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Optimizing sunflower yield: Understanding pollinator contribution to inform agri-environmental strategies

Lucie Mota [a,*], João Loureiro [a], José A. González [b,c], Violeta Hevia [b,c], Jorge J. Ortega-Marcos [d], Carlos Rad [e], Evan A.N. Marks [f], Sílvia Castro [a]

[a] Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[b] Social-ecological systems Laboratory, Universidad Autónoma de Madrid, Madrid, Spain
[c] Centro de Investigación en Biodiversidad y Cambio Global (CIBC-UAM), Universidad Autónoma de Madrid, Madrid, Spain
[d] Área de Investigación Aplicada y Extensión Agraria, Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario, El Encín, Alcalá de Henares, Spain
[e] Grupo de Investigación en Compostaje UBUCOMP, Universidad de Burgos, Facultad de Ciencias, Burgos, Spain
[f] Agroquímica y Medio Ambiente. Universidad Miguel Hernández, Elche, Spain

A R T I C L E I N F O

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Crop yield
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A B S T R A C T

Context: The agricultural intensification due to global increased food demand has harmed pollinator communities worldwide. However, some of the economically most important oilseed crops, such as the sunflower, depend on pollinators to produce seeds. While self-fertile varieties have undergone genetic selection to guarantee productivity, the pollinator-dependence levels and the economic contribution of pollinators have not been fully estimated.
Objective: Here, we aimed to explore floral and pollinator constraints limiting the agricultural yield of sunflower varieties most frequently used in the Iberian Peninsula.
Methods: Pollination experiments were undertaken to analyse the pollinator-dependence level of 12 varieties under controlled conditions and also under natural conditions in 23 fields of two Spanish agricultural regions. The selfing ability and economic contribution of pollinators were estimated by comparing bagged and open-pollination treatments.
Results: Our results showed that the degree of pollinator-dependence is highly dependent on sunflower variety, with impacts on production and productivity outcomes, e.g. individual plant yield values varied between 0.188 and 0.692. Several varieties could self-fertilize and produce seeds regardless of pollinators. However, outcrossing significantly increased seed set in most varieties with increments up to 0.341. Overall, a trade-off between the number and weight of seeds was observed. Under natural field conditions, pollinators significantly increased overall sunflower production, although differences were observed among regions (increment of 275 kg/ha in Burgos and 382 kg/ha in Cuenca), with an associated economic outcome.
Conclusions: The self-fertilization ability and the level of pollinator-dependence vary according to the intrinsic reproductive traits of the variety analysed. Although some varieties are able to produce seeds despite the absence of pollinators, the sunflower clearly benefits from insect pollination. The landscape context and the availability of pollinator communities influenced the final crop yield and the economic outcome.
Significance: Combining landscape-restoring interventions with the cultivation of self-compatible varieties during at least the first years of implementation may be a solid additional agri-environmental strategy to maintain production levels and economic outcomes, which may particularly mitigate effects of pollinator and biodiversity losses mainly in highly simplified agroecosystems.

* Correspondence to: Centre for Functional Ecology – Science for People & the Planet, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, Coimbra 3000-456, Portugal.
E-mail address: uc2007109365@student.uc.pt (L. Mota).

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1. Introduction

Increased global food demand, directly related to human population growth, has led to a higher need for agricultural production intensification worldwide. However, this increased demand faces a paradigm: while agricultural intensification is one of the most important causes of biodiversity loss of insect pollinators (Powney et al., 2019; Purvis et al., 2019; Wagner, 2020; Raven and Wagner, 2021), the percentage of pollinator-dependent crops, and consequently our dependence on biodiversity for food production, has increased (Aizen et al., 2008, 2009) as many crops rely to some degree on pollinators for producing seeds and fruits for both direct and indirect human consumption (Klein et al., 2007; Aizen et al., 2008, 2009; Siopa et al., 2024). According to Food and Agriculture Organization of the United Nations (FAO, 2022) "*three out of four crops across the globe producing fruits or seeds for human use as food depend, at least in part, on pollinators*." Recent studies estimate that 35 % of global agricultural land depends on animal pollination, ensuring the production of 87 of the leading food crops worldwide and at least 800 cultivated plant species (Klein et al., 2007; Nicholls and Altieri, 2013; Potts et al., 2016). In 2005, insect pollinators were estimated to be responsible for 9.5 % of the total economic value of agricultural production used directly for human food, which represented 153 billion Euros annually (Gallai et al., 2009; reviewed in Khalifa et al., 2021). As pollinators are crucial to the sexual reproduction of many plants, it is expected that the decline of pollinating species can lead to parallel declines of several plant species (Richards, 2001; Biesmeijer et al., 2006; Aizen et al., 2009), including many crops (McGregor, 1976), with expected ecological (Biesmeijer et al., 2006; Potts et al., 2010; Thomann et al., 2013; Powney et al., 2019) and economic consequences (Bauer and Wing, 2016; Majewski, 2014; Giannini et al., 2015; Reilly et al., 2020; Khalifa et al., 2021). However, despite substantial effort worldwide (e.g., Klein et al., 2007; Kremen and Merenlender, 2018; Allen-Perkins et al., 2022; Siopa et al., 2023, 2024), we still lack information on pollinator-dependence for many crops and varieties as well as, more specifically, on pollinator contribution in different landscape contexts (Bartomeus et al., 2014; Castro et al., 2021; Siopa et al., 2024).

