar trend for damage severity (percent achene intake, Figure 1B.).

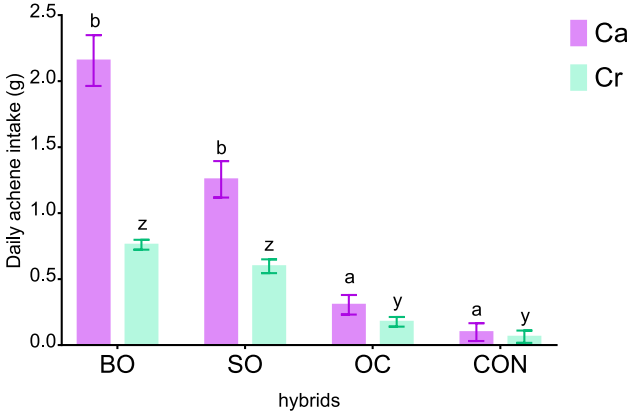
### Eared dove preferences of sunflower hybrids in cage trials

Overall, the absolute (Ca) and relative (Cr) achene intakes of each hybrid were significantly different among each other ( $H = 70.01$ ,  $p < 0.001$ ;  $H = 72.12$ ,  $p < 0.001$ , respectively), with a consistent pattern of decrease in achene intake (both in Ca and Cr) from BO (higher intake) to CON (lower intake, Figure 2). The general pattern of eared dove preferences (BO > SO > OC > CON) also was found on a daily basis (Figure 3). OC and CON hybrids were significantly less preferred than BO and SO

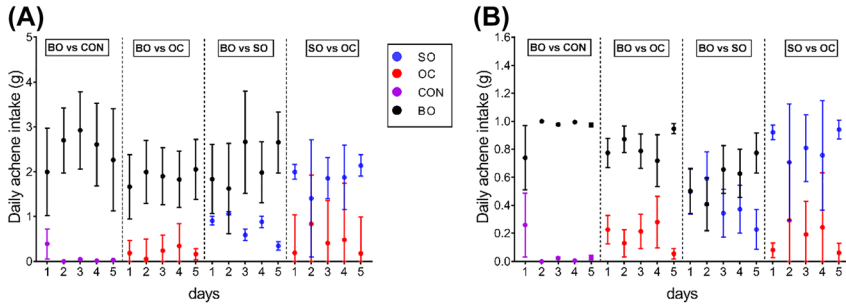


**Figure 1:** Damage incidence by eared doves (*Zenaida auriculata*) (A) and damage severity (B) evaluated in BO, SO, OC and CON hybrids at 15 and 30 days after plant maturity (DAPM) in the field experiment. Different letters represent statistical differences between DAPM and hybrids. Verticals lines correspond to standard errors.





**Figure 2:** Mean sunflower absolute and relative achene intake measured in BO, SO, OC and CON hybrids during the 5-h test trial of eared doves (*Zenaida auriculata*). Differences in letters above each bar denote significant differences ( $p < 0.05$ ) among the four hybrid types for absolute achene intake (Ca, Eq. (2)) and relative achene intake (Cr, Eq. (3)), a – b and y – z, respectively. Verticals lines correspond to standard errors.



**Figure 3:** Absolute (A) and relative (B) daily achene intake (Ca and Cr) in grams (mean  $\pm$  S.E.M.) measured in BO (black), SO (blue), OC (red) and CON (purple) hybrids during the 5-h test trial of eared doves (*Zenaida auriculata*).

hybrids ( $p < 0.01$  for BO vs. CON and BO vs. OC, and  $p = 0.01$  for SO vs. OC; Figure 3A and B). Instead, the relative achene intake (Cr) of BO and SO was not statistically different ( $p = 0.12$ ; Figure 3B). Finally, we observed not effect of test day or interaction of group  $\times$  day on Cr of each hybrid type during the tests ( $p > 0.05$  for all days and choices).

Eared doves ate slightly more total achenes (Tc) when smaller oilseed hybrids were offered at the same time (BO vs. SO:  $2.91 \pm 0.61$  g) compared to the other

paired-choice tests (BO vs. CON:  $2.60 \pm 0.75$  g; BO vs. OC:  $2.08 \pm 0.67$  g; and SO vs. OC:  $2.27 \pm 0.67$  g). However, these differences were not statistically significant ( $F_{(3,100)} = 0.33$ ,  $p = 0.8068$ ). Total achene intake ( $T_c$ ) of eared doves was relatively stable during the 5-days paired-preference trial [ANOVA: Group:  $F_{(3,100)} = 0.33$ ,  $p = 0.8068$ ; Day:  $F_{(4,100)} = 1.36$ ,  $p = 0.2549$ ; Group  $\times$  Day:  $F_{(12,100)} = 0.46$ ,  $p = 0.9293$ ]. Similarly, body mass of eared doves did not change during the 20-day test period, independently of the paired-choices that were offered [ANOVA: Group:  $F_{(3,19)} = 1.94$ ,  $p = 0.1670$ ; Cov (Initial weight):  $F_{(1,19)} = 51.60$ ,  $p < 0.0001$ , Coef: 0.92], indicating that day or test extension did not impact the health condition of eared doves.

## Discussion

Eared doves showed selective preferences for smaller oilseed hybrids (BO and SO), compared to confectionary (CON) and oilseed  $\times$  confectionary (OC), both in field and cage trials. This pattern of preference for oilseed hybrids compared to confectionary sunflower has been observed previously for eared doves (Rodríguez and Zaccagnini 1998; Zaccagnini 1985; Zuil and Colombo 2012) as well as with black-birds (Icteridae). Based on previous studies comparing sunflower achene consumption by birds, not including eared doves, we could expect preferences to be related to morphological (achene length, width and weight) or nutritive quality (oil and protein content) characteristics of achenes (Dedio 1995; Mason et al. 1989; Seiler and Rogers 1987).