Landscape simplification in intensely cultivated areas has been the most problematic cause of global biodiversity loss (Holzschuh et al., 2007; Garibaldi et al., 2011; Montero-Castaño and Vilà, 2012; Jonsson et al., 2015; Raven and Wagner, 2021) with the maintenance of pollinator communities and associated pollination services currently among the most significant concerns. Thus, several agri-environmental strategies have been proposed worldwide to preserve pollinators in agroecosystems. For example, in the US, the Agriculture Department and the Environmental Protection Agency annually discuss and propose actions for pollinator conservation and protection (e.g., the 2022 Annual Strategic Pollinator Priorities). In Europe, the Common Agricultural Policy (European Commission, EU) was created to improve agricultural productivity, maintain rural landscapes and conserve natural resources. Additionally, the EU also adopted the EU Pollinators Initiative with long-term strategic objectives. According to these objectives, agri-environmental strategies have been proposed and debated (European Commission, 2020), namely: the maintenance of natural and semi-natural patches near crop fields and management of uncultivated field boundaries (Mandelik et al., 2012; Blaauw and Isaacs, 2014; Söderman et al., 2016; Sutter et al., 2018; Kleijn et al., 2019; Albrecht et al., 2020; Mota et al., 2022), and the implementation of enhanced green infrastructures as flower strips and hedgerows on field margins using seed mixtures of melliferous plants (e.g., Scheper et al., 2015; Mota et al., 2022). Nevertheless, the implementation of agri-environmental strategies and desired impacts on biodiversity and its services require time following implementation to become effective. For example, the implementation of flower strips produces results, on average, after two years (Blaauw and Isaacs, 2014; Buhk et al., 2018; Albrecht et al., 2020), depending on the landscape configuration and conservation status (Kremen et al., 2018; Mota et al., 2022). Thus, in annual crops, especially, further knowledge to cope with low pollinator availability is needed to maintain productive systems.

Sunflower (*Helianthus annuus* L.), as an oleaginous annual plant, has a high agricultural and economic importance in Europe, mainly in the southwestern region, where it is one of the most important oilseed crops (Velasco et al., 2015). To obtain oils for industrial (biodiesel) and alimentary (both human and animal) uses, the sunflower crop is produced in 4.1 M ha in Europe, with Spain being the fifth greatest producer of biodiesel (Velasco et al., 2015). As an allogamic plant, sunflower needs to outcross to produce seeds, and is dependent on pollinators (Chambó et al. 2011). The honeybee is the primary pollinator, but some studies have shown a diversity of insects involved in sunflower pollination (Greenleaf and Kremen, 2006; Nderitu et al., 2008; Hevia et al., 2016), with managed and wild bees being considered the most efficient pollinators (Mallinger and Prasifka 2017a; Zaragoza-Trello et al., 2023). Additionally, the behavioural interactions of wild bees can indirectly increase pollen transfer efficiency by honeybees, improving sunflower productivity (DeGrandi-Hoffman and Watkins, 2000; Greenleaf and Kremen, 2006). Also, the review by Klein et al. (2007) concluded that the increase of seed set is significantly related to wild bees' abundance and diversity, and categorized the need for animal-mediated pollination for this crop as modest. In the case of sunflower fields, both the maintenance of semi-natural patches and the implementation of flower strips contributed to maintaining the taxonomic and functional traits of wild bees (Hevia et al., 2021) and improving sunflower productivity (Mota et al., 2022). However, the flower strips implementation was unsuccessful in depauperated landscapes, likely because they need extended periods to restore biodiversity (Mota et al., 2022). Interestingly, to overcome the dependence on pollinators, self-compatible and partially to strongly autonomous self-pollinated sunflower varieties began to be developed in 1976 (Fick and Zimmer, 1976; Fick and Rehder, 1977; Fick, 1978; Low and Pistillo, 1986). Currently, there are numerous routinely used cultivars, inbreds and hybrids (Gandhi et al., 2005). The identity of sunflower varieties and the developmental stages of sunflower florets will determine the degree of dependence and contribution of pollinators to sunflower yield. However, information on self-incompatibility and self-ability, either in the literature or at seed providers, is scarce or inexistent. Thus, identifying varieties less dependent on pollinators may be an additional agri-environmental strategy while restoration actions of highly depauperated landscapes, where sunflower is intensively cultivated, are developed.

Considering all this, the main objectives of this work were: 1) to characterize the selfing ability of different sunflower varieties and their degree of pollinator-dependence under similar edaphic, climatic and biotic conditions; and 2) to estimate the contribution of the pollinators to sunflower productivity under natural field conditions, i.e., exposed to existing pollinator communities. For objective 1, controlled pollination experiments were performed under common garden conditions to assess the selfing ability and pollinator-dependence of 12 sunflower varieties frequently used in the Iberian Peninsula by comparing pollinator exclusion and outcross treatments. For objective 2, in 23 fields in two regions of Spain, and using a similar set of sunflower varieties, the contribution of natural pollinator communities to sunflower productivity was evaluated by comparing pollinator exclusion and natural pollination treatments. Considering the development of new self-fertile sunflower varieties, we hypothesized that the sunflower varieties available on the market could vary in their selfing ability and, consequently, their degree of pollinator-dependence. Additionally, we hypothesized that the combined effect of selfing ability and available pollinator communities would produce different contributions of the pollinators to sunflower productivity.

2. Material and methods

2.1. Experimental design

Two experiments involving a total of 18 different sunflower varieties were prepared to address each of the objectives of this study. First, a common garden experiment at the Botanical Garden of the University of Coimbra (BGUC) was conducted to assess pollinator-dependence. For this, 12 sunflower varieties frequently used in the Iberian Peninsula (Supplementary Material - Table S1) were cultivated under the same biotic, climatic and edaphic conditions from May to October 2019. Seeds were sown directly in the soil in plots of 2 × 10 m, separated up to 30 cm apart, following the general sunflower cultivation recommendations. A total of 384 individuals were obtained, including 32 individual plants per variety (Section 2.2).

Second, the productivity of 16 sunflower varieties (some of them being the same as in the common garden experiment; Supplementary Material - Table S1) was estimated under natural field conditions to assess pollinator contribution to sunflower productivity. For this, 23 sunflower fields from two regions of Spain (Burgos and Cuenca) from June to October 2017 and 2018 (Supplementary Material - Table S1) were selected. The field experiment was conducted in a real production scenario with sunflower farmers of the region; thus, cultivation followed all the conventional management practices for sunflower and varieties selected by the farmers in each region. At each field, up to 32 individual plants were selected and marked at four distances from the field margin, namely at 0, 15, 30 and 60 m, with eight sunflowers per distance separated by 15 m each (Supplementary Material - Table S1) (Section 2.3). The number of heads per hectare were obtained from inter-row and individual plant distances.

2.2. Common garden experiment – sunflower pollinator-dependence

In the common garden experiment conducted at the BGUC to assess sunflower pollinator-dependence, all sunflower inflorescences were isolated under tulle bags before the first florets opened and during the whole flowering period to exclude pollinators and prevent pollen transfer among varieties. Each inflorescence received one of the following treatments: pollinator exclusion (hereafter bagged) or manually outcrossed (hereafter outcrossed) treatments. Inflorescences assigned to the bagged treatment (16 individuals per variety, 192 individuals in total) were bagged and left unmanipulated until seed maturation (Fig. 1A). Except for varieties PR63A40 and P64LL62, inflorescences assigned to the outcross treatment (16 individuals per variety, 160 individuals in total) were also maintained bagged to avoid pollen flow among varieties and manually pollinated with a minimum of three other inflorescences of the same variety bearing mature male florets. Pollinations were made by gently rubbing each inflorescence assigned to the outcrossed treatment with pollen-donor inflorescences of the same variety until the stigmas were covered in pollen. As the florets of the inflorescence open gradually across several days and acropetally within the inflorescence, pollinations were made daily until the last female florets in the treated inflorescence were open (Fig. 1B). Pins were applied to cardinal points of the inflorescence to avoid self-pollination through the contact of the bag with the florets (Fig. 1B).

In the case of PR63A40 and P64LL62 varieties, the outcross treatment was obtained through open pollination (i.e., each variety was left unbagged for outcrossing by pollinator insects during the day and bagged at the end of the day) as it was not possible to have sufficient pollen donors (Fig. 1C).

Sunflowers were harvested for seed set, seed weight and yield quantification on a per-individual-plant basis. Thus, in mid-October 2019 (when seeds were already mature), all sunflower infructescences were collected in individual paper bags, air-dried, and stored. The productivity of each infructescence was quantified in a quarter of the flower head. Only the external half of the infructescence was used to quantify the seed set and seed weight to avoid resource limitation within the inflorescence. The total number of florets (not fertilized; recognized as dried florets that did not evolve further), empty seeds (recognized by being frequently smaller and always having the seed coat collapsed as a sign of no embryo), and full seeds were counted. Finally, all full seeds were dried at 68 °C for 48 hours and weighed on a precision scale (up to milligrams). The response variables to calculate sunflower productivity were the total number of seeds (seed set, sum of empty and full seeds), the weight of 100 seeds (seed weight for simplification purposes) and yield. Sunflower yield was calculated by multiplying seed set with seed weight of 100 seeds.

2.3. Field experiment – pollinator contribution to sunflower productivity

In the field experiments to quantify pollinator's contribution to sunflower productivity under natural field conditions, the selected sunflowers (as described in Section 2.1) were marked to receive one of the following treatments: pollinator exclusion (hereafter bagged) or open pollination treatments, totalling 175 individuals (96 in Burgos and 79 in Cuenca) in 2017 and 176 individuals (96 in Burgos and 80 in Cuenca) in 2018 (Supplementary Material - Table S1). In the bagged treatment, inflorescences were isolated from pollinators under tulle bags as described in Section 2.2 and left unmanipulated until seed maturation. In the open pollination treatment, inflorescences were not bagged to allow exposure to pollinator communities for the entire flowering period. In mid-October 2017 and 2018 (when seeds were already mature), all selected sunflowers were harvested for seed set, seed weight and yield quantification on a per-individual-plant basis, as described in Section 2.2. In this experiment, only the external half section of the flower head was used to quantify the seed set and seed weight to

Fig. 1. Photographs of sunflower treatments at the common garden: A – bagged; B – outcrossed (here illustrated without the bag and with the pins to avoid self-pollination through the contact of the bag); C – open pollination (only for PR63A40 and P64LL62 varieties). The experiment was developed at the Botanic Garden of the University of Coimbra (July 2019).

guarantee that all the florets were pollinated, while avoiding resource limitation problems.

The economic contribution of pollinators to sunflower production was quantified under real conditions, i.e., for the field experiment. Two different methods were used to estimate the economic contribution of pollinators under natural field conditions in Burgos and Cuenca regions: 1) using the field data and 2) applying the guidelines proposed by Gallai and Vaissière (2009).

First, the value of productivity per field (kg/ha) was estimated for open-pollinated and bagged sunflowers using the following formula:

Sunflower productivity = average number of full seeds per head * average weight of a seed * number of heads per hectare

Then, the difference between the value of the productivity per field obtained from open pollination and the one obtained from bagged sunflowers was calculated to estimate the contribution of pollinators. Finally, this contribution value was transformed into an economic value using the average market price of sunflower seeds in Spain over the last ten years (from 2010 to 2020; 0.389€/kg). The estimations were made for both regions and extrapolated for Spain, using the average sunflower cultivated area during the same decade, according to the Statistical Yearbook of the Spanish Ministry of Agriculture.

Additionally, the contribution of pollinators was also estimated according to the guidelines developed by Gallai et al. (2009) and Gallai and Vaissière (2009). Using producer's price (389€/ton) and sunflower crop production data (72,869 tons in Burgos and 106,004 tons in Cuenca; 701,004 tons for the whole of Spain), the economic value of insect pollinators for both regions and the whole country was estimated.

2.4. Statistical analyses

Generalised linear mixed models (GLMMs) were used to explore the effect of pollination treatment (bagged *versus* outcross) in seed set, seed weight and yield of sunflower varieties growing under common garden conditions, including variety as a random factor. Additionally, because variety plays a key role in reproductive outcomes, we explored differences between variety and treatments nested within the variety. The seed set was arcsine transformed, and all response variables were analysed using a Gaussian distribution with an identity link function.

GLMMs were used to explore the effect of pollination treatment (bagged *versus* open pollination) in seed set, seed weight and yield of sunflower varieties growing under natural conditions (field experiment), including variety, region and year as random factors. Seed set was arcsine transformed and yield square root transformed. All response variables were analysed using a Gaussian distribution with an identity link function.

To quantify the contribution of pollinators under natural conditions in each field, we used a response ratio (R, according to Hedges et al., 1999), which was calculated for every sunflower opened to pollinators (open pollination) as:

$$R = \ln(\text{treatment}/\text{control})$$

treatment refers to the response variable (seed set, seed weight and yield) obtained for the plant under natural open pollination, and *control* refers to the mean value of the same response variable obtained in the absence of pollinators (bagged treatment). Values below and close to zero indicate no pollinator contribution for the response variable. Values significantly higher from zero indicate a positive effect of pollinator communities at a given location. Then, a one-sample t-test was used to understand if the contribution of the pollinators was statistically significantly different from zero at each field for each response variable.