In this study, eared doves preferred hybrids with the lower achene length, width and weight (BO and SO). In fact, morphological characteristics of the achenes seemed to be more important than the achenes' quality (in terms of oil and protein content) for explaining dove's preferences. We base this statement on the fact that the SO hybrids are smaller, thinner and lighter but had a similar oil content to OC, and a similar protein content with CON hybrids (both hybrids less preferred than the SO), indirectly indicating that achene morphological characteristic and not oil and protein were related to eared doves preferences. However, the most preferred hybrid (BO) had significantly higher oil and protein content than the less preferred hybrid (CON), indicating a potential confounding factor of achene quality and morphological characteristics on eared dove preferences in this case.

Hull size is also another morphological characteristic that could explain dove's preference for high-oil achenes (Greig-Smith and Crocker 1986). In this study, however, oilseed hybrids (BO and SO) had statistically similar hull percentage and hull-embryo ratio than the oilcon hybrid (OC). However, OC was

consistently less damaged (in the field) and preferred (in the cages) than the BO hybrid. Therefore, hull size apparently did not influence achene's preferences by eared doves. Additionally, eared doves showed generally lower preferences for stripped hybrids (SO, OC and CON) than black seed hybrids (in coincidence with results from Zuil and Colombo 2012), indicating eared doves also may use cues based on visual aspect or color to select sunflower seeds.

Whatever cues or learning mechanisms eared doves use to discriminate among hybrids in the field, it is clear that these birds prefer small achenes, in spite of other achene features. Moreover, our study results showed that eared doves preferred achenes primarily based on size and secondly on oil content. Seed handling time, a factor that influences feeding behavior of birds such as the red-winged blackbird on rice (Daneke and Decker 1988) and sunflower seeds (Mah and Nuechterlein 1991), it's not a factor we expected to influence feeding behavior of eared doves in sunflower achenes, because eared doves swallowed complete achenes. To our knowledge, this is the first study exploring eared doves' preferences for multiple kinds of sunflower hybrids, based on their achene's characteristics. Our findings are consistent with the food ecology of eared doves, given they mostly eat small achenes of annual plants (including both cultivated and wild plants, Murton et al. 1974). Additionally, they are consistent with empirical observations of reduced damage observed for 'resistant' sunflower hybrids in the field (Zuil and Colombo 2012).

Based on our results, farmers could plant hybrids with bigger achene sizes (e.g.; confectionary kind sunflower hybrids), in order to reduce eared doves damage in areas with high pressure from eared dove's populations. However, growers need to consider economic factors, including market value of achenes and additional plant features of hybrids such as head inclination and head-to-stem distance (Khaleghizadeh 2011; Linz and Hanzel 1997; Seiler and Rogers 1987; Yasumoto et al. 2012), in cases where "resistant" hybrids (i.e., confectionary kind) are not economically feasible. Additionally, farmers and growers should consider the influence of alternative foods for eared doves on the surroundings of the sunflower fields. In this study, alternative seed choices were available for eared doves in both the field and cage studies. This would be the most probable scenario in the field, given the multiple seeds eaten by eared doves on agroecosystems (Bucher and Nores 1976; Calvo Silvera 2006) and the wide range of movement of eared doves following availability of food (Bucher 1998). However, we cannot assure that damage will be reduced in a field setting where only a single hybrid is planted (even if less desirable by eared doves). Especially, considering that eared doves ate achenes of the four tested hybrids in this study, even when offered preferred choices. In spite of this, personal observations conducted on northern Argentina (an area with sunflower crops usually affected by eared dove damage)

suggest that when a unique hybrid (an striped oilseed hybrid, such as DK 4045) dominated the landscape, eared dove damages to sunflower tended to decrease (Zuil S., *per.obs.*). Therefore, it is possible to speculate that planting hybrids with bigger achene sizes (e.g.; confectionary kind sunflower hybrids) would help to reduce eared doves damage in areas with high pressure from eared dove's populations.

In this work, we added the novelty of a hybrid type (OC) that combine good agronomic characteristics and bird resistant traits, found in oil and confectionary hybrids, respectively (Zuil et al. 2016). The OC hybrids also have an excellent agronomic behavior, based on yield, grain number, grain weight and oil percentage (Zuil, *unpublished data*). Therefore, it could be an excellent alternative to striped oilseed hybrids (e.g., DK 4045), which are currently used to prevent eared dove's damage to sunflower crops in northern Argentina (Zuil, S., *pers. obs.*) and to confectionary hybrids, which have limited commercialization (Feoli and Ingarano, 2015). The confectionary market is much smaller compared to the oilseed hybrids because it is a specialty and, consequently, most of the production is backed up by a supply contract in which achene size determines the premiums.

## Conclusions

The combination of visual characteristics, size and nutritional value of achenes seems to be an encouraging path to determine preference and selection of sunflower hybrids by eared doves and, consequently, a potential tool for damage reduction in the field. The development of sunflower hybrids less prone (or more "resistant") to eared dove's damage may represent a cost-effective strategy to prevent damage at the field scale. Future research to develop these sunflower hybrids should explore additional phenotypic characteristics of sunflower plants, such as maturation rate, head curvature, and achene density, on hybrids of similar oil content and contrasting achene size to assess whether the interaction of such factors may influence bird damage. Additionally, analyzing the genotypic background and marking the specific genes that codify achene size in sunflower will help plant breeders to develop new bird-tolerant hybrids. Finally, including bird damage evaluations in relation to plant phenotypes into plant breeding programs could potentially help improve management to reduce eared dove damage to sunflower crops.

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