For all the statistical analyses described above, model validation was performed by visually inspecting the residuals to check heteroscedasticity and normality (Zuur et al., 2009). Statistical analyses were performed in R version 4.0.2 (R Core Team, 2014) using the packages 'car' with Type-III analysis of variance (Fox et al., 2015), 'nlme' for linear and nonlinear mixed models (Pinheiro et al., 2020), 'multcomp' for multiple comparisons after Type-III analysis of variance (Hothorn et al., 2017), and 'emmeans' for obtaining estimated marginal means (R Core Team, 2014).

3. Results

3.1. Pollinator-dependence levels in controlled conditions

Overall, treatment significantly impacted seed set ($F = 58.277$, $P < 0.001$) and seed weight ($F = 41.718$, $P < 0.001$), while no significant differences were detected in yield ($F = 3.1981$, $P = 0.0749$). Bagged sunflowers had a significantly lower number of seeds, but seeds were significantly heavier than outcrossed ones (Fig. 2A-B). Although not significantly different, the yield of outcrossing plants was higher ($P = 0.07$) than those of bagged plants (Fig. 2C), with values varying between 0.252 and 0.632 in outcrossing plants, and 0.188 and 0.692 in bagged ones.

Variety played a significant role in determining the reproductive outcome of sunflowers under controlled conditions (Supplementary Material - Fig. S1). Significant differences were observed between varieties for seed set, seed weight, and yield. In contrast, differences between treatments within each variety were observed only for seed set and seed weight (Table 1). In eight out of 12 varieties, the seed set was significantly higher in the outcross treatment than in the bagged treatment, with the outcrossed seed set increasing up to 34.1 % in comparison with the seed set in the bagged treatment (Fig. 3A). Seed weight showed an opposite pattern to that of the seed set. In four of the 12 varieties, seed weight was significantly higher in bagged treatment than in outcross treatment (Fig. 3B). Although the same trend was observed in the remaining varieties, no significant differences between treatments were observed (Fig. 3B). Finally, only one out of 12 varieties (Oleko) revealed significant differences in yield, with the bagged treatment having a surprisingly higher yield when compared with the outcross treatment. Most of the remaining varieties had higher yields after outcrossing than the bagged treatment, although only marginally significant for four varieties (Fig. 3C).

3.2. Pollinator contribution to sunflower productivity in natural field conditions

Overall, treatment significantly impacted the sunflower seed set ($F = 195.200$, $P < 0.001$), seed weight ($F = 23.493$, $P < 0.001$) and yield ($F = 83.650$, $P < 0.001$) (see Supplementary Material – Fig. S2). Bagged sunflowers had a significantly lower number of seeds, seed weight and yield than open-pollinated sunflowers (Fig. 4). However, the contribution of the pollinators varied across the 23 fields studied, but overall, similar trends for each of the studied variables were obtained within each field (Fig. 5). In Burgos, pollinators significantly increased sunflower productivity in five out of 12 fields, with an increment of 275 kg/ha, while no impact was detected in seven fields (Fig. 5). In Cuenca, pollinators significantly increased sunflower seed set in all fields and seed weight and yield in ten out of 11 fields, with an increment of 382 kg/ha (Fig. 5).

3.3. Economic contribution of pollinators to sunflower production

Estimations based on sunflower productivity obtained in the field and the FAO formula indicate that pollinators strongly contribute to the economy linked to sunflower production (Fig. 6). Overall, estimation values obtained through field data were higher than the ones obtained by FAO guidelines (Fig. 6). Using field data, sunflower pollination made by insects was estimated as 10 465 624 € and 24 425 892 €, in Burgos and Cuenca regions, respectively. For the whole of Spain, pollinators contributed 118 217 602 €, solely related to sunflower productivity.

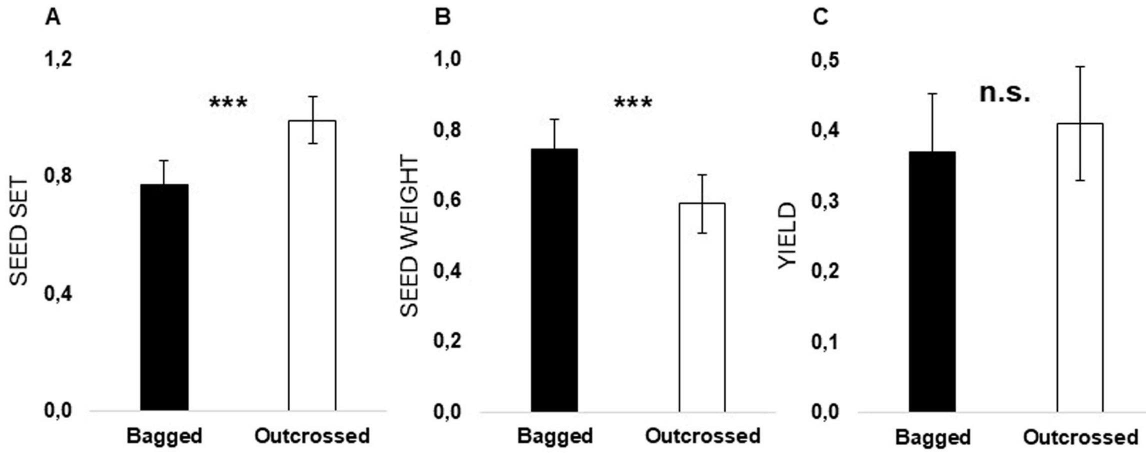

Fig. 2. Seed set (A), seed weight (B) and yield (C) for each treatment (bagged and outcross), combined across all varieties and obtained in controlled conditions in the Botanical Garden of the University of Coimbra. Seed set is the proportion of florets that developed into seed, seed weight is the weight of 100 full seeds (in mg), and yield is the product of seed set and seed weight. Values are given as estimated marginal means and 95 % confidence intervals. *** – significant differences at $p < 0.001$; n.s. – non-significant at $p < 0.05$.

Table 1
Effect of sunflower variety and treatment within variety (bagged and outcross) on seed set (proportion of florets that developed into seed), seed weight (weight of 100 full seeds, mg) and yield (product of seed set and seed weight).

Effect	Variety			Variety:Treatment		
Response variables	df	X^2	*P*	df	X^2	*P*
Seed set	11	7.9734	<0.001	12	5.2059	<0.001
Seed weight	11	9.9822	<0.001	12	7.5779	<0.001
Yield	11	11.9180	<0.001	12	2.4289	0.005

Applying the FAO formula, pollinators contributed 7 086 544 € in Burgos and 10 308 904 € in Cuenca. Extrapolating to the whole country, the estimation was 68 172 665 €.

4. Discussion

Pollination experiments under controlled and natural conditions enabled us to explore the floral (here represented as sunflower variety) and pollinator constraints limiting sunflower productivity. Our results show that selfing ability and, consequently, the degree of pollinator-dependence of sunflower varieties available in the market vary greatly and impact sunflower production and productivity costs. Several varieties can self-fertilize and produce seeds regardless of pollinators. However, outcrossing significantly increased seed set in most varieties. Interestingly, a trade-off was observed between the number of seeds and seed weight. Under natural conditions, pollinators significantly increased overall sunflower production, although differences were observed among fields. The results are discussed in detail below.

4.1. Sunflower pollinator-dependence levels

Intrinsic reproductive traits are major determinants of plant reproductive success (Fisher, 1930; Miller and Fick, 1997; Merilä and Sheldon, 2000). Thus, pollination experiments under controlled conditions allowed us to understand the reproductive behaviour of different sunflower varieties. Pollinator exclusion, obtained through the bagged treatment, impacted the reproductive variables analysed in this study differently. For most varieties (8 out of 12 varieties), the seed set after pollinator exclusion was significantly lower than outcrossed sunflowers. These results support previous observations that sunflowers need to outcross to produce more seeds (Chambó et al. 2011). Additionally, bagging experiments have been commonly used to quantify pollinator-dependence levels in many crop plants, namely those with high economic values worldwide, showing that insect-pollinated flowers develop more fruits and seeds compared with the bagged ones (Klein et al., 2007; Taki et al., 2009; Franceschinelli et al., 2013; Stanley et al., 2013; Giannini et al., 2015; Mulwa et al., 2019; Elisante et al., 2020). Here, the seed set obtained after bagged and outcross treatments showed that sunflower depends on pollinators, although bagged sunflowers produced some seeds in the absence of pollinators, being in accordance with the categorization as a modest pollinator-dependent crop made by Klein et al. (2007).

However, an opposite pattern was observed for seed weight, with bagged sunflowers having seeds significantly heavier than outcross ones (for 4 out of 12 varieties). The relationship between the seed set and the seed weight may be related to the allocation of the available resources of the plant (Gambín and Borrás, 2009). In nature, the relationship between seed set and seed weight may be negative, neutral or positive (reviewed by Gambín and Borrás, 2009), producing many small seeds or large ones, depending on the plant crop. In the case of sunflower, our results suggest a trade-off between the number and size of the seeds produced per sunflower head. This trade-off can be explained by morphological constraints related to the limited size of the inflorescence (Andrade et al., 2008), by photothermal conditions (Cantagallo et al., 2005), by evolutionary processes (Sadras, 2007) and/or by plant growth rates (Vega et al., 2001). Interestingly, trade-offs have been reported in other sunflower traits. For example, self-fertile hybrids have slightly lower oil contents and yields than male sterile plants because of the energy allocated for pollen production (Vear, 1984). Additionally, a previous review on trade-offs in grain crops suggested that in sunflower, selection for one inflorescence may have reduced seed number plasticity, while increasing seed size variability and its responsiveness to resource availability (Sadras, 2007).

Our pollination experiments also showed that sunflower varieties, to different degrees, are able to self-fertilize, producing seeds regardless of pollinators' absence. Autonomous self-fertilization (selfing ability) produced lower amounts of seeds with higher seed weight values, attaining statistically similar yield values as outcross pollination. Wild sunflowers presented sporophytic self-incompatibility (Ivanov, 1975; Fernandez-Martinez and Knowles, 1978) mainly due to cytoplasmic male sterility (Leclercq, 1969), thus requiring outcrossing for seed production. However, the domestication of sunflowers led to the selection and production of self-compatible cultivars, inbred lines and hybrids (Fick and Zimmer, 1976; Fick and Rehder, 1977; Fick, 1978; Gandhi et al., 2005) to reduce insect pollinator requirements and to maximize productivity (Onemli and Gucer, 2010). This selection

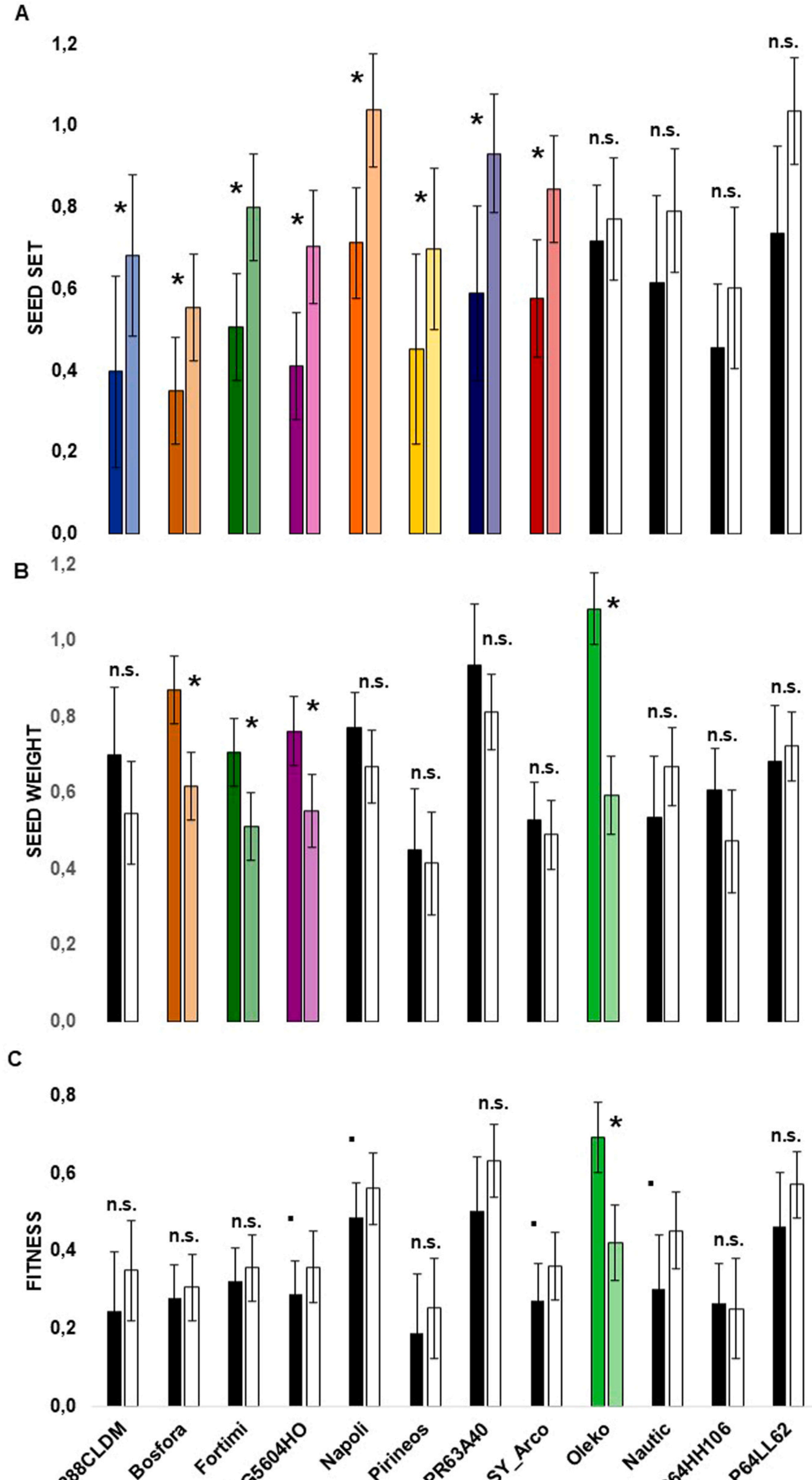

Fig. 3. Seed set (A), seed weight (B) and yield (C) of bagged (dark colour) and outcross (light colour) treatments made under controlled conditions in the Botanic Garden of the University of Coimbra for each sunflower variety. Seed set is the proportion of florets that developed into seed, seed weight is the weight of 100 full seeds (in mg), and yield is the product of seed set and seed weight. Values are given as estimated marginal means and 95 % confidence intervals. * – significant differences at $p < 0.05$ (also highlighted in bright colours); dot – marginal statistical differences between treatments at $p < 0.10$; n.s. – non-significant at $p > 0.10$.

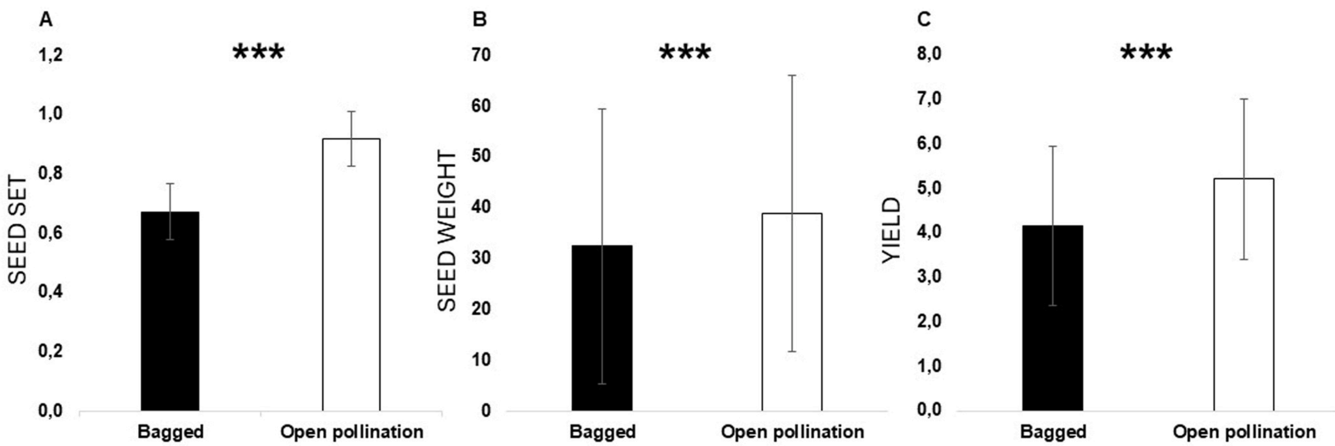

Fig. 4. Seed set (A), seed weight (B) and yield (C) for each treatment (bagged and open pollination) obtained in sunflower fields of Burgos and Cuenca regions. Seed set is the proportion of florets that developed into seed, seed weight is the weight of 100 full seeds (in mg), and yield is the product of seed set and seed weight. Values are given as estimated marginal means and 95 % confidence intervals. *** – significant differences at $p < 0.001$.

resulted in numerous varieties with a wide range of selfing abilities and pollinator-dependence, as shown here.

The reproductive success of the studied sunflower varieties showed no significant difference between bagged and outcross treatments (except for the Oleko variety) because of the seed number and seed weight trade-offs. However, it is important to note that outcross plants presented higher yield values than bagged ones overall ($P = 0.07$) and for most varieties (marginally significant for 4 out of 12). This small but consistent trend is expected to scale up at the field level and likely produce significant increases in sunflower production. Contrary to all expectations, Oleko had similar seed production between treatments and produced significantly heavier seeds after selfing, suggesting that this reproductive strategy is particularly advantageous for the productivity of this variety.

4.2. Pollinator's contribution to sunflower productivity

Pollinators are crucial for crop productivity (Aizen et al., 2008), and pollination experiments under natural field conditions allowed us to understand their contribution to the productivity of the sunflower varieties cultivated in two important regions of sunflower growth. Open-pollinated sunflowers produced significantly higher values of seed set, seed weight and yield than sunflowers excluded from pollinators, independently of the region analysed. These results confirm the importance of the pollinator's communities to increase the yield of sunflower fields as shown in previous studies (DeGrandi-Hoffman and Watkins, 2000; Chambó et al. 2011; Greenleaf and Kremen, 2006; Nderitu et al., 2008; Hevia et al., 2016) and in many other crops (e.g., Franceschinelli et al., 2013; Mulwa et al., 2019; Elisante et al., 2020; Castro et al., 2021).

Additionally, our results show that the contribution of the pollinators varied between sunflower varieties and field locations. In Cuenca, pollinators significantly and positively contributed to seed set (11 out of 11 varieties), seed weight (10 out of 11) and yield (10 out of 11), significantly improving sunflower yield. In Burgos, pollinator contribution was lower and significantly positive only in some sunflower fields, suggesting that most of the sunflower productivity results from self-fertilization in this region. Overall, the differences between fields result from a combination of a variety selfing ability and available pollinator communities, the latter depending on the local conditions (e.g., landscape features such as the presence of natural habitats) to sustain pollinator populations (Holzschuh et al., 2007; Garibaldi et al., 2011; Bartomeus et al., 2014). Indeed, differences in landscape context between the two regions studied have already been pointed out in a previous study (Mota et al., 2022). On the one hand, the landscape in Burgos has become highly homogeneous, with extensive agricultural fields and few natural vegetation patches which compromises the pollinator communities and their services. Consequently, pollination in this region has been described as poor, leading the sunflower productivity to rely mainly on selfing ability and likely compromising sunflower productivity (Mota et al., 2022). On the other hand, Cuenca has more seminatural patches richer in resources for pollinators near crop fields, forming a more heterogeneous landscape and allowing the maintenance of richer and more abundant pollinator communities that significantly contribute to sunflower productivity (Mota et al., 2022, and results herein).

4.3. Economic contribution of pollinators to sunflower production

Estimating the economic contribution of pollinators to sunflower productivity allowed us to better understand the importance of these insects to the regional (from 10 M€ to 24 M€) and national (118 M€) economy in Spain. The same pattern was obtained with both estimation approaches, with higher economic values in Cuenca than in Burgos. These results reveal that pollinators positively contribute to the sunflower industry, representing a crucial economic outcome in the order of millions of Euros in both regions studied, with a consequently significant impact at the national scale.

The contribution of pollinators depends on the sunflower variety and its self-compatibility, self-fertilization rate and pollinator-dependence level, as discussed above. Thus, the benefits of insect pollination to crop yield depend on intrinsic crop pollination requirements and the available abundance and diversity of pollinators (Mallinger and Prasifka 2017b; Mallinger et al., 2019). Thus, the economic contribution of pollinators can be highly variable and undervalued (Mallinger et al., 2019). The importance of wild pollinators, mainly wild bees, for sunflower pollination and productivity (DeGrandi-Hoffman and Watkins, 2000; Greenleaf and Kremen, 2006; Klein et al., 2007; Mallinger and Prasifka 2017a) may motivate pollinator conservation efforts in areas that depend on this service directly, such as agroecosystems. Even though the economic contribution of pollinators quantified here is considerably high, our estimates were based only on two regions and focused on only one pollinator-dependent crop. Consequently, the contribution of pollinators to the global economy is expected to be significantly higher (Potts et al., 2010). Further studies, including more assessments under natural field conditions and accounting for the variety, are necessary to fully quantify pollinators' contribution to food production.

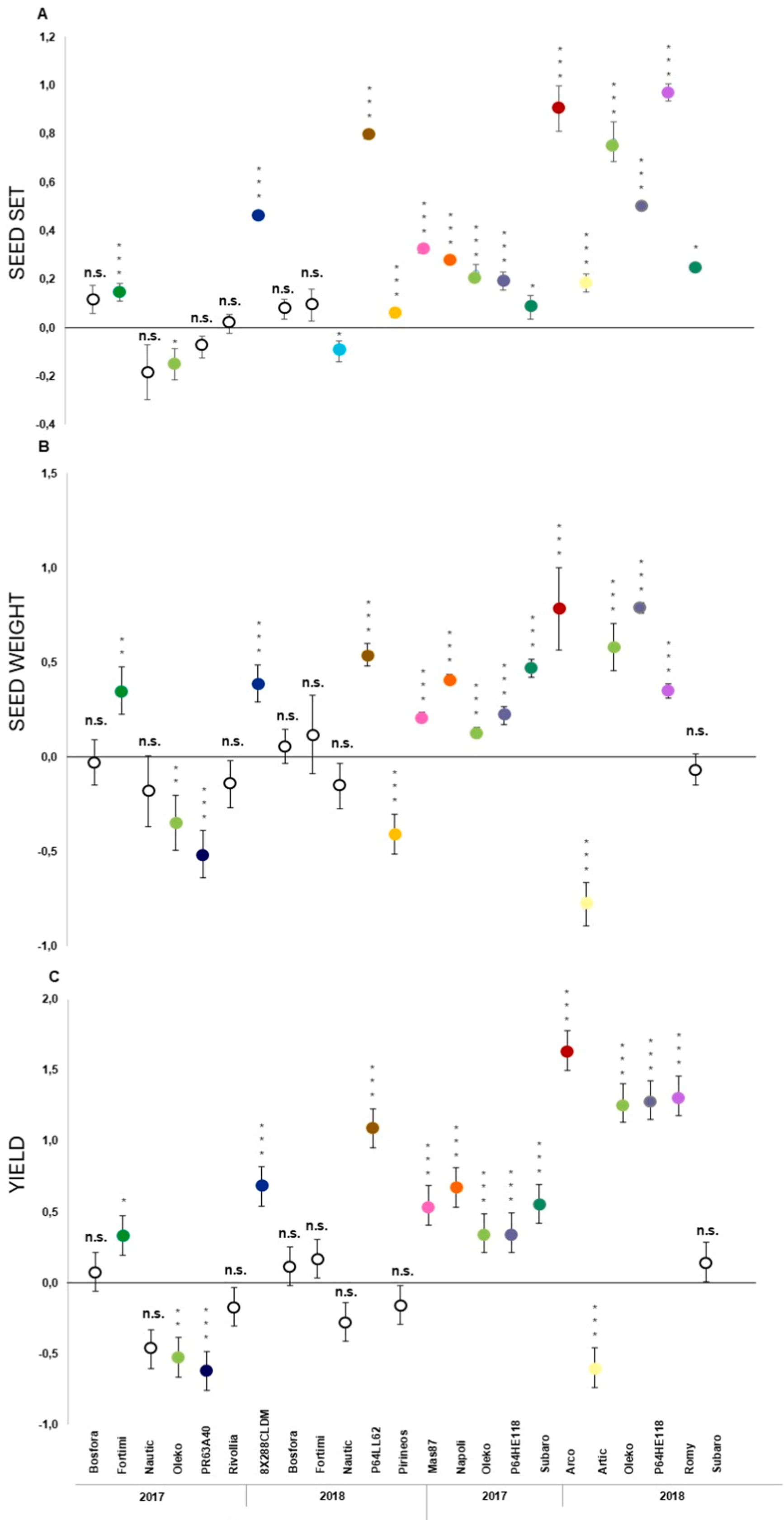

Fig. 5. Pollinator contribution (mean ± S.E. of the mean) for seed set (A), seed weight (B) and yield (C) for each sunflower variety. Pollinator contribution is given as R = ln (*treatment/control*), where *treatment* refers to the response variable (seed set, seed weight and yield) obtained in open pollination, and *control* refers to the mean value of the same response variable obtained in bagged treatment. Values significantly higher from zero indicate a positive effect of pollinator communities at a given location. *, ** and *** – significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively (also highlighted in bright colours); n.s. – non-significant at $p > 0.05$.

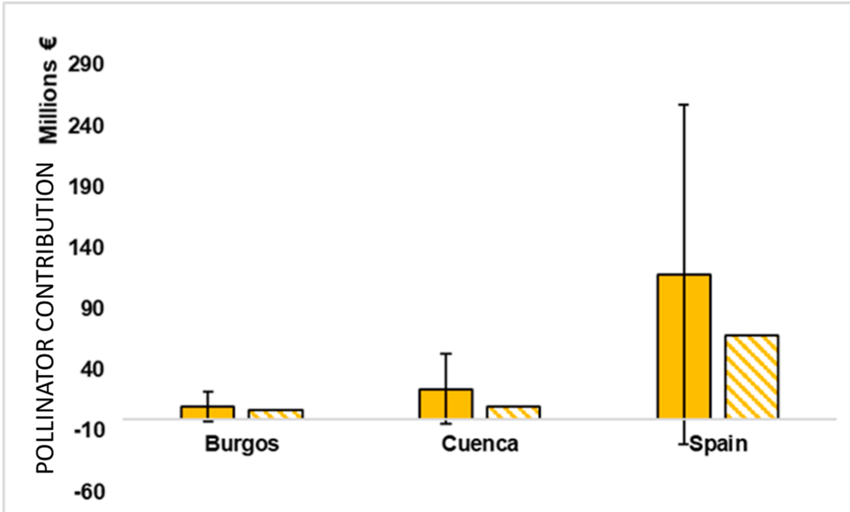

Fig. 6. Economic contribution of pollinators for sunflower productivity in Burgos, Cuenca and Spain, in Millions of Euros. Solid-filled bars indicate estimations based on field data, and are provided as estimated marginal means and 95 % confidence intervals; dashed bars indicate estimations based on Gallai et al. (2009).

4.4. Insights for policy making

Agri-environmental strategies have been proposed to improve pollinator populations, such as sowing floral mixtures and preserving natural and semi-natural vegetation patches near crop fields (Blaauw and Isaacs, 2014; Sutter et al., 2018; Albrecht et al., 2020; Mota et al., 2022). Despite the proven success of these actions, the effects on pollinators, pollination services and final crop yield may be observable only after two years of their implementation or longer, depending on the landscape context (Blaauw and Isaacs, 2014; Buhk et al., 2018; Albrecht et al., 2020; Mota et al., 2022). Thus, according to our results, in addition to the above-mentioned agri-environmental strategies, it is advisable to use self-fertile sunflower varieties while pollinator communities are growing and re-establishing in the agricultural area. This is particularly relevant in regions with more depauperated landscapes, such as Burgos, where the restoration of pollinator communities may take longer. Combining the preservation of natural and semi-natural vegetation patches and the implementation of wildflower strips near field crops, together with the selection of sunflower varieties which are self-fertile and less pollinator-dependent, at least during the first years of agricultural landscape restauration, can be a good agri-environmental strategy to maintain crop yield values.

5. Conclusions

The contrast between the decline of insect pollinators and the growing intensification of agroecosystems worldwide has led to the use of crop varieties less dependent on pollinators. However, in the case of most sunflower varieties tested here, production was lower in stronger self-fertile varieties than in ones that need outcrossing to produce seeds. In agreement with published findings (e.g., Gambín and Borrás, 2009; Sadras, 2007), a trade-off between seed number and seed weight was observed. In natural conditions, productivity also depended on the landscape context and pollinator communities, with pollinators contributing highly to the crop's economic outcome. In landscapes with intense agricultural use and fewer pollinators available, the cultivation of self-compatible varieties can be an excellent additional strategy to combine with the restoration of the agricultural landscapes, allowing the maintenance of crop yield and economic values. According to these results, further long-term studies are needed to understand better the reproductive behaviour of the sunflower varieties available in the market. By identifying the varieties that are able to produce seeds regardless of the pollinator communities and analysing the spatial landscape configuration, better agri-environmental strategies can be developed and locally tested to maintain crop productivity and the global economic outcome.

CRediT authorship contribution statement

Sílvia Castro: Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization. **Lucie Mota:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **José A. González:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **João Loureiro:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Jorge J. Ortega-Marcos:** Writing – review & editing, Methodology, Investigation, Data curation. **Violeta Hevia:** Writing – review & editing, Methodology, Investigation, Data curation. **Evan A.N. Marks:** Writing – review & editing, Project administration, Funding acquisition. **Carlos Rad:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition.

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Authors' contributions

All authors were involved in the design of the experimental field design and discussion of the results obtained; Lucie Mota and Sílvia Castro were involved in the experimental design in the BGUC; Lucie Mota was involved in the data collection and laboratory sample processing; Sílvia Castro, Lucie Mota, José González, Jorge J. Ortega-Marcos, Violeta Hevia performed data analyses; Lucie Mota with the help of João Loureiro and Sílvia Castro led manuscript writing, and all authors (including Carlos Rad and Evan A. N. Marks) contributed to the final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2024.109651.

Data availability

Data will be made available on request.

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